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7-8 July 1999, Manila Hotel

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CARRYING CAPACITY: FOOD PRODUCTION

The Philippines and Selected Asian Countries

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ABSTRACT

This paper reviewed and analyzed the indicators of carrying capacity within the context of the globe, Asia and the Philippines. It also extended the application of the methodology on subsistence level carrying capacity in the rice and corn producing sectors of the Philippines.

Given the dynamic and complex and nature of the population-natural resource/environment-development interactions, the paper recommends six imperatives that can assist the Philippines and Asian countries in dealing with the carrying-capacity issue. These include among others:

1. Creation of a Futures Center to coordinate futures oriented studies
2. Emphasis on the role of technology in alleviating poverty;
3. Incorporation of the carrying-capacity methodologies into the main stream of development/planning, policy analysis and formulation;
4. Setting up congruent policies related to carrying-capacity and the economy;
5. Development of an integrative development framework that can link the complex and dynamic interactions of carrying-capacity factors with the other determinants of sustainable human and national development; and
6. Special role of political governance to provide the political will in efficiently implementing administrative and legislative reforms.

Keywords: carrying capacity, population, demographics, food production, rice, corn, Philippines

1. INTRODUCTION

One of the most pressing, if not the most important issue confronting us today is food security. In fact, Lester Brown of the Worldwatch Institute argued that food scarcity will be the defining issue of the coming new era.

As early as the 1970s, one big question raised in "Probing our Futures: The Philippines 2000 A.D." posed a challenge to many: "Since the food problem is already critical today, will the world's seven billion people in year 2000 be adequately fed?" The study went on further to answer YES, if two considerations are met – food production triples and resources are more equitably distributed.

To respond to the challenge of emerging food crisis, the United Nations World Food Council pledged in 1974 to: "Create a world without hunger, a world in which no child would go hungry, no family needs fear its next day's bread, and no human being's future would be stunted by malnutrition."

We have seen through the past years how modern agricultural technology has somehow multiplied food production and eased the burden of hunger. However, the last two decades also showed that there is a growing imbalance between food and people. Everyday, some 219,000 people are added to the world's population (WHO, 1998). More so, there are almost one billion people in the world who go to bed hungry, who simply lack the means to purchase enough food (UNDP, 1995). The World Health Report also cited that among these people, around 19,000 among them, mostly infants and children die daily because of hunger and malnutrition.

But feeding these hungry people and the rest of the world's population does not only involve producing food. Man's prodigious need for food contributed to deforestation, soil degradation and species loss. The recent study of the Worldwatch Institute, "Beyond Malthus: Sixteen Dimensions of the Population Problem," revealed that the unbridled population growth, combined with the rising individual consumption, is pushing our claims on the planet beyond its natural limits. After nearly four decades of unprecedented expansion in both land-based and oceanic food supplies, the world is experiencing a massive loss of momentum.

People require at least 2,200 calories a day for metabolism and basic activities. Apparently, to meet these requirements, more food must be produced to respond to the need of the rising population. To produce more food, more resources are required. The combined effects of intensification and extensification, as estimated by the World Resources Institute (WRI, 1993), are that agricultural activities have removed about 15 percent of organic carbon from the world's soil. Some 70,000 sq. km. of farmland are being abandoned each year because of soil exhaustion (Preston, 1995).

In facing the challenge of food scarcity, Lester Brown's Tough Choices (1996) argued that the world is faced with at least six new constraints:

- The backlog of unused agricultural technology is shrinking, leaving the more progressive farmers fewer agronomic options for expanding output.
- Growing human demands are pressing against the limits of fisheries and the crop sector to supply beef, mutton, and milk.
- Demands for water are pressing against the limits of the hydrological cycle to supply irrigation water in key food growing regions.
- In many countries, the use of additional fertilizer on currently available crop varieties has little or no effect on yields

- Countries are already densely populated when they begin to industrialize risk losing cropland at a rate that exceeds the rise of land productivity, initiating a long-term decline in food production
- Social disintegration, often fed by rapid population growth and environmental degradation, is undermining many national governments and their efforts to expand food production.

Cognizant of this global problem, the international community, has held at least four international summits (during the 90s) to tackle and draw out solutions to these emerging global concerns—particularly that of food-population-environment problems. These summits include: The Earth Summit of 1992 or the UN Conference on Environment and Development (UNCED) held in Rio de Janeiro, Brazil; the International Conference on Population and Development (ICPD) in 1994 held in Cairo, Egypt; the World Summit for Social Development in 1995, in Copenhagen, Denmark; and the World Food Summit in 1996, held in Rome, Italy.

These past global summits all pointed out the urgent message for the need to reverse the negative current development trends in the world today, due to the interactions of population, environment, food (aqua-cultural) production and urbanization.

The world is indeed faced with a tough challenge of meeting the food requirements of the next generation without sacrificing mother earth. But we either **have to choose between the** *reproductive rights of the current generation* **and the** *survival rights of the next generation.*

This paper deals with the salient issues that await us all as we enter into the new millennium, particularly that of food-population-resources-environment problems. The objectives of this paper are:

1. To present the global development trends related to the environment, population, resources and food production;
2. To review the population/land carrying capacity methodology and compare selected carrying capacity indicators of the Philippines with other Asian countries; and
3. To recommend policy imperatives that can assist the Philippine and Asian countries in dealing with food carrying capacity.

2. CARRYING CAPACITY: THE BOTTOMLINE FOR POPULATION, RESOURCES, ENVIRONMENT AND DEVELOPMENT

Over the years, scientists have attempted several times to develop frameworks and models for examining the complex and dynamic relationship between population and the environment. The basic perspective probably was developed by Human Ecology through the works of Hawley (1950) and Duncan (1964). The basic assumption was that population constantly interacts with and adapt to their environments. This two-way adaptation is mediated by some form of organizations and technology. In

human ecology, this paradigm is known as population, organization, environment and technology or POET (Ness, 1994). However, despite the POET's paradigm, Ness further argued that there is no simple and direct relationship between population and environment. Identifiable forms of technology and social organizations mediate impact in both directions. It is only through this that either population or environment affect one another.

1.1 Defining Carrying Capacity

Biologists often apply the concept of "carrying capacity" to questions of population pressures on the environment. Carrying capacity is the largest number of any given species that a habitat can support indefinitely. When that maximum population level is surpassed, the resource base begins to decline—and sometime thereafter, so does the population (Postel, 1994).

An economist' perspective, Srinivasan (1988) viewed carrying capacity as the maximum population that can be sustained indefinitely in the future. But from the point of view of environmentalists Nebel and Wright (1998), the concept of carrying capacity refers to the number of a species that can be supported indefinitely without degrading the environment. They added that for human societies, it means the ability to meet food needs over the long term—that is, sustainably.

On the other hand, Brown (1994) cited that the earth's carrying capacity is shown by its capability to provide and sustain the basic needs of the present and future generation. He added that we are all depending on a finite environment where resources are easily depleted with an unabated use, hence the challenge lies on meeting present and future needs through sustainable resource utilization.

Biologist Garret Hardin, as cited by Nebel and Wright (1998), expressed that if ecology had a decalogue, the first commandment would be "Thou shall not transgress the carrying capacity."

2.3 Recent Developments and Models of Carrying Capacity*

2.3.1 Recent Developments

Modern thinking on the population-environment relationship reflects some continuity with Malthus' original formulation of population growth and environmental stress. A number of organizational, disciplinary, and methodological developments have both advanced and retarded systematic thinking about the population-environment relationship.

After the Second World War, the major issue that emerged was World Security. This was followed by massive physical reconstruction, which brought the creation of the International Bank for Reconstruction and Development (IBRD), popularly known as the World Bank; the Economic Commission for Europe (ECE) and the Economic Commission for Asia and the Far East (ECAFE).

*Condensed from Ness (1994).

By the 1960s, interest in development had increased considerably, partly legitimized by the agreement that security requires greater international cooperation. Thus, the United Nations Development Programme (UNDP), the Organization for Economic Cooperation and Development (OECD), and the U.S. Agency for International Development (USAID) were born. Later in 1965, when the population issue (family planning) started to emerge, the UN-ECAFE, now the Economic Commission for Asia and the Pacific (ESCAP), was created. This brought about large population programs mainly sponsored by the USAID.

As international population planning programs accelerated in the early 1970s, concerns for environmental degradation appeared in the horizon. This led to the creation of the United Nations Environment Program (UNEP) during the 1972 Stockholm Conference.

Thus, over the past four decades, both population and environment issues have come to occupy important and distinctive positions in the international and national arenas. Disciplinary development in both theory and methodology have paralleled organizational developments.

2.2.2 Theoretical and methodological developments

Demography has occupied the position of a special discipline for more than a century. In the West, it has developed principally within Sociology. During the 1960s, the developments of life tables and population theory which provided tools for population projections played an important role in linking population with other environmental issues. Improvements in observation and analysis technology in both coverage and accuracy have made demography a powerful analytical tool and had great impact on policy.

According to Ness, the theoretical methodological developments in environmental issues are much more difficult to document, primarily because the environment is so many things. It is located in a great variety of scientific disciplines, including agriculture, agronomy, atmospheric sciences, biology, forestry, geography, geology, limnology, meteorology, oceanography, physics, public health and zoology, plus all the social sciences. Each of these disciplines has developed its own specialized set of theories and methods. Each has also established a set of national and international organization that provide a political structure both binding the discipline together and cutting it off from others.

The observation from this near half-century of organizational, theoretical and methodological developments is twofolds. First, all of the individual disciplines have developed great powers of observation and analysis. Further, these analytical powers have often had substantial engineering potential, permitting us to intervene in human and natural processes with deliberate attempts to achieve highly specific goals. Sometimes, those goals have been laudable and sometimes the interrelations have been successful.

Second, however, the power of the disciplines has also made their practitioners unable, and often, unwilling, to attend to relevant developments in other disciplines.

Despite the deep divisions between population and environmental groups and disciplines, it is difficult to deny the relationship between population and environmental conditions in the real world. This empirical intrusion has led to some attempts to link the two in models and frameworks.

2.4 Carrying Capacity Models

There are at least six operational models that can illustrate the relationship between population and the environment. They are summarized below:

A. Bongaarts 1992. The model estimates the relative impact of population growth, GDP/cap, energy intensity and carbon intensity on CO_2 emissions and global warming. Bongaarts considers the world as a whole, then groups countries according to those with more and less developed economies. For time horizons, Bongaarts looks into the future, from 1985 to 2100.

B. Clark 1992. The Clark model also deals with the relative impact of population growth, GDP/capita, and energy intensity on CO_2 emissions. His analysis, however, examines the historical development in 12 countries over approximately the past 50 years.

C. Harrizon 1992. It presents a series of two sector calculations, using Commoner's 1972 Approach. Like Clark, Harrizon examines the relative impact of population growth, consumption, and technology on recent changes in a series of environmental conditions.

D. Meadows 1992. This is the updated WORLD3 model originally used in the 1972 Club of Rome's Limits to Growth study. It has five sectors, each with a number of indicators, dynamically related to each other with a range of positive and negative feedback loops. The study runs a number of extremely enlightening, different future scenarios.

E. IIASA. (International Institute of Applied Systems Analysis) presents a multisectoral work suggesting how multi-indicator societal, ecological, and economic subsystems are tied together. From this complex framework, a model of population and environment dynamics was developed specifically for Mauritius.

F. CIESIN. (Consortium for International Earth Science Information Network) is a multisectoral work for the human dimensions of global environmental change. It parallels the Brethernton "diagram" of atmospheric, oceanic, and terrestrial relations, which gave human activities the single small black box. The new CIESIN framework has been illustratively applied to sea level rise, human migration, and energy consumption.

The first five models are formal statements about population-environment relations, including data and calculations. The first three all attempt to estimate the relative impacts of population growth, technology, and consumption on one single environmental condition. These are all simple models in that they do not consider feedback process or linkages among the conditions that impact the environment. All

of these simple models reflect the basic human ecology proposition that some form of technology and organization mediate all population-environment relationships.

The fourth model is the more sophisticated, multisector dynamic model, WORLD3 used in the Limits to Growth study published in 1972. Meadows et al recently reexamined and slightly revised the model in a new edition. Beyond the Limits (1992).

The fifth is the IIISA model, being applied to Mauritius. This it the most developed of all the models and is probably the most appropriate for the more systematic empirical research on the problem.

The sixth and final model is a sophisticated multi-sector framework, from which we can work out options in specific sectors or arenas. In all of these multisectoral models, we can differentiate both population and environment by a number of characteristics. This will lead to much greater potential in tracing more complex connections.

2.5 Applications of Carrying Capacity (C-C) Models in the Philippines

During the past two decades, two C-C models were undertaken in the Philippines. One was national in scope and the other at a provincial level. The national C-C study was undertaken in 1978 under the "Population Resources Environment and the Philippine Futures (PREPF)), project, implemented jointly by the Development Academy of the Philippines (DAP), University of the Philippines School of Economics (UPSE), and the UP Population Institute (UPPI). The other Philippine C-C model was done in 1988 in the province of Palawan by the group of Dr. Candido Cabrido of the Population/Development Planning and Research Project of the National Economic Development Authority (NEDA). Salient points of these C-C models are discussed below.

2.5.1 Probing our futures: The Philippines 2000 A.D.

This study was perhaps the most pioneering C-C model of the Philippines in trying to project the World Situation and the Philippine Futures by the year 2000 using baseline data of 1975-1977, with the integrated components of population dimensions (fertility, mortality, spatial mobility, labor force and family formation); natural resources; education, nutrition and health; and income distribution. PREPF was a forward looking research that tried to identify not only historical trends and their implications for the future but also a feasible set of alternatives, preferred futures for the next Filipino generation, constrained only by the bias that scenarios of the future should be definite improvements over those of the present.

The major lessons learned from PREPF project was not the absolute accuracy of the projections made but rather the collective research efforts and the independent approach in delving into an exercise of projecting the futures under a Martial Law Regime. It was very "politically constraining" then to undergo into research activities

that might offend the regime. However, the research team of PREPF was able to forge a workable dialogue with the policy-makers at that time.

The recommendations of the PREPF study which are summarized below are still relevant to date.

A. A Futures Research Center

PREPF recommends setting up a national office to coordinate futures-oriented studies at the national level. The suggestion must also be broached to other ASEAN governments to consider setting up a similar center for futures-oriented studies at the regional level.

B. Politics

B.1 PREPF, in anticipation of the nation-state remaining as the viable international political unit till the year 2000, recommends the development of strong, viable nation-states through programs for political integration, social cohesion, and national development.

B.2 PREPF also anticipates increasing regional cooperation till the year 2000 and thus recommends increasing regional integration among nations.

B.3 Spell out policy packages based on empirical knowledge and the socio-political system.

C. Population

Population regulation policies should go hand in hand with a restructuring of the national society towards improving the whole society's quality of life.

D. Human Resources Development

D.1 Further studies on the objectives and context of our educational efforts, the efficient curricula, delivery systems, and the democratization of access to education.

D.2 The components of the education supplied should be closely matched to what are demanded. The work of the NMYC-Industrial Board (now TESDA Board) which links vocational training outside the school system to industry should be encouraged and expanded.

D.3 The self-employment assistance program for marginal workers, such as out-of-school youth, should be strengthened.

D.4 Equitable distribution for the opportunities of college education allowing the elite to produce a leadership whose values are consistent with those of society.

D.5 Categories of education (other than the conventional degrees in specific fields) should be demand driven.

E. Energy

E.1 In line with the national policy of self-reliance, policies on energy should be calculated to lessen the dependence on imported fossil fuel by accelerating the development of alternative energy resources (e.g., geothermal, solar, wind, nuclear, etc.) and encouraging the present national thrust towards oil exploration and development.

E.2 An important mechanism which can be used to moderate demand is the price system. Existing policies on price and tax schemes for petroleum products, other energy inputs, and energy-using equipment should be reviewed.

E.3 The growth of the private transport industry, the biggest energy-using sector, should be tempered to support the pollution control policies of the government. The mass transit system, being still the best alternative available to the car-riding public, should be seriously considered.

E.4 The proposal to build additional nuclear power plants to meet part of future power requirements should be re-evaluated in light of the large outlay of money that will be incurred and the dangers involved. Proper harnessing of the country's hydro resources will suffice to meet future power requirements.

F. Trade

F.1 Eliminate or reduce tariff and non-tariff barriers to products from less developed countries.

F.2 Diversify and expand sources of imports and markets for exports. Further develop tourism.

F.3 Encourage the export of labor until such time as the Philippine economy can absorb the labor supply.

F.4 Enhance the spatial mobility of the population, both within the country and abroad.

F.5 More research is needed on what would be a favorable commodity structure for the national economy.

G. Food

G.1 Greater efforts should be made towards the equitable distribution of food resources on both the national and international levels.

G.2 Philippine self-sufficiency in food should be achieved and maintained.

G.3 Health and nutrition programs should stress not only access to food but, more importantly, the mental development of young people.

H. Forestry

H.1 Priority should be given the reforestation of regions which are most deficient in forest cover and where serious water supply problems are occurring or are expected in the near future.

H.2 Multiple-use forest management should be pursued.

I. Fisheries

I.1 Technology diffusion (transfer) in fisheries should be carefully assessed and improved so that fishermen/fish farmers can avail of technological know-how to increase their production. Research on fishing gear technology and fishing boat construction should be pursued.

I.2 A Study of the microeconomics of important fishing gears and their relation to fishery resources in the country's different regions would be most useful.

I.3 One way to improve production is to encourage fishermen to undergo training in proper vessel and gear operation and fish catch handling and preservation. Fishermen's training centers should be put up in strategic regions of the country.

I.4 Extension service for and training of fishermen will have to be planned and implemented to help small-scale fishermen develop skills appropriate for the type of fishery obtaining in their own villages. Fishermen's associations and cooperatives can also help fishermen process and market their catches, and secure bank credit and government subsidy. If fishermen are properly organized, they can undertake fishing ventures on a commercial scale.

2.5.2 The Palawan C-C Model*

The Palawan C-C Model was a research activity under the Population/ Development Planning and Research Project (PDPRP) funded by the UNFPA. The major objective of the project was to integrate population with development planning. Under the PDPRP, three methods were developed to assess the population-carrying capacity of Palawan. These were: real demand, effective demand and income subsistence need (Cabrido, 1988).

Cabrido (1988) outlined four general procedures in assessing these three methods. These are:

a) *Classification of the ecosystems into major and subtypes on vegetation cover slope and dominant land use.* Ecosystems in the study were broadly classified into terrestrial and aquatic. Terrestrial ecosystems were then categorized into agroecosystem, forest ecosystem and urban ecosystem, while aquatic ecosystems were broken down into marine and freshwater categories. All areas considered too steep (30% slope) and marginal lands for crop cultivation were delineated for forestry. Future growth and expansion of urban areas were projected based on present trends and

* Two publications were reviewed for the Palawan C-C Model. These were: Candido Cabrido, Jr. 1988. Methods for determining the Population-Supporting Capacity of Ecosystems: Palawan Province NEDA, Manila; Candido Cabrido, Jr. 1994. Integration of Population Dimension in the Environment and Natural Resources Management Sector: Planning Framework, Tools, Techniques and Illustration Cases. NEDA, Manila. Needless to say, the discussions on this section were basically taken from these two research reports.

delineated as built-up areas. After deducting forest and urban areas, the remaining areas were assessed according to their land suitability.

b) *Assessment of the extent, suitability, yield and production of the various ecosystems.* The assessment focused on the lowland agroecosystem, both irrigated and rainfed, and the upland agroecosystem. Areas with slopes of 9% and 30% and elevation of more than 100 meters were considered as uplands. Two crops were studied: rice and corn. Potential grazing lands were likewise considered in the study. For the aquatic ecosystem, the fisheries production of marine, river and lake ecosystems was determined.

c) *Estimation of the potential net sustainable production of tile agroecosystems under different levels of inputs.* Sustainable production is considered in this study as the production level which will not harm the inherent productivity of the ecosystem (i.e., the rate of resource exploitation is equal to the rate of resource regeneration or renewal either through natural or artificial means). Net sustainable production was calculated by deducting from the sustainable production the amount of losses and wastage brought about by ecological stresses such as drought, flooding, typhoons, pests and diseases, water pollution and sedimentation, and other related factors. Likewise, losses caused by harvesting and post harvesting activities were accounted for.

d) *Calculation of the human population-carrying capacity of the potential net sustainable production.* Human population-carrying capacity of food was estimated under three separate considerations: real demand, effective demand and subsistence level. As a principle, food production should meet the standards of all three indices. Real demand is the minimum nutritional food requirements of the population whether they can buy it or not. Effective demand is the market demand for a particular type of food. Subsistence level is the level just above the poverty line (or poverty threshold level)—- when family income is just adequate to meet its basic needs.

Human population-carrying capacity in terms of real demand was calculated by deriving the calorie and protein values of the total output of food products, and dividing this with the calorie and protein requirements of the population.

Human population-carrying capacity in terms of effective demand was computed by determining the actual per capita consumption (in kilogram per unit time) of a particular food product, and using this figure to divide the total volume (i.e., net sustainable production) of the particular food product.

Human population-carrying capacity in terms of subsistence level was estimated by dividing the net income obtained from the sustainable production by the family's consumption cost, plus an allowance of 10-15

percent for savings. A positive ratio of one indicates that subsistence level is met. The subsistence level is established by using the poverty threshold level.

After assessing the income population-carrying capacity, the optimum population size, optimum population density and optimum farm size were determined. On the other hand, the results of the food population-carrying capacity determination provided the optimum crops (i.e., high energy crops) that are most ideal to grow in an area.

Some of the empirical findings of the Cabrido study include an estimation of the net sustainable production of lowland agroecosystems **(Table 2.1)** of Palawan; The sustainable population size and density and sustainable farm size for the different agroecosystems **(Table 2.2)**; Existing and population ceilings of the different agroecosystems **(Table 2.3)** and Population-supporting capacity of the agroecosystems in terms of real and effective demand **(Table 2.4)**

The calculations were also made for the rangeland and aquatic ecosystems. From this comprehensive study, Cabrido concluded the following:

a) Real demand and income population-supporting capacity are useful in monitoring agroeconomic and agroecological conditions, since they provide reliable indicators of the population of a certain area.
b) When the income supporting capacity is met while real demand lags behind this could mean any or several of the following: low production of food crops, high consumption (population growth rate exceeds population growth), high government subsidy, and/or shift from food to cash/ commercial crops. In this regard, there is a need to examine the existing policies/laws on food production, zoning and agricultural land use conversion.
c) Population-carrying capacity depends on the level of technology and management of inputs.
d) The results of the study indicate that the retention limit imposed by the Comprehensive Agrarian Reform Program (CARP) may create social inequities in some areas.
e) The income-population-carrying capacity of the lake and river ecosystems of Palawan is inadequate to sustain its present populations.
f) Overall, the aquatic ecosystem of Palawan has vast potential for supporting its projected population.
g) The accuracy of the results of the income population carrying capacity assessment depends greatly on the accuracy of farm budget data.
h) Palawan's uplanders could not be able to subsist if they were to depend mainly on their principal crops (rice and corn) in view of the low yield.
i) The study has shown that the methods developed could be put into practical use by the agroecological, land use and environmental planners for provincial, regional, national-level planning.

Table 2.1 Net Sustainable Production of Lowland and Upland Agroecosystems of Palawan Province

Agroecosystem	Dominant land use	Suitable area(ha)	Yield (t/ha)	No. of croppings per year	Total net sustainable prod.(tons)+
Lowland		136,459			
Irrigated	Rice	36,000	5.25*	2.0	378,000
			2.5**	2.0	180,000
Rainfed	Rice	100,459	5.25*	1.0	527,409
			1.6**	1.0	160,734
Irrigated	Corn	36,000	5.0t	2.0	360,000
			2.0*	2.0	144,000
Rainfed	Corn	100,459	5.0*	1.0	502,295
			1.5**	1.0	150,688
Upland		322,052			
	Rice	322,052	3.5*	1.0	1,127,182
			0.8**	1.0	257,641
	Corn	322,052	3.5*	1.0	1,127,182
			1.0**	1.0	322,052

* Potential or attainable yield
** Existing yield level
\+ Total net sustainable production was estimated on the basis of sustainable yield and number of sustainable croppings per year. sustainable yield and cropping intensity were calculated taking into account fallow period requirements and losses due to ecological constraints such as moisture and temperature stress, pests and diseases, and soil erosion.
Source: Cabrido, 1988

j) After further refinement and computerization of the income-population carrying capacity method, it is recommended that its application be expanded to cover other major crops such as sugar cane, coconut, cassava, sweet potato, vegetables and others.

2.5.3 Extending C-C methodology: The role of technology

The methodology developed by Cabrido was extended to estimate the human population-capacity in terms of subsistence level for rice and corn nationwide. The data used in the estimation came from the 1998-1999 Farming Systems Surveys of SIKAP-STRIVE Foundation.

The respondents in these surveys were classified into different levels of technology using per hectare yield as indicators: low technology means yields of less than 3 mt/ha; medium technology, from greater than 3 m.t./ha to less than 5 m.t./ha; and high technology, yields with 5.0 mt/ha and above.

Table 2.2 Sustainable Population Size and Density and Sustainable Farm Size for the Different Agroecosystems of Palawan Province: Critical and Optimum Levels

Agroecosystem	Net income (Plhalyr)	Sustainable population (no. of families)				Sustainable farm size (ha/family)	
		SIZE		DENSITY			
		C/MS	OS	C/MS	OS	C/MNS	OS
Lowland							
Irrigated rice	18854 *	21895	16421	0.6	0.45	1.64	2.87
	3344 **	3883	2912	0.1	0.08	9.27	16.2
Rainfed rice	9704 *	31450	23587	0.31	0.23	319	5.58
	(558) **	op	op	op	op	op	op
Irrigated corn	18236 *	21177	15882	0.58	0.44	1.69	2.95
	2636 **	3061	2295	0.08	0.06	11.7	20.6
Rainfed corn	9118 *	29548	22161	0.29	0.22	3.39	5.93
	17 **	58	43	0.0006	0.0004	1732	3031
	2499 ***	8101	6075	0 08	0.06	1.2	21
Upland							
Rice	6370 *	66176	49632	0 20	0.15	4.86	8.5
	(1244) **	op	op	op	op	op	op
Corn	6600 *	68565	51423	0.21	0.16	4.69	8.2
	100 **	1038	778	0.0032	0.0024	310	542

* Based on potential or attainable yield
** Based on existing yield level
*** Based on existing yield and low cost of production/investment
op overpopulated, an indication of the nonprofitability of culti-vating a given crop under a particular farming system
C/MS critical or maximum size
C/MNS critical or minimum size
OS optimum size
Source: Cabrido, 1988

The farm budgets were also generated by technology and net farm incomes by technology, were calculated using the standard cost and returns methodology. Average landholdings and cropping intensity were also based on the surveys while the subsistence level expenditures (Poverty threshold) by region came from the National Economic Development Authority.

Extending the formula of Cabrido (1988), the index of C-C at subsistence level for a crop was estimated as:

$$C\text{-}C = \frac{NFlit \times L \times Cl}{1.15 \times SE}$$

Table 2.3 Existing and Ceiling Population Levels of the Different Agroecosystems of Palawan Province

Agroecosystem	Present population		Ceiling population (no.of persons)	Optimum pop. size (no. of persons)
	(no. of families)	(no. of persons)		
Lowland				
Irrigated rice	6,700	40,200	131,370	98,527
Rainfed rice	24,100	144,600	188,700	141,525
Irrigated corn	6,700	40,200	127,062	95,296
Rainfed corn	24,100	144,600	177,288	132,960
Subtotal				
Rice	30,800	184,800	320,070	240,052
Corn	30,800	184,800	304,350	228,256
Upland				
Rice	4,250	25,500	397,056	297,792
Corn	4,250	25,500	411,390	308,542
Total				
Rice	31,250	210,300	717,126	537,844
Corn	31,250	210,300	715,740	536,798

Source: Cabrido, 1988

Where: C-C = carrying capacity at subsistence level
NFI = net farm income
L = average landholdings
CI = cropping intensity
SE = subsistence level expenditures or poverty threshold by region plus a 15% savings
i,t = province and technology

Intuitively, if the C-C ratio is greater than one, it implies that the subsistence level was met. Failure to meet subsistence level will result to impoverishment, impelling the population to exhaust the productivity of the ecosystem.

Preliminary results of the estimations are shown in **Tables 2.5** to **2.11** for rice and **Tables 2.12** to **2.14** for corn.

Table 2.4 Population-Supporting Capacity of the Different Agroecosystems of Palawan Province: Real and Effective Demand

Agroecosystem	Population-supporting capacity (no. of persons)			
	Real demand*		Effective demand**	
			Present	Projected
Lowland				
Irrigated rice	1) 1,203,904	1)	2,268,818	2,224,384
	2) 573,287	2)	1,251,818	1,059,230
Rainfed rice	1) 1,679,757	1)	3,667,881	3,103,592
	2) 511,924	2)	1,117,827	945,853
Irrigated corn	1) 500,996	1)	275,400,000	68,850,000
	2) 200,398	2)	110,160,000	27,540,000
Rainfed corn	1) 699,020	1)	384,255,000	96,063,750
	2) 209,705	2)	115,276,000	28,819,000
Subtotal				
Rice	1) 2,883,661	1)	6,296,699	5,327,976
	2) 1,085,211	2)	2,369,645	2,005,083
Corn	1) 1,200,016	1)	659,655,000	164,913,750
	2) 410,103	2)	225,436,000	56,359,000
Upland				
Rice	1) 3,589,996	1)	7,839,036	6,633,030
	2) 820,567	1)	1,791,772	1,516,115
Corn	1) 1,568,649	1)	862,294,000	215,573,500
	2) 448,184	2)	246,369,000	61,592,250
TOTAL				
Rice	1) 6,473,657	1)	14,135,735	11,961,006
	2) 1,905,778	2)	4,161,417	3,521,198
Corn	1) 2,768,665	1)	1.52×10^9	3.8×10^8
	2) 858,287	2)	4.72×10^8	1.79×10^8

1) Potential or attainable yield
2) Existing yield level
* Calorie basis
** Present actual per capita consumption per year: rice -110 kg; corn - 1 kg.
Projected consumption of rice is estimated tobe 130 kg/capita/year; corn - 4 kg/capita/year.
Calorie values used: rice= 341 kilocalories per 100 grams; corn= 149 kilocalories per 100 grams.
Protein valuesused: rice= 6.9 grams per 100 grams; corn=4.2 grams per 100 grams.
Source: Cabrido, 1988

Table 2.5. Rice Carrying Capacity In terms of Subsistence Level, Ilocos Norte, 1998.

TECHNOLOGY	Net Farm Income[a] (P/ha/season)	Average Land-holding[a]	Cropping Intensity[a]	Regional Poverty Threshold[b] (Annual)	Carrying Capacity in Terms of Subsistence Level[c]
IRRIGATED					
Transplanted					
Low Yield[d]	2,666	1.50	2.00	59,905	0.15
Medium Yield[e]	6,827	1.50	2.00	59,905	0.39
High Yield[f]	21,557	1.50	2.00	59,905	1.24
All Yield Levels	10,350	1.50	2.00	59,905	0.60
Direct					
Low Yield[d]					
Medium Yield[e]					
High Yield[f]					
All Yield Levels					
NON-IRRIGATED					
Transplanted					
Low Yield[d]	112	1.50	1.00	59,905	0.00
Medium Yield[e]	12,880	1.50	1.00	59,905	0.37
High Yield[f]	24,194	1.50	1.00	59,905	0.70
All Yield Levels	12,395	1.50	1.00	59,905	0.36
Direct					
Low Yield[d]					
Medium Yield[e]					
High Yield[f]					
All Yield Levels					

a Source: SIKAP/STRIVE Rice-Based Farming Systems Survey, December 1998 to March 1999
b annual per capita threshold x family household size of 5; Source: Statistical Yearbook, 1998
c (net farm income x average landholding x cropping intensity)/(regional poverty threshold + 15% savings)
d with palay yield of less than 3.0 mt/ha/season
e with palay yield of 3.0 but less than 5.0 mt/ha/season
f with palay yield of greater than 5.0 mt/ha/season

Rice

Six rice producing provinces (Ilocos Norte, Pangasinan, Isabela, Nueva Ecija, Iloilo, and North Cotabato) were subjected to the C-C analysis here. The rice production systems during the main crop year 1998-1999, were classified into water regimes (irrigated, rainfed) crop establishments (transplanted and direct seeded) and levels of technology (low, medium and high). The survey also provided the data on average landholdings per household and cropping intensity. Finally, the regional poverty

threshold level, i.e., the minimum household income to satisfy nutritional requirement of 2,000 calories per capita for a household size of five, came from NEDA.

Results from the estimates showed that in Ilocos Norte (Table 2.5), only those households with high yields from irrigated and transplanted rice had income that can sustain their minimum household subsistence requirements. This can be attested by the ratio of 1.24.

This same pattern was demonstrated in Pangasinan, except that direct seeded irrigated and transplanted technology rice were also economically sustainable with ratio of annual net farm income to poverty threshold of 1.28 (Table 2.6).

Table 2.6. Rice Carrying Capacity In terms of Subsistence Level, Pangasinan, 1998.

TECHNOLOGY	Net Farm Income[a] (P/ha/season)	Average Land-holdings[a] (ha)	Cropping Intensity[a]	Regional Poverty Threshold[b] (Annual)	Carrying Capacity in Terms of Subsistence Level[c]
IRRIGATED					
Transplanted					
Low Yield[c]	4,999	1.84	2.00	59,905	0.35
Medium Yield[d]	11,457	1.84	2.00	59,905	0.81
High Yield[e]	17,426	1.84	2.00	59,905	1.23
All Yield Levels	11,294	1.84	2.00	59,905	0.80
Direct					
Low Yield[c]					
Medium Yield[d]	18,137	1.84	2.00	59,905	1.28
High Yield[e]					
All Yield Levels					
NON-IRRIGATED					
Transplanted					
Low Yield[c]	304	1.84	1.00	59,905	0.01
Medium Yield[d]	12,241	1.84	1.00	59,905	0.43
High Yield[e]					
All Yield Levels	6,273	1.84	1.00	59,905	0.22
Direct					
Low Yield[c]					
Medium Yield[d]					
High Yield[e]					
All Yield Levels					

a Source: SIKAP/STRIVE Rice-Based Farming Systems Survey, December 1998 to March 1999
b annual per capita threshold x family household size of 5; Source: Statistical Yearbook, 1998
c (net farm income x average landholding x cropping intensity)/(regional poverty threshold + 15% savings)
d with palay yield of less than 3.0 mt/ha/season
e with palay yield of 3.0 but less than 5.0 mt/ha/season
f with palay yield of greater than 5.0 mt/ha/season

In Isabela, the irrigated, transplanted rice production systems from medium to high yields, had ratios of 1.5 and 2.26 respectively (Table 2.7). Incomes from non-irrigated transplanted rice were not sufficient to sustain expenditures higher than the household poverty threshold of P49, 365 per annum.

Nueva Ecija (Table 2.8) on the other hand, had high yield irrigated rice technology incomes that can cover subsistence level expenditures; Iloilo incomes from medium and high technology direct seeded irrigated and transplanted rainfed rice production had ratio greater than one; North Cotabato, only high yield irrigated transplanted had

Table 2.7. Rice Carrying Capacity In terms of Subsistence Level, Isabela, 1998.

TECHNOLOGY	Net Farm Income[a] (P/ha/season)	Average Land-holding[a]	Cropping Intensity[a]	Regional Poverty Threshold[b] (Annual)	Carrying Capacity in Terms of Subsistence Level[c]
IRRIGATED					
Transplanted					
Low Yield[c]	2,164	2.50	2.00	49,365	0.25
Medium Yield[d]	13,208	2.50	2.00	49,365	1.54
High Yield[e]	19,406	2.50	2.00	49,365	2.26
All Yield Levels	11,593	2.50	2.00	49,365	1.35
Direct					
Low Yield[c]					
Medium Yield[d]					
High Yield[e]					
All Yield Levels					
NON-IRRIGATED					
Transplanted					
Low Yield[c]	2,909	2.50	1.00	49,365	0.17
Medium Yield[d]	11,075	2.50	1.00	49,365	0.65
High Yield	17,019	2.50	1.00	49,365	0.99
All Yield Levels	10,334	2.50	1.00	49,365	0.60
Direct					
Low Yield[c]					
Medium Yield[d]					
High Yield[e]					
All Yield Levels					

[a] Source: SIKAP/STRIVE Rice-Based Farming Systems Survey, December 1998 to March 1999
[b] annual per capita threshold x family household size of 5; Source: Statistical Yearbook, 1998
[c] (net farm income x average landholding ₹ cropping intensity)/(regional poverty threshold + 15% savings)
[d] with palay yield of less than 3.0 mt/ha/season
[e] with palay yield of 3.0 but less than 5.0 mt/ha/season
[f] with palay yield of greater than 5.0 mt/ha/season

Table 2.8. Rice Carrying Capacity In terms of Subsistence Level, Nueva Ecija, 1998.

TECHNOLOGY	Net Farm Income[a] (P/ha/season)	Average Land-holding[a]	Cropping Intensity[a]	Regional Poverty Threshold[b] (Annual)	Carrying Capacity in Terms of Subsistence Level[c]
IRRIGATED					
Transplanted					
Low Yield[c]	(3,720)	2.50	2.00	64,185	(0.33)
Medium Yield[d]	2,457	2.50	2.00	64,185	0.22
High Yield[e]	24,531	2.50	2.00	64,185	2.20
All Yield Levels	7,756	2.50	2.00	64,185	0.69
Direct					
Low Yield[c]	(2,101)	2.50	2.00	64,185	(0.19)
Medium Yield[d]	5,132	2.50	2.00	64,185	0.46
High Yield[e]					
All Yield Levels	1,516	2.50	2.00	64,185	0.14
NON-IRRIGATED					
Transplanted					
Low Yield[c]	240	2.50	1.00	64,185	0.01
Medium Yield[d]	8,787	2.50	1.00	64,185	0.39
High Yield[e]					
All Yield Levels	4,514	2.50	1.00	64,185	0.20
Direct					
Low Yield[c]	283	2.50	1.00	64,185	0.01
Medium Yield[d]					
High Yield[e]					
All Yield Levels					

[a] Source: SIKAP/STRIVE Rice-Based Farming Systems Survey, December 1998 to March 1999
[b] annual per capita threshold x family household size of 5; Source: Statistical Yearbook, 1998
[c] (net farm income x average landholding x cropping intensity)/(regional poverty threshold + 15% savings)
[d] with palay yield of less than 3.0 mt/ha/season
[e] with palay yield of 3.0 but less than 5.0 mt/ha/season
[f] with palay yield of greater than 5.0 mt/ha/season

ratio greater than one; while South Cotabato's net farm incomes from medium technology irrigated and non-irrigated rice production, and high technology irrigated production system were higher than subsistence level expenditures.

In summary, from the rice data analyzed, it appears that only adoptors of the rice high technology yield in favorable areas (irrigated) had higher chance to generate net farm incomes to cover poverty threshold expenditures.

Corn

For corn, the same pattern demonstrated by the rice production systems across provinces was also manifested by the corn household samples. It was relatively more feasible to generate net farm incomes higher than the poverty threshold incomes, if the corn producing households adopt the production technology which can provide them with higher per hectare yield **(Tables 2.12 to 2.14).**

Table 2.9. Rice Carrying Capacity In terms of Subsistence Level, Iloilo, 1998.

TECHNOLOGY	Net Farm Income[a] (P/ha/season)	Average Land-holding[a]	Cropping Intensity[a]	Regional Poverty Threshold[b] (Annual)	Carrying Capacity in Terms of Subsistence Level[c]
IRRIGATED					
Transplanted					
Low Yield[e]	(321)	3.00	2.00	52,790	(0.04)
Medium Yield[d]	7,555	3.00	2.00	52,790	0.99
High Yield[e]					
All Yield Levels	3,617	3.00	2.00	52,790	0.47
Direct					
Low Yield[e]	2,213	3.00	2.00	52,790	0.29
Medium Yield[d]	10,281	3.00	2.00	52,790	1.34
High Yield[e]	18,246	3.00	2.00	52,790	2.38
All Yield Levels	10,247	3.00	2.00	52,790	1.34
NON-IRRIGATED					
Transplanted					
Low Yield[e]					
Medium Yield[d]	7,131	3.00	1.50	52,790	0.70
High Yield[e]	14,381	3.00	1.50	52,790	1.41
All Yield Levels	10,756	3.00	1.50	52,790	1.05
Direct					
Low Yield[e]	1,814	3.00	1.50	52,790	0.18
Medium Yield[d]					
High Yield[e]					
All Yield Levels					

[a] Source: SIKAP/STRIVE Rice-Based Farming Systems Survey, December 1998 to March 1999
[b] annual per capita threshold x family household size of 5; Source: Statistical Yearbook, 1998
[c] (net farm income x average landholding x cropping intensity)/(regional poverty threshold + 15% savings)
[d] with palay yield of less than 3.0 mt/ha/season
[e] with palay yield of 3.0 but less than 5.0 mt/ha/season
[f] with palay yield of greater than 5.0 mt/ha/season

Table 2.10. Rice Carrying Capacity In terms of Subsistence Level, North Cotabato, 1998.

TECHNOLOGY	Net Farm Income[a] (P/ha/season)	Average Land-holding[a]	Cropping Intensity[a]	Regional Poverty Threshold[b] (Annual)	Carrying Capacity in Terms of Subsistence Level[c]
IRRIGATED					
Transplanted					
Low Yield[c]	2,273	1.50	2.00	55,775	0.14
Medium Yield[d]	8,697	1.50	2.00	55,775	0.54
High Yield[e]	16,495	1.50	2.00	55,775	1.02
All Yield Levels	9,155	1.50	2.00	55,775	0.57
Direct					
Low Yield[c]	53	1.50	2.00	55,775	0.00
Medium Yield[d]	12,716	1.50	2.00	55,775	0.79
High Yield[e]	15,119	1.50	2.00	55,775	0.94
All Yield Levels	9,296	1.50	2.00	55,775	0.58
NON-IRRIGATED					
Transplanted					
Low Yield[c]	(2,343)	1.50	1.00	55,775	(0.07)
Medium Yield[d]					
High Yield[e]					
All Yield Levels					
Direct					
Low Yield[c]					
Medium Yield[d]					
High Yield[e]	20,584	1.50	1.00	55,775	0.64
All Yield Levels					

[a] Source: SIKAP/STRIVE Rice-Based Farming Systems Survey, December 1998 to March 1999

[b] annual per capita threshold x family household size of 5; Source: Statistical Yearbook, 1998

[c] (net farm income x average landholding x cropping intensity)/(regional poverty threshold + 15% savings)

[d] with palay yield of less than 3.0 mt/ha/season

[e] with palay yield of 3.0 but less than 5.0 mt/ha/season

[f] with palay yield of greater than 5.0 mt/ha/season

Table 2.11. Rice Carrying Capacity In terms of Subsistence Level, South Cotabato, 1998.

TECHNOLOGY	Net Farm Income[a] (P/ha/season)	Average Land-holding[a]	Cropping Intensity[a]	Regional Poverty Threshold[b] (Annual)	Carrying Capacity in Terms of Subsistence Level[c]
IRRIGATED					
Transplanted					
Low Yield[c]	222	2.50	2.00	52,445	0.02
Medium Yield[d]	11,076	2.50	2.00	52,445	1.21
High Yield[e]	21,129	2.50	2.00	52,445	2.32
All Yield Levels	10,809	2.50	2.00	52,445	1.19
Direct					
Low Yield[c]	1,011	2.50	2.00	52,445	0.11
Medium Yield[d]	8,619	2.50	2.00	52,445	0.94
High Yield[e]	19,487	2.50	2.00	52,445	2.14
All Yield Levels	9,706	2.50	2.00	52,445	1.06
NON-IRRIGATED					
Transplanted					
Low Yield[c]					
Medium Yield[d]	18,075	2.50	1.50	52,445	1.49
High Yield[e]					
All Yield Levels	18,075	2.50	1.50	52,445	1.49
Direct					
Low Yield[c]	2,887	2.50	1.50	52,445	0.24
Medium Yield[d]					
High Yield[e]	24,005	2.50	1.50	52,445	1.97
All Yield Levels	13,446	2.50	1.50	52,445	1.11

[a] Source: SIKAP/STRIVE Rice-Based Farming Systems Survey, December 1998 to March 1999
[b] annual per capita threshold x family household size of 5; Source: Statistical Yearbook, 1998
[c] (net farm income x average landholding x cropping intensity)/(regional poverty threshold + 15% savings)
[d] with palay yield of less than 3.0 mt/ha/season
[e] with palay yield of 3.0 but less than 5.0 mt/ha/season
[f] with palay yield of greater than 5.0 mt/ha/season

Table 2.12. Corn Carrying Capacity in Terms of Subsistence Level, Isabela, 1998.

TECHNOLOGY	Net Farm Income[a] (P/ha/season)	Average Land-holding[a]	Cropping Intensity[a]	Regional Poverty Threshold[b] (Annual)	Carrying Capacity in Terms of Subsistence Level[c]
OPEN POLLINATED					
Low Yield[d]	2,851	3.00	1.40	49,365	0.28
Medium Yield[e]	11,222	3.00	1.40	49,365	1.10
High Yield[f]					
All Yield Levels	7,037	3.00	1.40	49,365	0.69
HYBRID					
Low Yield[d]	2,142	3.00	1.40	49,365	0.21
Medium Yield[e]	11,534	3.00	1.40	49,365	1.13
High Yield[f]	17,257	3.00	1.40	49,365	1.69
All Yield Levels	10,346	3.00	1.40	49,365	1.01

[a] Source: SIKAP/STRIVE Rice-Based Farming Systems Survey, December 1998 to March 1999
[b] annual per capita threshold x family household size of 5; Source: Statistical Yearbook, 1998
[c] (net farm income x average landholding x cropping intensity)/(regional poverty threshold + 15% savings)
[d] with corn yield of less than 3.0 mt/ha/season
[e] with corn yield of 3.0 but less than 5.0 mt/ha/season
[f] with corn yield of greater than 5.0 mt/ha/season

3. CARRYING CAPACITY: GLOBAL TRENDS

Since the middle of the century, three factors have contributed most directly to the excessive pressures now being placed on the earth's natural systems: the doubling of the world population, the quintupling of global economic output, and the widening gap in the distribution of income (Postel, 1994).

3.1 The Population Challenge

During the 1950s, the world population was estimated at 2.5 billion. This increased to 5.9 billion in 1998 and projected to reach 9.4 billion by the year 2050 **(Table 3.1).**

This unprecedented increase in population (using the UN medium assumption) accompanied by rising individual consumption of goods and services is pushing the carrying capacity of mother earth beyond its natural limits.

The 3.6 billion population from 1950 to 2000 was a combined contribution of both developed and developing countries. The projected half century population increment of 3.3 billion in the next millenium, however, was projected to be attributed mainly to the developing countries, many of which are hard-pressed to satisfy even existing demands on resources (Brown et al, 1998).

Table 2.13. Corn Carrying Capacity in Terms of Subsistence Level, Bukidnon 1998.

TECHNOLOGY	Net Farm Income[a] (P/ha/season)	Average Land-holding[a]	Cropping Intensity[a]	Regional Poverty Threshold[b] (Annual)	Carrying Capacity in Terms of Subsistence Level[c]
OPEN POLLINATED					
Low Yield[d]	(1,167)	3.00	1.40	55,775	(0.10)
Medium Yield[e]					
High Yield[f]					
All Yield Levels					
HYBRID					
Low Yield[d]	53	3.00	1.40	55,775	0.00
Medium Yield[e]	15,067	3.00	1.40	55,775	1.30
High Yield[f]					
All Yield Levels	7,650	3.00	1.40	55,775	0.66

[a] Source: SIKAP/STRIVE Rice-Based Farming Systems Survey, December 1998 to March 1999
[b] annual per capita threshold x family household size of 5; Source: Statistical Yearbook, 1998
[c] (net farm income x average landholding x cropping intensity)/(regional poverty threshold + 15% savings)
[d] with corn yield of less than 3.0 mt/ha/season
[e] with corn yield of 3.0 but less than 5.0 mt/ha/season
[f] with corn yield of greater than 5.0 mt/ha/season

Given the variances in population growth rates among individual countries, the world can be demographically divided into two groups: countries that have achieved population stability and those that have not. The first group is composed mainly of nations from Europe and the industrial countries. On the other hand, the second group is composed of developing countries (e.g. Ethiopia, Pakistan, Nigeria) which are projected to triple their population over the next half century. The twenty largest countries ranked according to population are shown in Table 3.2.

3.1.1 Some dimensions of the population challenge

In the latest publication of the World Watch Institute, Beyond Malthus: Sixteen Dimensions of the Population Problem, Brown (Gardner and Halweil, 1998), enumerated at least sixteen development indicators affected by the population growth. Some of these indicators as projected by the above-mentioned authors, especially those relating to food and the environment, are discussed here.

3.1.1.1 Trends in resources/environment

Table 2.14. Corn Carrying Capacity in Terms of Subsistence Level, South Cotabato, 1998.

TECHNOLOGY	Net Farm Income[a] (P/ha/season)	Average Land-holding[a]	Cropping Intensity[a]	Regional Poverty Threshold[b] (Annual)	Carrying Capacity in Terms of Subsistence Level[c]
OPEN POLLINATED					
Low Yield [d]	5,273	3.00	1.40	52,445	0.49
Medium Yield [e]	8,777	3.00	1.40	52,445	0.81
High Yield [f]					
All Yield Levels	7,025	3.00	1.40	52,445	0.65
HYBRID					
Low Yield [d]	5,632	3.00	1.40	52,445	0.52
Medium Yield [e]	9,791	3.00	1.40	52,445	0.90
High Yield [f]	17,873	3.00	1.40	52,445	1.65
All Yield Levels	15,525	3.00	1.40	52,445	1.43

[a] Source: SIKAP/STRIVE Rice-Based Farming Systems Survey, December 1998 to March 1999
[b] annual per capita threshold x family household size of 5; Source: Statistical Yearbook, 1998
[c] (net farm income x average landholding x cropping intensity)/(regional poverty threshold + 15% savings)
[d] with corn yield of less than 3.0 mt/ha/season
[e] with corn yield of 3.0 but less than 5.0 mt/ha/season
[f] with corn yield of greater than 5.0 mt/ha/season

Fresh Water

Population growth affects the supply of fresh water. As population increases, the per capita availability of fresh water declines. The World Watch Institute estimates that water availability per person from the hydrological cycle will fall by 74 percent between 1950 to 2050. As the growing demand for water collides with the limits of supply, countries typically satisfy rising urban and residential demands by diverting water from irrigation. To substitute for water, countries import grains, the cheapest way to import water. The water scarcity would affect agriculture output in terms of lower irrigation water supply. Already China and India are feeling the pinch. If total irrigated areas remain at roughly 263 million hectares until 2050, the figure is projected to fall to 0.0280 hectares per person in 2050 - declining by 38 percent (Figure 3.1)

Biodiversity

As human population has surged during the century, populations of other numerous species have tumbled almost at the point of extinction. The leading causes of today's species losses such as habitat alteration, invasion by exotic species, pollution and over hunting/fishing are all function of human activities. These activities have pushed the percentage of mammals, amphibians, and fish that are in immediate danger of extinction into double digits (Table 3.3).

Table 3.1 World Population, 1950, with projections to 2050.

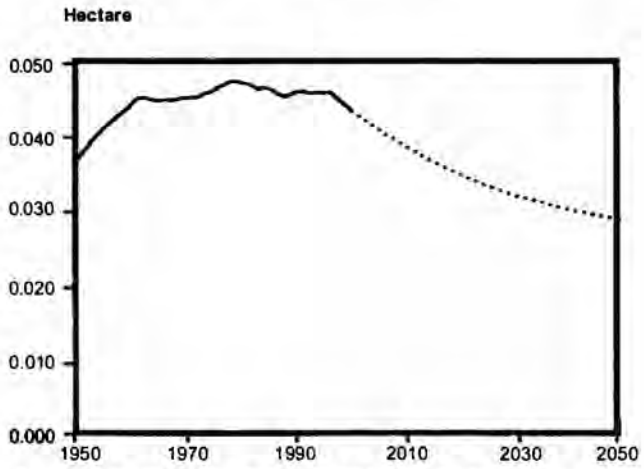
Year	World Population	Half-Century Increase (billion)
1950	2.5	
200	6.1	3.6
2050	9.4	3.3

Source: Postel, N. (1998) as cited by Brown, Gardner and Halweill (1998)

Table 3.2. The 20 largest Countries Ranked According to Population Size, 1998. with Projections to 2050

	1998		2050	
Rank	Country	Population (million)	Country	Population (million)
1	China	1,255	India	1,533
2	India	976	China	1,517
3	United States	274	Pakistan	357
4	Indonesia	207	United States	348
5	Brazil	165	Nigeria	339
6	Pakistan	148	Indonesia	318
7	Russia	147	Brazil	243
8	Japan	126	Bangladesh	218
9	Bangladesh	124	Ethiopia	213
10	Nigeria	122	Iran	170
11	Mexico	96	The Congo	165
12	Germany	82	Mexico	154
13	Viet Nam	78	Philippines	131
14	Iran	73	VietNam	130
15	Philippines	72	Egypt	115
16	Egypt	66	Russia	114
17	Turkey	64	Japan	110
18	Ethiopia	62	Turkey	98
19	Thailand	60	South Africa	91
20	France	59	Tanzania	89

Source: United Nations, World Populations Prospects 1996.



Source: *From Sandra Postel (1998) as cited by Brown, Gardner, and Halweil (1998).*

Figure 3.1 Global Irrigated Area Per Person, 1950-96, with Projections to 2050.

Climate Change

Carbon emissions from fossil fuel burning have expanded at nearly twice the rate of population. The destabilization of the world's climate threatens more intensive heat wave, more severe droughts and floods, storms, and intensive forest fires. The atmospheric concentrations of carbon dioxide, the principal greenhouse gas are projected to increase 30 percent over their pre-industrial level (Figure 3.2).

Energy

In the past half-century, global demand for energy grew twice as fast as population as industrial nations burned coal, oil and natural gas to fund their economies. Over the next half century of the third millenium, energy demands are projected to continue expanding beyond population growth, as developing countries try to catch up with industrial nations (Figure 3.3).

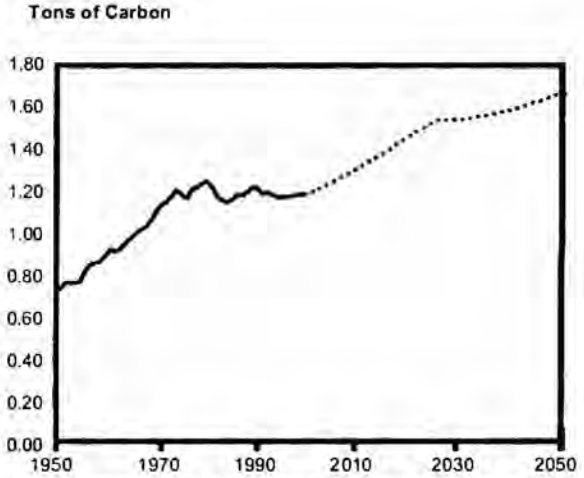
Waste

Another dimension of population growth is its implication to waste. Disposal of garbage and industrial wastes will become a problem with increases in population. The estimate of half kilo of municipal waste per day, at today's population, implies a total of 824 million tons of waste to be disposed off annually in developing countries.

Table 3.3. Share of Species Worldwide Classified as Threatened

Species	Share of Species That is		Total Share of Species Threatened with Extinction
	In Immediate Danger of Extinction	Vulnerable to Extinction	
Birds	4	7	14
Mammals	11	14	25
Reptiles	8	12	20
Amphibians	10	15	25
Fish	13	21	34

Source: Postel N. (1998) as cited by Brown, Gardener and Halweil (1998)



Source: From Marland et. al. as cited by Brown, Gardner, and Halweil (1998).

Figure 3.2 Global Carbon Emissions Per Person, 1950-95, with Projections to 2050.

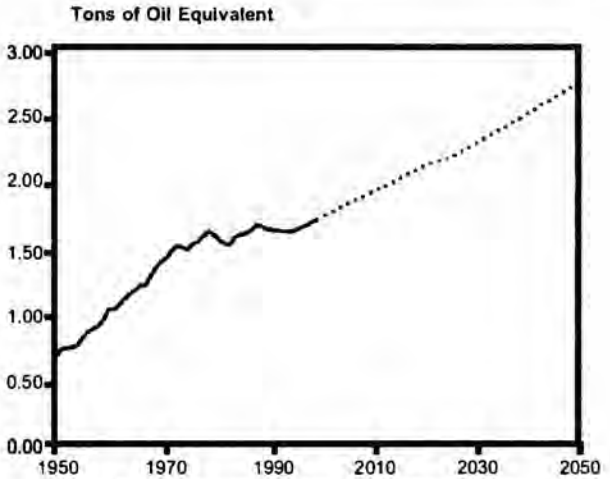
3.1.1.2 Output

Ocean Fish Catch

From 1950 to 1988, fish catch reached its peak in 1988 at 88 million tons. However, since 1988, growth in the catch has slowed down falling behind population, and projected to decline in 2050 (Figure 3.4). As we end the 20th century, over-fishing has become the rule, not the exception. The fivefold growth in the human appetite for seafood, has pushed the catch of most oceanic fisheries to their sustainable limits. Marine biologists estimate that the oceans cannot sustain an annual catch of greater than 93 million tons, at the current take (FAO, 1996).

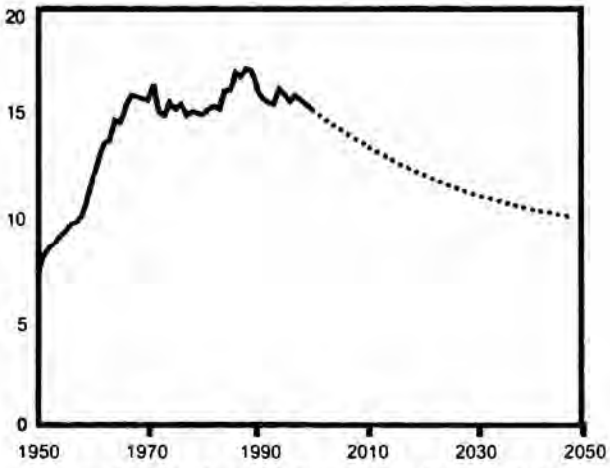
Grain Production

From 1950 to 1984, growth of grain production exceeded population growth raising the harvest per capita from 247 kg to 342, a gain of 38 percent (Figure 3.5). The slower growth in the world grain harvest since 1984 is due to the lack of new land and



Source: Brown, Gardner, and Halweil (1998).

Figure 3.3 Global Energy Use Per Person, 1950-95, with Projections to 2050.



***Source:** FAO, Yearbook of Fishery Statistics 1996, as cited by Brown, Gardner, and Halweil, 1998.*

Figure 3.4 Global Fish Catch Per Person, 1950-95, with Projections to 2050.

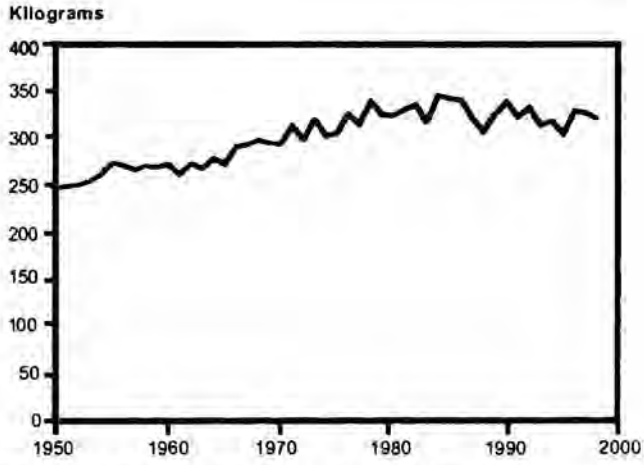


Figure 3.5 Global Grain Production Per Person, 1950-98

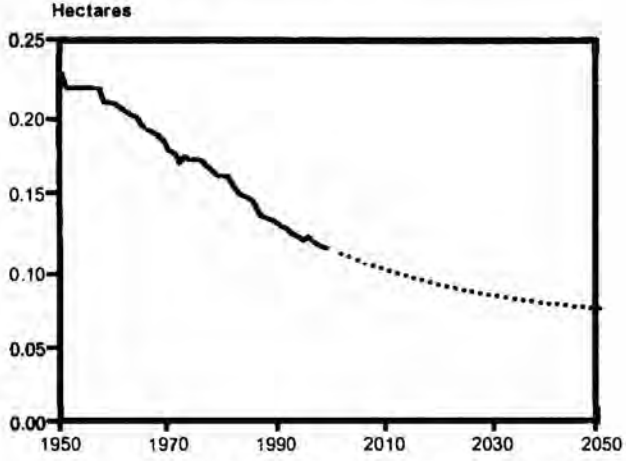
to slower growth in irrigation and fertilizer use. As a result of population increase, grain area per person has fallen half since 1950, from 0.24 to 0.12 hectares (Figure 3.6).

Meat Production

World meat production increased from 44 million tons in 1950 to 211 million tons in 1997. In per capita terms, world meat production expanded from 17 kilograms in 1952 to 36 kilograms in 1997. Expanding meat production in the future would depend on the expansion of grain production and to some extent soybean production. Assuming a feed conversion ratio of 3 kilograms of grain per kilogram of meat produced, this would require more than 900 million tons of additional grain for feeds in 2050, an amount equal to half of current world grain consumption. The expansion therefore of meat production in the future will be limited by volume of grains produced.

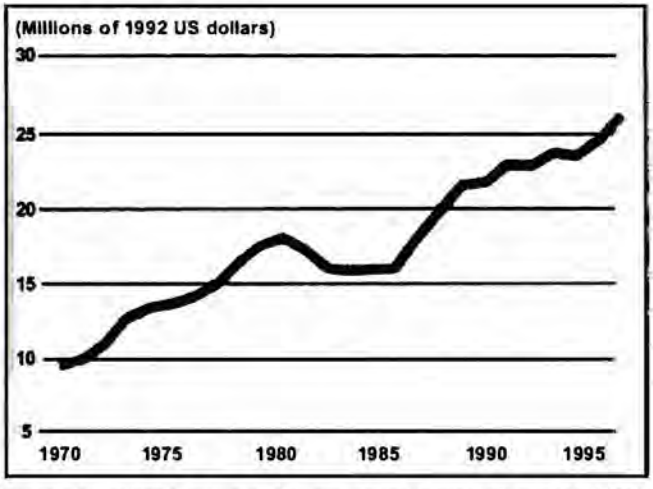
3.1 Quantum Leap in Economic Output

Since the past three decades, total global output or Gross domestic product of the world has expanded from roughly US $9.4 trillion to more than US $ 25 trillion (Figure 3.7). Although the industrial countries account for a major share in this output, economic growth in the developing countries has likewise followed the trend. This growth is expected to continue well in the middle of the next century (World Resource Institute (WRI), 1998-1999). The liberalization of both trade and investment across borders has helped fuel this economic growth. International trade alone increased from US $308 million in 1950 (UNDP, 1994) to US $ 6,225 trillion in 1995 (WRI, 1998-1999).



Source: USDA 1998, cited by Brown, Gardner and Halweil (1998).

Figure 3.6 Global Harvested Area Per Person, 1950-95, with Projections to 2050.



Source: The World Bank, World Development Indicators 1997, on CD-ROM (The World Bank. Washington D.C. 1997), as cited by WRI, (1998)

Figure 3.7 Gross World Product, 1970-95.

Along with this quantum leap in output was the corresponding heavy increases in energy consumption accelerating two-fold from 3,230 trillion kilograms of oil equivalent in 1965 to 6,490 trillion in 1991.

3.3 The Widening Gap in Incomes Between the Rich and the Poor

The final driving force that is currently putting excessive pressure on planet earth is the disparity in incomes among nations.

In 1960, the richest 20 percent of the world's people absorbed 70 percent of global income; by 1991, the wealthy's share had climbed to nearly 85 percent. The poorest 20 percent, meanwhile, saw their share of global income drop from an already meager 2.3 percent to just 1.4 percent. The ratio of the richest fifth's share to the poorest thus grew from 30 to 1 in 1960 to 61 to 1 in 1991.

4. CARRYING CAPACITY INDICATORS: PHILIPPINES AND SELECTED ASIAN COUNTRIES

The Asian region occupies a special place, in any discussions related to carrying capacity. The region as a whole comprises 60 percent of the current world population (China and India alone accounted for 38 percent of the world's population in 1998) and will continue to share a substantial percentage even in 2050.

The green revolution in rice, the staple food of majority of the people of the world and characterized by technology induced crop intensification occurred in Asia. Due to population pressure, the region is no exception in facing the challenges of deteriorating soil and aquatic resources, declining per capita crop land and fertilizer use, and increasing urbanization.

Technology likewise has reached its plateau. Although production from agricultural biotechnology adoption is already an acceptable reality in the West, it has not yet permeated the Asian economies. Likewise, Asia is not homogenous in terms of culture, religion as well as political governance. It is therefore of great interest for scientists, agriculturists, demographers and policy makers to examine closer the carrying capacity trends in Asian countries and see how individual countries in the region are coping up with the challenge of population growth in relation to their efforts in sustaining their resource endowments given their development goals.

For our analysis, the selected countries of Asia under study are divided into two groups based on levels of income according to the recent classification of the World Bank (WB). The middle income Asian countries include Indonesia, Malaysia, Philippines, and Thailand. On the other hand, those considered low-income countries include Cambodia, China, Lao PDR, Myanmar, Vietnam, Bangladesh, India, Pakistan, and Sri Lanka. The C-C trend indicators being compared across countries include GNP, population, food security, index of food production, land use and cropland, indicators of the environment and human development indicators.

4.1 GNP

In the 1998 Asian Development Bank (ADB) Annual Report, the 1997 data on gross national product (GNP) per capita for the countries under study, were reported as follows: Malaysia had the highest at US$4,530; Thailand US$2,740; Indonesia and the Philippines with GNP/capita of US$1,110 and US$1,200, respectively; China and Sri Lanka at US$800 range; Pakistan US$500; while the others had GNP/capita ranging from US$300 to US$ 400 (Table 4.1)

The gross domestic products (GDPs) in these countries were also growing robustly between 1985 to 1995 (no available data yet for comparison after the 1997 financial crisis) with China and Thailand topping the list with 9.6 and 9.0 percent growth per year, respectively; Malaysia, Indonesia and Vietnam, within 6-7% range per year; Lao PDR, India, Pakistan and Bangladesh, from 4-5% range; while Sri Lanka (3.8%) and the Philippines (3.4%) had the lowest GDP growth rates (Table 4.1).

In terms of how the per capita income is distributed using the Gini ratio by country, Sri Lanka and Lao PDR had the lowest (most evenly distributed income) Gini ratio of 30. This was followed by Pakistan, India, Bangladesh, Vietnam, Indonesia and China – all within the range of 31 to 38 Gini ratios. Thailand, Malaysia and the Philippines had the most unevenly distributed per capita income with Gini ratios ranging from 52-45 (Table 4.1).

4.2 Population

Asian population is projected to reach 5.4 billion in 2050. China, India, Pakistan, Indonesia and Bangladesh belong to the top ten countries with high population growth in this projection. The population of India, China, Pakistan and Indonesia would account for 68 percent of the Asian population and 40 percent of the world in 2050 (Table 4.2).

4.3 Food Security

The indicators of food security, as used in this analysis are calorie and protein availabilities. This seems to be overly simplistic because effective purchasing power, instead of supply availability is a more powerful indicator. Superimposing the recommended daily allowance (RDA) of the Philippines of 2052 kilocalories and 50 grams for protein, as divisors in the supply available calorie and protein data would yield us a rough national picture of calorie and protein adequacy.

The analysis showed that from 1982-84, only Cambodia (87%) and Bangladesh (95%) had inadequate calorie intakes. In terms of protein nutrient adequacy, Cambodia, Bangladesh, Sri Lanka and Indonesia had protein inadequacy in their diets. For the period 1992-1994, most of Asian countries had improved their nutrient supply availability. However, Cambodia and Bangladesh deteriorated (Table 4.3).

Caution should be noted at this stage in the estimation of percent nutrient adequacy by country. The first point is relative to the method in estimating supply availability. The method is a straight supply-disappearance method using the food

Table 4.1. Per Capita GNP of Selected Asian Countries, 1997.

	Per Capita GNP[a]	Annual GDP[b] Growth 1985-1995	GINI Coefficient[c]
	(US$)	(%)	
Middle Income			
Indonesia	1,110	7.2	32
Malaysia	4,530	7.4	48
Philippines	1,200	3.4	45
Thailand	2,740	9.0	52
Low Income			
Cambolia	300	.	.
China	860	9.6	38
Lao PDR	400	5.2	30
Myanmar	—	.	.
VietNam	310	6.3	36
Bangladesh	360	4.0	35
India	370	5.2	32
Pakistan	500	5.1	31
Sri Lanka	800	3.8	30

...Data not available.

[a] Source: Country sources; ADB data file; and World Bank, official communications, February 1999, as published by ADB Annual Report, 1999.

[b] WRI, 1998-1999.

[c] Gini coeficients measure the equality of distribution (0, perfectly equal; 100, perfectly unequal). From WRI, 1998-1999.

balance analysis. It simply is the arithmetic of food production plus imports, minus export, minus waste and non-food uses. The net effect is total food supply available for human consumption. Divide this by population size and we get per capita availability per year. Then convert this into daily per capita calorie and protein availability and divide it by the RDA to calculate the food supply adequacy (Figure 4.1).

There is nothing wrong with the methodology per se. However, the caution lies in the interpretations of food supply adequacy as an absolute indicator of nutrient adequacy, because this data indicator does not reflect actual consumption. A better indicator should be the actual food consumed from food nutrition consumption surveys.

To illustrate our point, let's take the Philippine data in Table 4.3 which all indicated that the Philippines was adequate in caloric intakes (1982-1984, 105 percent and 1992-1994, 115 percent) and protein intakes, 105 percent for 1982-1984 and 112 for 1992-1994. Data from the Food Nutrition Research Institute (FNRI) during the period 1982 to 1993 would indicate that in terms of calorie intakes, the Filipinos had more than 100

Table 4.2 Population Trends and Projections, World, Asia and Selected Asian Countries, 1950-2050

	Population (thousands)			
	1950	1998	2025	2050
World	2,523,878	5,929,839	8,039,130	9,366,724
Asia	1,402,021	3,588,877	4,784,833	5,442,567
Middle Income				
Indonesia	79,539	206,522	275,245	318,264
Malaysia	6,110	21,450	31,577	38,089
Philippines	20.988	72,164	105,194	130,511
Thailand	20,010	59,612	69,089	72,969
Low Income				
Cambodia	4,346	10,751	16,990	21,394
China	554,760	1,255,091	1,480,430	1,516,664
Lao PDR	1,755	5,358	10,202	13,889
Myanmar	17,832	47,625	67,643	80,896
Vietnam	29,954	77,896	110,107	129,763
Bangladesh	41,783	124,043	179,980	218,188
India	357,561	975,772	1,330,201	1,532,674
Pakistan	39,513	147,811	268,904	357,353
Sri Lanka	7,678	18,840	23,934	26,995

Source: United Nations Population Division and International Labour Organization, as cited by the World Resources Institute, 1998

percent adequacy from 1987 to 1989 but not in 1993 which was only 87.8 percent adequacy (Table 4.4).

Variances of percent adequacy would also differ once the data are disaggregated into urban, and rural (Table 4.5); by region (Table 4.6); by educational attainment of meal planner (Table 4.8); and by household size (Table 4.9).

The point made here is, national level data when it comes to interpreting food security using the disappearance approach has no meaning. It has to be balanced by actual consumption nutrition surveys and must be disaggregated at different levels of intervention points.

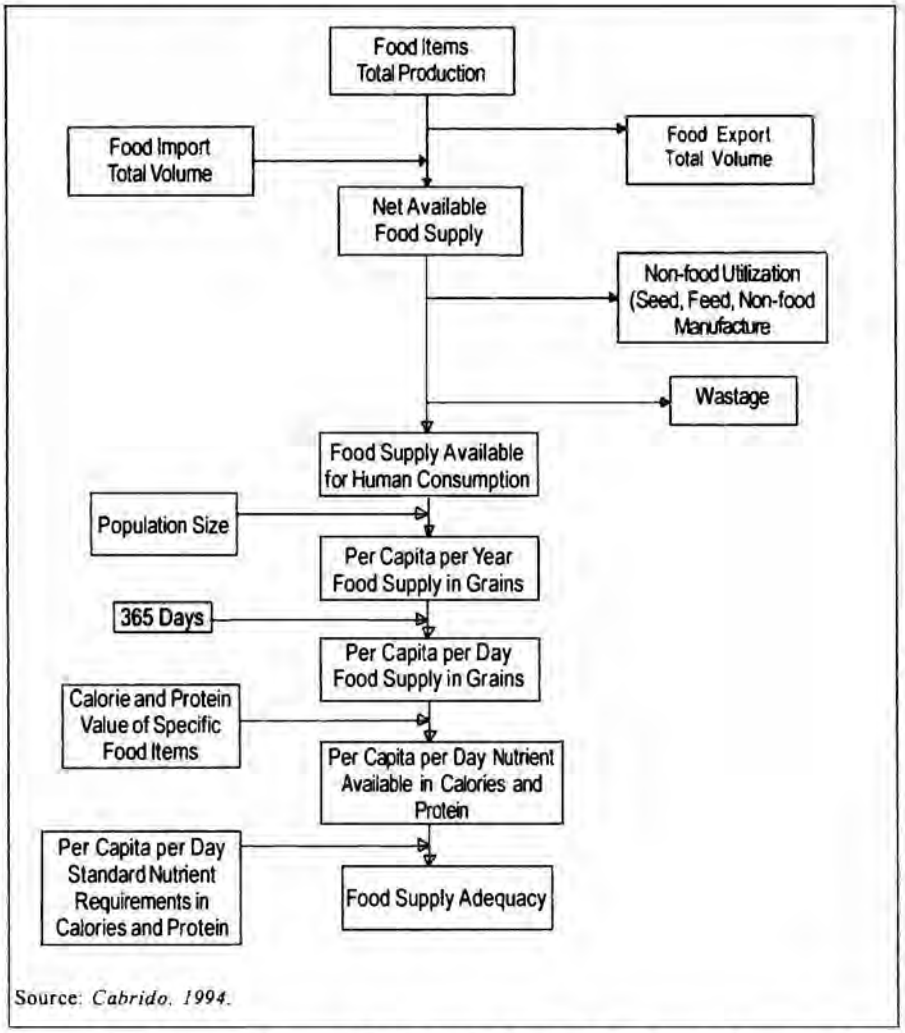
4.4. Food Production Index

Using the 1989-1991 period as the base, the indices of both agricultural and food production had improved from 1984 to 1996, both in terms of total and per capita indices. In terms of food import dependency ratio, Malaysia had the highest at 53.1

Table 4.3 Food Security Status, Per Capita Calorie and Protein Supply Availability, Selected Asian Countries.

COUNTRY	Average Daily Per Capita Calorie Supply (kilocalories)		Average Daily Per Capita Protein Supply (grams)		Calorie Supply-RDA Ratio [1] (Percent Adequacy)		Protein Supply-RDA Ratio [1] (Percent Adequacy)	
	(1982-84)	(1992-94)	(1982-84)	(1992-94)	(1982-84)	(1992-94)	(1982-84)	1992-94)
Low Income								
Indonesia	2,331	2,609	49	62	114	127	98	124
Malaysia	2,706	2,782	58	65	132	136	116	130
Philippines	2,161	2,370	51	56	105	115	102	112
Thailand	2,215	2,365	49	53	108	115	98	106
Low Income								
Cambodia	1,777	1,805	43	43	87	88	86	86
China	2,681	3,082	59	68	131	150	118	136
Lao PDR	2,148	2,106	55	55	105	103	110	110
Myanmar	2,563	2,619	65	66	125	128	130	132
Vietnam	2,246	2,302	50	55	109	112	100	110
Bangladesh	1,954	2,023	42	44	95	99	84	88
India	2,157	2,397	54	58	105	117	108	116
Pakistan	2,177	2,399	53	61	106	117	106	122
Sri Lanka	2,295	2,242	47	48	112	109	94	96

[1] based on an RDA of 2052 for calorie and 50 grams for protein
Basic Source of Data: Food and Agriculture Organization of the United Nations, as cited by the World Resources Institute, 1998.



Source: *Cabrido. 1994.*

Figure 4.1. Schematic Outline of the Food Balance of the Food Balance Analysis Methodology

%, followed by Sri Lanka (30.4%); Pakistan (14.1%); Bangladesh (12.3%); and the Philippines (11.4%). The others had less than 6% food import dependency ratios (Table 4.10.)

These food production trends appear very optimistic. The latest projections by the International Food Policy Research Institute (IFPRI) for Asia by 2020 are more pessimistic. In its original base projections, IFPRI (Impact, 1995) reported a positive net trade of rice for Asia in 2020. This supply source would come from Thailand, Vietnam, Myanmar, India and China. On the other hand, Philippines, Bangladesh,

Table 4.4 Per capita daily calorie and protein intake and percent adequacy (national average), Philippines, 1982-1993

ITEM	1982	1987	1989	1993
Total Calories (Kcal) Consumed	2,152.0	2,089.0	2,123.0	1,684.0
Recommended Daily Allowance (RDA)	1,919.0	1,919.0	1,919.0	1,919.0
Percent Adequacy	112.0	108.8	110.6	87.8
Total Protein (grams) Consumed	54.5	53.8	54.9	49.9
Recommended Daily Allowance (RDA)	47.0	47.0	47.0	47.0
Percent Adequacy	116.0	114.0	116.8	106.2

Source: FNRI, 1995.

Table 4.5 Mean one-day per capita energy and protein nutrient intake and percent adequacy by urbanization: Philippines, 1993.

	ENERGY			PROTEIN		
Group	Intake (kcal)	Recommended Dietary Allowance	Percent Adequacy	Intake (g)	Recommended Dietary Allowance	Percent Adequacy
Philippines	1684	1919	87.8	49.9	47.0	106.2
All Urban	1673	1922	87.0	50.8	47.2	107.6
Metro Manila	1651	1926	85.7	52.2	47.2	110.6
Other Urban	1681	1921	87.5	50.2	47.2	106.4
Rural	1696	1915	88.6	49.1	46.8	104.9

Source: FNRI, DOST Fourth National Nutrition Survey: Philippines, 1993.

Malaysia and Indonesia would be importers of rice ranging from 224,000 m.t. to 1.5 million mt (Table 4.11).

In cereals trade, IFPRI's revised projections were more pessimistic with Asia as a net importer of around 117 million mt of cereals. China, Pakistan and Indonesia will be the major importers of cereals ranging from 41 million mt (China) to 11 million mt (Indonesia). In the medium category, cereal imports of 4 to 7 million mt will be imported by Bangladesh, Philippines and Malaysia (Table 4.12).

These revised projections of IFPRI support what Lester Brown calls the "old" and "new era" of food security (Brown, 1996). Brown argues that earlier projections made by IFPRI, the World Bank and the FAO were optimistic because they have not included the 'S-curve effects' of technical change and resource endowments (IFPRI, 2020 vision, 1995). Indicators for the "new era" (from 1990 into indefinite future) of food security would include among others, declining per capita

Table 4.6 Mean one-day per capita energy and protein nutrient intake and percent adequacy by region: Philippines, 1993.

Region	ENERGY			PROTEIN		
	Intake (kcal)	Recommended Dietary Allowance	Percent Adequacy	Intake (g)	Recommended Dietary Allowance	Percent Adequacy
NCR	1651	1926	85.7	52.2	47.2	110.6
Ilocos	1732	1925	90.0	49.4	47.8	103.3
CAR	1839	1945	94.6	50.8	48.1	105.6
Cagayan Valley	1741	1928	90.3	47.4	47.5	99.8
Central Luzon	1758	1933	90.9	51.0	48.0	106.2
Southern Tagalog	1709	1910	89.5	48.9	46.8	104.5
Bicol	1618	1875	86.3	46.0	45.1	102.0
Western Visayas	1587	1903	83.4	47.2	46.5	101.2
Central Visayas	1640	1905	86.1	54.2	46.0	117.8
Eastern Visayas	1696	1923	88.2	49.4	47.2	104.7
Western Mindanao	1699	1945	87.4	53.0	47.8	110.9
Northern Mindanao	1652	1926	85.8	49.4	47.2	104.7
Southern Mindanao	1679	1947	86.2	50.2	47.5	105.7
Central Mindanao	1688	1910	88.4	49.6	46.3	107.1
ARMM	1759	1901	92.5	46.9	46.9	100.0

Source: FNRI, DOST Fourth National Nutrition Survey: Philippines, 1993.

production of grain and seafoods, inadequate grain stocks, rising grain prices, shrinking irrigated area and fertilizer used per person, diminished backlog of unused technology and a new politics of food from surpluses before 1990 to scarcity and competition among importers from 1990 and beyond (Table 4.13).

4.5 Land Use

Trends in land use from 1982 to 1994 by countries are shown in Table 4.14. In terms of population density and cropland percentage to total land area, Bangladesh had the highest at 9,224 people per 1000 hectares of land and 71 percent of cropland, respectively. However, in terms of absolute hectarage of cropland across countries,

Table 4.7 Mean one-day per capita energy and protein nutrient intake and percent adequacy by annual per capita income: Philippines, 1993.

Annual Per Capita Income (In Pesos)	Distribution of Households	ENERGY		PROTEIN	
		Intake (kcal)	Percent Adequacy	Intake (g)	Percent Adequacy
Less than P3000	22.6	1559	82.6	44.0	96.5
3000-5999.99	25.7	1622	85.0	47.0	101.3
6000-8999.99	16.1	1680	87.2	50.2	106.6
9000-11999.99	10.7	1725	88.7	51.8	107.5
12000-14999.99	7.2	1746	90.3	53.9	112.8
15000-17999.99	4.5	1822	93.7	55.7	115.3
18000 and over	13.2	1956	100.0	61.6	125.2

Source: FNRI, DOST Fourth National Nutrition Survey: Philippines, 1993.

Table 4.8 Mean one-day per capita energy and protein nutrient intake and percent adequacy by education of meal planner: Philippines, 1993.

Education of Meal Planner	ENERGY		PROTEIN	
	Intake (kcal)	Percent Adequacy	Intake (g)	Percent Adequacy
No Formal Schooling	1802	94.0	49.1	100.6
1-7 Years	1634	84.8	48.0	101.5
8-11 years	1658	87.0	49.2	106.7
12 and over	1862	96.8	57.1	120.5

Source: FNRI, DOST Fourth National Nutrition Survey: Philippines, 1993.

Table 4.9 Mean one-day per capita energy and protein nutrient intake and percent adequacy by household size: Philippines, 1993.

Household Size	ENERGY		PROTEIN	
	Intake (kcal)	Percent Adequacy	Intake (g)	Percent Adequacy
1-2	2165	109.0	67.0	121.8
3-4	1882	97.2	57.0	118.0
5-6	1734	91.0	51.4	111.0
7-8	1601	83.4	47.0	100.4
9 and above	1544	80.4	45.0	96.2

Source: FNRI, DOST Fourth National Nutrition Survey: Philippines, 1993.

Table 4.10 Index of Agricultural and Food Production, and Food Import Dependency Ratio, Selected Asian Countries, 1986-1996.

COUNTRY	Index of Agricultural Production (1989-91=100)				Index of Food Production (1989-91=100)				Food Import Dependency Ratio (%)
	Total		Per Capita		Total		Per Capita		
	(1984-86)	(1994-96)	(1984-86)	(1994-96)	(1984-86)	(1994-96)	(1984-86)	(1994-96)	(1988/90)
Medium Income									
Indonesia	84	115	91	107	91	107	83	115	5.7
Malaysia	78	115	88	102	79	109	70	122	51.3
Philippines	90	117	100	105	100	106	90	119	11.4
Thailand	91	110	99	105	100	101	92	106	3.8
Low Income									
Cambodia	73	119	85	103	85	102	73	118	3.2
China	82	140	88	136	88	136	81	144	4.1
Lao PDR	91	114	106	98	107	98	92	114	5.6
Myanmar	111	139	123	127	121	127	110	139	0.9
Vietnam	82	126	98	87	97	86	82	112	1.8
Bangladesh	89	104	98	96	96	96	87	104	12.3
India	83	114	92	105	92	104	83	114	1.8
Pakistan	79	119	92	104	94	109	80	125	14.1
Sri Lanka	105	108	112	103	113	103	107	108	30.4

Source: Food and Agriculture Organization of the United Nations, as cited in World Resources, 1998.

Table 4.11 IFPRI Projections of Rice, 2020.

	Area/No. (000 ha)	Yield (kg)	Production	Demand	Food	Feed	Net Trade
				000, mt			
World	149,995	3,258	488,727	488,727	438,867	5,272	0
Developed	4,253	4,669	19,856	19,304	16,365	9	552
Developing	145,742	3,217	468,871	469,423	422,502	5,263	(552)
Asia	127,250	3,303	420,293	410,434	369,998	5,263	9,858
Middle Income							
Indonesia	11,216	3,756	42,126	43,590	38,553	856	(1,464)
Malaysia	659	2,560	1,687	2,943	2,804	58	(1,256)
Philippines	3,466	3,009	10,428	10,652	9,419	796	(224)
Thailand	8,707	1,787	15,559	8,193	8,325	326	7,366
Low Income							
Cambolia	...	...	...	...	...	...	...
China	30,039	4,905	147,341	145,004	131,593	2,443	1,336
Lao PDR	...	...	...	...	...	...	...
Myanmar	5,943	2,776	16,496	13,516	13,516	0	2,980
Viet Nam	6,507	3,268	21,398	18,527	15,926	214	2,871
Bangladesh	10.027	2,586	25,931	27,026	24,881	0	(1,095)
India	42,030	2,743	115.283	112,798	101.817	454	2,486
Pakistan	2,384	2,603	6,205	4,737	4,230	0	1,459
Sri Lanka	...	...	...	...	...	...	...

Source: (Rosegrant, Sombilla and Perez, 1995) Impact Model, IFPRI.

Table 4.12 IFPRI Projections of Cereals, 2020.

	Area/No. (000 ha)	Yield (kg)	Production	Demand	Food	Feed	Net Trade
				000, mt			
World	739,284	3,369	2,490,722	2,490,722	1,269,838	926,691	0
Developed	273,117	4,047	1,105,401	877,214	192,932	536,428	228,187
Developing	466,167	29,172	1,385,321	1,613,508	1,076,907	390,263	(228,187)
Asia	269,399	3,435	925,501	1,042,781	729,822	236,322	(117,282)
Middle Income							
Indonesia	14,534	3,631	52,766	63,554	49,217	9,266	(10,788)
Malaysia	681	2,558	1,742	9,091	4,481	4,009	(7,349)
Philippines	6,839	2,908	19,886	25,072	15,111	9,213	(5,186)
Thailand	10,141	2,171	22,018	17,760	7,373	8,597	4,259
Low Income							
Cambodia	...	...	...	...	...	...	...
China	89,137	5,037	448,949	490,053	293,716	170,653	(41,104)
Lao PDR	...	...	...	...	...	...	...
Myanmar	6,529	2,638	17,221	14,364	14,094	211	2,858
Viet Nam	7,055	3,231	22,798	20,515	17,324	709	2,283
Bangladesh	10,747	2,569	27,606	31,865	29,356	19	(4,260)
India	101,242	2,480	251,044	251,888	218,723	8,325	(846)
Pakistan	12,747	2,636	33,599	49,865	44,320	2,885	(16,266)
Sri Lanka	...	...	...	...	...	...	...

Source: (Rosegrant, Sombilla and Perez, 1995) Impact Model, IFPRI., Revised Projections.

Table 4.13 Indicators of Food Security in Old and New Eras

Indicator	Old Era (roughly 1950 to 1990)	New Era (roughly 1990 into indefinite future)
Grain production per person	Rising: up 40 percent from 1950 to 1984	Failing: down 15 percent from 1984 to 1995
Seafood catch per person	Rising: double from 1950 to 1989	Falling: down 7 percent 1989 to 1995; will fall as long as population growth continues
Grain prices	Declining in real terms from 1950 through 1993	Rising: will fluctuate, but around rising trend 1993 onward
Grain stocks	Abundant, often excessive	Low, often inadequate
Idled cropland	Cropland idled throughout this period	Little or no cropland idled after mid-nineties
Grainland per person	Shrinking slowly until 1981, then more rapidly	Shrinking rapidly as long as population growth continues
Irrigated area person	Expanding: up 28 percent 1950 to 1979	Shrinking since 1979: will continue as long as population growth continues
Fertilizer use per person	Rising: up fivefold 1950 to 1989	Shrinking since 1989: will not rise much as both grainland and irrigation water per person shrink
Effect of climate change	Effect beginning to show as temperatures rise	More intense heat waves likely to plague efforts to expand output
Backlog of unused technologies	Huge at beginning of era, but diminishing over time	Greatly diminished: no dramatic advance in prospect
Politics of water	Gradually intensifying as period progressed	Intense competition among countries and between countryside and city
Politics of food	Dominated by surpluses; competition among exporters for access to markets	Dominated by scarcity; competition among importers for access to to supplies

Source: Lester Brown, Tough Choices, 1996.

Table 4.14 Trends in Land Use, Selected Asian Countries, 1982-1994

COUNTRY	Land area (000 ha)	Pop'n Density per 1,000 hectares	Domesticated as a % of land area (a)	Land Use (000) hectares: Crop		Permanent Pasture		Forest & Woodland		Other Land	
					Percent Change Since		Percent Change Since		Percent Change Since		Percent Change Since
		1996	1994	1992-94	1982-84	1992-94	1982-84	1992-94	1982-84	1992-94	1982-84
Medium Income											
Indonesia	181,157	1,107	23	31,146	19.9	18,800	1.2	111,516	-2.6	26,695	-8.1
Malaysia	32,855	625	24	7,536	46.6	281	8.9	22,428	0.0	2,790	-46.4
Philippines	29,817	2,324	36	9,320	5.0	1,280	14.3	13,600	15.6	5,617	-30.3
Thailand	51,089	1,149	42	20,488	6.7	800	14.3	14,833	-3.7	14,968	-5.1
Low Income											
Cambodia	17,652	582	30	3,832	81.9	1,500	158.5	12,200	-7.3	120	-93.4
China	929,100	1,321	53	95,145	-3.6	400,000	12.5	128,630	-1.1	305,324	-11.4
Lao PDR	23,080	218	7	900	18.5	800	0.0	12,560	-4.1	8,820	4.7
Vietnam	32,549	2,310	22	6.738	2.3	328	5.1	9,650	-3.9	15,833	1.4
Bangladesh	13,017	9,224	71	8,849	-3.1	600	0.0	1,891	-1.3	1,677	45.2
India	297,319	3,177	61	169,569	0.5	11,424	-4.8	68,173	1.2	48,136	-2.1
Pakistan	77,088	1,817	34	21,323	4.7	5,00	0.0	3,477	15.1	47,288	-2.9
Sri Lanka	6,463	2,801	36	1,889	1.3	440	-1.8	2,100	20.2	2,034	-15.6

Source: Food and Agriculture Organization of the United Nations and United Nations Population Division, as cited in World Resources, 1998

India had the highest at around 170 million hectares. From 1982 to 1994, cropland areas had generally increased with the exception of China, Bangladesh and Myanmar. The highest percent expansion in cropland during the same period was Cambodia followed by Malaysia and Lao PDR. In terms of permanent pasture, Cambodia had also the highest percentage increase. However, in terms of magnitude of permanent pasture and forest and woodland, China had the highest at 400 million hectares and 129 million hectares, respectively (Table 4.14).

In terms of per capita cropland hectarage during the period 1982-1994, the trend was generally declining, with the exception of Malaysia and Cambodia. Irrigation trends as a percentage of cropland, were slightly increasing during the period, except for Indonesia, Malaysia and Cambodia (Table 4.15).

4.6 Environmental Trends

Average annual rate of deforestation was highest in the Philippines at 3.5%; followed by Pakistan, 2.9%; Thailand, 2.6% and Malaysia, 2.4%. The other countries

Table 4.15 Trends in Per Capita Cropland vs. Irrigation, Selected Asian Countries, 1984-1992

COUNTRY	Cropland				Irrigated Land as a Percentage of Cropland	
	Total Hectares (000) 1984	Hectares Per Capita 1984	Total Hectares (000) 1994	Hectares Per Capita 1994	1982-84	1992-94
Middle Income						
Indonesia	25,934	0.16	30,171	0.16	17	15
Malaysia	5,300	0.35	7,604	0.39	6	5
Philippines	8,920	0.17	9,370	0.14	16	17
Thailand	19,331	0.38	20,445	0.35	18	22
Low Income						
Cambodia	2,110	0.29	3,838	0.39	5	4
China	98,746	0.09	95,782	0.08	45	52
Lao PDR	810	0.23	900	0.19	16	16
Myanmar	10,061	0.27	10,076	0.23	10	11
Vietnam	6,590	0.11	6,758	0.09	26	28
Bangladesh	9,132	0.09	8,700	0.07	20	37
India	169,078	0.22	169,700	0.19	24	29
Pakistan	20,330	0.21	21,510	0.16	76	80
Sri Lanka	1,872	0.12	1,883	0.11	29	29

Source: Food and Agricultural Organization of the United Nations, United Nations Population Division

with deforestation rate of slightly one percent per annum, include Sri Lanka, Vietnam, Myanmar, Cambodia and Indonesia (Table 4.16).

As a percentage of total land area, Cambodia, Thailand and Sri Lanka had the highest protected areas ranging from 13 to 16%. Those with percentage range of 3.0 to 10 percent include Indonesia, Malaysia, Philippines, China, India and Pakistan. The others had national protected areas less than three percent of total land area (Table 4.16).

There is also a growing trend in the increase of Carbon Dioxide (CO_2) emission in Asian countries. In 1995, Malaysia had the highest per capita CO_2 emission at 5.3 mt, followed by Thailand and China with 3.0 m.t. and 2.7 m.t., respectively. The other countries had relatively low CO_2 emissions per capita ranging from 0 to 1.5 m.t. (Table 4.16).

Table 4.16 Environmental Indicators of Selected Asian Countries, 1990-1995.

	Average Annual Rate of Deforestation[a] (as % of forest area)	National Protected Areas[b] (as % of total land area)	Per Capita Carbon Dioxide Emissions[c] (metric ton)
	1990 - 1995	1994[d]	1995
Middle Income			
Indonesia	1.0	9.7	1.5
Malaysia	2.4	4.5	5.3
Philippines	3.5	4.9	0.9
Thailand	2.6	13.1	3.0
Low Income			
Cambolia	1.6	16.2	0.0
China	0.1	6.4	2.7
Lao PDR	▬	▬	0.1
Myanmar	1.4	0.3	0.1
Viet Nam	1.4	3.1	0.4
Bangladesh	0.8	0.8	0.2
India	0.0[d]	4.8	1.0
Pakistan	2.9	4.8	0.6
Sri Lanka	1.1	13.3	0.3

...Data not available.

[a] Positive figures indicate deforestation rates while negative figures indicate reforestation rates.

[b] Refers to all protected areas at least 1,000 hectares listed in categories I-V of the International Union for Conservation of Nature and Natural Resources.

[c] Refers to carbon diozide emissions from fossil fuel burning and cement manufacturing.

[d] The number 0.0 means the magnitude is zero or less than half of the unit employed and not known more precisely.

4.7. Human Development Index

One of the most innovative approaches initiated by the UNDP, measuring a people-centered development, was the human development index (HDI). The HDI value is an aggregate measure of life expectancy, access in safe water, infant mortality, daily calorie supply, child malnutrition, adult literacy, mean years of schooling, possession of radios, real GDP per capita and GNP per capita (UNDP, 1994).

Table 4.17 shows some selected indicators and HDI value by country. Of the Asian countries studied, Thailand and Malaysia had the highest HDIs close to 0.800, followed by Sri Lanka, China and the Philippines with over 0.600. (Figure 4.2). The other Asian countries had HDI not higher than 0.450.

Table 4.17 Human Development Indicators of Selected Asian Countries, 1998.

	Adult Literacy Ratea %		Life Expectancy at Birth (years)		Population In Povertyb %	HDIc
	Female	Male	Female	Male		
Middle Income						
Indonesia	78	90	66	63	39	0.586
Malaysia	78	89	74	69	10	0.794
Philippines	94	95	69	64	38	0.621
Thailand	92	96	72	67	13	0.798
Low Income						
Cambolia	53	80	55	52	30	...
China	73	90	71	67	7	0.664
Lao PDR	44	69	52	50	46	...
Myanmar	78	79	63	60	....	0.406
Viet Nam	91	97	69	66	51	...
Bangladesh	26	49	58	58	36	0.309
India	38	66	65	62	36	0.382
Pakistan	24	50	62	63	34	0.393
Sri Lanka	87	93	75	70	35	0.665

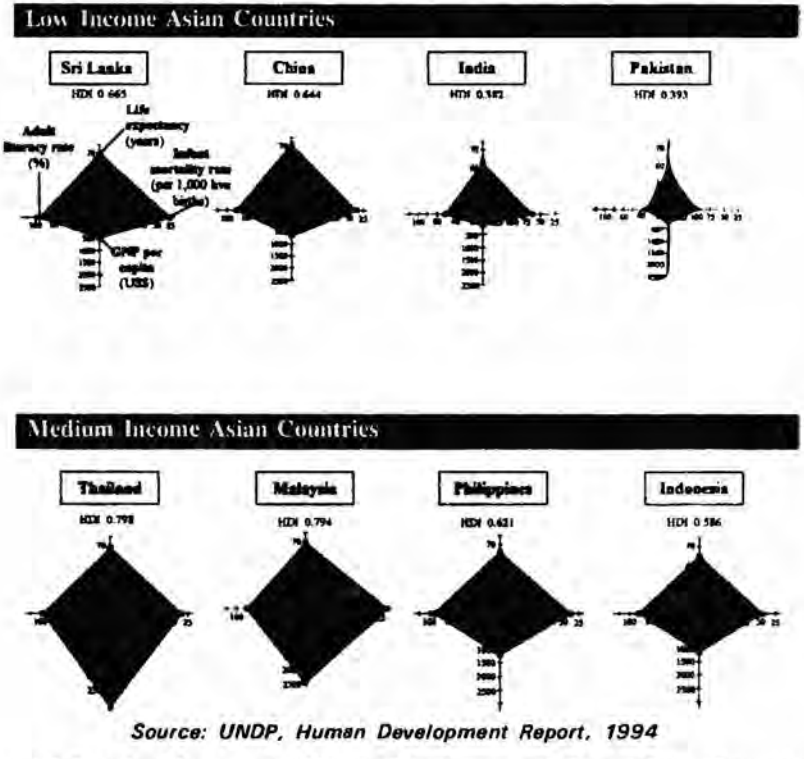
... Data not available.

[a] Refers to population of 15 years old and over.

[b] Refers to the headcount or proportion of the households (population) falling below the poverty line to total households (population). Data relate to years 1990 through 1998.

[c] HDI, human development index, an overall measure of the quality of life. See UNDP, 1994.

Sources: United Nations Educational, Scientific and Cultural Organization, Statistical Yearbook 1996 and past issues; World Bank, World Development Indicators 1998; Economic and Social Commision for Asia and the Pacific, Asia-Pacific in Figures 1997; Directorate-General of Budget, Accounting and Statistics, Statistical Yearbook; and country sources.



Source: UNDP, Human Development Report, 1994

Figure 4.2 Levels of Income and Human Development, Selected Asian Countries.

5. INTEGRATING MULTI CARRYING-CAPACITY CONCERNS: THE ROLE OF PUBLIC POLICY AND GOVERNANCE

5.1 Suggested Development Framework

The interactions of population pressure with the environment, natural resource endowments and the stakeholders of the development process are dynamic and complex warranting the need for integration within the context of human and sustainable development. A suggested development framework that links the multi C-C concerns of population, technology, socio-economic factors, management of the environment/natural resources, government policy and governance is schematically shown in Figure 5.1. This framework which was first introduced in 1990 (ADB, Economic Policies for Sustainable Agriculture, 1990) was modified to accentuate the role of government policies and governance.

The framework identifies several distinct but interrelated factors that determine the nature and strength of the linkages of population and environment with the other determinants of human and sustainable development.

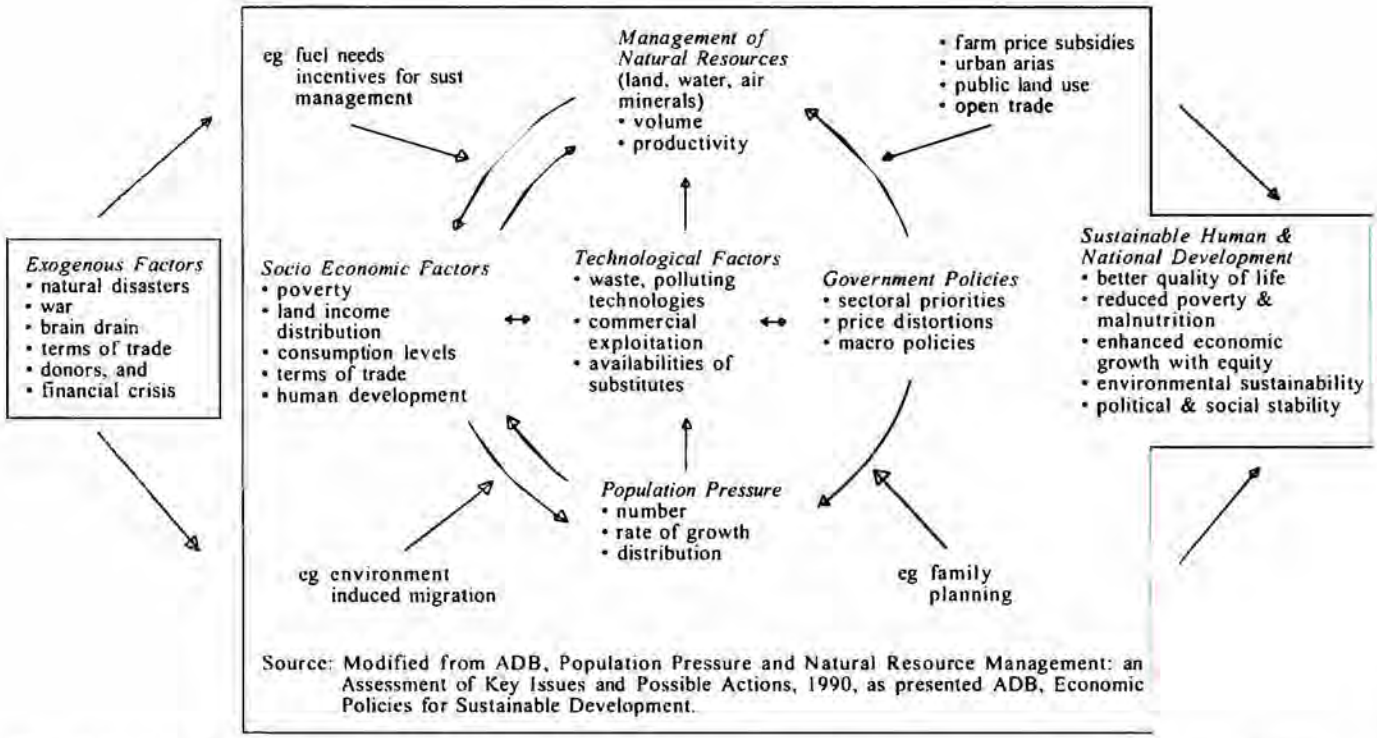


Figure 5.1 A Development Framework Linking Population, Technology and Natural Resources with other factors of Human and Sustainable Development

5.1.1 Exogenous factors

Largely, exogenous factors are those related to natural disasters (floods, storms, droughts among other), war, donors, and assistance from other countries. It can also include those of economic in nature, such as exodus of professionals (brain drain) in search for better employment opportunities; deteriorating terms of trade because of fluctuations of supply-demand conditions in external trade; and economic recession brought forth by external factors such as the Asian financial crisis of 1997.

5.1.2 Socio-economic factors

Within the context of human and sustainable development, the socio-economic factors summarize man's state of development: his/her levels of income, socio-cultural aspirations, poverty levels, level of distribution of assets and incomes, access to opportunities in education, jobs, access to basic support services, pattern of consumption and socio-cultural-religious values as a society. The socio-economic factors are the driving force (demand) in the dynamic interactions of population growth and the environment.

5.1.3 Technological factors

Technology can be simply defined as the state of art in doing things. The supply and availabilities of technologies that can shift the production function curves of most economic activities can, at the extreme, have indirect effects or negative externalities detrimental to the environment and the major stakeholders of the development process. The prevalence of waste from pollution producing technologies of industrial countries are examples of these. On the other hand, technologies that are environment friendly can prolong and sustain the life span of some environmental resources thus, creating the external economies whose benefits can be internalized by the same stakeholders.

5.1.4 Population

The dimensions of the population issue such as fertility, mortality, spatial mobility, the labor force and family formation are part of the demographic factors that should be incorporated in the process of demographic transmission and the analysis of the dynamics of population-environment/resources–sustainable development interface. In most C-C models, population is often considered as a stock variable (e.g. cattle population in a ranch) rather than a flow variable (e.g. human being with socio-cultural aspirations). In a human-sustainable developed paradigm, population should be treated beyond the concept of simply a biological organism that simply needs food but a homo sapiens with basic multiple socio-cultural needs.

5.1.5 Management of resource

Mankind has a bad history of managing his natural resources (land, water, air and minerals). Monitoring the level of productivity and degree of preservation and conservation should be part of resource and environmental management. Largely, most governments in the world assign the public sector to manage their natural resource endowments and the environment. The major argument for this is that natural resources and the environment are public goods and therefore should be managed by the public sector. However, the public sector, especially in the developing countries, had dismal records in efficiently managing their natural resources and environment. This brings to focus the need for new modalities (e.g. greater participation of endogenous people in natural resource management) in managing the natural resources and the environment.

5.1.6 Government policies

In the early stage of development, the public sector assumes the major function of defining developmental goals, allocating resources across sectors, and prescribing policies to sustain economic growth. Policies prescribed by the government can be sectoral in nature (those related to a particular sector such as agriculture, industry, or specific commodities) or the macroeconomic types (e.g. trade, monetary, and fiscal policies) dealing with national aggregates.

There is a special and unique role of government policies in the population-natural resource and environment interactions. Government policies provide the directional mechanism and legal framework by which the dynamic interactions of stakeholders take place. Macroeconomic policies, which define resource allocation across sectors, may or may not lead to efficient management of natural resources sector.

Some of the current policies adopted by most Asian countries whose impact on the exploitation of natural resources/environment need to be monitored and analyzed. Some of those are those related to openness in trade like: the General Agreement on Tariff (GATT), World Trade Organization (WTO), Asian Pacific Economic Conference (APEC) and the ASEAN Free Trade Agreement (AFTA) for ASEAN member countries.

To accentuate the role of government policies in the population-environment/ natural resources, a closer look at the GATT-WTO provisions on Agreement on Agriculture; Agreement on Application of Sanitary and Phytosanitary Measures; and Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS). General provisions of these agreements (Sereno, 1999) are expanded below:

a) Agreement on Agriculture

The Agreement on Agriculture consists of three major obligations:

1) the obligation to reduce domestic subsidies or support and to reduce current export subsidies as well as to refrain from granting new export subsidies,

2) the obligation to tariffy products previously subject to quotas; and
3) obligation to observe due notice and transparency requirements in imposing allowable export prohibition and restrictions.

b) Agreement on Application of Sanitary and Phytosanitary (SPS) Measures

The intent of the agreement is to ensure that sanitary and phytosanitary measures are not used as disguised form of non-tariff barriers. Members are encouraged to harmonize sanitary and phytosanitary measures by applying international standards and guidelines whenever applicable. Members may apply more than the international standards if there are scientific justification for such higher standards. International standards are defined as follows

1) for food safety, the standards, guidelines and recommendations established by the Codex Alimentarius Commission;
2) for plant health, the international standards, guidelines and recommendations developed under the auspices of the International Office of Epizootics;
3) for animal health and zoonoses, the standards. guidelines and recommendations developed under the auspices of the Secretariat of the International Plant Protection Convention in cooperation with regional organizations operating within the framework of the International Plant Protection Convention; and
4) for matters not covered by the above organizations, appropriate standards, guidelines and recommendations promulgated by other relevant international organizations open for membership to all members, as identified by the WTO's Committee on Sanitary and Phytosanitary Measures.

c) Agreement on Trade-Related Aspects of Intellectual Property Rights

The Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) requires the Members to make available:

"... patent protection for any inventions, whether products or processes, in all fields of technology, provided that they are new, involve an inventive step, and are capable of industrial application."

Members may exclude from patentability inventions, the prevention within their territory of the commercial exploitation of which is necessary to protect ordre public or morality, including to protect human, animal or plant life or health or to avoid serious prejudice to the environment, provided that such exclusion is not made merely because the exploitation is prohibited by law.

Members may also exclude from patentability:

a) diagnostics therapeutic and surgical methods for the treatment of humans and animals;
b) plants and animals other than micro-organisms, and essentially biological processes for the production of plants or animals other than the protection of plant varieties either by patents or by an effective sui generis system or by any combination thereof."

5.1.7 Governance

Governance is incorporated in the model because it is the most notable operational link that can facilitate and direct the dynamic and complex linkages and interactions of population, the environment and natural resources sector with the various stakeholders to a sustainable human and national development of a country. Given the dismal performance record of less developed countries in management of the natural resource endowments, the role of a good government cannot be overemphasized.

Governance simply means the quality of relationship between the government and its citizens whom it exists to serve. Another definition, is the "manner in which power is exercised in the management of a country's social and economic resources for development. Put more simply, governance means the way those with power use that power" (ADB, 1998).

Governance has both political and economic dimensions. The economic dimension of governance, sound development management (e.g. in the natural resource sector) is "the core of sustainable development". The four key components of governance (in natural resource management) need to be taken into account. These are accountability, transparency, predictability and participation. (ADB, 1998).

Accountability is the capacity to call officials of government to account for their actions. Effective accountability includes answerability and consequences. Government officials are answerable for their actions. They should respond whenever some citizens question their actions. These responses should be followed by predictable and meaningful consequences . . . not simply for compliance but for effective interactions.

Transparency entails low-cost access to relevant information, which are reliable and relevant. Economic and financial information is a must for the public to have access to.

Predictability results from laws and regulations that are clear, known in advance and uniformly and effectively enforced. Predictability of government economic actions is also needed as an indicator on which the private sector can rely to make its own production, marketing and investment decisions.

Participation is needed to obtain reliable information and to serve as a reality check and watchdog for government action. Past experience has shown that a strong civil society plays a critical role in advancing good governance. In natural resource

management, for example, there are positive results in allowing positive results in allowing wider participation of indigenous people in natural resource conservation.

6. CONCLUSIONS AND RECOMMENDATIONS

As the next millennium unfolds, we see a new world with an endangered future: a world entangled with the pressing challenges of population growth, deteriorating natural resource base and environment, technology plateaus, widening gaps of incomes between the rich and the poor and accelerated urbanization. This global scenario is likewise pervasive in developing countries, particularly Asia, whose indicative carrying-capacity trends have also already reached their limits.

Unlike in the old world where natural resources were still in abundance, stock of technologies readily available and human inhabitants of planet earth relatively smaller, we have to face the future with the greatest challenge of providing food security, the most basic quality of life indicator, to the human inhabitants of mother earth. This demographic transition for the next century is central to the survival of humankind.

Given the pessimistic scenario reviewed by this paper, the following recommendations are made.

6.1 The Establishment of Futures Center

This recommendation was earlier made by DAP/UPSE/UPPI in the PREPF study, to set up a national office (and probably an Asian Regional Office) to coordinate futures oriented studies at the national and Asian level. This idea should be brought across the ASEAN member countries for adoption considering that some negative impacts of population-natural resource interfaces (e.g. industrial pollution, global warming, forest fires) respect no political boundaries.

6.2 Emphasis on the Role of Technology

Appropriate environment friendly technologies have a major role in enhancing carrying capacities of less developed countries. First, technology, in the long term, if properly adopted and managed, lowers the per unit cost of food production. This will enable the poorer segments of society afford food supplied in the market. Since food commodities (e.g. rice) are wage goods in most developing countries of Asia, lower food prices, in effect, augment the effective purchasing power of the poor. This is regardless of where the food supply comes from.

The second point to be made on the role of technology is that anybody who is opposed to the generation, adoption and commercialization of appropriate technology is also anti poor. Technology and poverty alleviation are two sides of the same coin. Given the current depletion of the backlogs of technologies, in contrast to their availability and abundance in the past, developing countries have to fast tract technology generation and commercialization if they want to eradicate hunger and poverty.

6.3 Incorporation of Carrying-Capacity Methodologies into the Main Stream of Planning, Policy Analysis and Formulation

Methodologies on carrying developed over the years, are rich in scope and approaches. Yet they are isolated and fragmented and not incorporated in main stream development planning and policy formulation. These methodologies should be applied not only to the global and national levels but also at the local levels, considering that some C-C concerns are local-specific in nature. Indicators should be disaggregated at the local levels for a better treatment of their specific solutions.

6.4 Setting Up Congruent Policies

Macroeconomic and sectoral policies provide the prioritization guides in the development process. Since policies have general resource allocation mechanisms, they should be set in a consistent manner to avoid unnecessary sectoral imbalances and negative effects in their implementation. As the world becomes a global village, much is expected for more congruence and consistencies of government policies. Although the dynamic features of policy setting (e.g. the GATT-WTO Agreement) makes it difficult at times for developing countries to anticipate, still LDCs should devote vigorous research efforts in the understanding the likely consequences of these policies (laws, agreements, Executive and Administrative Orders) across sectors.

6.5 The Need for an Integrative Development Framework

Considering the complexities of the interactions of the concerns on population, natural resources and environment, an integrative development framework is imperative to link all these concerns more consistently. An indicative integrative framework (suggested earlier) that incorporates, socio-economic factors, demography, management of resources, set of policies and governance within the context of a sustainable human and national development, should be examined more closely.

6.6 Special Role of Political Governance

Political governance as discussed earlier, describes the quality of relationship between the governed (citizens) and their government. With a strengthening of its four pillars of transparency, accountability, predictability and participation, policy and administrative reforms related to carrying-capacity issues will have higher probability of being implemented more efficiently. In particular, economic governance, or sound development management is at the core of sustainable human and national development.

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THE SAFETY OF NOVEL FOODS

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ABSTRACT

This paper focuses on the food safety assessment and issues of foods derived by modern biotechnology, those produced by manipulation of living organisms at the cellular and molecular levels. Some of the food safety concerns of modern biotech or genetically engineered foods are: allergenicity, possible gene transfer from GMO to microorganisms. International organizations such as FAO-WHO and OECD have held expert consultations to establish food safety assessment procedures. Several countries such as the US, the European Union and Japan have established their food safety procedures for modern biotech foods. In the Philippines, the biosafety guidelines of the National Committee on Biosafety of the Philippines (NCBP) covers R&D activities in contained facilities up to controlled field tests. The author recommends the issuance of a clear government policy on GMOs so that regulatory agencies can have a framework within which rules, guidelines and procedures can be established. Since several agencies will be involved, the author further recommended the establishment of an overarching body that can span departmental responsibilities.

Keywords: modern biotechnology, genetically modified organisms, genetically engineered organisms, biosafety, food safety

Food is a basic necessity and the concern on the safety of food is universal. General awareness on food safety has been heightened through information technology. When any food poisoning outbreak occurs, particularly if it involves a vulnerable group such as children, the news is immediately disseminated to all parts of the world. Reports of the incidents have dramatically increased in the last few years, so that the ordinary consumer has started asking difficult questions such as: Is this a new phenomenon? Does this mean that the population is becoming

more susceptible? Or, are we creating new substances or re-engineering microorganisms which have some harmful effects on men and making them stronger and more resistant? And finally, what is the impact of globalization on the safety of the food supply?

Competent bodies and individuals will have to find the answers to all these questions. But today I propose that we look more closely at the specific concern on the use of new technologies to create new substances or engineer organisms vis-a-vis their impact on the safety of the food supply. The application of new technologies can result in "novel foods" which has been defined by the European Commission in its novel Foods Regulation (EC/258/97) to include the following categories: (1) Food consisting of or containing genetically modified organisms (GMOs), or produced by use of GMOs without containing them; (2) Food with a chemically modified molecular structure; (3) Food made from microorganisms, fungi, or algae, or food from plants or animals produced by new breeding methods; and (4) Food produced by an unusual procedure that leads to a change in the composition or structure of the food so that the nutritional value, the metabolism, or the amount of undesirable substances differs from that of conventional food.

One technology which has been receiving a lot of attention is biotechnology which is really not new, if we consider its classical definition: any technique that uses living organisms (or parts of organisms) to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses. Fermented foods and alcoholic beverages are examples of products of traditional biotechnology. For the purposes of this paper, however, we shall limit the discussion to modern biotechnology which is narrowly confined to the manipulation of living organisms at the cellular and molecular levels. That is, this paper will deal only with the first category of novel foods.

Modern biotechnology has made possible the production of plant varieties with specific traits such as resistance to crop pests, delayed ripening and improved nutritional quality. Within the past few years, a variety of foods produced using modern biotechnology have been approved in many countries. In the U.S., for example, seven new genetically engineered plants have successfully completed the USFDA's safety assessment process. These include three varieties of delayed ripening tomatoes, a virus-resistant squash, potatoes resistant to the Colorado potato beetle and herbicide-tolerant soybeans. However, just as with any new means of food production, while there are anticipated benefits, there are also potential human health risks that must be considered when foods are developed using modern biotechnology.

Biotechnology and Food Safety

"Food Safety" is defined as providing assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use. In the global market, the multilateral trade agreements established under the auspices of the World Trade Organization (WTO) recognize the standards and related texts of the

Codex Alimentarius Commission (CAC) as international points of reference for food safety. At present, however, there are no Codex standards that deal directly with the safety aspect or other technical requirements of genetically modified foods. But the current Medium Term Plan of the CAC has identified the work of relevant Codex Committees on various aspects of biotechnology to be of high priority.

When the subject of biotechnology and food safety was first introduced to the Commission, it realized the complexity of the matter and it also recognized the fact that much work had already been done on this topic. Therefore, the CAC at that time urged FAO and WHO to hold a joint meeting of experts who would recommend the appropriate strategy to deal with the safety of foods produced by biotechnology.

At least two such consultative meetings have been held, one in 1990 entitled "Expert Consultation on Strategies for Assessing the Safety of Foods Produced by Biotechnology" and another in 1996 entitled "Biotechnology and Food Safety". Some of the recommendations of the 1990 meeting include those addressed to national regulatory agencies to establish, maintain and enforce comprehensive food regulations for foods produced from biotechnology, that are based on sound scientific principles and data. The 1996 meeting, on the other hand, focused on the drawing up of recommendations for international guidelines for safety assessment of foods and food components which have been produced by techniques that change the heritable traits of an organism.

Food Safety Considerations

Food safety considerations regarding organisms produced by techniques that change the heritable traits of an organism such as rDNA technology, are basically of the same nature as those that might arise from other ways of altering the genome of an organism, such as conventional breeding techniques. These include the following:

1. Direct consequences (e.g., nutritional, toxic or allergenic effects) of the presence in foods of new gene products encoded by genes introduced during genetic modification.
2. Direct consequences of altered levels of existing gene products encoded by genes introduced or modified during genetic modification.
3. Indirect consequences of the effects of any new gene product(s), or of altered levels of existing gene product(s), on the metabolism of the food source organism leading to the presence of new components or altered levels of existing components.
4. Consequences of mutations caused by the process of genetic modification of the food source organism, such as the interruption of coding or control sequences or the activation of latent genes, leading to the presence of new components or altered levels of existing components.

Safety Assessment Principles

"Substantial equivalence," as the guiding principle in the assessment of genetically modified foods and food ingredients, was first articulated in 1993 by a Committee of the Organization for Economic Cooperation and Development (OECD). This concept was subsequently endorsed by the 1996 Joint FAO/WHO Consultative Meeting of Experts. Substantial equivalence embodies the concept that if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety, i.e., the food or food component can be considered to be as safe as its conventional food or food component counterpart. This is established by a demonstration that the characteristics assessed for the GMOs or the food derived therefrom are equivalent to the same characteristics of the conventional foods or food ingredients already available in the food supply, within the natural variation for such characteristics. Examination of the GMO-derived food or food ingredient for substantial equivalence is not a safety assessment in and of itself, but can lead to its categorization into one of three possible groupings.

The first is when substantial equivalence is established, in which case the food is regarded to be as safe as its conventional counterpart, and if the latter has been judged to be safe, then no further safety consideration need to be taken with regard to the GMO-derived food or food component.

The second group comprises those foods which have been demonstrated to have substantial equivalence to their conventional counterparts, except for specific new traits or gene products. The safety assessment for these foods should then focus on the new traits or products arising from the inserted gene. An example is the herbicide-tolerant soybean which derived its inserted gene from agrobacterium sp. CP4. The gene expresses the enzyme 5-enolpyruvylshikimate-3 phosphase synthase (CP4 EPSPS). The safety assessment of the GMO will have to include a thorough evaluation of the toxicity and allergenicity of this expressed protein.

In the third category are genetically modified foods which have no substantial equivalence to conventional foods. This does not mean that they are unsafe, but each must undergo a comprehensive testing program which will have to be designed on a case-by-case basis.

Food Safety Issues

Concerns on the safety of foods derived through modern biotechnology arise from the direct and indirect consequences of altering the genome of an organism, which can also take place in the conventional breeding techniques. These had been touched on earlier. But let us focus on two of the more prominent issues.

Allergenicity

Observations show that while there are not a large number of potential allegens in the food supply, recent food introductions (such as the kiwi fruit) have proven

to be additional sources of food allergens. There is a need therefore to pay particular attention to allergenicity when assessing the safety of foods produced through modern biotechnology. True food hypersensitivity is an immunologic, i.e., Immunoglobulin E- mediated, reaction to allergens in foods. Virtually all allergens are proteins.

Predicting the potential allergenicity of foods from genetically modified plants, animals and microorganisms requires the examination of a number of parameters which are common to many food allergens. Examples of these parameters are molecular weight (most food allergens have molecular weights between 10,000 and 40,000) and amino acid sequence homology to known allergens. The amino acid sequence of many allergens is readily available and the amino acid sequence of the gene product can be compared against these known databases.

Other physico-chemical attributes which may be useful in the assessment of the allergenicity potential of proteins expressed by the introduced genes are: (a) vast majority of allergens are resistant to heat and digestion juices; (b) allergens are usually the major proteins of the food; (c) allergens tend to be stable to processing (e.g., allergens in peanut butter, soybean meal).

In the identification of potentially allergenic gene products, an important criterion to consider is the source of the transferred genetic material. A gene transferred from a source known to be allergenic should be assumed to encode for an allergen, until proven otherwise. If the protein's amino acid sequence and other physico-chemical attributes raise concerns about the allergenic potential of the molecule, a series of additional tests is conducted, the first of which is a serological test to determine whether or not it is recognized by serum from individuals with known allergies. Confirmatory skin prick testing followed by an oral food challenge should be done following protocols for the use of human subjects.

Gene Transfer from Genetically Modified Organisms

The most relevant food safety issue concerning gene transfer is the potential consequence of the transfer of an introduced gene from a GMO to microorganisms in the intestinal tract, such that the gene can be successfully incorporated and expressed and thus impact on human safety.

The greatest concern in connection with possible gene transfer from the GMO to the gut microorganisms has to do with antibiotic resistance. Antibiotic resistance genes are present in transgenic plants as a result of their use as marker genes to facilitate identification of genetically modified cells or tissues during development.

The potential for transfer and expression of antibiotic resistance genes from transgenic plants to gastrointestinal microflora is of concern, as this could potentially affect the therapeutic efficacy of antibiotics. While the likelihood of transfer of an antibiotic resistance marker from plants to microorganisms in the gut is remote considering the complexity of steps required for gene transfer and expression, the USFDA believes that the use of antibiotic marker genes in crops should still be

evaluated on a case-by-case basis taking into account such information as: (1) whether the antibiotic is an important medication; (2) whether it is frequently used; (3) whether it is orally administered; (4) whether it is unique; (5) whether there would be selective pressure for transformation to take place; and (6) the level of resistance to the antibiotic present in the microbial populations. If a careful evaluation of the data suggests that the presence of the marker genes or gene product in the food compromise the use of relevant antibiotic(s), the marker gene or gene product should not be present in the finished food. The USFDA also notes that certain antibiotics are the only drugs available to treat certain clinical conditions (e.g., vancomycin for the treatment of certain staphylococcal infections). Therefore, marker genes that encode resistance to such antibiotics should not be used in transgenic plants used as food source.

There is greater probability of gene transfer from genetically modified microorganisms to microorganisms in the gut, as there are well known mechanisms of transfer of genetic material between microorganisms. The likelihood of maintenance of the transferred gene in a recipient organism increases if the gene confers to the microorganism a selected advantage. If this is so, the possible health consequences need to be assessed, based on the function and specificity of the gene.

Although the insertion of marker genes is a necessary part of the selection process, it should be noted that such genes can be removed at a later time. Moreover, there are recent developments that demonstrate the feasibility of using alternative marker systems not dependent on antibiotic resistance.

Regulations of the U.S., the European Union and Other Asian Countries on the Safety of Genetically Modified Foods

The United States

In the U.S., the regulatory authority for most foods is the USFDA while the USDA is responsible for meats, poultry and egg products. The FDA's authority is embodied in the Federal Food, Drug and Cosmetic Act of 1938. Fruits, vegetables, cereals, oils, milk, fish and shellfish may be introduced without pre-market approval although the agency may take legal action for violations of the Act. But producers of food additives must get pre-market approval for all additives that do not qualify for Generally Recognized as Safe (GRAS) exemptions.

The USFDA maintains that it has sufficient authority to regulate foods developed by biotechnology, such as foods derived from new plant varieties, under the Federal Food, Drug and Cosmetic Act, particularly the adulteration provisions of Section 402(a)(1) and the food additive provision (Section 409). It was this latter provision that was invoked in the evaluation of a specific substance in Calgene's Flavr Savr tomato. To develop this tomato, Calgene used recombinant DNA techniques to introduce an antisense polygalacturonase (PG) gene into the tomato. The sense PG gene, normally present in tomatoes, encodes the enzyme PG, which is associated with the breakdown of pectin (a constituent of the tomato cell wall)

and the resulting softening of ripe tomatoes. The antisense PG gene encodes a messenger RNA that suppresses the production of the PG enzyme. The result is a tomato that remains on the vine longer for enhanced flavor. In developing the Flavr Savr tomato, Calgene used a marker gene for kanamycin resistance, that encodes the enzyme, aminoglycoside-3'-phosphotransferase II (APH(3')II), to identify plant cells carrying the antisense gene. APH(3')II inactivates the antibiotics kanamycin and neomycin, and its presence in plant cells permits cells to survive and grow in the presence of these antibiotics, unlike normal cells which are killed by these antibiotics. This allows scientists to select transformed cells that have successfully taken up the desired gene. The USFDA, in addition to evaluating the safety and conducting a nutritional assessment of the tomato per se, also looked at APH(3')II enzyme, the only new substance in the Flavr Savr tomato, as an additive.

In 1992, the USFDA issued a policy statement clarifying its legal and regulatory framework for oversight of food derived from new plant varieties, developed by both conventional and new breeding techniques such as rDNA. The policy paper stressed the Agency's stand that irrespective of the method by which a food or food ingredient is produced, all products must meet the same stringent safety standards. The Agency recognizes that many of the food crops currently being developed with gene splicing techniques do not contain substances that are significantly different from substances already in the diet, and thus would not require pre-market approval as a food additive. However, the 1992 policy paper makes it clear that the USFDA will require pre-market approval as food additives for proteins (or other substances such as fatty acids and carbohydrates) produced by introduced genes if the protein differs substantially in structure and function from the many proteins that comprise conventional foods.

The European Union

Together with the Council Directive on the Deliberate Release of Genetically Modified Organisms into the Environment (90/220/EEC), the so-called "Novel Food" Regulation (EC 258/97) is part of a legislative framework for biotechnology in the EU. The regulation provides a scheme for those responsible for placing foods on the market and also for the control authorities to identify those cases where there is need to scientifically evaluate a food which is being offered for sale in the European Union for the first time. The underlying principle is a pre-market safety assessment of novel foods and food ingredients, either through a simplified notification procedure or a more stringent authorization procedure. The authorization procedure starts with the submission of a request to one of the Member States that leads to a Union-wide decision on the product. If foods and food ingredients produced from, but not containing GMOs (i.e., the inserted genes and their expressed proteins) can be shown to be substantially equivalent to existing foods, their placing on the market only requires a notification to the EU Commission. However, if objections are raised, the application will be shunted to an authorization procedure.

Japan

In Japan, the Guidelines for Safety Assessment of Food and Food Additives Produced by rDNA Techniques were established in February 1996, based on the OECD concept of "substantial equivalence". The assessment focuses on:

1. Properties of hosts, vectors, inserted genes;
2. Properties of the genetically modified crop, with emphasis on:
   (a) toxicity and allergenicity of the expressed protein;
   (b) difference from host in terms of composition, amino acid profile, fatty acid profile, presence of anti-nutrients, etc.

Since 1996, the Ministry of Health and Welfare has confirmed the safety of 22 genetically modified crops, clearing the way for the entry of these crops and their products into the Japanese market. To date, however, no genetically modified crop is grown commercially in Japan, but field trials are on-going.

It may be noted that while countries with regulations on the safety of GM foods have invoked the principle of "substantial equivalence" in the establishment of their regulations, their operationalization of this principle can differ. Let us take the difference between the US and EU regulations as a case in point. The US does not impose a pre-market approval on a GM food, except when it contains a new substance which warrants its regulation as a non-GRAS additive and therefore would require a pre-market approval. The EU, on the other hand, requires all such foods to go through a pre-market assessment procedure.

The difference is understandable, considering that there are cultural elements involved in the setting up of regulations which have to be acceptable to the community in general. Surveys have shown that Europeans are more suspicious of anything regarded as interference with nature, while Americans are more inclined to accept products of new technologies.

Labeling of Genetically Engineered Foods

Aside from the safety of novel foods, the labeling of novel foods is another issue that may not be easy to resolve, since products derived from GMOs or processed using GMOs may be indistinguishable from the conventional product. Examples are starch products derived from genetically modified (GM) maize, oil from GM soya bean, or beer prepared using enzymes derived from GMOs. These materials may be further processed before reaching the consumer, e.g., starch into glucose syrups which may in turn be used in jams. Thus, the tagging of every ingredient in a food product as consumed may be a formidable task indeed. There are some consumer groups, however, who believe that this can be done if GM crops are segregated from the point of agricultural production and monitored as they go through the food chain.

The issue on labeling, which should not be confused with food safety issues, has to do with public perceptions of risk and the information that should be provided on the product label to enable the consumer to assess the risk. Labeling requirements are in keeping with the principle that the consumer has the right to know the product and to choose whether or not to buy the product based on the information given to him.

Labeling requirements in the EU differ radically from those in the U.S.

The European Union

The EC Regulation on Novel Foods requires the labeling of products if the food contains or consists of a GMO. It further stipulates that the consumer must be informed if the novel food or food ingredient is no longer equivalent to the corresponding conventional food because of differences in the composition, nutritive value or use of the food. The regulation, however, does not say how the labeling is to be carried out and discussions are underway on the labeling scheme to be adopted. One particular aspect that is being looked at is the labeling requirement for foods containing or contaminated with genetically modified materials (i.e., the transgene and expressed proteins) at very low levels. In this regard, the usefulness of setting detection thresholds is being considered. The detectable limit for identifiable genetically modified material is currently 0.1%. It has been suggested that a practical threshold for GM foods might be 2%.

The United States

The USFDA, on the other hand, does not require, as a rule, labeling to describe the technique used in the development of a new plant variety, be it conventional or the use of biotechnology. However, if the technique used significantly changes the composition of a food, then labeling will be required. For example, if a tomato variety is developed without any Vitamin C, then it will have to be labeled to disclose the radical difference from the traditional varieties. The USFDA policy also requires that foods to which potential allergens have been added must be so labeled. Thus, tomatoes bred to contain a peanut protein would need to be labeled to disclose the presence of the peanut protein, unless it had been conclusively demonstrated that the new tomato was not allergenic to those allergic to peanuts. Notwithstanding this general policy of not requiring the labeling of GMOs, the USFDA allows producers or manufacturers to label a food as being a GMO (or not) if such information is deemed useful to the consumer or if it gives the product some marketing advantage.

The Wirthlin survey conducted for the International Food Information Council (IFIC) in February 1999, which asked 1000 US adult consumers about their attitudes toward food biotechnology, showed that four out of five US - based consumers support the current USFDA's labeling policy for GM foods that requires that these foods be labeled only if they had been significantly changed.

Japan

In Japan, labeling of GM foods is not required at the present time, since existing laws cannot enforce the labeling of GM foods whose safety and quality are equivalent to existing ones. However, in response to the request of consumer groups to make the labeling of GM foods mandatory, the authorities have started to re-examine regulations on food labeling.

A difficulty that arises in requiring food products derived from or containing GM materials to be properly labeled is the identification of these products. Research and regulatory laboratories are developing tests, usually based on polymerase chain reaction (PCR) that will enable such products to be clearly identified, in anticipation of the full implementation of national and regional labeling requirements.

Philippine Regulations on GMOs

In the Philippines, except for the biosafety guidelines of the National Committee on Biosafety of the Philippines (NCBP), which covers R&D activities in contained facilities up to the controlled field release of GMOs, I am not aware of regulations that relate directly to the safe consumption of foods or food ingredients from GMOs. This situation is similar to that in other ASEAN countries such as Thailand. At the Regional Symposium on Genetically Modified Foods held in Bangkok in March 1999, a Thai scientist (Dr. Saipin Maneepun) stated that this lack has adversely affected Thailand's trade with the EU. It has deterred its control agencies from ascertaining which portion of its soybean imports is genetically modified, and therefore, Thailand cannot comply with the requirement of EU for a certification that its exported soybean products do not contain genetically modified material.

I think the time has come for us to make hard decisions on genetically modified foods and food ingredients. As a start, we must have a clear national policy on GMOs. Should such materials be allowed for human consumption? If government deems it wise to ban such materials, a clear and unequivocal statement should be made so that even research activities of academic institutions and the rules regulating such activities can be made to comply with such a policy.

But if government decides to allow the entry and commercial production of GMO-derived foods and food ingredients under certain conditions, those conditions will have to be explicitly spelled out. For example, the policy could disallow the production and use of certain GM crops in a particular part of the country, not for food safety reasons, but because it is deemed that such a prohibition is necessary in order to preserve the rich biodiversity of that area.

Once a public policy on GMOs is in place, regulatory agencies can then have a framework within which rules, guidelines and procedures can be established, and the individual applications for clearances of developers and producers of GM foods/ food ingredients can be considered.

Since its creation in 1990, the NCBP has been functioning without a broad public policy on GMOs to guide it in dealing with individual applications for clearances for R&D activities, including the planned release of GM plants, on a case-by-case basis. Indeed, the NCBP has been doing a yeoman's job of regulating R&D on GMOs inspite of this policy vacuum.

The government entities that will be mainly responsible for the setting up and implementing of regulations on the safety of GM foods will be the pertinent bureaus of the Department of Agriculture and the Bureau of Food and Drugs (BFAD) of the Department of Health, with the participation of the Department of Trade and Industry, as these will definitely have implications on trade. The NCBP, however, should be ready to provide scientific inputs, such as results of the activities conducted during the development of the novel food, to assist the relevant regulatory agency in evaluating applications for clearances.

Since there will be many agencies involved, each one regulating a particular aspect of GM foods, it may be worthwhile to consider the establishment of an overarching body that can span departmental responsibilities. Such a body will be in the best position to effectively oversee the enforcement of existing or future regulations on GM foods and the close monitoring of GM technologies on human health and the environment.

There are some initiatives to put policies in place on the regulation of GMOs, but these are generally limited in scope. An example is Senate Bill 1313 entitled "Genetically Modified Organisms and Substances Ban Act" filed by Senator Gregorio Honasan in late 1998. The proposed legislation would make illegal the release of any genetically modified organism into the environment. It is silent, however, on the importation of GMOs.

While we should welcome initiatives such as those of Sen. Honasan to establish national policies on GMOs, such policies should be based on a careful weighing of our options, as each option would have potential risks as well as benefits.

Ultimately, whatever policy and regulatory decisions are made should be acceptable to the people, and therefore should have taken account of common values, as well as the wealth of scientific and technical knowledge already existing on the matter. These are time-bound. A decision made at a particular point in time will have to be revisited when additional knowledge is gained or when cultural values have changed.

The Philippines is fortunate in that other countries have gone ahead in the setting up and enforcement of regulations on the safety of GM foods. We should learn from the experiences and benefit from the know-how of the advisory and regulatory bodies of these countries if we wish to fast-track the development of a regulatory system to deal with this important category of novel foods.

Conclusion

Recombinant DNA technology has the potential for making a difference on the food security and economies of all countries, especially those that are agriculture-based. However, issues on the safety of foods derived from GMOs will have to be addressed in order to bring down the barriers to their acceptance before we can fully benefit from this technology. The safety assessment of foods derived from GMOs requires up-to-date legislation, and a food control system as well as trained manpower for the effective implementation of the laws and regulations. This is true not only for the Philippines but for all countries in the world. In this era of globalization, when raw material producers, processors and consumers of all regions of the world are interconnected, it is imperative that proper safety assessment of food and food components produced by genetic modification be practiced world-wide. We should do no less in the Philippines, otherwise we may find ourselves consuming food that had been rejected for being unsafe elsewhere. Or equally damaging, we may be rejecting as unsafe food commodities or products that can add to our food supply. Therefore, we should develop the capacity to screen and safety-test GMOs, as well as manage their release and use.

While it is of utmost importance to ensure that the food supply is safe, it is just as important to ensure the adequacy of that supply. To be able to do this, we should not be afraid to explore new technologies or non-traditional sources of foods.

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CRITICAL ISSUES AND STRATEGIES FOR THE DEVELOPMENT OF MAJOR AGRICULTURAL CROPS

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ABSTRACT

This paper is a comprehensive review of the critical issues and strategies to develop the major agricultural crops in the country. It has been recognized that the crops sector dominates Philippine agriculture and could serve as a logical and potent springboard for the country to achieve global competitiveness. Among the major agricultural crops are rice, corn, sugarcane, coconut, fruits such as banana and mango, vegetables, and fiber such as abaca, root crops. The impact of globalization on Philippine agriculture is discussed. The Philippine Government has recommended activities and policies to modernize agriculture by the AFMA.

Keywords: Philippine agriculture, crops, modernization, production, world trade, global competitiveness, rice, corn, sugarcane, coconut, banana, mango.

I. INTRODUCTION

The new world order has placed the Philippines in a situation where its capability and viability to produce a wider range of products and services and offer opportunities comparable to those of the rest of the world are challenged. Amid this prevailing socioeconomic landscape that speaks of market forces, competition, and free flow of trade in goods and services, modernization of the production sector plays a prominent role.

In addressing the demands of global competitiveness as well as the promise of economic development, the new administration of President Joseph Ejercito Estrada has identified modernizing agriculture as a major fore that will fuel the nation's economy and ensure the social well-being of all Filipinos. The goal is for the government to spare no effort in empowering the agriculture sector to develop and sustain itself under the principles of food security, poverty alleviation and social equity, people empowerment, global competitiveness, and sustainability.

The new administration has, indeed, ushered in a greater appreciation of the crucial role of agriculture in economic development, with globalization and food security as the central concerns. While it is convinced that a liberal agricultural trading regime based on the interplay of market forces is the best way to kick-off agriculture on the path to progress and prosperity, a bigger challenge is providing the Filipino people at all times with access to enough food for an active, productive, and healthy life.

At the center of the Estrada Administration's agricultural modernization thrust is the fusion of the goals to meet the food requirements of the present and future generations of Filipinos, and to attain profitable production of world-class agricultural products that globalization will not only be competitive globally but flourishing locally – a decent source of livelihood for two-thirds of the Filipino poor who live in the rural areas, and a renewed hope of food that is accessible, available, nutritious and safe, and affordable to all.

A. Patterns of Agricultural Growth

Every new administration of this nation has set out to make economic development a reality – changing policies and experimenting with strategies, but the dream has remained elusive. One major setback to the realization of this dream is the status of the agriculture sector in the country, which speaks of minimal growth and low priority.

Among Asian countries as well as world averages, the growth of the Philippine agriculture sector has been less than satisfactory (Table 1). The poor performance of Philippine agriculture is the primary cause of the high incidence or rural poverty at 47% in 1994 and 44.4% in 1997. Low purchasing power in the rural sector is a serious drag to the development of consumer and industrial goods sectors.

Table 1. Comparative average annual growth of the agriculture sector (%)

Country	1980-90	1990-97
Vietnam	4.3	5.2
China	5.9	4.4
Pakistan	4.3	3.8
Thailand	4.0	3.6
India	3.1	3.0
Indonesia	3.4	2.8
Malaysia	3.8	1.9
Philippines	1.0	1.9
All middle income	3.5	2.3
World 2.8	1.8	

Note: Agriculture includes crops, livestock and poultry and fisheries.
Source: World Bank, World Development Report 1998/99.

In the sixties the spurt in agricultural growth has not brought about by an increase in productivity per hectare of land but by expansion of land put under cultivation. In the late seventies productivity was achieved in grains due to the Green Revolution technologies and the massive subsidies extended by the Marcos Administration to ensure the success of its Masagana 99 rice production program. In the middle and until the latter part of the eighties the economy was hit hard by a politico-economic crisis. And in the nineties agricultural productivity continued to decline as a result of the combined effects of past neglect, exhaustion of our natural resource base, and weather-rooted factors such as the El Niño phenomenon.

Table 2 shows the lackluster performance of Philippine traditional crops, rice and corn, as compared to our Asian neighbors. Except for Thailand, our productivity in rice was lowest among selected Asian countries. In corn, the Philippines' yield was lowest at 1.72/ha. In 1995, the country imported about 263,250 metric tons (MT) of rice and 208,020 MT of corn. In 1996, importation rose to 862,380 MT of rice and 402,340 MT of corn. Importation has since continued to rise due to drought in certain parts of the country caused by El Niño, reaching as high as 2.0 million MT in 1998. Currently, the country is already importing substantial amounts of agricultural commodities, including sugar, except for coconut and its byproducts.

The performance of the crops subsector, as compared to the livestock, poultry and fishery subsectors, waned over the last 30 years. From 6.7% per annum in the early 1970s, it slid to negative growth in the early 1990s. Although there were some fast-effect and high-value crops, their size relative to the crops subsector was too small to effect a reversal. However, the influence of the crops subsector on total agricultural remains very substantial.

Table 2. Comparative average annual growth of the agriculture sector (%)

Crop/Country	1964-65	1979-81	1991	1993	1994
Rice					
Taiwan	3.65	4.24	5.66	--	--
Japan	5.15	5.59	5.86	4.59	6.77
Korea	3.33	5.51	6.01	5.81	6.10
Philippines	1.25	2.18	2.83	2.80	2.90
Thailand	1.61	1.89	2.00	2.20	2.40
Vietnam	1.02	2.12	3.09	3.50	3.60
Indonesia	-	3.26	4.35	4.38	4.34
Corn					
Taiwan	2.10	3.04	4.56	-	-
Philippines	0.68	0.97	1.30	1.43	1.72
Thailand	2.19	2.20	2.38	2.04	3.17
Indonesia	-	1.46	2.15	2.18	2.18

Source: FAO Production Yearbook, various issues.

Under the Ramos Administration, from January to June 1998, the agriculture sector posted a considerable 7.15% reduction in aggregate output. Livestock and poultry farms came up with moderate output increases. Fishery production was slightly lower than in 1997. The big slump in crop production brought down agriculture to its worst performance record in 20 years. In terms of gross value of output at current prices, the sector earned P245.1 billion indicating a 1.94% reduction from the 1997 level.

The effects of El Niño on crop farms translated into a 14.61% contraction in aggregate output in 1998. Rice and corn production decreased by 26.57% and 43.62%, respectively. With the exception of tobacco, abaca, mungbean, onion and rubber, all other crops registered output losses in the first six months of 1998. The crops subsector grossed P127.6 billion at current prices, down by 9.68% from the 1997 level.

B. Emerging Global Challenges

The emerging global economic environment presents tremendous opportunities and challenges for Philippine agriculture as it crosses into the next century.

The entry of the Philippines into the World Trade Organization (WTO) in 1995 lifted practically all quantitative import restrictions but provided higher tariffs on sensitive agricultural products through the year 2004. Prior to this, the country had made commitments under the ASEAN Free Trade Agreement (AFTA) regarding accelerated tariff reductions. In late 1999, discussions on various trade agreements will continue, so must the safety net measures be adequately and concurrently put in place to make producers productive or make them diversify to other activities. This leads to the strategic question: How should we prepare and enable Philippine agriculture to meet the rigors of global competition?

With the country's low agricultural productivity, specifically in the crops subsector, trade liberalization in agriculture is a virtual prescription for economic suicide. At the present level of productivity, the agriculture sector will encounter serious difficulties in adjusting to the new trading arrangement particularly when its full impact is felt in year 2004. While it is true that a liberalized trading regime offers many opportunities for growth, only those that are competitive would be able to take advantage of these opportunities.

Unless the government addresses the basic and structural weaknesses of agriculture, the sector will be unable to supply the food requirements of the present and future generations of Filipinos, much less take advantage of the opportunities presented by the WTO. This will require radical changes not only in terms of priorities but in the manner by which the new administration fosters the development of the countryside. This will involve the modification of our thinking, attitude, policies, institutions and expenditure priorities so that Philippine agriculture will be prepared for the keener competition that will surely happen when the full implementation of the new global trade agreement is realized.

To this, modernizing Philippine agriculture is the new administration's response to globalization, and its strategy toward a hunger-free nation. Hence, under

the Agriculture and Fisheries Modernization Act (AFMA) passed in 1997, global competitiveness and food security are two major concerns. The AFMA has been dubbed as a landmark legislation *"prescribing urgent related measures to modernize the agriculture and fisheries sectors of the country in order to enhance their profitability and prepared said sectors for the challenges of globalization through an adequate, focused and rational delivery of necessary services"*.

Among the goals and objectives of AFMA in the area of global competitiveness include increase in the volume, quality and value of agriculture production for domestic consumption and for export, reduction in post-harvest losses, increase in the number/types and quality of processed products, and a wider level of entrepreneurship among farmers. It recognizes that in a liberal trade environment, significant improvement in agricultural productivity, product quality and production cost driven by technological change is necessary.

In the area of food security, AFMA seeks to meet the food requirements of the present and future generations of Filipinos in substantial quality, ensuring the accessibility, safety, availability and affordability of food to all, either through local production or importation or both, based on the country's existing and potential resource endowment and related production advantages.

II. STATUS OF MAJOR AGRICULTURAL CROPS

Although growth and structural change within the crops subsector is slow primarily due to lack of diversification, it continues to dominate the agriculture landscape and hence, remain as the logical and potent springboard for achieving global competitiveness and food security in the country.

In this paper, analysis of individual agricultural crops was limited to a selection of dominant crops which make up a significant proportion of the subsector in terms of area planted, volume of production, and value of produce; and which carry tremendous potential in terms of meeting the government's goals of food security and global competitiveness.

Major crops that are deemed responsive to the country's thrust for food security and global competitiveness include rice, corn, rootcrops (camote and cassava), legumes (peanut and mungbean) and vegetables (onion, tomato and cabbage), and crop commodities where the country is a net exporter such as sugarcane, coconut, fruits (banana, mango, and pineapple), abaca, and other fruit crops (exported as processed).

A. Rice (Palay)

When one speaks of food security in the Philippines, it usually means sufficiency in rice. Rice is a dominant crop in the country because it is a major staple of the Filipino diet. The dominance infuses the crop with a significant socio-political mystique which ensures that this subsector is favored by policy and heavily supported by public investments, or at least more investments than other crops.

As a staple food of over 90% Filipinos, the attainment of rice self-sufficiency remains an important policy objective of the government. Estimated per capita consumption is at 103kg/yr, contributing an average of 35% of the total calorie intake. About 70% Filipinos are dependent on rice cultivation and marketing for their livelihood.

1. Production

Rice occupies 3.17 million ha (irrigated and rainfed) of land with an average yield of 2.7 MT/ha. Production volume is at 8.55 million MT (Table 3). Central Luzon (Region 3), Cagayan Valley (Region 2), Southern Tagalog (Region 4) and Western

Table 3. Status of rice production in the Philippines, 1994-1998

RICE	1994	1995	1996	1997	1998
Hectarage	3,651,530	3,758,691	3,951,136	3,842,270	3,170,042
Production (MT)	10,538,054	10,540,649	11,283,568	11,268,963	8,554,824
Yields/ha (MT/ha)	2.89	2.80	2.86	2.93	2.70
Areas of production	Top 4 regions: Region 3, 2, 4, and 6 Top 5 provinces: Nueva Ecija, Isabela, Pangasinan, Iloilo, Cagayan				
Major problems	• Low yield stagnation (and a possible yield decline) due to declining hectarage; limited use/availability of good seeds; varietal constaint; insufficient production and post production inputs; uncertainties in climate; depleted natural resource base; and high post-harvest losses. • Deterioration of irrigation systems (given low and decreasing investments in systems maintenance). • Increased pests (insects (insects, weeds) and diseases in some areas. • Inadequate funding for research, development and extension (RDE).				
Major problems encountered and constraints to marketing	• Wide gap between farm gate price of palay sold by farmers and retail price of milled rice traded by wholesalers and retailers. • Increase in price of milled rice does not translate to increase in the price of palay at the farm level. • Gap in the wholesale to farm margn has increased dramatically over the years. • High cost of transport and distribution due to lack of physical infrastructure such as farm-to-market roads and port facilities.				

Note: Refer to Appendix Table 1 for 1990-98 data on estimated rice production, area harvested and yield per hectare by region. Appendix tables are available from NAST.

Figure 1. Rice area harvested , by region ('000 ha) 1998

Visayas (Region 6) contribute the largest share of the total rice production (Figure 1). The five top rice producing provinces are Nueva Ecija, Isabela, Pangasinan, Iloilo and Cagayan (Figure 2). Rice production contributes 17% to the Gross Value Added (GVA) in agriculture, with the total industry valued at P11 billion.

From 1970 to 1977, the country achieved increases in production because of the combined effect of increases in area harvested and yield. The quest for self-sufficiency and food security became part of an increasingly common agenda, and led to a worldwide Green Revolution. It was perhaps greenest in Asia, with the Philippines under the Masagana 99 rice program. The period was characterized by the expanded utilization of high-yielding varieties and availability of subsidized credit under the rice program. While before, exports had been sporadic and consisted mostly of re-exports influenced by policies on import duties, for a brief and heady time starting 1978 the Philippines became a rice exporter. That same year, however, area harvested began to stagnate. Among the problems encountered under the program were loan repayment, post-harvest operations, and marketing.

The political upheaval in the 1980s caused dislocations in the bureaucracy, eventually resulting in reduced production growth. Production was highest in 1994

Figure 2. Top five provinces in area harvested to rice ('000 ha) 1997

because of the relatively higher yield per hectare (2.89 MT/ha) compared with the previous year, plus an 11% expansion in area harvested. Almost the same production level was obtained in 1995 attributed to a slight increase of about 3% in area planted to rice. Yield declined by about the same percentage.

Over the years, irrigated and rainfed rice production in the country has been fluctuating, dipping to a low 8.55 MT in 1998 (Appendix Table 1) primarily attributed to weather-rooted factors such as the El Niño. In general, the country has yet to achieve further production increases given the current low yield of 2.7 MT/ha compared with the potential yield in on-farm trials of 6 MT/ha. This low yield can be attributed to the decline in area planted to rice; limited use/availability of good seeds; insufficient irrigation; varietal constraint; insufficient production and post-production inputs; uncertainties in climate; depleted natural resources base; high post-harvest losses; and inadequate funding for research, development and extension.

Out of the total 9.9 million ha devoted to agriculture in the country, only 39% is planted to rice (Appendix Table 2). Regions 4 and 5 had the highest agricultural

land area, but only 30% area planted to rice. For the whole country, only 35% of the area planted to rice are covered by irrigation as of 1997.

2. Gap Between Production and Consumption

In determining the rice (palay) requirements of the present and future generations, the Policy Action Group of the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD) considered three main uses of palay – as seeds, for human consumption and for buffer stock requirements for 90 days. Using the Bureau of Agricultural Statistics (BAS) data from 1991-1998, the trend was used to project the 1999-2004 palay requirements. Human consumption of rice was computed based on per capita consumption and the increasing population.

The computed total consumption of palay based on the requirements for seeds, actual consumption and buffer stock for the whole country reached as high as 13.9 million MT in 1998, 15.3 million MT in 1999, and 17.9 million MT in year 2004 (Appendix Table 3). Compared with the country's estimated palay production (Appendix Table 1), self-sufficiency in rice is far from being realized given an estimated deficiency of 5.4 million MT in 1998, 4.8 million MT in 1999 and 6.9 million MT in year 2004 (Appendix Table 4, Figure 3).

Meanwhile, under the Agrikulturang Makamasa Rice Component of the Department of Agriculture (DA), the projected total rice (milled) requirement for the year 1999 was pegged at 8.12 million MT. This figure was based on the current population of 72 million Filipinos multiplied by an annual per capita consumption of 103 kg, plus wastes, seed requirement, industrial and other uses. Compared with the estimated total supply of 7.21 million MT milled rice in 1999, a deficit of 900,000 MT was obtained. To narrow the gap between production and consumption toward self-sufficiency in rice, production needs to increase yearly by 1.65 million MT milled rice per year – driven by technological advancement through research and development (R&D), complete support from all sectors, and a genuine political will by all stakeholders.

In the past, importation was done to meet the country's rice (palay) consumption. The volume of importation ranged from 6,000 MT in 1991 to 2 million MT in 1998.

3. Marketing

From the farm to the end users, rice is distributed primarily by the private sector, with the government handling a very small portion of the produce that enters the marketing system. From the farm to the consumption point, the movement of rice produce through private traders is made possible by numerous market intermediaries such as local assemblers, assembler-wholesalers, millers, wholesalers, wholesalers-retailers, and retailers performing different marketing services. The grains marketing arm of the government, the National Food Authority (NFA), can purchase only 5-10% of the total rice production due to limited budget.

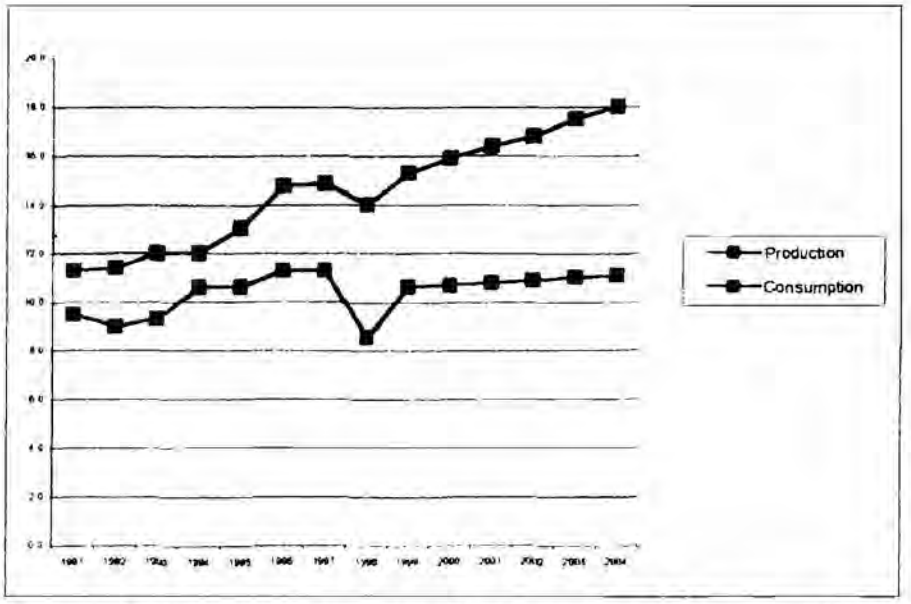

Figure 3. Production and consumption of palay in the Philippines, actual (1991-1998) and projections (1999-2004).

Given this marketing scheme, a major constraint is the wide gap that exists between farm gate price of rice sold by farmers and the retail price of milled rice traded by wholesalers and retailers. Increase in price of milled rice does not translate to increases in the price at the farm level. In fact, over the years, the gap in the wholesale to farm margin has increased dramatically. There is also the problem of high cost of transport and distribution due to lack of physical infrastructure such as farm-to-market roads and port facilities.

B. Corn

Just like rice, growing and eating corn has become a way of life for most Filipinos. One-third of the farmers in the country are engaged in corn production and most of them are small holders. As the second most important crop in the Philippines, about 12 million Filipinos prefer white corn as their main staple. Thirty percent (30%) of the total production is consumed as food in the Visayas and Mindanao. Meanwhile, 70% serves as an important input to the swine and poultry industries and some industrial applications. Corn is also processed into high value products, such as corn starch, corn oil, glutten and snack foods. About 60-70% of feed formulations use corn as the major ingredient. Fifty-four percent (54%) of total corn production comes from yellow corn, which comprises one-third of the total corn area.

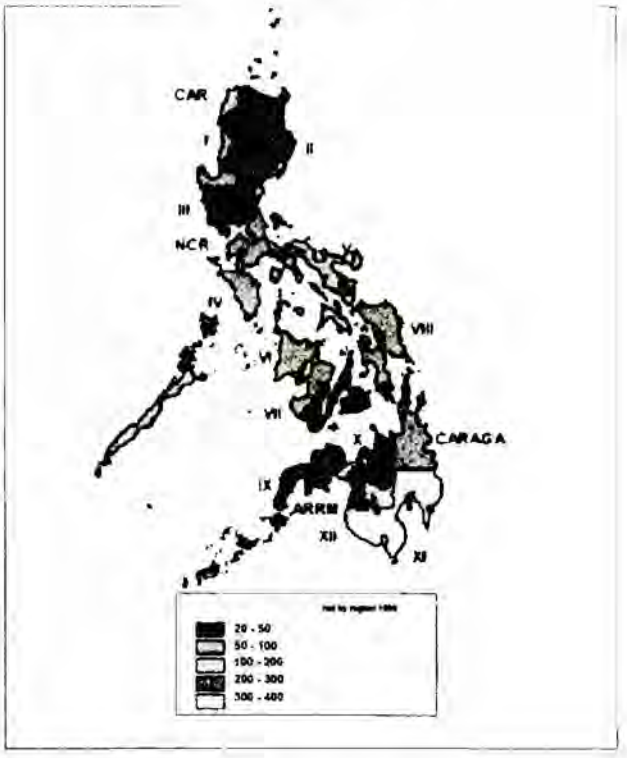

Figure 4. Corn area harvested ('000 ha) by region 1998

1. Production

Corn is largely grown in Mindanao which occupies about 60% of the entire area harvested to the crop. The combined output from Mindanao represents 70% of the country's total corn production. The top corn producing regions in the country are Northern Mindanao (Region 10), Southern Mindanao (Region 11), Central Mindanao (Region 12), the ARMM, and Cagayan Valley (Region 2) Figure 4).

On a national average, corn production in the Philippines is inefficient. Mean yield has been extremely low at 1.62 MT/ha in 1998, as compared to major corn producing countries in the world. Despite the low yields attributed to marginal white corn areas planted to traditional varieties, prime yellow corn areas have demonstrated high yield growth rates due to the adoption of high yielding open-pollinated and hybrid varieties. The yellow corn technology demonstrated an annual yield growth rate of 15% over the past 25 years, in contrast to the white corn technology which exhibited only 1.4% annual growth rate during the same period.

Domestic corn production decreased from its peak of 4.85 million MT in 1990 to a record low of 3.82 million MT in 1998. On the other hand, the composite

demand for corn for food, feed and other industrial uses is projected to growth annually by 3.94% for the period 1995-2004. In fact, the demand from 1995 to year 2000 is 6.3 million MT, while total corn supply at present growth level is only 5.2 million MT. If current productivity and profitability levels do not improve, the production-consumption gap is expected to worsen by the year 2000. On the average, some 400,000 MT of corn have been imported annually since 1990, mainly to meet the requirements of the livestock and poultry sector.

As a result of poor profitability, area harvested to corn declined drastically from 1990 (3.8 million ha) to 1998 (2.3 million ha.) This decline can be traced mainly to the shift to white corn areas to yellow corn and to the production of high value crops. In major corn producing provinces, farmers have exited from about 670,000 ha of white corn lands, while shifting to only about 150,000 ha for yellow corn production. To a greater extent, traditional and marginal white corn areas may have likewise been left idle as farmers seek employment in urban areas.

The corn industry is faced with varied problems that hinder government efforts to achieve corn self-sufficiency. These include low level of adoption of improved corn technology, especially the use of open-pollinated and hybrid varieties (as a consequence of inadequate supply of seeds), declining public investment in corn research and development (R&D), inefficient delivery of extension services, lack of credit support, and inefficient corn marketing and distribution system.

Table 4 summarizes the status of corn production in the Philippines, while Appendix Table 5 presents ten-year (1990-1998) data on estimated corn production, area harvested and yield per hectare by crop type by region.

2. Marketing

The protection of the corn industry through quantitative restrictions has discouraged the transformation of the corn sector into a more efficient industry. Overall, average product costs the farmgate level stood of about 10% higher than CIF prices of imported corn. This non-competitiveness of domestic corn can be attributed to a confluence of constraining factors, such as: 1) low adoption of modern corn production technologies; 2) high cost harvest losses; and 3) high transport and marketing costs due to inadequate infrastructure.

The production and marketing inefficiencies plaguing the corn sector have adversely affected farm profitability over the years. The absence of clear and consistent corn import policies continues to depress farmgate pries to levels that do not afford corn farmers reasonable returns.

Marketing agents of corn include farmers, local assemblers, local millers, grain wholesale dealers, wholesale millers, feed millers and retailers. Imported corn competes with local produce when its arrival coincides with peak harvest season. Seasonality of domestic corn supply causes price fluctuations within the year. Other marketing problems include increase of prices of farm inputs, insufficiency of funds of the NFA to support its floor price scheme, and inadequate transport.

Table 4. Status of rice production in the Philippines, 1994-1998

CORN	1994	1995	1996	1997	1998
Hectarage (ha)	3,005,820	2,692,332	2,735,723	2,725,875	2,354,208
Production (MT)	4,519,246	4,128,510	4,151,332	4,332,417	3,8 3,184
Yields/ha (MT/ha)	1.50	1.53	1.52	1.59	1.62
Areas of production	Regions 2, 10, 11, 12, ARMM				
Major problems encountered and contraints to production	• Inadequate postharvest processing, and storage • Decline in area harvested to corn • Limited access to available support systems (credit support, crop insurance, etc) • Low adoption of recommend technologies • Very limited investment investment to corn research, development & extension • Threats: competition from other countries, high population growth rate, decreasing land area devoted to corn				
Major problems encountered and constraints to marketing	• Imported corn competes with local producer when its arrival coincides with peak harvest reason. • Seasonality of domestic corn supply causes price fluctuations within the year. • Other problems are increase in prices of farm inputs, insufficiency of funds of the NFA to support its floor price scheme, and inadequate transport and infrastructure.				

3. Globalization and the Corn Sector

The corn sector has catapulted itself into a new era with the Philippines' commitment to regional and international trade agreements. Under the General Agreement on Tariffs and Trade – World Trade Organization (GATT-WTO), the country is required to provide a minimum access quota or volume for imported corn of 130,000 MT starting 1995 and increasing to 217,000 MT in 2004 at 35% tariff. Quantities imported over these levels carry higher tariffs of 100% in 1995 and declining to 50% in 2004.

Private sector importation of corn substituted in mixed feeds, i.e., feed wheat and barley, are allowed and carry lower tariff rates. Likewise, in-quota imports of swine and poultry under GATT-WTO enter the country at 30% to 40% tariff, respectively. Out-quota tariff rates for the two commodities are slightly higher at 40% and 65% for 1998, declining to 35% and 50% by 2000.

Globalization implies that both the corn and livestock industries must economically survive in trade with declining tariff protection of its domestic product. Domestic corn can be price competitive with corn imported under out-quota tariff rates. However, import-quality differentials and the entry of low-price corn substi-

tutes continue to exert downward pressures on farmgate prices of domestic corn. High domestic corn prices, on the other hand, adversely impact on the competitiveness of the local livestock and poultry industry, in view of the lower tariffs on meat and poultry imports. The economic survival of both industries can only be met if adequate quality corn can be produced and sold at prices that ensure both farmers profitability and the competitiveness of the livestock and poultry sector. Hence, a shift to a new paradigm of agricultural development strategy for the corn sector is imperative.

C. Sugarcane

The sugar industry has been and will continue to be a mainstay of the Philippine economy, with more than half a million workers and another two to five million people directly and indirectly depending on it. Sugarcane is the fourth major agricultural crop of the country, next to rice (palay), coconut and corn. The sugar industry provides direct employment to about 556,000 workers in the agricultural sector with 25,000 mill workers.

In terms of world production, the average area harvested and production volume (sugar) for 1990/91-1994/95 were 21.11 million ha and 74.9 million. MT, respectively. Leading producers for the same period were India, Brazil, China, Cuba and Thailand, with the Philippines ranking ninth in terms of area harvested (0.37 million ha) and 11th among the world producers with 1.9 million MT.

1. Production

Currently, the Philippine province of Negros Occidental accounts for 56% of total production, mainly because its regular monsoon rains and low typhoon incidence complement its good soil. Sugarcane growing, however, is widely dispersed: 17 provinces located in eight regions grow sugar. Other top-producing provinces include Negros Oriental, Bukidnon, Tarlac and Davao del Sur. It is grown on a wide variety of soil types, from sandy loams to clay loams and from acidic volcanic soils to calcareous sedimentary deposits.

Area planted to sugarcane fluctuated from 1992 to 1996, averaging at 345,920 ha/year. It increased by 24% from 302,000 ha in 1995 to 375,1000 in 1996. However, it again declined to 330,486 ha in 1998 (Appendix Table 6). Largest area planted to sugarcane in 1998 was in Western Visayas (Region VI) at 184,402 ha (Figure 5).

Average production of sugarcane from 1992 to 1996 was 22,165,320 ton cane (TC)/year. Production in 1996 was 23,142,000 tc, up by 33% from 1995. However, it again declined to 17,347,886 TC in 1998 (Appendix Table 7). Western Visayas led domestic production in 1998 (9.65 million TC), followed by Central Visayas (2.25 million TC) and Southern Tagalog (1.63 million TC). Table 5 summarizes the status of sugarcane production in the Philippines. *(Volume of production is expressed in ton cane (TC) or sugarcane harvested from the farm, as differentiated from metric ton (MT) for sugar.)*

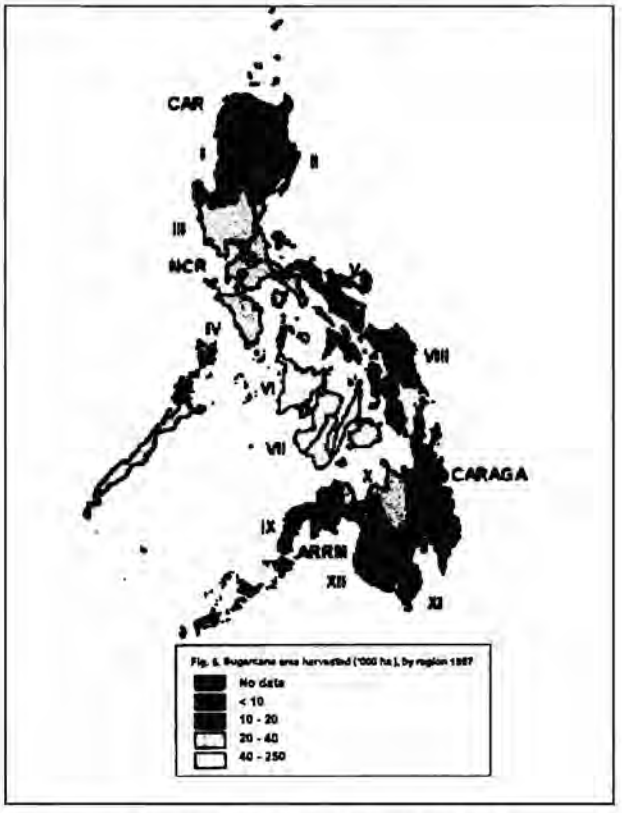

Figure 5. Sugarcane area harvested ('000 ha) by region 1998

The 1990s have been marked by periods of declining productivity, mostly due to poor farm cultivation and poor harvesting schedules in addition to insufficient incentives for development and limitations on land ownership, transfer, and lack of mortgage value due to the Comprehensive Agrarian Reform Program (CARP). The scientific community, however, attributes these low figures to years of government neglect in research and development, as well as inadequate extension services.

A study conducted in 1995 by the Madecor Group, a consulting company, identified the reasons for the continuing decline in sugar farm productivity. Some of the reasons cited were the decline in piculs sugar per ton cane or PSTC, brought about by the lack of high-yielding cane varieties, diseases, outdated farm practices, improper fertilization, and post harvest losses, among others. Meanwhile, Ledesma (1997) identified constraints to sugarcane production as follows: 1) lack of irrigation facilities; 2) limited adoption of newly released varieties; 3) insufficient and improper fertilizer application; 4) poor ratooning characteristics of current varieties; and 5) inadequate control of pests and diseases. Among the industrial constraints

Table 5. Status of sugarcane production in the Philippines, 1994-1998

RICE	1994	1995	1996	1997	1998
Hectarage (ha)	401,635	302,005	395,640	375,181	330,486
Production (MT)	24,695,188	17,774,401	23,142,220	22,273,095	17,347,886
Yields/ha (MT/ha)	61.49	58.85	58.49	59.37	52.49
Areas of production	Regions 6, 7 and 4 Provinces: Negros Occidental, Negros Orienta, Bukidnon, Tarlac, Davao				
Major problems	• Insufficient supply of outstanding varieties suitable to specific areas • Inefficient distribution of high-yielding varieties • Inappropriate fertilizer recommendation • Poor adoption of recommended technologies/cutlural management practices • High production cost • Planter-miller sharing system that hinders production of quality canes • Low priority on research, development and extension (RDE)				
Major problems encountered and constraints to marketing	• Increasing cost of production (high prices of sugar compel industrial users to use cheaper sugar imports) • Lack of credit assistance for small producers • Implementation of the Comprehensive Agarian Reform • Law that reduces incentives to investment Low tariff protection • Depressed domestic sugar prices (due to imported sugar) • Postproduction handling losses • Inefficient mills				

Note: Refer to Appendix Table 6 and 7 for 1990-98 data on area harvested to sugar and volume production by region.

he identified include: 1) milling and processing losses; and 2) handling and transportation losses.

As of 1995, there were 208,618 sugarcane farms in the country with average production of 63 ton cane per hectare (TC/ha). The 39 sugar mills in the country have a combined rated capacity of 183,328 MT per day. The nine refineries have a combined capacity of 82,500 50-kilogram bags (Lkg) per day. At constant prices in 1996, sugarcane had a 6% share in value of production of agricultural crops with P7.5 billion out of P125.2 billion.

Sugar and its by-products are commonly used in ingestible commodities such as candies, sweets, softdrinks and bread. It can also be used to produce solvents, plastics, plasticizers, fiberboards, building materials, surfactants, additives, and fuel.

By-products include cane tops, bagasse and molasses which can be used as components of swine and poultry feeds.

The Philippine sugar industry has the potential to increase productivity by nearly 30%. Such an increase could lead to annual raw sugar output being 30-45% higher by the year 2000, and the sugar industry could contribute an estimated US$104 million a year more to the Philippine economy.

Without productivity increases, sugar production looks set to fall due to rising costs elsewhere in the economy. Output is estimated to be lower by the year 2000. Meanwhile, increasing incomes and population are projected to cause Philippine sugar consumption to be between 60% and 75% higher by the turn of the century. Projected domestic demand for sugar is 2.12 million MT by 2000 and 2.95 million MT by 2010. There will be a production gap if the current production remained static at 1.9 million MT. If production declines, more of the sugar consumed will have to be imported. By year 2000, imports could make up nearly 45% of the total amount of sugar consumed. However, if productivity does increase, imports could account for only 1-10% of total consumption. The challenge can only be met by increasing the sugar yield per hectare or the productivity of the land, at the same time reducing the cost of production.

2. Marketing

The Philippine sugar industry does not exist in a vacuum and cannot be understood apart from a broader national and global contexts within which it functions. Issues such as currency valuation, interest rates, taxation, disincentives for economic diversification and investment, infrastructure development, wage rates, agrarian reform, and pricing policies all directly affect the sugar industry. Internationally, no sugar producer can avoid the impact of preferential agreements, quotas, subsidies, tariffs and trade consumption.

Sugar (centrifugal and refined) is among the country's principal agricultural exports, sharing 9.98% of the US$1.36 billion export value in 1996. Export volume and value equally grew by 107% from 153,210 MT in 1995 to 317,700 MT in 1996; and from US$65.88 million in 1995 to US$136.2 million in 1996, respectively.

Average wholesale and retail prices of raw sugar in 1993 were P476.14/Lkg and P11.54/kg, respectively. Wholesale price of refined sugar was P722.81/Lkg, and retail price was P16.49/kg for the same year. Centrifugal sugar was sold at P13.60/kg in 1996, an increase of 52% from P8.95/kg in 1992.

Import of refined sugar tremendously increased from 4,539 MT in 1994 to 48,401 MT in 1995. Value likewise increased form 2.3 million to $25.3 million during the same period.

The Philippines supplies 13.5% of the US sugar demand under the latter's tariff rate quota system – the third largest share, next only to the Dominican Republic and Brazil. Meanwhile, sugar imports have started to make inroads in the domestic market due to trade liberalization under the GATT and the Association of Southeast Asian Nations (ASEAN) Free Trade Are (AFTA) agreement.

At the GATT Uruguay Round, the Philippines offered perhaps the biggest reduction in bound tariffs for out-quota imports over the life of the agreement. Recently, the sugar industry has sought a modification of the government's tariff commitments, including raising the final out-quota bound rate under the GATT-WTO. Leaders of the sugar industry have asked government to continue with the 65% tariff rate until the year 2003 to allow the sector more time to upgrade its efficiency, including additional investments in rehabilitation and modernization of sugar mills and refineries, as well as for government to correct the disincentives to agricultural investments.

At risk of decline and demise are nearly P100 billion in investments and the livelihood of 4,000 sugarcane farms that employ half a million Filipinos. Sugar mills with investments worth at least P50 billion will be in jeopardy as cane supply dwindles and the increasing domestic demand for sugar is met by imports of cheap, subsidized or even dumped refined sugar. The Philippine sugar industry admits the need to metamorphose and change its shape, character and destiny – not only through tariffs, but through investment and policy climate favorable to restoring sugar as a viable business, with competitive prices as the key to producing more.

D. Coconut

The coconut industry is a pillar in Philippine economy. It earns an annual average of US$700 million from exports alone. Moreover, the Philippines remains as the world's leading supplier of traditional coconut products, with a market share of 40.3% of world coconut production, 60.4% of world copra and coconut oil supply, and 59% in laurics output. The industry has strong processing sectors and stakeholder associations – 94 oil mills, 66 oil refineries, 11 desiccated coconut (DCN) plants, 11 activated carbon plants, and 16 oleochemical plants and food processing industries. It benefits 1.6 million farmers, 1.9 million farm workers, and 20 million people directly or indirectly. It occupies about one-fourth of the total area harvested to agricultural crops. It also contributes to the sustainable management of environments as the palm protects the soil from erosion and nutrient loss.

Lately, however, the coconut industry has been characterized as a sunset industry because of erratic and declining production, and its seeming inability to meet the challenges brought by the domestic economic climate, new competitors, and changes in international trading regulations.

With the reduction in tariffs under the GATT, domestic support and export subsidies will lead to greater market access. For the coconut industry, the goal is global competitiveness – enhancing the country's edge in the export market. The opportunities being brought about by trade liberalization must be maximized, while protecting the industry from the adverse impact of the opening of markets.

1. Production

World production of coconut was 43.275 million MT in 1994. The average growth rate was 1.92% from 1980 to 1949. Major producing countries are in Asia and the Pacific where coconut is primarily a smallholder crop, with Indonesia and the Philippine leading the production.

Here in the Philippines, coconut is a major crop in 67 provinces, occupying 3.16 million ha in 1998 (distributed in Mindanao, 1.7 million ha; Luzon, 0.9 million ha; and Visayas, 0.6 million ha) with 240 million trees bearing an annual average of 7 billion nuts. It is widely grown in Mindanao which occupies over half of the total coconut hectarage and produces the largest proportion of outputs. Major producing regions are Southern Tagalog, Bicol, Eastern Visayas, Western Mindanao, and Southern Mindanao (Figure 6, Appendix Table 8).

From 1990 to 1998, the total area planted to coconut was highest in 1997 at 3.31 million ha (Appendix Table 8). There is, however, a continuing reduction trend in area planted to coconut, especially in Mindanao, due to the shift from coconut to other high-value crops, conversion of coconut lands to other more profitable uses (residential, commercial or industrial), and cutting of coconut trees for coco lumber. The shift in land use to other high value crops is significant in Mindanao where more lands are planted to banana, durian and other exotic fruits and commercial crops. Land conversion and cutting of coconut trees for lumber are prevalent in Southern Tagalog as a result of industrialization.

Through the years, coconut production has been on the downtrend and this could be attributed to the declining hectarage and yield. The ban on copra exportation in the early 1980s may have affected the industry's production during this period. With the lifting of the export ban in 1986, production surged to a record high of 14.33 million MT. In 1994, production fell by 23% from the 1986 level, with 11.2 million MT of nuts. This was attributed to senility of old trees, conversion of coconut farms to other uses in the Mindanao and CALABARZON areas, and typhoons in Visayas and Luzon. Production slightly increased in 1995 (12.18 million MT) and 1997 (12.11 million MT), but reached a low of 10.9 million MT in 1998 (Appendix Table 9).

Among the reasons identified for the decline in productivity of coconut in the country area: 1) low productivity of old senescing trees and failure to implement replanting program; 2) non-utilization of improved farming practices to increase yield; 3) limited supply of improved and high-yielding varieties; 4) prevalence of typhoons in coconut-growing regions in Luzon and Visayas; 5) poor post harvest handling causing low quality copra and copra meal; 6) poor soil conditions; 7) inadequate funds to develop industry, scientific manpower is inadequate, and capability for RDE is low. Table 6 summarizes the status of coconut production in the Philippines.

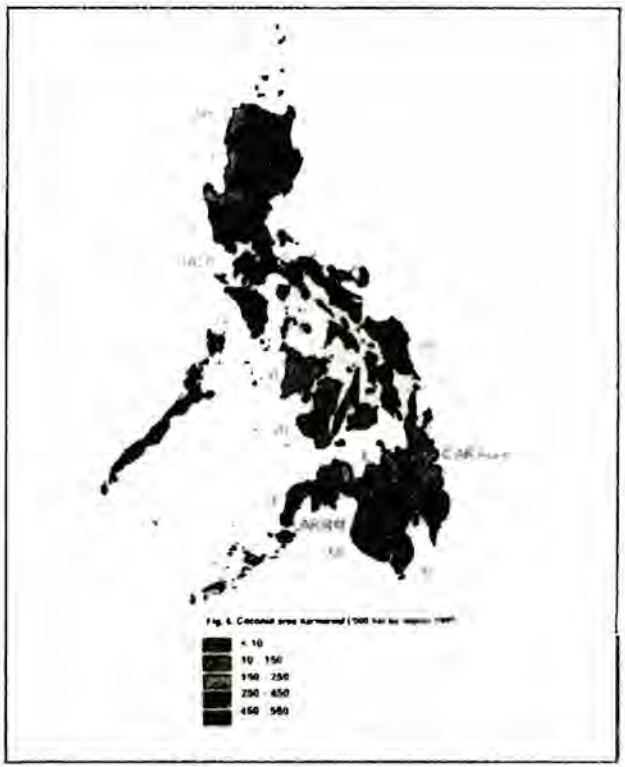

Figure 6. Coconut area harvested ('000 ha) by region 1997

2. Uses, Consumption and Marketing

Coconut goes through a marketing system that has developed within the industry. The nuts are harvested by hired workers and brought to accessible areas, preferably near the road. They may be sold to barangay or town buyers who are normally involved in fresh nut and copra trading. The various users of copra get their supply from these buyers. The users are the millers, oil miller/refiners, and oil miller/refiner/manufacturers who may also be involved in the domestic sale and export of coconut products. For their raw materials, desiccated coconut manufacturers buy fresh nuts from the buyers.

Under this marketing scheme, farmgate prices fluctuate and are generally low, sharing arrangements are inequitable, and farm worker wages are low. The whole trading and marketing system works to the disadvantage of majority of the poor coconut farmers.

The Philippines used to export copra as a major item. Starting in 1980, however, copra became a minor export product. In 1995, it accounted for only about 1% of the total volume of coconut export in copra equivalent. For the last ten years,

Table 6. Status of coconut production in the Philippines, 1994-1998

COCONUT	1994	1995	1996	1997	1998
Hectarage (ha)	3,082,651	3,064,457	3,149,035	3,314,416	3,115,832
Production (MT)	11,206,997	12,183,088	11,368,111	12,118,452	10,905,328
Yields/ha (MT/ha)	3.64	3.98	3.61	3.66	3.50
Areas of production	Southern Tagalog, Bicol, Eastern Visayas, Western Mindanao, and Southern Mindanao				
Major problems encountered and constraints to production	• Low productivity of old trees and failure to implement replanting program • Non-utilization of improved farming practices Limited supply of improved and high-yielding varieties • Prevalence of typhoon in coconut-growing regions • Poor harvest handling • Poor soil conditions • Inadequate funds for RDE				
Major problems encountered and constraints to marketing	• Farmgate prices fluctuate and are generally low; sharing arrangements are inequitable; farm worker wages are low; the whole trading and marketing system works to the disadvantage of majority of the poor coconut farmers • Industry is basically export dependent; domestic utilization is less competitive in the world oils and fats trade • Development of new world sources of lauric acid fats has posed a threat to the industry.				

Note: Refer to Appendix Table 8 and 9 for 1990-98 data on area harvested to coconut and volume of production by region.

coconut oil (CNO) has become the dominant export product of coconut, accounting for about 74% in 1995. About 13% was exported as DCN, coco chemicals, and copra.

Twenty percent was used locally for the production of commercial edible cooking oil; coco chemicals; base material for shortening, laundry soap, and detergent; home made oil; and food. Other products with potential in domestic/foreign market are coir fiber and dust from the husk; charcoal and activated carbon from shell; beverage, vinegar, plant culture media, growth hormones for coconut water; and food products from coconut meat.

In 1995, coconut production was 2.65 million tons (copra terms). In 1997, Philippine coco chemical capacity was expected to grow by 63% (310,500 tons) with the entry of the Prime Oleochemicals, Inc., with production capacity of 120,000 tons. In the next five years, the composition of coconut exports is seen to change, with the Philippines exporting mostly processed or finished products.

From 1989 to 1995, the major markets for CNO value-wise are USA (43%); Europe (41%); and non-traditional destinations (16%). Japan is the main market for coconut shell charcoal, while USA and Japan are the major markets for activated carbon. Exports of coco chemicals followed a downward trend from the recorded 154,000 tons in copra equivalent in 1987 to 43,000 tons in 1991 following an expanding domestic market. It recovered in 1992 and registered a peak of 88,000 tons in 1993.

Prices of coconut products follow the international market trends. From 1980 to 1991, average domestic and export prices in nominal terms of copra, coconut oil, copra meal and DCN fluctuated. Such trend is attributed to fluctuating world coconut oil prices, and in response to the supply position in the Philippines which is basically controlled on a yearly basis by the weather.

The export-dependent nature of the industry is a valid concern among stakeholders, with domestic utilization of coconut as lowest among coconut-producing countries. Another concern that threatens the industry is the less competitive price of coconut oil compared to that of other vegetable oils in the world oils and fats trade, and the development of new world sources of lauric acid fats besides coconut oil. Lower tariff rates on imported coconut and coconut oils are also expected to increase their inflow into the country, thus the need to make local production more competitive.

The projected market outlook for coconut in the near term remains bright. It is and will continue to be a sunrise industry, especially with the government's sustained development efforts to increase coconut production at globally competitive prices.

E. Fruits and Vegetables

Fruits and vegetables are relatively cheap sources of healthy foods which impact the nutritional state of Filipino consumers. While there are specialty fruits and vegetables for the high-end markets, there are more than can easily be grown for home consumption or marketed to the average consumers. The main thrust of the government is to promote market driven-based production system for fruits and vegetables that are technologically cost efficient and competitive in local and export markets, and home consumption-based production system for indigenous, nutritious crops.

Three factors led to the development of the Philippine fruit export industry: large investments and technology transfer by multinationals, principally in banana and pineapple; keen interest from Japanese traders and consumers; and export liberalization. The industry classifies major fruits and nuts into two categories. *Currently exported fruits* are banana, pineapple, mango and papaya. *Potential exports* include citrus, durian, cashew, pili, mangosteen and jackfruit.

Meanwhile, the growth and development of the vegetable industry in the last four decades contributed significantly to improvements in both the nutritional well-being and economic status of the Filipino. The Philippines enjoys a comparative advantage in the production of select fresh vegetables and vegetable seeds. Its

favorable climate makes it possible to produce over 30 kinds of fresh vegetable all-year round and grow several vegetable seeds from crops that require different climate for flowering. Recent developments in the domestic and export markets open bright opportunities for vegetables.

This section will touch on two major fruit exports – banana and mango, and leading vegetable crops – onion and mungbean.

1. Banana

The Philippines ranks fifth in the international banana trade. The country is the only supplier of banana chips in the world, accounting for 95% of the annual export receipts of processed bananas over the past five years. Banana chips are exported to 32 countries, with the USA and EEC as the major importers. Under the GATT, the banana industry can take advantage of the lower tariff in the export market.

Banana is a widely grown fruit in the country, planted as a component of the farming system or as a main crop in large plantations in Mindanao. It is an important source of cash income for small farmers who constitute 75% of the banana growers. A total of 5.9 million farm households are dependent on the banana industry.

Of the 80 distinct Philippine banana cultivars, Lakatan, Latundan, Bungulan and Saba are popularly grown for the local market. Meanwhile, Cavendish is produced for fresh fruit export market. At present, Senorita and Lakatan are gaining acceptance in major importing countries. Likewise, Saba is the chief source of banana chips and catsup.

Banana is the leading fruit crop in terms of area, volume and value of production. A total of 337,082 ha were planted to banana in 1998. Major areas of production are in Southern Tagalog, Western Visayas, Western Mindanao, Northern Mindanao, Southern Mindanao, Central Mindanao, CARAGA, and ARMM (Appendix Table 10).

Total production in 1997 was 3.4 million tons valued at P10.74 billion (Appendix Table 11, Table 7). Top producing region is Southern Mindanao, accounting for 1.6 million tons of total production. The national average yield was 10.56 t/ha while big plantations produced about 40 t/ha per year.

Bananas are consumed fresh or processed as chips, catsup, wine, flour, puree and other food/feed preparations. About 66% of production is being consumed locally, while 34% is exported. About 73% of the local consumer fruit intake consists of banana. Per capita consumption averaged about 22kg/year in 1993-1997.

Export earnings from fresh and processed banana averaged about US$244.13 million/year (1993-1997). About 1.14 million MT of fresh banana and 19,094 MT of chips were exported in 1997. Japan, Hong Kong, and the Middle East countries are the major export markets.

Domestic market for fresh banana is dominated by middlemen and traders, while the export industry is handled mostly by multinational corporations. In small-scale farms, bananas are sold on a finger count basis; in commercial plantations, these are sold by weight.

Table 7. Status of banana production in the Philippines, 1994-1998

COCONUT	1994	1995	1996	1997	1998
Hectarage (ha)	362,542	322,008	326,913	338,277	337,082
Production (MT)	3,192,620	3,489,453	3,304,060	3,391,150	3,560,763
Yields/ha (MT/ha)	9.80	10.87	10.13	11.16	10.56
Areas of production	Southern Tagalog, Western Visayas, Western Mindanao, Northern Mindanao, Southern Mindanao, Central Mindanao, CARAGA, and ARMM				
Major problems encountered and constraints to production	• Widespread occurrnce of diseases, particularly, banana bunchy top and mosaic • Unreliability of raw materials supply for the banana chip industry				
Major problems encountered and constraints to marketing	• Poor/lack of transport system, market information as well as stiff competition from other countries whole trading and marketing system works to the disadvantage • Perishable nature of banana limits farmers' bid to command favorable price for produce • Poor condition of farm-to-market roads, transportation and port facilities, post harvest and storage losses • Inadequate financing program for banana at the farm and market levels.				

Note: Refer to Appendix Tables 10 and 11 for 1990-98 data on area harvested to banana and volume of production by region.

Among the problems encountered b the industry include: 1) widespread occurrence of diseases, particularly banana bunchy top and mosaic; 2) poor/lack of transport system, market information as well as stiff competition from other countries; 3) unreliability of quality raw materials supply experienced by the banana chip industry; and 4) high production cost.

In terms of price trend, local prices fluctuate considering the perishability and availability of the fruit. Lakatan fruits command selling prices than Latundan and Bungulan. In terms of marketing assistance, there is inadequate financing program for banana at the farm and market levels.

2. Mango

The Philippines is the world's eight leading mango producer. It takes advantage of the lower tariff in its export market. Japan's effort to reduce its tariff of dried mangoes from 603% and offer General Systems of Preference (GSP) privileges to allow Philippine mangoes to enter Japan duty-free the GATT is a big boost to Philippine exports.

Mango is the country's third most important fruit crop in terms of production area, volume and value. The 'Carabao' variety is one of the world's best; other

important varieties are 'Pico' and 'Katchamita'. The industry supports some 2.5 million farmers and farm family members.

Mango thrives best in areas with at least five months of dry period. Area planted to mango in 1998 was 92,939 ha. Leading mango production areas are Ilocos, Cagayan Valley, Southern Tagalog, Western Visayas and Central Luzon (Appendix Table 12). Total volume produced in 1998 was 950,074 MT valued at US$51,376,000, with Ilocos Region leading at 290,169 MT. From 541,662 MT in 1994, production rose to 1,028,121 MT in 1997 and 917,471 in 1998 (Appendix Table 13). Table 8 summarizes the status of mango production in the Philippines.

The fruit is eaten fresh as dessert or as relish depending on fruit maturity. It is also processed into dried, puree, juice, nectar, chutney, pickle, and mango scoops; roll, powder, halves or scoops in light syrup; as used as flavoring for ice cream, bakery products, and confectioneries.

Philippine mango export is expected to increase by 13% because of growing market demand and world population. Domestic consumption is estimated at 16.3 kg/person annually.

In 1998, 32,513 tons fresh mango fruits worth US2$24 million (FOB) was exported to Hong Kong, Japan, Australia, United Kingdom, Canada and other countries. Export from dried and other processed mango products was 40,000 tons valued at US$27,642,249 (FOB).

Marketing is done by middlemen who are contract-buyers, agents, assembler-wholesalers, wholesalers-exporters, and wholesaler-retailers. Fruits for local markets are packed with or without newspapers as liners in bamboo baskets, while those for export are placed in carton boxes or wooden crates.

Among the common problems in mango production are as follows: 1) poor orchard management, especially lack of pruning, inadequate nutrition, indiscriminate use of chemicals, and use of inferior quality planting materials; 2) lack of crop zoning, which weakens necessary support services and infrastructure; 3) weak integrated pest management technology; 4) lack of an effective technology transfer program for growers; 5) insufficient pre-and post-harvest facilities. In terms of marketing, following are some concerns; 1) inadequate financing and credit support and defective marketing system; 2) lack of standard quality control; and 3) limited promotion and expansion of potential markets.

3. Onion

Onion is a dry season crop usually planted as a second crop to rice. It is usually grown in Nueva Ecija in Central Luzon (8,010 ha) and in the Ilocos Region (4,299 ha). Area planted with onion in the Philippines has doubled significantly from 5,854 ha in 1992 to 12,769 ha in 1998 (Appendix Table 14).

Central Luzon accounted for 60% (51,245 MT) of the total onion production of 87,666 MT in 1998, while Ilocos region shared 36% (34,834 MT) (Appendix Table 15). There is a significant increase in the volume of production from 1990 (61,470 MT) to the 1998 level.

Table 8. Status of mango production in the Philippines, 1994-1998

MANGO	1994	1995	1996	1997	1998
Hectarage (ha)	64,960	80,393	87,680	91,899	92,939
Production (MT)	541,662	595,138	932,730	1,028,121	950,074
Yields/ha (MT/ha)	8.34	7.40	10.64	11.19	10.22
Areas of production	Ilocos Region, Cagayan Valley, Southern Tagalog, Western Visayas, Central Luzon				
Major problems encountered and constraints to production	• Poor orchard management, especially lack of pruning, inadequate nutrition, indiscriminate use of chemicals, and use of interior quality planting materials • Lack of crop zoning, which weakens necessary support services and infrastructure • Weak integrated pest management technology • Lack of an effective technology transfer program for growers • Insufficient pre-and post-harvest facilities.				
Major problems encountered and constraints to marketing	• Inadequate financing and credit support and defective marketing system • Lack of standard quality control • Limited promotion and expansion of potential markets				

Note: Refer to Appendix Tables 12 and 13 for 1990-98 data on area harvested to mango and volume of production by region

Return on investment (ROI) in onion is high, making it one of the more profitable crops in the country. ROI for native onion is 2.47; for red creole, 2.06; and for yellow granex, 2.04 (Batang et al as cited by Librero and Rola 1996). Onion production through the years, however, is marked by fluctuations because of changes in weather conditions, pests and diseases, area harvested and yield. The occurrence of long dry season enhances the incidence of pests and diseases that significantly affects onion yield.

The marketing system of onion is basically competitive because of the presence of many buyers and sellers interacting in the market. Farmers sell onions based on variety and size, with no strict standards being followed by both farmers and traders. The abundance of intermediaries has led to increased marketing cost.

There is a virtual lack of technology on the production of quality onions, particularly the yellow granex variety. Seeds are still being imported. Development activities on varietal improvement to lengthen shelf-life, higher solid components for processing, and alternative storage methods should be pursued. People venturing into onion growing should be cautious as the industry is sensitive to oversupply. Adequate storage facilities are needed to prolong shelf life and avoid price drops during the harvest season. Table 9 summarizes the status of onion production in the Philippines.

Table 9. Status of onion production in the Philippines, 1994-1998

UNION	1994	1995	1996	1997	1998
Hectarage (ha)	7,559	8,693	9,806	11,888	12,769
Production (MT)	73,635	88,427	83,322	85,393	87,666
Yields/ha (MT/ha)	9.74	10.17	8.50	7.18	6.87
Areas of production	Central Luzon and Ilocos Region				
Major problems encountered and constraints to production	• Fluctuation in production due to changes in weather conditions, pests and diseases, and decline in yield. Occurrence of long dry season enhances the incidence of pests and diseases. • Lack of technology on the production of quality onions, particularly the yellow granex variety. Seeds are still being imported. • Inadequate R&D on varietal improvement to lengthen shelf-life and higher solid components for processing, and on alternative storage methods. • Lack of financing and high cost of inputs				
Major problems encountered and constraints to marketing	• Inefficient marketing and distribution systems. Abundance of intermediaries lead to increased marketing cost. • The industry is senstive to oversupply. Adequate storage facilities are needed to prolong shelf life and avoid price drops during the harvest season.				

Note: Refer to Appendix Tables 14 and 15 for 1990-98 data on area harvested to onion and volume of production by region.

Traditionally, onion has the biggest share in export earning. In 26 years, it accounted for 34% of the vegetable industry's foreign exchange earnings. From the 1970s, fresh onion exports showed fast growth. The record in volume (18,246 tons) and value (US$6.8 million) was reached in 1986. In 1996, export of fresh onion (including shallots or native onion) reached 27,227 tons, worth US$11.4 million. Japan got 87% of the onions, followed by Hong Kong, Singapore, Thailand, and the United Kingdom. Indonesia was the major exporter of shallots, absorbing 73% of the total. Dried and pickled onions, though in minimal quantities, were also exported to Singapore and Japan.

4. Mungbean

Of the 13 million ha devoted to agricultural production in the country, only 0.12 million ha are planted to legumes (BAS 1989-1996). Volume of production is low at 77,000 MT annually during the past 17 years, while demand has grown to more than 100-200%. To meet the demand, importation was resorted. In the last six years, local supply of legumes (groundnut and mungbean) meet only about half of the demand.

Legumes are usually grown in combination with rice and corn either as an inter-crop (corn with peanut) or in rotation (corn-legume; corn-corn-legume; rice-legume; rice-rice-legume).

Mungbean is a poor man's meat and, thus, has become part of the Filipino's eating habit. It is harvested all year-round in different areas of the country, thus, seed supply is not much of a problem.

Unlike other legumes which experienced declining trends in area and volume of production for the past nine years (1990-1998), there was not much change in area (35,000 ha) and volume (25,000 MT/year) of mungbean (Appendix Tables 16 and 17). Its status as a subsistence crop helped prevent the decline in area of production. The slight increase in mungbean yield (10%) between 1980-1997 may have been the result of high adoption of BPI Mg9 or "Taiwan Green" a drought resistant mungbean variety with a higher yield of 1.0MT/ha. The Ilocos region has the largest area planted to mungbean (11,897 ha) and the highest volume of production (12,197 MT) as well.

Among the major constraints to mungbean production are occurrence of pests and diseases, inadequate supply of seeds, high cost of production inputs, bad weather and natural calamities, lack of water or irrigation system, inadequate transportation, poor soil condition, and losses due to thieves and stray animals. Marketing constraints include price instability, lack of marketing information, high transport cost/poor transport system, and lack of market outlet. Table 10 summarizes the status of mungbean production in the Philippines.

F. Roots

Among the crops with great potential to fulfill the food security requirements of the country are the rootcrops, particularly cassava and sweetpotato – indigenous, nutritious crops that require minimal technology, labor and inputs. Lately, however, cassava and sweetpotato have emerged from their traditional image of being poor man's crops into important industrial and food crops. Both grow easily even under poor conditions and are major crop components in mixed cropping systems in the uplands.

1. Cassava

The Philippines is one of the major producers of cassava, along with Thailand, Indonesia, India, China and Vietnam. In 1997, the national production of cassava reached 1,958,004 MT, the highest level attained during the past 10 years. Yields range from 8-20 t/ha in the uplands to 20-40 t/ha in plantations. The national average, however, was 8.25 t/ha in 1998.

Production area in the country reached 216,474 ha in 1998 (Table 11), with the ARMM having the largest area followed by the Bicol region and Eastern Visayas. These accounted for 57.9% of the area planted to cassava nationwide. ARMM, Bicol and Western Mindanao were the top cassava producers, with yields of 869,278 MT, 226,883 MT, AND 216,626 MT, respectively.

Table 10. Status of mungbean production in the Philippines, 1994-1998

MUNGBEAN	1994	1995	1996	1997	1998
Hectarage (ha)	34,006	34,860	35,453	36,420	34,629
Production (MT)	24,218	26,651	26,792	27,468	27,670
Yields/ha (MT/ha)	0.71	0.76	0.76	0.75	0.80
Areas of production					
Major problems encountered and constraints to production	• Occurrence of pests and diseases in mongo farms • Inadequate supply of seeds • High cost of inputs • Bad weather and natural calamities, lack of water or irrigation system, inadequate transportation, poor soil condition and losses due to thieves and stray animals imported.				
Major problems encountered and constraints to marketing	• Price instability Lack of marketing information • High transport cost/poor transport system • Lack of buyer or market outlets				

Note: Refer to Appendix Tables 16 and 17 for 1990-98 data on area harvested to mungbean and volume of production by region.

About 44% of production is consumed as food. Cassava can either substitute for or supplement rice and corn in daily meals. It is the most important food crop in Lanao, Zamboanga, and Sulu where grated cassava is the staple of the Muslim population. Substantial production volume is processed into various industrial products such as starch and its derivatives. Increasing volumes are processed into dried chips which are either exported to Europe or utilized domestically as source of energy in aqua and livestock feeds.

2. Sweetpotato

The Philippines ranks eight in the world in terms of sweetpotato production. From 667,807 MT in 1994, production declined to 568,102 MT in 1998 (Table 11). Current productivity level is at 4.44 MT/ha.

Production area in the country reached an averaged of 145,718 ha from 1993 to 1996. In 1998, however, it declined to 127,977 ha. Bicol region has consistently led domestic production in terms of volume and hectarage, contributing 25% and 19.9% respectively, of the total production in 1996. Next to Bicol region in Eastern Visayas, sharing 14.7% in production volume and 17.6% in area harvested in 1996, followed by CARAGA and Central Visayas. The value of production was pegged at P3.17 billion in 1996.

Sweetpotato is utilized mostly as food in traditional forms (such as boiled, roasted, fried) and as unprocessed feeds. At present, sweetpotato can be made

Table 11. Status of cassava and sweetpotato production in the Philippines, 1994-1998

CASSAVA	1994	1995	1996	1997	1998
Hectarage (ha)	212,877	225,751	228,343	230,522	216,474
Production (MT)	1,890,509	1,905,903	1,910,775	1,958,004	1,786,714
Yields/ha (MT/ha)	8.88	8.44	8.37	8.49	8.25
Areas of production	ARMM, Bicol, Western Mindanao				
Major problems encountered and constraints to production	• Farmers-processors do not receive optimum returns on investments because of poor processing and low quality products. • Traders offer low price for fresh and processed product because of lack of quality control by farmers and processors. The unstable supply and low quality of dried chips are some of the problems, especially in marketing for industrial uses. • Lack of postharvest facilities and the continuous importation of cassava starch and glucose have also been cited as problems in the cassava industry				
SWEETPOTATO	1994	1995	1996	1997	1998
Hectarage (ha)	146,111	145,236	141,006	141,701	127,977
Production (MT)	667,807	667,946	654,231	631,431	568,102
Yields/ha (MT/ha)	4.57	4.60	4.64	4.46	4.44
Areas of production	Bicol, Eastern Visayas, CARAGA and Central Visayas				
Major problems encountered and constraints to production	• Low productivity due to inefficient production and low adoption of technology • Incidence of pest and diseases • Low dry matter content even of recommended varieties • Use of marginal lands for most sweetpotato production • Insufficient postharvest facilities, tools and equipment • Weak farm producers-market linkage • Poor social/cultural acceptability of sweetpotato products				

into semiprocessed products, flour/starch, catsup, fruit-like products, jam, snack chips, and beverage. Sweetpotato starch is used for the manufacture of paper, ink, paint, chemical products, feed stuff and accelerant. The by-products from starch processing can be used for alcohol and organic fertilizer production.

Sweetpotato has a potential demand of 648,000 MT/year from human consumption alone at 9 kg/capita consumption. There is a growing demand in the local and export markets for sweetpotato and its products. Initiatives/enterprises for the production of starch and flour for various high-value processed products are now

in place. Demand for sweetpotato as energy source in commercial feeds and as raw materials for alcohol production has created growing need to further increase production in the country.

Domestic market still remains as the major outlet for fresh sweetpotato. Increasing volume in the future is expected to cater to emerging local and international markets as ingredient for animal feeds and flour/starch.

F. Abaca

The Philippine abaca has remained a viable source of export earnings contributing an average of US$50 million from 1985 to 1995. It still dominates the world market supply of 85%. Being a consistent dollar earner and contributor to the upliftment of the socioeconomic condition of the people, abaca is identified as the flagship commodity of the Eastern Visayas region.

In 1996, abaca production in the country reached 70,400 MT valued at P1.27 billion. In 1998, it slightly increased to 71,276 MT. Area of production is 106,299 ha in 1998 (Table 12), with a ten-year national average yield of 0.93 MT/ha. Eastern Visayas is noted as the largest abaca producer in the country, followed by Bicol.

Products derived from abaca are ropes and other cordage products; fibercraft products such as bags, hats, place mats and other cottage industries; and abaca pulp.

Demand for raw abaca fiber in the world market increased by 6.6% from 18.7 T mt in 1994 to 19.32 T mt in 1995. Raw fiber production decreased from 48,915 MT to 45,541 MT (1994-1995). Export earnings from abaca fibers and manufacturers reached US$94.5 million in 1995 from US$82.4 million in 1994. The rising demand for abaca fiber can be attributed to the GATT ratification and new markets and growing popularity.

III. PHILIPPINE AGRICULTURE: TRADING INTO THE FUTURE

As the country grapples over the propriety of opening up its agricultural markets to foreign competition, so unfolds the World Trade Organization's (WTO) process of continuing the negotiations to further globalize agricultural markets in the world, as well as to continue to process of installing rules-based trading regime for agriculture. Besides weighing the net advantages of deepening market access commitments, the country will have to assess if it is in its advantage to adopt the proposed new rules on agricultural global trading.

Following the ratification by the Philippine Senate of the GATT Uruguay Round Final Act, the government acceded to the WTO in 1995 as one of the organization's founding members. Under this trade treaty, the Philippines agreed to not only increasing open its agricultural markets to foreign competition but also to legally enable the rules governing agricultural trade as defined in the treaty.

The Uruguay Round Final Act integrates for the first time agricultural trade under the rules and discipline of the GATT. In the past, agricultural trade has been distorted, both in favor of countries which have been able to subsidize their re-

Table 12. Status of abaca production in the Philippines, 1994-1998

ABACA	1994	1995	1996	1997	1998
Hectarage (ha)	103,127	105,641	116,845	112,456	106,299
Production (MT)	66,410	64,833	70,431	67,110	71,276
Yields/ha (MT/ha)	0.64	0.61	0.60	0.60	0.67
Areas of production	Eastern Visayas and Bicol				
Major problems encountered and constraints to production and marketing	• Low fiber yield and lack of supply of quality fiber due to: use of mixed varieties, lack of planting materials of high-yielding varieties, problem of drying especially during rainy season, and use of antiquated production and post harvest processing practices. Pest and disease infestation • Lack of capital for the establishment of plantations • Low farm gate price of fibers • Lack of field technicians				

spective agricultural sectors, and at the expense of the non-subsidizing agricultural exporting countries like the Philippines. Under the GATT, this distortion will be corrected gradually by agreed-upon reductions in farm and export subsidies as well as by the tarrification of the quantitative import restrictions (QRs) on agricultural products.

In 1996, Congress passed Republic Act 8178 to legally enable the country's market access commitments. This law lifted all QRs in agriculture except rice. It replaced import regulations with the highest possible tariff protection – 100%. Any one can import agricultural products as the country's tariff binding rates, which are supposed to go down to 50% in 2004 in accordance with the WTO agreement.

All these are in line with GATT's objective to increase world trade by improving the access of goods and services of its member-countries to the markets of other member-countries. It also aims to set stable rules for the exchange of goods and services among countries, making the conduct of world trade more transparent and predictable. However, there are opposing views to the benefits of the GATT, especially in Philippine agriculture.

Since the Philippines does not subsidize its agricultural sector as much as the developed countries, the lifting of QRs on agricultural products under the GATT is seen by some as a bane to local production. QRs are protective devices in favor of local producers against competition from imported products which are generally perceived to be dumped or subsidized by the trading partners. The lifting of the QRs on agricultural products, therefore, exposes Filipino farmers to stiffer competition in the local agricultural markets posed by more efficient or still heavily subsidized foreign farmers. The problem is aggravated by the fact majority of the country's population are small farmers who depend on agriculture for their livelihood.

With this possible loss in markets, farm income may decline. As a consequence, the access of farm families to food supply would be impaired, exacerbating the country's undernutrition problem. Critics of GATT also claim that the country's ability to provide food to its growing population will also be impaired because of the shift to high value crops for export to replace traditional crops as rice and corn.

Trade liberalization under the GATT-WTO is indeed, a double-edge sword – it can make or break Philippine agriculture. Its long term goal is to make domestic producers more competitive in the world market through exposure and access to better technology, improved production efficiency and higher product standards. However, the preconditions to the achievement of these in the domestic situation rest upon the resources especially of the small farmers, and the so-called safety net measures in the form of infrastructure and institutional support from the government that would facilitate farmers' access to these free trade opportunities.

In the next millennium, globalization will be a central concern. Filipino consumers will enjoy access to a wide range of goods and services. At the same time, however, Filipino farmers may fear – validly – that globalization is a threat to their livelihood.

Provided that all stakeholders are true to their commitments, modernizing Philippine agriculture through the AFMA, with the corresponding substantial increase in public investment, is what will cushion our small farmers from the fall.

A. Key Features of the Agricultural Accord under the GATT

1. Conversion of all Quantitative Restriction (QRs) Imposed on Agricultural Products Into Tariffs

QRs are measures imposed by a government to deny entry or restrict the amount of agricultural products imported from other countries. Under the GATT, all these restrictions will be lifted by member-countries. However, they will be allowed to impose tariff rates equivalent to the level of protection enjoyed prior to the removal of such restrictions.

2. Reduction of Tariffs on Agricultural products

All member-countries are required to "bind" or set a maximum limit on tariffs to be imposed on all agricultural products. Developing member-countries will then reduce these limits by a minimum of 10% for each tariff line and by a simple average of 24% for all tariff lines within ten years starting 1995 until year 2004. Developed member-countries, on the other hand, will reduce these limits by a minimum of 15% for each tariff line and by an average of 35% for all tariff lines within a six-year time frame starting in 1995.

3. Reduction of Domestic Subsidies

Domestic subsidies are measures implemented by a country to reduce the costs of production or increase the net revenues received by agricultural producers

in its domestic market, thereby encouraging the production of these commodities beyond what is economically efficient. Unfair subsidies will be phased out through reduction of aggregate measure of support (AMS). The AMS, which is the monetary value of subsidies given to agricultural producers of a given commodity, will be reduced by 20% over six years by developed countries and 13% over 10 years by developing countries.

4. Reduction of Export Subsidies

Export subsidies are payments made by the government to its domestic producers to permit them to reduce their cost of production, thus enabling them to compete more effectively in world trade. Prevalent in developed countries, these subsidies are provided to encourage the export of farm products which are domestically-produced in large amounts as a result of the domestic product support.

Developed countries are to lower their respective export subsidies by 21% over six years while developing countries are required to cut the same by 14% over 10 years.

5. Market Access Commitments

To promote transparency in agricultural trade, use of non-tariff measures (NTMs) such as QRs will not be expanded, and existing QRs on farm products will be tarrified. However, contracting parties can postpone their compliance for at most the length of the implementation period from 6 to 10 years. For countries facing extreme political difficulties with respect to sensitive agricultural products, this requires the granting of minimum access of 1% of consumption and the implementation of effective production restraining measures.

6. Harmonization of Sanitary and Phytosanitary Measures

Sanitary and phytosanitary measures necessary to protect human, animal, or plant life or health will be harmonized among GATT member-countries.

B. Impact on Philippine Agriculture

1. Removal of QRs

The Philippines is required to lift all existing quantitative restrictions on all agricultural products, except rice. At present, the country imposes import restrictions and licensing regulations on corn, livestock, poultry and meat products, onions, garlics, potatoes, cabbage and coffee and coffee by-products. In lieu of these restrictions, higher tariff rates equivalent to at least double the final applied rates in 1995 will be imposed.

For critical agricultural products, the increase will be more substantial and reach the maximum allowable limit of 100% under the Philippine Tariff and Customs Code. For example, the tariffs on corn imports has been increased from 20% to 100%.

In recognition of food security concerns, developing member-countries like the Philippines have been allowed the flexibility of retaining quantitative restrictions for staples. The Philippines chose to avail of this privilege; hence, import restrictions on rice will remain in place for the next ten years. However, in exchange for this privilege, the country will be required to allow the importation of rice equivalent to 1% of our domestic consumption or about 59,000 MT in 1995. This level of importation will increase to 4% of our domestic consumption or about 239,000 MT in 2004. The Philippines reserves the right to allow the NFA to exclusively import such quantities of rice for its buffer stocking.

2. *Reduction of Tariffs*

For agricultural products whose quantitative restrictions will be lifted, the Philippines will bind or set maximum limits on tariffs at a minimum of twice the existing tariff rates. For critical agricultural commodities like corn and livestock and poultry products, the bound tariff rate is 100%. These bound tariff rates will be reduced to levels equivalent to at least ten percentage points higher than the existing rates within ten years starting 1995.

In case of corn, whose tariff rate stands at 20%, the initial bound rate is 100%. This rate will be reduced to 50% by 2004. On the other hand, the initial bound rate for meat of swine, whose present tariff rate is at 20%, will be 100%. This will be reduced by 40% by 20004.

For agricultural products which do not enjoy protection through quantitative restrictions, the initial bound rates will be ten (10) percentage points higher than existing tariff rates. These bound rates will then be reduced by the minimum requirement of 10% by the year 2004.

For selected agricultural commodities whose tariffs are currently bound under the GATT Tokyo Round of Negotiations, the initial bound rates will be maintained at their existing levels. These rates will then be reduced by the minimum requirement of 10% by the year 2004.

These reduction in bound rates will enable the Philippines to comply with the requirement of reducing tariff rates by an average of 24% for all tariff lines within the 10-year time period between 1995-2004.

3. *Reduction of Domestic Subsidies*

The Philippine currently provides domestic subsidies to its rice, corn, coconut and sugar sectors. These subsidies take the form of production support measures such as fertilizer, certified seed, and planting material subsidies, as well as price support mechanisms. The computed AMS for any of these sectors fall below the *de minimis* level for developing countries of 10% of the total value of production. In fact, the rice sector, which is the most heavily subsidized in the Philippines, merely receives an AMS for roughly 5% of its value of production. As such, the Philippines is not obligated to reduce its budgetary outlays for domestic support to any of these sectors.

C. Impact on Rice, Corn and Sugar

If the country provides the appropriate infrastructure and implements the necessary policy reforms that further enhance the competitiveness of its agribusiness exports, there is a tremendous potential to significantly increase estimated income and employment benefits under the GATT.

1. Rice

No major changes will be required by the GATT Uruguay Round for the rice sector. Under the provision for special safeguards for staples, the country has opted to retain the quantitative restrictions on rice. Moreover, the amount of rice to be imported in exchange for this privilege is minimal. The MT that we are committing to import in 2004 is also 22.7% less than our computed average of rice imports.

Furthermore, a change in the NFA's mandate away from price support toward buffer stock management for food security objectives will not be contrary to the GATT since food security programs are exempt from reduction commitments. There is, therefore, sufficient flexibility in the Agreement to allow either the maintenance of the status quo in government policies and programs in rice or a change in policy direction.

2. Corn

The Philippines has committed to set the maximum limit on corn tariff at 100% and to reduce this rate to 50% in 2004. Since, historically, the domestic wholesale price of corn has been above world prices by an average of 50%, the committed initial tariff rate of 100% will serve as adequate protection to domestic producers from corn imports.

In addition to maintaining adequate protection on corn under the GATT, significant improvement is foreseen as a result of the greater transparency and predictability to trade policies affecting the feed/livestock subsector. Under the regime of quantitative restrictions, both corn farmers and feed users face a great deal of uncertainty on whether importation will be allowed, what the timing of the importation will be, and at what amounts. These uncertainties will be reduced with the policy changes earmarked for the corn sector, thereby encouraging more investment in corn production and agro-processing which will lead to a more stable supply of corn. For farmers, complementary policy measures such as allowing corn exports will further reduce uncertainties, thus, contributing to a more competitive and dynamic industry.

3. Sugar

The reduction in domestic support and export subsidies extended by developed countries, like the US and the members of the European Union (EU), will most likely raise the price of sugar in the world market. However, the estimated increase will be rather small at 1%.

From 1980 to 1983, the average world price of sugar has been estimated at 10.5 centers per pound while Manila prices hovered at 16.5 cents per pound. Given this large discrepancy between the world price and our domestic price for sugar, it does not seem likely that there would be an appreciable opening up of new opportunities for our sugar producers as a result of the GATT, despite the slight increase in the world price of sugar. However, with the special safeguards in the GATT that allows for temporary increases in tariffs during import surges, an advantage for our sugar producers will be the minimal adjustments they will have to undertake in response to increased imports.

More significantly, the Philippine access to the US sugar market will be affected by the GATT since the US committed to phase out its sugar quota system by 2001 in compliance with the GATT rules. The volume of Philippine sugar exports to the US in 1992 was 155,000 MT, valued at US$100 million.

It is important to note that the US phase out of its quota system is a commitment under the GATT, and that this commitment will be implemented as soon as the Agreement is ratified by the US Congress. This clearly spells out the need to formulate an action plan to ensure the efficiency and competitiveness of the Philippine sugar industry within the GATT implementation table.

IV. STRATEGIES AND OPTIONS

Open trading policies have underpinned strong economic growth in South-East Asia. If the Philippines is to participate in the growth of this region and respond to changes occurring elsewhere in the world, it must become a more open and liberal economy. But then again, the question remains; How should we prepare and enable Philippine agriculture, particularly the crops subsector, to meet the rigors of global competition at the same time secure food self-sufficiency for the nation? Following are some strategies and options:

A. Second Green Revolution

Green Revolution swept Philippine agriculture in the early 1970s in response to the rice crisis. Covering rice, Masagana 99 introduced a package of technology for rice that was supported by a non-collateral, supervised credit scheme for farmers, and which required close supervision of farm operations by the government's farm management technicians. However, while it resulted in surpluses and paved the way for the country's entry into the world market, second-generation problems involving postharvest and marketing became more pronounced. Viewed as too dependent on chemical inputs, problems in loan repayment, postharvest operations, and marketing persisted in the early 1980s even upon introduction of program improvements.

Currently, the pressure is increasing for a second green revolution that would increase yields of major agricultural crops without destroying the environment. This second green revolution will focus on the needs of the poor, increase productivity of

small farms using low agricultural inputs, and promote environmentally sound policies and practices. It should be different from the first where higher yields from high-yielding crop strains and chemical inputs cannot be sustained over time.

The essence of a second green revolution is a dramatic improvement in productivity, if agricultural production growth is to at least keep pace with growth inn food demand, and with the rigors of global competition. It will have to be more complex and will have to consider other important development challenges such as decreasing farm size and environmental degradation. According to Librero (1996), chemical inputs which characterized the first green revolution are now less acceptable given the growing awareness of the adverse environmental effects of such technologies. Also, unlike in the first green revolution, the concern should go beyond mere increasing agricultural output, but greater output should be achieved through highly efficient and competitive means. The second green revolution should also be greater in scope to focus not just on rice but on other commodities such as corn, fruits and vegetables which have gained critical importance in view of the country's membership to the WTO.

The burden of realizing a second green revolution, Librero (1996) added, is placed on science and technology (S&T) – an aggressive program of technology generation and transfer, as well as a policy environment that will ensure the availability of the much needed infrastructure and support services. Following are general policy recommendations to pave the way for a second green revolution:

- Implement an ambitious public investment program for agricultural infrastructure and support service.
- Correct the built-in bias against the rural areas in the allocation of budget for road construction, repair and maintenance.
- Increase government support to research and development (R&D) in agriculture and natural resources. Recommended is a gradual but steady increase in R&D budget as percentage of GVA from agriculture until the recommended level of 1-2% of GVA is reached.
- Enhance the partnership among farmers, the local government intervenors, and scientists. The participation of farmers in major aspects of development work is crucial to ensure the success and sustainability of development programs.

B. Biotechnology

For Philippine agriculture to survive and be competitive in this present world order, the country would have to increase and sustain production of high quality, low cost agricultural products amid a rapidly decreasing land area resource through the processes of biotechnology R&D. Biotechnology is certainly an extremely important component of the menu of answers that is needed to respond to growing concerns on food production for an expected population explosion in the future, amid a depleted natural resource base and deteriorating environmental conditions.

As food demand grows and world resources dwindle, new technologies that could address these concerns the soonest possible time should be given serious consideration. Biotechnology offers the opportunity to identify the value inherent in nature, and use this to meet expanding demand for nutrition and health in a way that preserves the environment. Many experts believe that it will not be possible to feed future world population while at the same time protecting the natural environment, until the full potential of biotechnology has been realized in world agriculture.

One major issue affecting Philippine development endeavors is how to catch up with its developed neighbors amid the present world order characterized by tremendous advances in economic, information and agricultural development. With GATT expected to level the playing field in international trade, it also threatens it dislocate many of the country's traditional products. Developing countries like the Philippines will have to compete with developed countries in the world market, especially in agriculture. The challenges and potential benefits of biotechnology in meeting these challenges are enormous.

1. Biotechnology in Crop Improvement

Following are some of the more recent biotechnology R&D in the country, particularly on crop improvement.

- *Tissue culture.* Among the tools of biotechnology applied to crop improvement, tissue culture for micropropagation has been the most established. Advantages derived from the technology include; uniformness, vigor, early maturity, and cleanliness. Crops which have been successfully micropropagated in the country include orchids, banana, bamboo, rattan, abaca, sugarcane, rootcrops, and forest trees. In crop improvement, tissue culture is used to generate variability for a desired trait. Tomato tolerant to salinity, as well as banana and avocado variants with resistance to diseases have been developed. For wide hybridization, success has been achieved in the embryo culture of makapuno. To facilitate the development of homozygous lines, anther culture of rice to generate doubled haploid lines is now being utilized by the Philippine Rice Research Institute (PhilRice). Tissue-culture tecniques for genebanking purposes have been used for banana, abaca, sweet potato, yam taro, garlic and taro.
- *Genome mapping, markers and marker-assisted selection.* The Institute of Plant Breeding (IPB) has pioneered in the area of genome mapping, DNA and protein markers for biodiversity and identity, and marker-assisted selection (MAS) for various crops such as mungbean, cowpea and mothbean, potato, banana, abaca, coconut and mango. Genetic analyses of pathogens such as bacterial wilt and insect pests such a the Asiatic corn borer have also been done.
- *Genetic engineering.* Although still at an early stage, plant transformation using genetic engineering is now being done in the country.

BIOTECH in collaboration with PhilRice is developing rice varieties with resistance to stem borer by using the proteinase inhibitor gene, and to greenleaf hopper and brown plant hopper using snowdrop lectin gene. PhilRice is also involved in transforming rice with the XA21 gene for resistance to bacterial sheath blight and tungro disease.

2. Biotechnology for Global Competitiveness

Under the program "Biotechnology: Pole-Vaulting Philippine Agriculture into the 21st Century", genetic engineering is being applied to improve productivity and quality toward enhancing global competitiveness of five major crop commodities – coconut, corn, banana, mango and papaya.

- *Coconut.* Through genetic engineering, the medium chain fatty acid content in coconut oil will be increased. These are the fatty acids which make coconut oil unique and high-valued in industrial applications.
- *Corn.* Transgenic corn varieties resistant to Asiatic corn borer are being developed by incorporating borer-resistance gene from *Bacillus thuringiensis* or *Bt.* This in effect is expected to reduce yield loss from 75% to 5% or a gain of about P6.14 billion. The solution is environmentally-friendly since less pesticides shall be used, and it is compatible with other integrated pest management systems. In this genetic engineering strategy, the gene from the Bt will be incorporated into the corn plant which will then develop resistance against corn borer.
- *Banana.* The development of transgenic banana varieties resistance to banana bunchy top virus (BBTV) and mass propagation of virus-free plantlets appear to be the best solution to cope with the disease. From the use of BBTV-resistant plants, yield loss can be reduced from 90% to 10%. With the availability of resistant plants, pesticide use shall be lessened, as well as the risk of farmers from exposure to harmful chemicals. In effect, this will stabilize banana production in the country. The process shall involve incorporating a part of the virus into the banana plant in a vaccine-like manner. The resulting transgenic banana plant will then have resistance to BBTV.
- *Mango and Papaya.* Through the development of transgenic papaya and mango varieties, early ripening of these fruits can be delayed from one week to seven weeks. Through biotechnology, shelf life can be increased, thereby retaining the high quality of these fruits while in transit. This will allow mango and papaya growers in the Philippines to export more and capture other foreign markets. Through genetic engineering, the production and activity of the gene responsible for ripening of mango and papaya will be inhibited or lowered.

  Developing transgenic papaya resistant to PRSV is seen to suppress papaya ringspot virus (PRSV) infection from 100% to 10%, minimizing the yield loss from 40% to 5%. This would mean a recovery of

about 34,200 tons valued at P136 million. Ultimately, with the available virus resistant papaya, the papaya industry in the Philippines will be revived. Under the Biotechnology Program, local papaya shall be genetically engineered using local PRSV strains. The resulting transgenic papaya will then have resistance against PRSV.

3. Philippine Biotechnology Issues and Concerns

The power of biotechnology to help the Filipino farmer achieve his utmost productive capability is immense. The R&D community in the Philippines does not doubt biotechnology's merits. Yet, certain issues must be addressed to fully actualize its advantages.

- *Biosafety.* The Philippines is the first Asian country to adopt biosafety rules and regulations. The National Committee on Biosafety of the Philippines (NCBP) headed by the Department of Science and Technology (DOST) Undersecretary for Research has the responsibility to set biosafety rules in the country to approve or disapprove, and monitor the conduct of researches involving genetic manipulation by genetic engineering. Each institution conducting biotechnology researches should have its own institutional biosafety committee (IBC) to screen proposals and monitor researchers with regard to biosafety concerns. In May 1998, the NCBP approved the "Guidelines on Planned Release of Genetically Manipulated Organisms (GMOs) and Potentially Harmful Exotic Species (PHES)".

  The biosafety regulations were set up to study the possible impacts of the proposed releases of GMOs on public health and safety, occupational safety, biodiversity, agricultural productivity, and the quality of the environment.
- *Intellectual Property Rights.* The local development of transgenic crops further emphasizes the need for a law on plant variety protection and for the protection of intellectual property rights, in general. It should be noted that gene constructs in transformation make use of several genes or DNA key elements which are patented. Local researchers and their respective agencies should be assisted by concerned entities in the DOST in the negotiation for the use of these patented genes/elements.
- *Transgenic Crops in IPM and Farming Systems.* One controversial issue regarding transgenic crops is whether they will require a package of high input technology such as chemical fertilizers and pesticides – or whether they fit into the existing farming systems of the country.

  Transgenic crops will require integrated pest management (IPM) and should fit well in a farming system. For some transgenic crops like the Bt corn, insecticide inputs will be reduced tremendously. However, the package of technology developed in other countries may not necessarily be appropriate in the Philippines. Thus, for local conditions,

this point should be well studied alongside the development and testing of transgenic crops in order to provide the farmer with the proper technology package.

- *Acceptance of genetically engineered products by Filipino consumers.* In the Philippines, there exists an ensuing debate between oppositionists groups led by nongovernment organizations (NGOs) and other parties such as the R&D community, on acceptability of genetically engineered food. In general, NGO networks in the country seem to have a negative attitude toward genetic engineering in agriculture as they advocate free choice for consumers and producers. In the case of transgenic rice, political decision makers also have reservations concerning the sustainability of Bt rice as against the practice of alternative pest management. Hence, there is an urgent need for education and promotion of the potential benefits of genetic engineering to the attainment of the goals of global competitiveness and food security.

C. Modernizing Philippine Agriculture Through the AFMA

The goal of the AFMA is to empower the agriculture and fisheries sectors of the country to develop and sustain themselves under the principles of poverty alleviation and social equity, food security, global competitiveness, sustainable development, people empowerment, and protection from unfair competition. Through the AFMA, the Department of Agriculture (DA) in collaboration with other agencies of the government shall transform the present agriculture and fisheries sectors into one that is dynamic, technologically advanced, and competitive, yet centered on human resource development guided by the sound principle of social justice. It shall spearhead the improvement of Philippine agriculture and fisheries to one that is able to compete in an increasingly interdependent world, and to stimulate rural income and employment opportunity toward eradicating poverty.

Under the AFMA, the new banner program for agricultural development has been dubbed as *Agrikulturang MakaMASA* to reflect its preferential option for the poorest, often neglected segments of the population — the farmers and fisherfolk, who will be empowered to enhance their productivity and competitiveness in the global market.

Three of the components of the program are on major agricultural crops – rice, corn and high-value crops. These programs shall provide the national directions and framework for an increased productivity in the crops subsector, and shall harness a favorable environment conducive to increased agricultural investments and global competitiveness. Detailed in the respective programs are strategies and policy imperatives, which include provisions of production support services, research and development, irrigation, other infrastructure (such as postharvest, machinery, farm-to-market roads), rural financing, marketing and support services, communication and advocacy, and training and extension.

V. RECOMMENDATIONS

The crops subsector continues to dominate the Philippine agriculture landscape and hence, remains as the logical and potent springboard for achieving global competitiveness and food security in the country. Agricultural crops were valued at P276 billion in 1997, 57% of which was accounted for by rice, corn, coconut and sugarcane, with the balance of P118.2 million attributed to other crops led by banana, pineapple, mango, cassava, sweet potato, vegetables, coffee and other fruits.

Agricultural modernization is imperative to enable the country's major crop commodities to achieve strong competitiveness as the domestic market is opened-up, deregulated and liberalized. The enactment of Republic Act 8535 or AFMA provides a legislative framework for modernizing agriculture – strengthening and redirecting the government bureaucracy toward market orientation coupled with improved production and extension services. With Agrikulturang MakaMASA as its banner program under the AFMA, the Estrada Administration hopes to meet its goals of enhancing the profitability, and prepare the agriculture sector for the challenges of the twin goals of global competitiveness and food security through an adequate, focused and rational delivery of necessary support services.

Following are some general recommendations toward modernizing Philippine agriculture, in general, and realizing the full potential of major agricultural crops in the country, in particular:

- *Productivity-enhancing technology through R&D.* This involves the generation of productivity enhancing technologies and product diversification/value added technologies through R&D. R&D expenditures must be increased to at least 1% of GVA. Private sector investment in R&D should be encouraged.
- *Tariff and trade policies.* Appropriate tariff and trade policies are necessary to attain highest possible gains from trade amid the GATT-WTO.

  A balance between domestic production and importation must be maintained, consistent with the objective of growth, equity and efficiency. For domestic production, budget support must be for activities with the highest social returns, including exploring less favorable areas for production and generation of agro-climatic zone specific technologies.
- *Provision of infrastructure and other support services.* This includes public investment in farm-to-market roads, irrigation systems, flood control and drainage, post-harvest facilities such as mechanical dryers and storage facilities, and other support services.
- *Credit.* This involves the provision of immediate sources of credit to address lack of working capital owing to problems such as timeliness in release and access to credit, and high interest rates and transaction

costs. Among the measures toward this end are: performance rebates on production loans to farmers; strengthening credit institutions; innovative credit schemes; and supervised credit schemes. Better cooperative management should be promoted to ensure financial viability and easy access to credit. In supervised credit, farm plans and other investments programs should be established.

The bankability of agricultural enterprises and credit-worthiness of small farmers through stronger cooperatives should also be enhanced.

- *Assured source of quality seeds/planting materials and other production inputs.* Seeds/planting materials are important to production and hence, must be obtained easily at reasonable prices. Efforts to improve/ reengineer planting materials that are high-yielding and tolerant to drought and pests and diseases should be enhanced. Sustainable, production-enhancing inputs are likewise vital to production, and hence should be readily accessible/affordable.
- *Cultivation of available land to be use and sustainability of resource base.* Most recent land use studies should be reviewed and put to use for this purpose. Government resources, being limited, should be directed to crop areas for optimum use. Sustainability of resource base should also be ensured.
- *Calamity mitigation.* This includes the development of strategies to cope with typhoon/flooded environment/drought, reduction of crop losses through effective crop protection strategies, and reduction of production costs and post harvest losses. This also includes coping with global climate change though modeling studies to investigate and predict the effects and implications of adverse climatic changes to plant growth/yield.
- *Extension, technology promotion and training.* This entails the development of a critical mass of researchers, extension worker's farmers, local government managers, and policy makers to support improved crop production toward food security and global competitiveness; enhancement of extension services through the LGUs and extension support services through various channels; strengthening of agricultural education and training activities for farmer's education; enhancement of extension delivery system; and strengthening linkages among agencies and organizations concerned with agriculture.

A liberal trade environment under the GATT necessitates significant improvement in agricultural productivity, product quality and production cost. Improving productivity means increasing the level of output produced from a given level of input. Productivity can also be expressed in monetary terms per unit of cost or area or per producer. Improvement in yield, however, does not necessarily lead to reduction to make prices of agricultural products more competitive. Equally important is the need to improve product market products in a highly competitive environment.

Following are sets of R&D recommendations for specific crop commodities to address these concerns.

To achieve self-sufficiency through a competitive rice industry with emphasis on key production areas:

1. Develop location-specific, pest and stress-tolerant varieties and hybrids with good grain quality through conventional breeding and genetic engineering.
2. Evaluate, modify and validate land leveling equipment.
3. Develop seedling management; crop establishment methods; and protocol on plant, water and nutrient management for hybrids.
4. Formulate/modify NPK recommendations including organic-inorganic combinations.
5. Develop/refine simple and reliable diagnostic tools.
6. Develop and promote knapsack sprayer, evaporation suppressants, seedling/transplanting implements for hybrid rice cultivation, flour mill and wine presser.
7. Fabricate, test and promote harvester, thresher, dryer and equipment for handling wet paddy.
8. Standardize food processes to fortify rice products and improve shelf life.
9. Increase people's awareness using tri-media and other information materials.
10. Train core trainors, researchers, DA technicians and farmers.

To achieve self-sufficiency in food, feeds and other raw materials from corn:

1. Variety development and seed production:
   - Breeding for corn for high efficiency in N-utilization, resistance to pest and diseases, high yield and early maturity.
   - Breeding for special maize types.
   - All-Philippine varietal improvement program and variety testing.
   - Seed production.
2. Production and post-production technology development and dissemination:
   - Assessment of soil fertility levels in key corn producing areas.
   - On-farm research on various component technologies for corn production.
   - Promotion of technologies on water management.
   - Promotion of cost-efficient farm machinery implements.
   - Communication support for enhanced corn technology adoption.
3. Socioeconomics and policy studies:
   - Benchmark and impact assessment and policy needs of corn programs.

- Assessment of corn marketing schemes in key corn producing areas and development of efficient market strategies.
- Evaluation of credit needs and capital formation and development of innovative financial assistance program for corn enterprises.

To promote sustainable agro-industrial development of the coconut industry:

1. Support establishment of seedgardens.
2. Support coconut planting, replanting and rehabilitation.
3. Promote coconut-based farming systems.
4. Continue hybridization and genetic trial of promising cultivars and hybrids through conventional techniques and application of DNA marker technologies.
5. Support to improve production package of technologies (POT) and post-harvest practices.
6. Develop and promote utilization of non-traditional coconut by-products.
7. Enhance human resource capability, infrastructure and S&T services in research, information and technology transfer.

To meet the strategic sugar reserve requirements of the country as well as produce adequate amount to compete in the domestic and export markets:

1. Develop location-specific high-yielding varieties.
2. Establish micropropagation laboratories to produce tissue-cultures plantlets.
3. Improve dispersal system of high-yielding varieties.
4. Generate location-specific fertilizer recommendations.
5. Develop integrated pest management (IPM) strategies
6. Develop pest-resistant varieties.
7. Generate location-specific production technologies.
8. Package and massively disseminate site-specific technologies.

Amid the prevailing socioeconomic landscape that speaks of market forces, competition, and free flow of trade in agricultural products, ensuring a policy environment more supportive of agricultural development, in general, and of agricultural exports, in particular, is necessary. The following policy reforms are proposed:

- Provide of adequate public investments to support the agriculture sector.
- Encourage the flow of credit from institutional sources to rural areas.
- Make the exchange rate reflect the true value of the peso.
- Provide better access to more and lower-priced farm machinery, transport facilities, as well as greater variety of packaging materials.

- Reform transport policies and eliminate monopolies and other regulations creating inefficiencies in agricultural marketing.

The conditions necessary for this country to survive and win the global economic arena are increasingly being understood. In an era of trade liberalism within the framework of a borderless economy, Filipinos must come to grips with cutting edge agricultural technologies and policy reforms to be globally competitive – and to attain our vision of a hunger-free nation.

For Appendix Tables of this paper, please contact NAST Secretariat, 2/F Philippine Science Heritage Center, DOST, Taguig, Metro Manila; nast@dost.gov.ph or nast@mozcom.com.

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MAJOR ISSUES, POLICIES AND STRATEGIES ON FISHERIES

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ABSTRACT

This paper describes the status of the fisheries sector – its resources, contribution to economy, and fisheries production. The major concern in this sector is the need to maintain a delicate balance between requirements for increased production and the need to conserve and protect the resources for long term sustainability. Some of the environmental issues facing the fisheries sector are resource depletion, overfishing, destructive fishing, siltation and pollution. Moreover, there are socioeconomic issues of poverty in the coastal areas and policy issues including the need for strong fisheries regulation and enforcement. The paper discusses government programs to address the food needs of the country while conserving and protecting the fisheries resources.

Keywords: fishery, production, overfishing, aquaculture, coastal areas

I. INTRODUCTION

The primary policy of the Philippine Fisheries Code of 1998 states food security as the overriding consideration in the use, management, development, conservation and protection of fisheries in order to address the food needs of the population.

With this premise, the national government under the Estrada Administration launched a new program called "Agrikulturang MakaMASA. Its fisheries component, or "Agrikulturang MakaMASA – Fisheries, aims to develop and management the country's fisheries for food security, contribute to the socio-economic upliftment of subsistence fisherfolk nationwide, and promote environmental protection for sustained aquatic productivity over the long term period.

II. BRIEF OVERVIEW OF THE FISHERIES SECTOR

A. *The Philippine Fisheries Resources*

An archipelago with 7,100 islands, the Philippines has an extensive coastline more than 17,000 km in length and about 28 million hectares of coastal waters. The country exercises authority over 2.2 million sq km (or 220 million ha) of territorial ocean waters in its exclusive economic zone (EEZ), over seven times its land area. Much of the coastal shelf lies within the so-called "municipal waters" presently defined as those coastal waters extending out to 15 km from the shore.

B. *Contribution to Economy*

The Philippines is an important producer of fish in the world, ranking 13th among the 51 top fish-producing countries in 1996, with its total production of about 1.8 million metric tons[1]. In 1998, the fishing industry contributed 2.8% (US$ 8.80 B) to the country's Gross Domestic Product (GDP) of US$ 65.10 Billion at current prices. On the other hand, the fisheries sector contributed 3.9% (US$ 0.85 Billion) to the GDP which totalled US% 21.70 Billion at constant prices.

In terms of the Gross Value Added in Agriculture, Fishery and Forestry Sector, the fisheries industry accounted for 16.50% (US$ 1.80 Billion) at current prices and 20.1% (US$0.85 Billion) at constant prices. Fisheries contributed the largest share next to agricultural crops.

On the country's external trade, the regional financial crisis had its toll on our fish and fishery trade products. Although the quantity of exports slightly increased by 6.7% from 173,887 mt in 1997 to 185,560 mt in 1998, the value declined by 3.6% from US$ 549.8 M to US$ 530 M in the same period.

Although not a dominant player in the national economy, fisheries is nevertheless an important sector. Fish continues to be the principal source of protein of the country's population. The fisheries sector even provides direct and indirect employment to over one million people or about five percent of the national labor force.

C. *Fisheries Production*

Based on the preliminary estimates of the Bureau of Agricultural Statistics (BAS), the total fish production for 1998 rose to 2.79 metric tons valued at US$ 2.1 B, compared to 2.77 million metric tons valued at US$2.7 B in 1997. An increase of 0.72 percent and 8.7 percent was recorded in terms of quantity and value, respectively. The fish production was mainly contributed by aquaculture fisheries, 34.4 percent, followed by commercial fisheries, 33.7 percent, and municipal fisheries, 31.9 percent. In terms of total value, the highest share came from commercial fisheries, 33.9 percent, followed by aquaculture fisheries, 33.1 percent and municipal fisheries, 33 percent.

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[1] At the 1997 and 1998 exchange rate of US$1=P29.47 and P40.89, respectively.

III. KEY ISSUES IN THE FISHERIES SECTOR

The major concern in the fisheries sector is the need to maintain a delicate balance between the requirements for increased production, which contribute to food security, and the need to conserve and protect the resources for long-term sustainability. Other interrelated key issues and concerns which the government should address include the following:

A. Resource Management and Environmental Issues

1. *Resource Depletion in the Coastal Areas and Inland Waters*

   The current problems in the coastal areas and inland waters are overcrowding, illegal fishing, resource and habitat degradation (fish stock, coral reefs and mangroves), pollution and intense competition between user groups. All of these reflect the lack of effective resource management activities in these areas.

2. *Overfishing*

   Competition between the municipal and the commercial fishing sectors is due to the finite nature of marine resources combined with an "open-access" principle. When excessive levels of fishing effort lead to resource depletion, fisherfolk are forced to employ more efficient fishing technology, increasingly finer meshed nets, poisons and other destructive fishing methods to meet their short-term needs at the expense of resource sustainability.

3. *Destructive Fishing*

   Dynamite or blast fishing, fishing with poison and/or destructive gears are particularly damaging to fisheries resources. In addition to endangering the safety of the user and causing structural damage to coral reef areas, blast fishing tends to indiscriminately kill aquatic organisms essential to continued marine productivity.

4. *Siltation/Pollution*

   Deforestation in upland areas has dramatically increased sediment loads in associated inland water systems in many parts of the Philippines. A number of bays, lakes and river systems have been particularly damaged with increased siltation and pollution from mine tailings and agricultural wastes.

B. Socioeconomic Issues

Poverty in the coastal areas is caused by high population density in most nearshore areas; resource depletion of most municipal waters due to overfishing; weather problems which limit the time spent at sea by the fisherfolk; poor post-harvest handling and inefficient distribution practices which lower product value; and lack of alternative income-generating opportunities in the countryside. The fact is, the municipal fisherfolks are in a vicious poverty cycle wherein fish catch per fisherfolk is declining and can no longer support a viable livelihood.

C. Policy Issues

1. *Need for Strong Fisheries Regulation and Enforcement*

With the implementation of the Philippine Fisheries Code of 1998, fishery law enforcement nationwide needs strengthening, in close coordination with local government units and national law enforcement agencies. Generally, the main problems in effective implementation are: lack of appropriate equipment, operating funds and trained manpower.

2. *Fisheries Information*

To make intelligent policy, program and investment decisions, an appropriate, useful and timely fisheries data is required. Currently, knowledge about both traditional and nontraditional fisheries resources of the country is grossly inadequate to properly shape the longer-term fisheries management and development policies. The statistical system, a potential source of knowledge on the resource as well as its use, needs to further expands its coverage, and be more accurate and comprehensive.

3. *Revision of Lease and Licensing Fees*

Licenses or rents are imposed by the government for access to and for the use of public resources for fisheries exploitation in order to appropriately manage publicly owned assets and to obtain fair owned assets. However, at present the license fees and rent levels are too low to directly substantiate, the two objectives. Therefore, there is an urgent need to review and revise license rates and related fees based on completed studies.

D. Fisheries Institutional Issues

1. *Need for Institutional Strengthening*

To further develop and manage the Philippine fisheries sector, it is important that the recently approved Philippine Fisheries Code of 1998

be implemented effectively and efficiently nationwide. This is to ensure effective promotion of fish production, judicious management of fisheries resources, provision of extension, research and support services to the fisheries sector. In addition, institutional strengthening efforts must be directed at preparing the local government units in assuming fisheries resource management responsibilities.

2. *Need for Human Resources Development*

There is an urgent need to continuously upgrade the technical capabilities and skills of the fisheries staff and field personnel of concerned agencies in the implementation of fisheries programs and projects, particularly the local government units, in terms of management, conservation, research and extension.

3. *Access to Credit*

At present, access to credit for fisherfolk and small fishfarmers is still constrained by the lack of conduit banks in the rural areas and the reluctance of commercial banks to lend to small entrepreneurs because of high intermediation costs, lack of sufficient collateral, and limited potential for business growth. Moreover, there is a need to strengthen the capabilities of NGOs and community-based credit cooperatives serving as financial intermediaries in project identification and financial management to effectively assist the fisheries industry, particularly the fisherfolks to gain access to credit facilities.

E. Fisheries Industry Issues

1. *Post-Harvest*

Although the government has started fisheries infrastructure programs to upgrade post-harvest infrastructure and techniques nationwide, its implementation need to be expedited to minimize fisheries production loss. Most losses being incurred by the municipal fisherfolk are inadequate access to post-harvest technologies and facilities as well as difficulties in placing their products in the higher-priced urban markets. With regards to exports, the commercial sector must also conform with the specific quality standards of each importing country.

2. *Aquaculture Productivity*

Overall production from municipal fisheries is unlikely to the increase substantially in the future considering that majority of the fishing areas in the country are overfished. The country will therefore depend more on aquaculture to meet its rising domestic consumption requirements.

Therefore, there is a need to improve aquaculture productivity through the introduction of improved aquaculture technologies within ecological limits, and the provision of extension services and credit access to the industry.

IV. DEVELOPMENT AND MANAGEMENT POLICIES AND STRATEGIES

A. Declared Policies in the Fisheries Sector

The declared fisheries policies of the State under the Philippine Fisheries Code of 1998 (Republic Act 8550) are:

- To achieve food security as the overriding consideration in the use, management, development, conservation and protection of fishery resources in order to provide the food needs of the population. A flexible policy towards the attainment of food security shall be adopted in response to changes in demographic trends for fish, emerging trends in the trade of fish and other aquatic products in domestic and international markets, and the law of supply and demand;
- To limit access to the fishery and aquatic resources of the Philippines for the exclusive use and enjoyment of Filipino citizens;
- To ensure the rational and sustainable development, management and conservation of the fishery and aquatic resources in Philippine waters including the Exclusive Economic Zone (EEZ) and in the adjacent high seas, consistent with the primordial objective of maintaining a sound ecological balance, protecting and enhancing the quality of the environment;
- To protect the rights of fisherfolk, specially of the local communities with priority to municipal fisherfolk, in the preferential use of the municipal waters. Such preferential use, shall be based on, but not limited to, Maximum Sustainable Yield (MSY) or Total Allowable Catch (TAC) on the basis of resources and ecological conditions, and shall be consistent with our commitments under international treaties and agreements;
- To provide support to the fishery sector, primarily to the municipal fisherfolk, including women and youth through appropriate technology and research, adequate financial assistance, production, construction of post-harvest facilities, marketing assistance, and other services. The protection of municipal fisherfolk against foreign intrusion shall extend to offshore fishing grounds. Fisherworkers shall receive a just share for their labor in the use of marine and fishery resources.

- To manage fishery and aquatic resources, in a manner consistent with the concept of integrated coastal area management, in specific natural fishery management areas, appropriately supported by research, technical services and guidance provided by the State, and
- To grant the private sector the privilege to use fishery resources under the basic concept that the grantee, licensee or permittee thereof shall not only be a privileged beneficiary of the State but also an active participant and partner of the government in the sustainable development, management, conservation and protection of the fishery and aquatic resources of the country.

B. Agrikulturang MakaMASA – Fisheries Programs

The Makapagpabagong Programa Tungo sa Maunlad at Masaganang Agrikultura at Pangisdaan. (Agrikulturang MakaMASA -- Fisheries Program), 1999-2004 is designed to provide national directions and framework to develop and manage the country's fisheries resources for food security and ensure socio-economic upliftment of subsistence fisherfolk.

This program will serve as guide for LGUS, RFUs and other concerned agencies and organizations in the implementation of management and development interventions in the fisheries sector. Development efforts shall be focused on the expansion and revitalization of productivity programs as well as the provision of support activities such as research and extension. Management efforts on the other hand, shall cover the conservation, protection and sustained management of the country's fishery and aquatic resources to ensure its long-term sustainability.

1. *Goals and Objectives*

The goals and specific objectives of the Agrikulturang MakaMASA-Fisheries Program, 1999-2004 are to:

- Contribute to national food security at all times;
- Ensure the rational and sustainable development, management and conservation of fishery and aquatic resources in Philippine waters including the EEZ and adjacent high seas;
- Reduce poverty incidence in the coastal areas; and
- Enhance people empowerment in the fisheries sector.

Specifically, the objectives formulated under the Program seeks to:

- To improve aquaculture productivity within ecological limits;
- To optimize use of offshore fisheries and deep-sea resources;
- To improve product quality and reduce post-harvest losses;
- To provide a favorable policy environment conducive to increased investment and global competitiveness and people participation.

- To conserve, protect and sustain management of the country's fishery and aquatic resources; and
- To alleviate poverty among municipal fisherfolks and provide supplementary livelihood.

2. Strategies

The overall strategies as specified under the Program are to:

- Produce quality fish broodstock, seeds and fingerlings;
- Promote production-intensifying but cost reducing technologies within ecological limits;
- Improve production-marketing systems to become more efficient and effective;
- Empower local government units (LGUs) to assume primary responsibility for food security and resource management within their respective areas;
- Provide technical support for LGUs to help them attain the target yield;
- Develop complementation and counterparting schemes with the LGUs;
- Conserve and protect the country's fisheries and aquatic resources;
- Direct national government support to strategic areas;
- Help the private sector avail of trade and fiscal incentives.
- Promote fisherfolk organizations; and
- Tap the expertise of private/state universities and colleges (SUCs) in accessing appropriate technologies, providing a forum for research extension linkages, and assisting in the evaluation of programs.

3. Major Fisheries Projects and Activities

There have been several fisheries projects and activities initiated by the government, non-governmental organizations, the academe, people's organizations and local government units to address the issues on food security and socio-economic upliftment of municipal fisherfolk, to wit:

A. *Fisheries Production*

Increase in fish production shall come from aquaculture including increase in productivity of brackishwater and freshwater fishponds, swamp/marsh fisheries and sea cages in coastal areas. Technical assistance and extension services shall be provided, fisheries technologies disseminated and fishfarms rehabilitated/improved.

B. *Conservation and Management*

Implementation of integrated coastal and marine resource management activities, strict enforcement of fisheries laws, rules and regulations and rehabilitation of habitats shall be undertaken to ensure a rational and properly utilized, managed, conserved, protected and sustained Philippine fisheries and aquatic resources in the coastal and other marine areas as well, and address the problems of environmental degradation and destruction of fishery resource base.

C. *Fisheries Post-Harvest and Infrastructure*

The Maka-MASA – Fisheries Program shall focus on activities that will help reduce post-harvest losses, protect the health of consumers, increase fisheries exports establish appropriate product standards and implement quality inspection procedures for fishery products.

In addition, the Program through the Philippine Fisheries Development Authority (PFDA) shall provide fisheries infrastructure facilities to reduce post-harvest losses. The infrastructure facilities shall include the establishment and/or improvement of regional and municipal fish ports/landings, ice plant and cold storages and other post harvest and marketing support facilities such as municipal processing plants.

D. *Fisheries Training and Extension Services*

Fisheries training and extension services/technical assistance on aquaculture, marine fisheries and post-harvest technology to fisherfolk nationwide shall be provided in coordination with the LGUs to ensure that results of scientific research studies reach the desired clientele.

E. *Fisheries Information and Marketing Support*

In order to create awareness among Filipinos, particularly the fisherfolks, a well-coordinated information campaign on fisheries education, marketing, resource management, fisheries laws, rules and regulations and fisheries technologies shall be undertaken.

F. *Research and Development in Fisheries*

An integrated approach to research that includes a unified, updated, area-based and client-responsive R&D fisheries program will be pursued. This program aims to ensure the sustainability of

appropriate fisheries technologies which the fisheries industry can adopt as well as enhance its global competitiveness.

G. *Rural Finance in Fisheries*

Per the Agriculture and Fisheries Modernization Act 1997 (RA 8435) and the Philippine Fisheries Code of 1998 (R.A 8550), provision of credit and credit guarantee and mobilization of rural savings as support mechanisms are directed to: a) improve income diversification among marginal coastal fisherfolk; b) intensify aquaculture productivity within ecological limits; c) support commercial/deep sea fishing within ecological limits; and d) support small and medium scale enterprises (SMEs) engaged in fisheries in pursuit of a modernized and self-sufficient fisheries sector.

H. *Program Organization and Management*

As one of the components of the Department of Agriculture's new banner program, the MakaMASA – Fisheries Program shall focus on the attainment of food security and poverty alleviation, in cooperation with local government and other stakeholders engaged in developing and managing their respective localities. The Program shall be managed through a Program Committee chaired by the Secretary of Agriculture, co-chaired by the Undersecretary for Fisheries and Aquatic Resources. The MakaMASA -- Fisheries Program Directorate shall be created with the BFAR Director as Chairman and the heads of all concerned agencies as members. The Program Directorate shall oversee program implementation and ensure proper coordination among concerned agencies and units. At the field level, the local government units shall provide extension services, facilitate implementation of fisheries development and management projects and provide linkage with the private sector, cooperatives/organizations, NGOs, fishing communities and other stakeholders.

V. FUTURE THRUSTS AND DIRECTIONS OF FISHERIES SECTOR

The Philippine Fisheries Code of 1998 provides the tall mandate to modernize the Philippine fishery industry while promoting the development, conservation, management, and protection of the country's vast fisheries and aquatic resources. We envision in the next millennium that the fisheries sector shall be dynamic, technologically advanced and internationally competitive in a fair market regime with the transformation guided by the sound practices of resource efficiency, sustainability, equity and active private sector participation.

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GLOBAL PERSPECTIVE ON CEREALS – FOCUS ON RICE"

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ABSTRACT

Rice is considered by many governments as a strategic commodity due to its importance in ensuring national food security and in generating employment and income for the poor in society. Since the mid-1960s, rice production has increased by 2.5% yearly with nearly 80% of the growth in production to be due to increases in yields brought about by the gradual use of modern cultivars. However, poverty and food insecurity still affects 1.3 billion people, 70% of whom live in Asia. The paper discusses the main factors affecting demand for rice in the region: income, urbanization and population growth. In one nation, demand may fall as incomes rise, but this may be outweighed by population growth elsewhere. The paper further noted that in the mid-1980s, the growth in rice yield has failed to outpace population growth in a large number of countries. It discusses the factors that contribute to the slowing growth in rice yield: labor and wages, water availability, competing demand for land, incentive to sustain interest of farmer in rice farming, trade liberalization, limitation of technological process.

Keywords: rice, sustainability, trade liberalization, green revolution, food security

OVERVIEW OF FOOD SECURITY SITUATION

In talking to you today about a global perspective on cereals and rice, I especially wanted to focus on food security and look at some of the issues and challenges we face it we are to ensure there is enough food for everyone well into the next millennium.

As you all know, rice is the staple food and principal crop in most of Asia, with other cereals playing a smaller, but still significant role. From the Philippines in the east to Eastern India in the West, and Central and Southern China in the north

to Indonesia in the South, rice accounts for 30 to 50 per cent of agricultural production and 50 to 80 per cent of the calories consumed by people.

Because of its importance in ensuring national food security, as well as generating employment and income for the poor in society, rice, unlike other cereals, is regarded by many governments as a strategic commodity. Maintaining self-sufficiency in rice production and ensuring stability in rice prices, therefore, remain important political objectives of the governments of most Asian nations.

Partly because of such policies, Asia has done remarkably well in meeting the food needs of the region's growing population over the past three decades. Since the mid-1960s, rice production has increased by an impressive 2.5 per cent year; keeping pace with population and income growth induced changes in per capita food consumption. Nearly four-fifths of the growth in this production was due to increases in yields, made possible by the gradual replacement of traditional varieties by modern cultivars. These were mostly developed in rice research stations, supported by public investment.

In addition, the downward trend in real rice prices, experienced in many Asian countries since the late 1970s because of increased production, has contributed greatly to poverty alleviation by allowing the rural landless, and the urban laboring class, to acquire more food from the market (See Graph 1). However, poverty and food insecurity is still widespread in many low-income Asian countries. Recent World Bank estimates indicate that nearly 1.3 billion people still live in poverty, while 840 million suffer from hunger, 70 per cent of them living in Asia. It should also be noted that this situation is expected to get worse before it gets better, because of the continuing impact of the Asian economic crisis in Southeast Asia, especially in countries such as Indonesia.

Therefore, a key question for the future is whether Asia, as the most heavily populated region of the world, will be able to sustain favorable food supplies, and further improve food security for low-income households over the next few years. Many factors adversely affect Asia's food security position. These include: food production performance; population growth; income growth and distribution; and available foreign exchange to import food.

Emerging trends involving these factors indicate that many countries remain vulnerable to food security problems. Failure to do the right thing now will further exacerbate the precarious food security situation of these nations, especially in the low-income countries that still contain a large section of the Asian population.

While other cereals will play a role in answering the food security question, obviously rice is the most important crop. It is important, not only as the predominant source of energy in Asian diets, but also as a vital source of income and employment for rural people. For the rest of my presentation I would like to focus on the supply and demand issue of rice, as well as look at how science and research may cause an impact on the global perspective for this crop.

EMERGING TRENDS IN RICE-CEREAL DEMAND

I would like to begin by looking at some of the emerging trends in rice-cereal demand starting with incomes.

The Income Effect

An important factor that influences the per capita consumption of any staple food is the income level of the consumer. At low-income levels, meeting energy needs is the most basic concern of any individual. Staple foods, such as starchy roots, rice, wheat and coarse grains provide the cheapest source of energy. Thus, poor people spend most of their income on such food.

In rice producing regions, the extreme poor often make do with coarse grains and sweet potatoes because they lack purchasing power. As incomes grow, per capita rice consumption increases, with consumers substituting rice for coarse grains and root crops. But as incomes increase beyond a threshold, people can afford to have a high-cost, balanced diet containing foods that provide more proteins and vitamins, such as vegetables, fruits, fish and livestock products. From that income threshold, per capita rice consumption starts declining.

The above pattern of changes in food consumption with economic growth is amply demonstrated by the experience of Japan, South Korean, and Taiwan which made transitions from low to a high-income levels within a relatively short period of time. In South Korea, the per capita consumption of rice increased from 318 grams per day in mid-1960s, when it was a low-income country, to 386 grams by 1979 when it reached the middle income level. Since then, the per capita consumption of staple grains which consist mostly of rice has been declining (Table 1).

You can see there has been a substantial reduction in the consumption of cereals and root crops, but a surge in the demand for livestock products, fish, fruits

Table 1. Changes in food consumption pattern in South Korea, 1974-75 to 1992-94.

Food item	1974-76	1992-94	Change
Cereals	686	513	-173
Roots	101	43	-58
Vegetables	373	511	138
Fruits	63	229	166
Sugar	23	88	65
Oils and fats	11	35	24
Fish	132	181	49
Meat and eggs	32	124	92
Milk	12	58	46
Total	1433	1782	349

Source: FAO 1996: Food Balance Sheets, 1992-94 average.

and vegetables over the past two decades. We can expect similar changes to occur in other Asian countries as they move along the same path of economic development.

However, in South Asia, particularly in the Philippines and Vietnam, 30 to 50 per cent of the people who live in poverty do not have adequate incomes to buy food they need. With economic growth and a reduction in poverty, per capita rice consumption is expected to further increase in these countries, since the poor can then afford to satisfy their unmet demand for a staple food. The expected rice demand in such nations, which constitute about 40 per cent of the regional total, will easily overcome the downward pressure on demand from the middle- and high-income industrialized countries whose share is a mere 10 per cent.

Urbanization

The other force that will affect the demand for rice is urbanization. As people move from rural to urban areas, their energy needs correspondingly decreases as they move from more physical jobs to more intellectional ones. Also, the cost of meeting basic non-food needs, such as education, health care, transportation and recreation services, is higher in urban areas; therefore a smaller share of the family budget is available for staple food.

The consumption composition between staple and non-staple food also changes because of greater nutritional awareness of the importance of a balance diet, and the widespread practice of eating outside the home. So, for the same level of income, the per capita consumption of rice is generally lower in urban areas compared to rural districts. The evidence from Thailand and Bangladesh as shown in Table 2 demonstrates this.

In Asia, the urbanization level is still low but will continue to grow over the next two decades (Table 3). Thus, the demand for rice will decline because of a larger proportion of people living in urban areas.

Urbanization also increases the demand for high quality food and processed products. Urban consumers may not increase their consumption of rice with an increase in income, but they will pay premium prices for preferred varieties, particularly if they meet certain health standards (such as produced under organic farming or are non-transgenic) and have improved nutritional quality.

Table 2. Per capita consumption of rice (kg/yr) by income groups and regions, Thailand and Bangladesh.

Ranks in the	Thailand			Bangladesh	
Income scale	Rural	Semi-urban	Urban	Rural	Urban
Bottom 25%	151	133	97	148	141
Middle 50%	146	125	89	179	147
Top 25%	134	125	83	175	142

Source: National Household Expenditure surveys.

Table 3. Projection of populations residing in urban areas in selected countries. (In percent of total population)

Country	1960	1990	2010	2020
Japan	56	77	81	84
South Korea	28	74	91	93
Thailand	13	19	31	35
Indonesia	15	31	54	57
China	19	26	43	51
India	18	26	34	41
Bangladesh	5	16	28	36

Source: United Nations.

In Japan and Korea we have seen a surge in demand for high-quality non-glutinous rice over the past two decades and a drastic fall in the consumption of standard quality rice. The same pattern of change in the composition of demand is in progress in China after the liberalization of the food market, with a growing demand for Japonica rice at the expense of low-quality hybrid Indica rices. The demand for rice-based products, such as noodles, and rice cakes is growing with urbanization and women's involvement in activities outside home.

Population growth

Given the per capita consumption level, the increase in total demand depends on the number of mouths to be fed. In the developed world, the total demand for cereals has decreased as many of them have achieved stationary population. But most Asian countries still have populations growing at 1.5 to 2.8 per cent per year. (except Japan, South Korea, China and Thailand). However with increasing economic prosperity, population growth will slow down as experienced be developed nations during the early stages of development.

According to UN projections, population growth in most Asian countries over the period 1995-2025 will be reduced to almost half of the level experienced by these countries since the mid-1960s (Table 4).

However, due to the expanded base of population (from 3.5 billion in 1995 to 4.8 billion in 2025), the absolute increase in the number of people over the next three decades will remain as large as ever from 1.21 billion to 1.45 billion. Ironically, it is in the poverty-stricken regions, where the per capita rice consumption is expected to increase and population growth will also be the fastest. In South Asia, for example, the population is projected to increase by 732 million over 1995-2025, compared to 670 million over 1965-95. It is only in East Asia that the additional number of mouths to be fed is going to be substantially lower in the future compared to what they have been in the past.

Table 4. Population projections (millions) for Asia, 1995-2020.

Country	1995	2020	% change	Annual growth
China	1220	1449	19	0.69
India	929	1272	37	1.26
Indonesia	197	264	34	1.17
Bangladesh	118	171	45	1.50
Vietnam	118	171	45	1.50
Thailand	58	68	16	0.61
Myanmar	45	64	42	1.41
Japan	125	124	-1.0	-0.04
Philippines	68	100	47	1.56
South Korea	45	52	16	0.58
Asia	3147	4121	31	1.08

Source: Asia includes countries in South, Southeast and East Asia.

Source: United Nations.

Projected growth of demand

The International Food Policy Research Institute (IFPRI) has developed a Model for Policy Analysis of Agricultural Commodities and Trade (IMPACT) for developing long term projections of the demand and supply balances for major food items. It specifies a set of country or regional sub-models, each with a particular structure within which supply, demand and prices for the commodities are determined. The demand projections for rice for Asian regions obtained from a revision of the model that incorporates the most recent population projections by the UN (1997), and slower growth in East and Southeast Asia due to the recent economic turmoil, show demand for rice growing at only 1.02 per cent per year. This implies an increase of 31 per cent over the 1998-2025 period (Table 5).

The projected growth in demand is substantially lower than the historical growth in rice production of 2.5 per cent per year over the past three decades. This is mainly because of East Asia, where it is projected to grow at only 0.6 per cent per year. However, the low-income poverty stricken countries of South and Southeast Asia are expected to experience strong growth in the demand for rice. The countries in Asia where the demand is expected to grow rapidly over the next 25 years are: Philippines (65%); Malaysia (56%); Bangladesh (51%); India (46%); Vietnam (45%); Myanmar (42%); Indonesia (38%).

An important point to note in this context is that although the demand is quantity terms may relax, the market for quality rice will expand rapidly following growth in the size of the middle class and with urbanization, as demonstrated by Japan and South Korea.

Table 5. Projected growth of demand for rice in Asian regions.

Region	1993	2020	Percent increase 1993-2020	Annual rate growth of demand	Annual rate of growth of population
East Asia	143.2	163.3	14.0	0.58	0.60
Southeast Asia	73.6	103.8	41.0	1.29	1.17
South Asia	101.7	150.2	47.7	1.46	1.40
Asia	318.5	417.3	31.0	1.02	1.02

Source: IFPRI updated IMPACT model.

Therefore, the main factors affecting demand for rice in the region will continue to be income, urbanization and population growth. In one nation, demand may fall as incomes rise, but this may be outweighed by population growth elsewhere.

Let us now look at the supply situation.

Emerging trends in supply

The impressive growth in rice production during the first two decades of the green revolution (1966-86) generated a sense of complacency in Asia's ability to feed itself. Recent trends in rice production growth, however, raise serious concerns regarding the sustainability of these past achievements (Fig. 1).

The growth in rice yield has slowed considerably since the mid-1980s, and has failed to outpace population growth in a large number of countries (Table 6). Several factors that I will now discuss suggest that this is perhaps the beginning of a long-term trend more than a cyclical downswing.

The growing scarcity of agricultural inputs

The availability of labor, water and land for rice cultivation depends on economic growth. Competing demand for these inputs from other economic activities affects their relative scarcity and prices, and thereby changes relative profitability, depending on the use intensity these inputs in a particular activity.

Labor and wages. Economic growth brings dramatic changes in the structure of employment, the adoption of labor saving technology and increases in labor productivity. With opportunities for more remunerative employment elsewhere, workers move out of low productive, poorly paying food production activities. Although the agricultural sector tries to address the problem of labor shortages by adopting labor saving technologies, it cannot compete with the manufacturing and services sectors which enjoy strong markets and can easily diversify and adjust according to the change in demand.

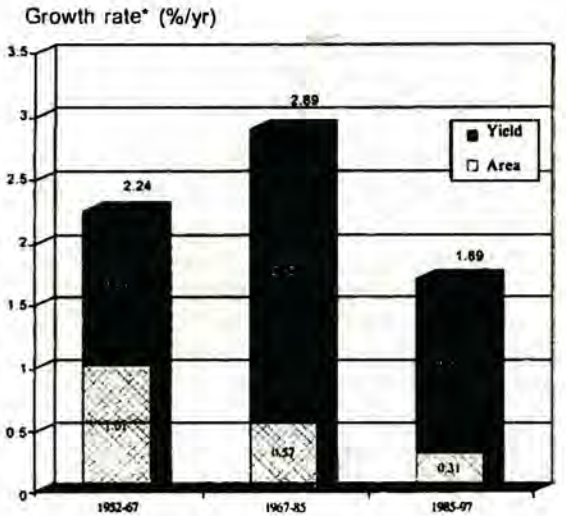

Figure 1. Growth in rice production and productivity: pre- and post-green revolution period.

Table 6. Population projections (millions) for Asia, 1995-2020.

Country	Growth rate in production (% yr-1) 1966-86	1986-96	1966-86	Growth in yield (% yr-1) 1986-96
South Korea	2.48	-2.62	2.35	-0.53
China	3.20	0.66	2.97	1.38
Indonesia	5.18	2.44	3.95	1.06
Philippines	3.77	2.07	3.95	1.06
Vietnam	2.98	5.41	1.85	2.94
Thailand	2.53	1.07	0.52	1.61
India	2.49	2.99	1.86	2.46
Bangladesh	2.03	1.62	1.59	2.22

Note: Growth rate estimated by fitting semi-logarithmic trend lines to the time series data taken from FAO electronic database.
Source: Dr. Mahabub Hossain

The other disadvantage of agriculture in competing with other sectors is caused by constraints in increasing farm size. This is particularly the case in Asia, where there is extreme population pressure on land and exorbitant land prices. Therefore, productivity differences continue to grow with manufacturing increased by 4.3 times during the 1966-90 period, compared to only a 1.2 times increase in the agricultural sector. The total agricultural labor force increased from 4.5 to 6.1 million persons between 1966 and 1975. However, it started to decline in absolute terms and reached 3.2 million by 1990.

Labor scarcity is reflected in the price of labor – the wage rate. According to the 1996 World Development Report, East and Southeast Asia, which experienced more than five per cent per year growth in per capita incomes, saw their real wage rates increase by 170 per cent over a 20-year period. In contrast, in South Asia, where economic growth was moderate, the real wage rate increased by only 50 per cent.

Water availability. Water resource development has been the key to increasing rice production in virtually all Asian countries where land is a scarce factor of production. Water has generally been regarded as an abundant resource for humid Asia. But with rapidly increasing populations, per capita availability has been declining and the substitution of water for scarce land has taken place to meet growing food needs. As a result, the perception of abundant water has been changing.

Many Asian nations now face emerging water resource problems which include:

(a) The stress of meeting human and industrial needs in exploding urban centers;
(b) The plateauing of full economic exploitation of irrigation potential in many regions;
(c) And, the expansion of coastal salinity because of reduced river flows during the dry season.

As populations increase and economic development intensifies, satisfying public needs for drinking water, sanitation and industrial activities have to be accorded higher priority in the allocation of water resources. Almost all Asian governments now face hard decision-making choices involving long-term plans for the regulation, allocation and utilization of water resources.

In absolute terms, annual water withdrawals are by far the greatest in Asia, where agriculture accounts for 86 per cent of total annual withdrawals compared to 38 per cent in Europe and 49 per cent in North and Central America. The per capita availability of water resources declined by 40 to 60 per cent in most Asian countries over the 1955-1990 period and are expected to decline further.

The scope for further conversion of rainfed land to irrigated plots, which was a major source of past growth in rice production, is also becoming limited. Irrigation has increased substantially, as other options for irrigation development are already being used. In addition, environmental concerns such as adverse effects of irrigation and flood control projects on waterlogging, salinity, fish production and

the quality of ground water, have been growing. Already, there has been a drastic decline in investment for the development and maintenance of large-scale irrigation projects in many Asian countries.

Competing demand for land. Economic prosperity and industrial progress is leading to rapid urbanization. As more and more people move to live in large cities. Most of the additional increase in population beyond 2000 will be found in urban areas. By 2025, 53 per cent of the people in Asia will live in urban areas compared to 30 per cent in 1990. An important implication of growing urbanization is that fertile agricultural land must be diverted to meet the demand for housing, factories and roads.

As the frontier of cultivable land was closed long ago, the per capita availability of arable land has been declining rapidly with growing population. China now supports 17 persons per hectare of arable land; the figure is 13 for Bangladesh, 11 for Vietnam and 8 to 10 for India, Indonesia, and the Philippines. Only Thailand, Myanmar and Cambodia have favorable endowments of land, with 2 to 4 persons per hectare. The population pressure is reflected in the high cropping intensity for food grain production. The cropped area under food grains per unit of arable land is 148 per cent for China, 132 per cent for Bangladesh, 112 per cent for India, and 108 per cent for Vietnam, compared to about 60 per cent for Thailand and Myanmar.

The area under rice cultivation is also expected to decline with economic prosperity and urbanization, as the demand for land for non-agricultural uses will grow. There will also be economic pressure to release rice land in favor of vegetables, fruits and fodder, whose markets become stronger with economic progress. In China, the rice harvested area declined from 37 million hectares in 1976 to 32 million in 1992; in the Philippines, it declined from 3.7 to 3.2 million hectares within the same period.

Future growth in rice production must occur on smaller land, use less labor, and water. The downward pressure of input availability on the growth of supply is thus obvious. Rice yields labor and water productivity must grow at a rate faster than increase in the demand for rice, to maintain a favorable demand-supply balance and to release resources for other economic activities.

Sustaining farmer's incentives in rice cultivation

Despite the impressive increases in land productivity, countries and regions that have achieved high yields are finding it difficult to sustain producers' interest in rice farming. Because rice farming is a highly labor intensive activity, the growing labor scarcity and higher wages have pushed up the cost of rice production and reduced profits and farmers' incomes. But it is not only the wage laborers who are tempted to move to non-farm urban and rural occupation. Even small scale rice farmers find it more attractive to leave rice farming and join the farm-farm labor force because of increasing wage rates as shown in Table 7.

Table 7. Long-term trend in wages rates, (US$/day) selected Asian countries.

	1961	1971	1981	1991
Bangladesh	0.46	0.44	0.86	1.39
Philippines	1.39	0.59	1.51	2.16
Korea	0.82	1.86	10.84	32.59
Japan	1.22	8.19	24.16	51.93

Source: IRRI World Rice Statistics, 1993-94.

Efforts are underway to improve the competitiveness of rice farming by:

(a) Improved farm management practices that increase efficiency in the use of non-land inputs and increase total factor productivity.
(b) Increased use of capital to replace labor through the mechanization of farming operations so that labor productivity can be continually raised when no further increases in land productivity are possible.
(c) Use of price mechanisms to transfer income from relatively well off rice consumers to low income rice producers, so the balance between rural and urban incomes can be maintained.

In spite of these policies, in sustaining farmer interest in rice cultivation has remained a major challenge in fast growing Asian nations. In countries and regions where yield levels are high, such as Japan, South Korea, Java in Indonesia, as well as Punjab and Tamil Nadu in India, the scope for increasing profitability through the efficient use of inputs has almost been exhausted.

As labor accounts for only one fourth of the cost of rice production, the substitution of capital for labor increased farmers' incomes only up to a point, particularly when the average farm size was small. Land prices remained high and increased over time due to the extreme pressure of population and growing land demand for housing and industrial purposes. In South Korea, rural wage rates and land prices increased by 18 per cent per year from 1970 to 1990, when machinery and fertilizer prices rose by just seven per cent.

As the cost of rice cultivation continued to increase due to rising opportunity cost of labor and land, governments had to continually raise rice prices and increase farm subsidies to maintain a balance between rural and urban household incomes. The protection of domestic rice industries encouraged high-cost domestic production. A study on costs and returns conducted by the FAO shows that in the late 1980s, the cost of producing rice in Japan was about 17 times higher than in Thailand and Vietnam and about 10 times higher than the USA (see Table 8). Thus, with economic growth, the comparative advantage in rice production shifts to low-income countries.

Table 8. A comparison of domestic rice prices and the cost of production in selected countries, 1987-89.

Country	Paddy yield (t/ha)	Cost of production (US$/T)	Domestic farm gate price of paddy (US$/T)
Japan	6.5	1,987	1,730
Korea	6.6	939	957
USA	6.3	220	167
Vietnam	4.6	100	130
Thailand	1.8	120	141
Philippines	2.6	124	160
Indonesia	5.8	118	132
Bangladesh	4.6	138	180

Source: IRRI for Bangladesh and Vietnam. For other countries, FAO.

The impact of trade liberalization

The implementation of the GATT Uruguay Round of Agreements may further dampen incentives for rice production, particularly in middle-and high-income countries. They will not be able to compete with the low-income economies of Asia, where the wage rates and opportunity cost of family labor is low, or with large land-surplus countries in the developed world (e.g., Australia, USA, Italy). These nations will reap economies of scale because of the large size of their rice farms. If the domestic market is opened for competition, the price of rice will decline substantially, providing incentives to consumers to buy imported food staples, and forcing farmers to abandon rice cultivation in favor of more lucrative economic activities.

An important way of gaining competitive strength in the face of rice trade liberalization is the consolidation of tiny holdings into large-scale farms, as rural households migrate to urban areas leaving their land behind. The so-called "smart farming" of large-scale holdings as currently practiced in the developed world, and the vertical integration of the rice industry (production, processing and marketing managed by the same farm), may contribute to more efficient use of large-scale machinery. It may also lead to a reduction in the number of part-time farmers that are now tied-up in the supervision of numerous tiny rice farms.

The main constraint to the consolidation of holdings in Asia is, however, exorbitant land prices that prohibit the development of an active land market. At existing land prices, the rate of return in rice farming from an investment in land will be substantially lower than the return on an investment in other enterprises. Thus, for Asia, we cannot expect any consolidation of holdings of the same scale as happened in North America and Europe during the early sages of their development.

Because of the forces mentioned above, middle-and and high-income countries will not be able to generate any exportable surplus, even when domestic rice consumption declines with growing economic prosperity. Rather, rice area and production will decline as domestic production is adjusted in line with the downward trend in demand. In Japan, the peak rice harvest was 18.8 million tons in 1967; it has recorded a secular downward movement since then, reaching 12 million tons in 1997. In Taiwan, the peak reached 3.6 million tons in 1976, with the present level of production at less than 2 million tons. South Korea is going to follow the same trend soon. These countries could have maintained their peaks by exporting any surplus over their domestic needs. However, this did not happen because they could not compete in the world market due to their high cost of production in the domestic market.

The exploitation of excess capacity

Any expected drop in global rice production on account of the high-income countries of Asia could be compensated for by an increase in rice production from outside Asia. There is some potential for an expansion in the rice area in the humid tropics of Africa and Latin America. The FAO estimates there are 20 million hectares of potentially suitable rice land in the river valleys of West and Southern Africa, of which only 15 per cent is currently cultivated.

In tropical South America, rice cultivation can be extended to an additional 20 million hectares. The exploitation of this potential will, however, require a substantial increase in price, as well as the capacity of the countries to invest in the reclamation of new land and the development of marketing infrastructure. The unit cost of production and the marketing margin is many times higher in Africa and Latin America than in Asia. Also, the demand for rice has been growing faster in other continents compared to Asia (at more than 4.0 per cent per year in Africa) so the exportable surplus available for Asia from these continents could be quite small.

Eastern India also has considerable excess capacity in rice. However, with the alleviation of poverty and the high growth of population, Eastern India may need to exploit any excess capacity to meet its own growing internal demand. Only Myanmar and Cambodia could generate additional exportable surplus' to meet potential shortages in other Asian nations. The exploitation of such potential would, however, require substantial investment for land reclamation, the expansion of irrigation systems, technologies for the improvement of rice quality, and the development of marketing infrastructure.

Also, additional exports from Myanmar and Cambodia may not add much to the world rice market, as exports from Thailand and Vietnam are likely to decline over the long run. Thailand's comparative advantage in generating an exportable surplus is its favorable land endowment. However, this advantage is being gradually eroded due to rapid increases in farm wages and opportunity cost of family labor, a process that Japan, Taiwan and South Korea have already gone through in

the past three decades. Vietnam has almost fully exploited its potential for increasing rice cropping intensity and production, and may have to reduce exports in the future to accommodate growing internal demand.

Technological progress: Running out of steam

One of the most important factors behind the recent slowdown in the growth of rice production (See Table 9) is that the technological progress needed to improve rice cultivation is running out of steam.

The increases in rice yields in the past originated mainly from:

a) The gradual adoption of modern varieties on existing irrigated land.
b) Public and private investment in the expansion of irrigation areas to support the diffusion of modern varieties and improved farming practices.

The green revolution was successful mostly in irrigated ecosystems where yields increased from 3.0 to 5.8 ton/ha over the past three decades while the yield growth remained moderate in rainfed systems. Today, however, almost the entire irrigated land are of the region has been covered with modern varieties. In addition, the yields of the best farmers are already approaching the potential that scientists were able to attain with today's knowledge in that particular environment. In addition, with the intensive monoculture of rice in the irrigated systems and high doses of chemicals, natural resources are becoming stressed.

Table 9. The race between population and rice production.

Country	Rice harvested area (000 ha)	Population growth (%/year)		Growth in (%/year)	
	1994	1965-85	1985-95	1965-85	1985-95
India	42,034	2.2	1.9	2.4	2.4
China	30,373	1.9	1.1	3.3	0.6
Indonesia	10,646	2.3	1.6	5.7	2.0
Bangladesh	9,912	2.8	1.9	1.8	1.9
Thailand	8,482	2.6	1.4	2.5	0.0
Vietnam	6,500	2.3	2.3	2.5	3.9
Myanmar	6,477	2.2	2.3	2.8	2.6
Brazil	4,446	2.4	1.6	1.8	1.2
Philippines	3,350	2.8	2.4	3.9	1.5
Japan	2,212	1.0	0.3	-0.5	-0.6
Madagascar	1,180	2.7	2.9	1.5	1.5
South Korea	1,160	1.8	0.9	2.0	-1.7
Malaysia	665	2.5	2.3	2.1	1.8
World	146,452	1.9	1.6	3.1	1.4

Agronomists have also noted yield declines in the experimental farms that tested the effect of intensive rice farming on yields and the soil's nitrogen supplying capacity. In the humid tropics, maximum achievable yields at the farmer level are lower than 6.0 tons per hectare because of increased pest pressure, frequent cloudy days with below optimal sunshine, and susceptibility of the crop to floods, droughts and strong winds.

In regions with good irrigation infrastructure, this potential yield level is about to be reached. Further research is needed to shift the yield frontier for irrigated systems and develop appropriate crop and resource management technologies to sustain high yields.

There are some technologies in the pipeline which may help raise land productivity and input-use efficiency in the irrigated ecosystem, and thereby contribute to further increases in rice supplies (Khush, 1995). These will help, but will not provide all the answers. Please allow me to briefly detail two. In 1989, IRRI began to design a new rice plant type, one that would make it possible to grow an irrigated rice crop with up to 30 per cent higher yield. It is also designed to increase nutrient efficiency with fewer numbers of larger panicles per plant, to reduce unproductive tillers and to increase photosynthesis efficiency through erect and thick leaves. The field evaluation of the breeding lines has just begun. The new plant architecture also needs to be matched with agronomic practices such as planting method, nitrogen application and weed control. Work is also needed to develop resistance to insects and diseases and to improve grain quality. Therefore, it may take another five to 10 years for this technology to reach the rice farmers.

Another technology which is within reach of the farmer is hybrid rice for the tropics (Virmani, 1994). Hybrids have a yield advantage of 15 to 20 per cent over the currently inbred high-yielding varieties. Rice hybrids were developed earlier in China. Increases in rice yields in China in the 1975-1990 period were largely due to the diffusion of hybrid varieties to 50 per cent of the country's rice area. Chinese hybrids, however, are not suitable for the tropical climates of Southeast and South Asia. IRRI scientists have already developed other suitable hybrid lines for the tropics, which are now being used by scientists in India and Vietnam to develop varieties for release to farmers. The main constraint in any rapid expansion of hybrid rice among the small farmers is the development of infrastructure for the production and distribution of seeds. Farmers will need to change seeds every season, which is an unconventional practice.

It should also be noted that the potential for raising yields in the rainfed ecosystems is still vast, as the current yields is only about 2.0 tons per hectare. Across nearly 45 per cent of arable Asia, rice is grown under rainfed conditions. Indeed, this ecosystem is the dominant one in the low-income countries of Asia, where the demand for rice is projected to remain strong. If rice science succeeds in developing appropriate technologies, this ecosystem could make a major contribution to any future growth in rice production.

However, rainfed ecosystems are subjected to the vagaries of nature such as droughts, floods, typhoons and erratic monsoons. Traditional low-yielding varieties have developed traits that enable them to withstand temporary submergence in water and prolonged droughts that cause large year to year fluctuations in yields for this ecosystem. Rice scientists have had limited success in identifying these traits, and incorporating them into high-yielding modern cultivars. Where the rainfall is unreliable and the drainage is poor, farmers still grow traditional varieties, and use fertilizers in sub-optimal amounts on modern varieties, due to the uncertainty of returns from investments. This is the main factor behind the low-yields and the large yield gap in the rainfed ecosystems.

If rice research succeeds in incorporating traits that help withstand such abiotic stresses, improved systems management help avoid these stresses, modern varieties will be adopted more extensively in the rainfed system. This will help increase the stability of yields and reduce risks in rice cultivation; thereby providing incentives to farmers to apply inputs in optimal amounts that will contribute to further increases in yields and rice production.

CONCLUSION

Let me say clearly that IF we cannot meet most of the challenges that I have just outlined to you, then the food crisis in Asia that are now just a distant memory could return to haunt us all. The global outlook for rice and cereals is quite clear: there will not be enough for every one unless more resources are committed to research so problems faced by most of the developing world can be overcomed.

This is no time for complacency, we should not be resting on our laurels as scientists. There is still a large unmet demand for food not just in Asia but in many other parts of the world. There are other issues involved in achieving food security, many of them political, but increasing production or output is one sure way to avoid potential problems in the future.

If the region succeeds in alleviating poverty, the demand for rice will increase much above the level induced by population growth. The amount of arable land per capita is already low, and farm size has been declining due to rapid increases in population and the slow absorption of labor in the nonfarm sector.

In addition to these problems, the rainfed areas which account for half the area planted to rice have benefited little from the Green Revolution. This is because scientists have had limited success in developing varieties that could adapt to the difficult natural and environmental conditions such as drought, floods, temporary submergence and soil salinity common in much of the region. The conversion of rainfed land to irrigated ecosystems has also been slow due to the high and rising cost of irrigation, the difficult physical conditions for irrigation development, and growing environmental concerns over irrigation projects.

Thailand, Myanmar, and Cambodia to have considerable excess capacity to meet potential shortages in other countries in South and Southeast Asia. If rice

prices go up due to tightness in the world market, farmers will be encouraged to increase rice production by investing in irrigation and adopting high-yielding varieties. But achieving food security through trade may not be possible due to foreign exchange constraints in low-income, food-deficit countries.

Also, since rice production is a major rural economic activity and land and labor cannot be easily diverted to other economic activities during the monsoon season, the poor would not have the economic capacity to acquire the imported food if rice productivity remains stagnant. Any increase in rice prices would only aggravate the poverty situation in these countries, unless the economic condition of small farmers and the landless improve.

Therefore, Asian countries must continue to focus on technological progress that reduces the cost of production per unit of output, and thereby maintains the profitability of farmers while keeping prices affordable to the urban and the rural poor. The conflicting interests of the rice producers and consumers can only be reconciled by strengthening the infrastructure of science and technology and thereby creating a firm base for cost-reducing technological progress.

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GLOBAL PERSPECTIVE ON PESTICIDES

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ABSTRACT

World food supplies will have to be doubled several times by 2025 to ensure food in sufficient quantity, due to increase in population, greater urbanization and spending power. Among the technology packages which need to be developed to increase agricultural production to during the next three decades are: germplasm, crop protection products, fertilizers, irrigation systems, technical equipment, management concepts such as integrated pest management, integrated crop management and precision farming. The annual losses due to pests especially for rice, wheat, barley, maize, potatoes, soybeans, cotton and coffee are estimated to be 50% of the total crop area worldwide. Pre-harvest losses caused by insect pests constitute 15%, pathogens ~ 13%, weeds ~ 14%, and post harvest losses, an additional 10%. Presently, pesticides are used only in about 1/3 of the total cropped area in the world, 75% of which is in North America, Europe and Japan, and 25% in the developing world. Studies show that for each US$6.5 billion in pest control save approximately US$26 billion in crops, based on direct costs and benefits. Thus, to produce more food, it is important that crops be protected against diseases, pests and weeds. It is therefore the ultimate strategy of crop protection companies to produce improved, pest-specific chemicals that are less harmful to human health and the environment and less likely to affect non-large species than earlier broad spectrum chemicals. Companies that offer farmers solutions that make use of synergies between chemical crop protection and biotechnology will have an important competitive advantage.

Keywords: crop protection, pesticides, weeds, pathogens, insect pests, biotechnology, integrated pest management

Food security is a global concern. The 1995 world food production was only 0.26% higher than the global food demand. This describes the situation of the present world food security.

It is generally agreed that world food supplies will have to be double several times by 2025 to ensure food in sufficient quantity, not only because of the increase in population but also as a result of greater urbanization and spending power. In the past, world agriculture was technically in a position to produce enough healthy food for the growing population by gradually introducing yield-increasing technologies, such as high-yield seeds, crop protection products, fertilizers and improved irrigation systems. Due to many different factors, however, more than 840 million people throughout the world are at the present time still undernourished. A prediction of the USDA (1998) shows that the number of malnourished will rise from the current level of 840 million people to 1140 million in 2008.

"Meeting the needs of the present without compromising the ability of future generations to meet their own needs" is the definition of sustainable development as written in the Brundtland commission report in 1987.

Sustainable agriculture, a paradigm that rests on the wise management and preservation of agriculture's resource base – water and land – offers us our only hope of ensuring food security as we enter the next millennium.

More land was cultivated in the last 140 years than during the last 40,000 years put together, when agriculture did begin (Dobson, 1997). Furthermore, we have to learn to accept the notion that we have to produce twice as much food within the next 30 to 40 years than in the whole of the last 10,000 years added together (Kern, 1998). One twentieth of all the people who ever lived on the earth are living today (Kaku, 1998).

What are the technology packages in agricultural production?

The key question is the following: What are the technology packages that the stakeholders in the food-production chain need to develop over the next three decades: germplasm, crop protection products, fertilizers, irrigation systems, technical equipment, management concepts such as integrated pest management, integrated crop management or precision farming?

What can we expect from different agro technologies within the next 30 years?

Full utilization of all technologies in crop production, including modern biotechnology, will play a decisive role in increasing yield for the preservation of sustainable global self-sufficiency. At the moment 1.7 billion people are "fed" by fertilizers, 1.67 billion people by irrigation, and 2.4 billion people by good agricultural practice including fertilizers, 2.4 billion by irrigation, 2.25 billion by good agricultural practice and 1.6 billion (19% of the global population) by bioengineering of crops. The quantitative impact of biotechnology on regional agricultural food production is forecasted for 2025: Africa, 6%: Latin America, 17%; Asia, 20%; developed countries, 28%.

What has happened during the last 30 and the last 60 years of agricultural production?

From 1930 up to 1995, the cotton growing area has remained unchanged, but cotton production has tripled.

Since 1950, global harvested rice area has been enlarged by 40% only, even though global rice production increased by more than 300%. This situation underlines very well the importance of agro technologies, because during the last 40 years it was possible to increase the productivity by a factor of 7.5.

Since 1980 a global reduction of cereal acreage through yield increases was possible at a level of 34%. In contrast, in Africa, an extension of cereal acreage by 24% was necessary due to insufficient yield increases.

How big are the annual crop losses from pests?

Yudelman et. al. (1998) have reviewed the estimated annual losses from pests, especially for rice, wheat, barley, maize, potatoes, soybeans, cotton, and coffee, i.e. 50% of the total crop area worldwide. Pre-harvest losses are caused by insects (15%), pathogens (13%), weeds (14%) and additionally 10% by post harvest losses. Without pest control the overall worldwide losses from pests were as high as 50%. A comparison of global crop loss estimates from different sources has shown that since the 1940s the pest losses today are as high as they were 50 years back (Pimentel, 1991).

Where and how much pesticides are used in the world?

At the moment pesticides are used only in about one-third of the total cropped area in the world (Oerke et al., 1995). More than 75% of pesticides are used in North America, Europe and Japan on a crop land area of only 45%, whereas in developing countries with 55% of the global cropland only 25% of global pesticides are used. The "top ten" country markets are USA, Japan, France, Brazil, Germany, China, Canada, Italy, Argentina and Australia.

The average amount of chemical crop protection products applied to arable cropland differs greatly from one country to another. Japan applies about 17.5 kg. active ingredient per hectare, but application rates in Europe and the US are below 4 and 2 kg a.i./Ha. Developing countries, e.g. Indonesia, India, and Africa, apply much less at 0.2 to 1 kg. a.i./Ha. Limited inputs for agricultural production results in generally lower yields, especially in developing countries.

Two examples describe the different trends. In Germany, the pesticides use per hectare decreased from 5.8 kg./ha to 3.0 kg./ha within the period of 1990 to 1995. In Taiwan, the overall pesticide consumption increased from 10,000 tons to nearly 50,000 tons within the period 1965 to 1995 (Chen, 1997).

Between 1960 and 1998 the world pesticide market increased by a factor of 3, from US$ 900 million to US$ 28 billion.

Ten top crops currently make up 65% of the total world market. There are important market potentials in orchards, vineyards, cereals, rice, horticulture, vegetables, rape, maize, soybeans and cotton.

Herbicides account for approximately 50% of the global crop protection market. The world insecticide market accounts for about US$ 6.8 billion, with 52% of this in the Asia-Pacific region. The most important crops in the insecticide markets are cotton, horticulture, vegetables, rice and fruits.

What are the costs/benefits from pesticides?

Pimentel (1997) made an assessment of the total estimated environmental and social costs from pesticides in the US. In general, each US$ 6.5 billion in pest control save approximately US$ 26 billion in crops, based on direct costs and benefits (Pimentel et al., 1991, 1997; USBC, 1994). With the use of IPM/ICM strategies, proper use of available pesticides as well as the development of new pesticides with improved qualities, it will be possible to optimize the benefits of crop protection products.

If mankind is forced to produce more food, feed and fibers, it is vitally important that crop plants should be protected against diseases, pests and weeds. The goal in reducing high losses is to enhance food security. It is estimated that, without crop protection, only half of what we produce would be actually available for harvesting. In other words, to achieve the same harvest volume without crop protection, it would be necessary, in theory, to double the agricultural acreage. The need for increased food production can only be met with the aid of agricultural inputs, including chemical crop protection products. The benefits of crop protection products by far outweigh their direct private and social costs, since high yields, sparing use of resources and environmentally benign technologies will not only secure our food supplies but will also maintain the biodiversity of our planet. Further conversion of forests into arable land can and must be avoided.

What is an ideal insecticide?

We focus on insecticides as an example. Today we have insecticides based on around 40 different chemical classes and more or less different modes of actions (Naumann, 1998).

The scientific challenge for a new one is described as follows:

An ideal insecticide should be highly researched, but low priced; superior to other products, but not more expensive; highly selective, but big in market size; with long life in the market, but without creating resistance problems; broadly active on pests, but does not leave crop and environmental residues; fast-acting on pests, but preferably not as a neuro-toxicant; mobile in plants, but immobile in soil.

Which are essential challenges to develop an ideal insecticide?

Combining 20 essential factors for an ideal insecticide, such as target spectrum, crop spectrum, selling price, turn over, market size, innovation, application, mode of action, mechanism of action, resistance, cross-resistance, mechanism of resistance, resurgence, selectivity, IPM/ICM-ability, acute toxicity (LD_{50}), fish toxicity, water solubility, registration costs and future perspectives, we can conclude, that there are excellent chances to improve the quality of new insecticides and to reach the level of an ideal insecticide. This is one of the research challenges for the chemical industries at the beginning of the next millennium.

How is AgrEvo realizing scientific innovations?

New chemicals

The R&D Strategy is focused on new technologies. An innovative technology in combinatorial chemistry was developed and used to rationalize the synthesis of many new molecules. Protocols and competence were developed to establish a high-through put screening in vivo Micro/Nano screening, where some milligrams of active ingredient are enough to test 100,000 to 1,000,000 compounds per year. Investments were made on new targets sites and new modes of action, leading to entirely new classes of molecules.

Forty percent of the R&D budget accounted for approximately 12% of sales invested in research and development of new chemicals, 30% in the maintenance of existing products, new formulations, re-registration and new indications, and 30% held in reserve for biotechnology.

Our goal is to launch two to three new active ingredients per year.

Nevertheless, as mentioned earlier, the new technology – biotechnology genetic engineering – will significantly contribute to crop protection and/or crop production in the future. Therefore, it is essential to reflect and to analyze the potential of both technologies, what is technically possible, in which time, at what price as well as what is socially acceptable.

Rice as an example (of low and high potential zone) will show some parameters, which are important for setting research targets to support sustainable agriculture, sustainable development, and finally sustainable socio-economy. Five areas are included: seeds, water irrigation, fertilizer, crop protection products and biotechnology.

Which plant production problem can be solved by using which technology?

Seeds: for higher yields, higher conversion rate of sun energy, better defense system of rice against pathogens; for rice plant/ insect pest interaction, plant herbivore interaction, better utilization of nutrients; for rainfed rice, better plant "architecture", tolerance of greenhouse gases (CO_2, CH_4, etc.), tolerance to temperature, and increase of feed value of secondary products.

Water irrigation: to avoid salinity and contamination, optimized use.

Fertilizer: to avoid methane production, optimized use.

Crop protection products: for improved pesticides, broad spectrum fungicide, broad but selective insecticide, combat-resistant baiting insects, systemic activity of insecticides, highly flexible "one shot" herbicides, development and implementation of IPM/ICM.

Biotechnology/Genetic engineering: for new qualities or properties, resistance against hoppers, stem borers and weevils, resistance against virus diseases, resistance against fungus diseases, minimizing use of nutrients, gene mapping, gene discovery -- useful for rice plants, rice plants with low CH4 transport potential, improved level of vitamins, improved level of iron, temperature tolerant or insentive rice plants, increase of the feed value of secondary products.

At the moment a wide spectrum of genetically optimized crops are grown in the world, especially in North and South America. It is forecasted that in the year 2003 nearly 60% of all grown crops are GMO-crops in North America (corn, soybean, cotton, potato, rape, sugar beet). Most of them are pest resistant or herbicide tolerant. The final result is a reduction in the number of pesticide applications and last but not least a significant reduction of chemicals.

Pesticides: Future trends

Nevertheless, a changing crop spectrum and a changing pest spectrum will need new pesticides. Therefore, continuous development will be important and essential. Pest control methodologies are expected to change substantially in developed and in developing countries, but pesticides will remain a major tool in the future.

A very good summary is given by Yudelman et al. (1998):

In developing countries, however, if current trends persist, chemical pesticides will continue to grow over the next 25 years. There will have to be substantial growth in food production to provide food security for their population. Production of pesticides within developing countries is also increasing, with both multinational and local manufacturers (India's and China's export will be more than US$ 500 million).

In developed countries the major manufacturers of pesticides are investing heavily in research to produce improved, pest-specific chemicals that are less harmful to human health and the environment and less likely to affect non-target species than the earlier broad spectrum pesticides.

Rates of pesticides use have dropped from 2 to 5 kilograms per hectare to 0.01 to 0.2 kilogram per hectare. The same is true for the number of applications using ICM. The industry is also moving towards improved delivery and application systems. Most companies are also diversifying to other pest management solutions, including IPM/ICM, precision farming and biotechnology.

It is the ultimate strategy of crop production companies to develop and implement new technologies down to farmer level. Companies such as AgrEvo, which offer farmers new solutions that make use of synergies between chemical

crop protection and biotechnology, will have an important competitive advantage. Companies providing all tools of integrated crop production systems will be in the pull position.

Yardstick for agricultural progress

At the end let's take a look at the yardstick of agricultural progress and AgrEvo's contributions. In recent history, let us begin with the discovery of photosynthesis by Jungenholz in 1779. From then on until the invention of the first photosynthetically active herbicide, Atrazine, in 1957, 178 years have passed. Between the first discovery of the cell nucleus by Brown in 1833 and the establishment of the first seed company in 1926, 93 years have passed. The innovation concerning fertilizers made by Liebig in 1840 was intensively used after World War II. Between the first report on chemical pest control of the Colorado Potato Beetle in 1877 and the synthesis of the first insecticide in the year 1939 by Muller, there was a span of 62 years, The time span between the genetically engineered organism in 1980 and the first commercial introduction of a genetically modified plant, the insect resistant Bt-cotton, was a mere 46 years.

These simple examples suggest that changes are not progressing in a linear way, but in a positive, non-linear acceleration. Fortunately, the increasing speed of implementing agro technologies is in line with the exponential growth of the world food demand and safeguards world food security, today and in the future. Within this context we have an optimistic vision of what the future can be.

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COMMUTATIVE CENTRALIZER ALGEBRAS AND RELATED STRUCTURES[1]

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ABSTRACT

Let $M(x)$ be a completely reducible matrix representation of a group G over a field K. The set $C(M)$ of matrices T over K such that $TM(x) = M(x)T$ for all $x \in G$ forms an algebra over K called the centralizer algebra of M. In this paper we investigate the centralizer algebras of permutation representations. We prove a sufficiency condition for the commutativity of centralizer algebras of semi-direct products and also obtain several commutativity results coming from some classes of finite groups using character theory and other techniques. Finally we show how these algebras arise in various contexts and discuss some related structures.

Keywords: *representation, centralizer algeba, Hecke algebra, group algebra, Bose-Mesner algebra, permutation character, symmetric group.*

1. INTRODUCTION

One of the most fundamental results in representation theory is Schur's Lemma which says that if $M(x)$ is an irreducible matrix representation of a group G over an algebraically closed field, then the only matrices which commute with all the matrices $M(x), x \in G$, are the scalar mutiples of the unit matrix.

It is interesting to find that the converse is also true, that is, if the only matrices that commute with $M(x)$ are the scalar multiples of the unit matrix, then $M(x)$ is irreducible. This result is part of a more general situation.

Let $M(x)$ be a completely reducible matrix representaion of a group G over

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a field K. The set $C(M)$ of all matrices T over K satisfying $TM(x) = M(x)T$ for all $x \in G$ forms an algebra over K, called the *centralizer algebra* of M.

We recall that an algrebra is a vector space which also an (associative) ring in the usual way. It is a subalgebra of the complete matrix algebra $\mathcal{M}_m(K)$, where m is the degree of M. It is not difficult to prove that if A and B are equivalent representations, then their corresponding centralizer algebras are isomorphic.

In this paper we investigate centralizer algebras of transitive permutation representations and prove several commutativity results. Commutativity is a much desired condition that facilitates the investigation of the algebra and its representation theory. We shall consider a more general structure called a Hecke algebra and introduce the notion of a Gelfand pair. We conclude by discussing related structures arising from these algebras.

2. HECKE ALGEBRAS

In this section we shall consider a more general structure called a Hecke algebra, a particular case of which is our previously defined centralizer algebra. let us start with a brief historical background.

In 1937 E. Hecke introduced certain endomorphisms of the space of entire elliptic modular forms, now called Hecke operators (Hecke, 1937). About two decades later, G. Shimura defined an abstract Hecke algebra that gave an algebraic setting for different kinds of Hecke operators (Shimura, 1959).

Shimura's construction involved a group G and a subgroup H of G. Letting $H\backslash G/H = \{HgH : g \in G\}$ denote the set of (H, H)-double cosets of G, a product of two double cosets is defined as an integral linear combination of all double cosets. The abstract Hecke algrebra, $\mathcal{H}(G, H)$ is the free Z-module with $\{HgH\}$ as a basis. If H is a normal subgroup of G, $\mathcal{H}(G, H) \cong Z[G/H]$.

In the 1960s important work was done by mathematicians such as C.W. Curtis and N. Iwahori who considered Chevalley groups and various distinguished subgroups. They defined Hecke algebras in terms of generators and relations and used the notion of generic algebras. See (Curtis, Iwahori, Kiloyer, 1971) and (Curtis, Reiner, 1981). Currently there is much interest in cyclotomic Hecke algebras which treat complex reflection groups (Malle, 1998). The study of Hecke algebras continues to this day. The theory has a wide range of applications from number theory to representation theory as well as the structure theory of finite groups and other algebraic objects.

Today there are several closely related definitions of Hecke algebras, not all equivalent. We give a particularly simple one. Recall that an idempotent in an algebra $\mathcal{R}$ is a nonzero element $e \in \mathcal{R}$ such tha $e^2 = e$.

Definition 1. *(Hecke algebra)*

The subalgebra $e\mathcal{R}e = \{ere : r \in \mathcal{R}\}$ of $\mathcal{R}$ is called a *Hecke algebra.* The identity of $e\mathcal{R}e$ is the idempotent e.

Remarks.

1. Some authors refer to these algebras as *Iwahori-Hecke* algebras in honor of Iwahori, who did much of the pionering work in this area.
2. One point of the study of these algebras is that $e\mathcal{R}e$ is often easier to understand than the algebra $\mathcal{R}$.

Example 1.

Let K be a field and $M_n(K)$ be the algebra of $n \times n$ matrices, with $1 \leq m \leq n$. Let e be the $n \times n$ matrix whose i, j-entry is 1 if $1 \leq i = j \leq m$ and 0 otherwise. Then $eM_n(K)e$ consists of the matrices whose i, j-entry equals 0 unless both i and j lie between 1 and m. Hence, $eM_n(K)e \cong M_m(K)$.

To prepare for our next examples, we need to review the concept of group algebras. In this paper, we consider only finite groups.

Example 2.

Let $G = \{g_i : i \in I\}$ be any finite group, and R any commutative ring with unity. Let RG be the set of all formal sums $\Sigma_{i \in I} \alpha_i g_i$, $\alpha_i \in R$ and $g_i \in G$, where all but a finite number of the α_i are 0. We define equality of formal sums if and only if all coefficients are equal.

Next, addition in RG is defined as:

$$\sum_{i \in I} \alpha_i g_i + \sum_{i \in I} \beta_i g_i = \sum_{i \in I} (\alpha_i + \beta_i) g_i;$$

and multiplication by:

$$\left(\sum_{i \in I} \alpha_i g_i\right)\left(\sum_{i \in I} \beta_i g_i\right) = \sum_{i \in I} \sum_{g_j g_k = g_i} (\alpha_j \beta_k) g_i .$$

In the above product, we formally distribute the sum $\Sigma_{i \in I} \alpha_i g_i$ over the sum $\Sigma_{i \in I} \beta_i g_i$ and rename a term $\alpha_j g_j \beta_k g_k$ by $\alpha_j \beta_k g_i$, where $g_j g_k = g_i$.

Definition 2. *(group ring, group algebra)*

Under the operations above, $(RG, +, .)$ is a ring, called the *group ring* of G over R. If k is a field, the kG is called the *group algebra.*

We shall focus on group algebras kG, where k is a field of characteristic zero. In this case, scalar multiplication is difined by

$$\alpha\left(\sum_{i \in I} \alpha_i g_i\right) = \sum_{i \in I} \alpha \alpha_i g_i, \ \alpha \in k .$$

The formal sum $1 \cdot e$, $1 \in K, e \in G$ is the identity in kG. The set of formal sums $\{g^* = 1 \cdot g, g \in G\}$ is linearly independent and is a basis of kG over k. The map $g \rightarrow g^*$ is an isomorphism of G into kG, and we will identify G with its image under this isomorphism.

Suppose that H is a subgroup of G. The element $e = \frac{1}{|H|}\Sigma_{h\in H} h$ of the group algebra kG is clearly an idempotent. So that if $H = \{1\}$,then $ekGe = kG$ and if $H = G$, we have $ekGe = \{\alpha e : \alpha \in k\}$.

We can identify kG with the set of all k -valued functions on G, where

$$\sum_{g\in G} \alpha_g g \longrightarrow f: G \rightarrow k,$$

where the function f maps the group elements g to α_g. Under the correspondence, addition of functions in G is defined by

$$(f_1 + f_2)(g) = f_1(g) + f_2(g).$$

As for multiplication,

$$(\sum_{x\in G} \alpha_x x)\ (\sum_{y\in G} \beta_y y) = \sum_{z\in G}\ (\sum_{xy=z} \alpha_x \beta_y) z = \sum_{z\in G} \gamma_z z,$$

where

$$\gamma_z = \sum \alpha_x \beta_{x^{-1}z}.$$

So, multiplication of functions is defined by convolution, i.e. $f_1 f_2 = f_3$, where

$$f_3(z) = \sum_{x\in G} f_1(x) f_2(x^{-1}z).$$

Under the above identification of kG with $\{f: G \rightarrow k\}$, we see that we can identify the Hecke algebra as follows:

$$ekGe = \{f: G \rightarrow k\} : f(h_1 g h_2) = f(g)\ \forall h_1, h_2 \in H\}.$$

Thus the Hecke algebra $ekGe$, where $e = \frac{1}{|H|}\Sigma_{h\in H} h$ is the subalgebra of k-valued functions that are constant on the (H, H)-double cosets of G.

Remark.

In the above construction, if one takes G to be a transitive permutation group and H a subgroup stabilizing a point, then the Hecke algebra $\mathcal{H}(G, H)$ is naturally isomorphic to the centralizer algebra of the permutation representation.

For our first results, we will use the following proposition proved in (Balmaceda, 1996).

Recall that an anti-automorphism of a group G is a bijection ϕ from G onto itself with $\phi(g_1g_2) = \phi(g_2)\phi(g_1)$ for all $g_1, g_2 \in G$.

Proposition 1. (Balmaceda, 1996)

Let G be a finite group and $H \leq G$. Suppose there exists an anti-automorphism σ of G satisfying $(HgH)^\sigma = HgH$ for all $g \in G$. Then $\mathcal{H}(G, H)$ is commutative.

W now use this result to obtain the next two theorems.

Theorem 2.

Let K be a finite group and put $G = K \times K$. Let $H = \{(a, a) | a \in K\}$. Then (G, H) is a Gelfand pair.

Proof. One can check that the map σ, where $(a, b)^\sigma := (b^{-1}, a^{-1})$ is an anti-automorphism of G fixing every double coset.

The above result can also be proved using character theory. However, the proof given above is probably the shortest. In the next application we prove a result on semi-direct products of groups. We recall its definition.

Definition 4. *Semi-direct Product*

Let two groups H and N be given as well as a homomorphism $\tau : H \rightarrow \mathrm{Aut}N$, $H \rightarrow \tau_h$. We define a product on $N \times H$ via

$$(n, h) \cdot (n_1, h_1) := (n\tau_h(n_1), hh_1).$$

Thus we obtain a group structure on $N \times H$, the semi-direct product of $N \times H$, which is denoted by $N \times_\tau H$.

Due to the embedding $N \rightarrow N \times_\tau H$, $n \rightarrow (n,e)$, we can regard N as a normal subgroup of $N \times_\tau H$. Moreover, H can be embedded into $N \times_\tau H$ via $H \rightarrow N \times_\tau H$, $h \rightarrow (e, h)$.

Theorem 3.

Let $G = N \times_\tau H$ be the semi-direct product of N and H. Then (G, H) is a Gelfand pair, whenever N is abelian.

Proof. From the definition of the product, each right coset relative to H contains a unique representative of the form (n, e), $n \in N$. A straightforward calculation shows that the double coset $H(n, e)H$, $n \in N$ is the disjoint union of the right costs $H(m, e)$, $m \in \{\tau_h(n) | h \in H\}$.

Suppose now that N is abelian and consider the map $\phi : G \rightarrow G$, where

$$(n, h) \rightarrow (\tau_h^{-1}(n), h^{-1}) = (n^{-1}, h)^{-1}.$$

Nontrivial examples are obtained by taking particular proper subgroups of G. See (Curtis, Iwahori, Kilmoyer, 1971) and its references. For instance, a deep result shows that if $G = GL(n, q)$, the group of all invertible $n \times n$ matrices over a finite field of order q and H is the subgroup of all lower triangular matrices, then $e\,kGe \cong kS_n$, the group algebra of the symmetric group of degree n.

We can generalize the above construction in the following way.

Example 3.

Let ψ be an irreducible character of a subgroup H of G. Then the element

$$e = \frac{\psi(1)}{|H|} \sum_{h \in H} \psi(h^{-1})\, h$$

is an idempotent. One can define the Hecke algebra $H = e\,kGe$. Our previous example is the special case where ψ is the trivial character.

This construction is important for the representaion theory G. We recall that every character X of G can be regarded as a character of kG. Also if X is an irreducible character of kG, the restriction of X to $\mathcal{H}$ is either zero or is an irreducible character of $\mathcal{H}$. Furthermore, every irreducible character of $\mathcal{H}$ is the restriction of some irreducible character of kG.

The reader may consult the book (Curtis, Reiner, 1981) for more details of the Hecke algebra and representation theory.

3. COMMUTATIVITY AND GELFAND PAIRS

In this section we consider the centralizer or Hecke algebra of k-valued functions on G that are constant on the (H, H)-double cosets of G defined in Example 2, and prove several results concerning commutativity of these algebras. As before let us denote these algebras by $\mathcal{H}(G, H)$. We have the following definition.

Definition 3. *Gelfand pair*

If $\mathcal{H}(G, H)$ is commutative, we call (G, H) a *Gelfand pair*.

The terminology comes from the work of (I.M. Gelfand, 1950) on Lie groups in the 1950s who proved that if G is a Lie group and H is a compact subgroup of G, the algebra of all integrable functions $\phi(g)$ satisfying the condition that $\phi(h_1 g h_2) = \phi(g)$ for almost all g and all $h_1, h_2 \in H$ is commutative. In this algebra, the product of two functions is given by convolution, i.e., $\phi = \phi_1 \times \phi_2$, where

$$\phi(g) = \int \phi(g g_1^{-1}) \phi_2(g_1) dg_1,$$

and addition is as usual.

Direct calculation shows that ϕ is an anti-automorphism of G satisfying $\phi(H) = H$ and $(H(n, e)\ H)^{\phi} = H(n, e)\ H$. An application of Proposition 1 completes the proof.

The above theorem can be applied to any semi-direct product. For example, one can take the dihedral groups D_n of order 2_n defined by

$$D_n = < a, b \mid a^n = b^2 = e, bab = a^{-1} >.$$

Then D_n is the semi-direct product of the cyclic groups generated by a and b. If one takes $H = < b > = \{e, b\}$, then using the above theorem, we see that $\mathcal{H}(D_n, H)$ is commutative.

In representation theory, the study of Hecke algebras arises in the investigation of induced representations and their irreducible decompositions. The following theorem is known (Curtis, Riener, 1981).

Proposition 5.

Let G be a transitive permutation group and H be the stabilizer in G of a point. then $\mathcal{H}(G, H)$ is commutative if and only if the permutation character is a sum of distinct irreducible characters.

If π is the permutation character of G, then $\pi(g)$ is the number of points fixed by the action of the element g of G. Furthermore if G is transitive, we know that $\pi = (1_H)^G$, the character of G induced from the trivial character of H. Hence to determine if (G, H) forms a Gelfand pair, we need to know the decomposition of $(1_H)^G$ into irreducible characters of G.

Out last results in this paper involve Gelfand pairs in wreath product subgroups of symmetric groups. We will use the approach using permutation characters. Before we state and prove the next theorem, we define the following. Let n be any even integer, say $n = 2k$. If $\lambda = (\lambda_1, \ldots, \lambda_r)$ is a partition of the integer k, then $(2\lambda_1, \ldots, 2\lambda_r)$ is a partition of n. We denote this partition of n by 2λ and call 2λ an even partition.

Consider the subgroup $S_2 \wr S^k$ of the symmetric group S_{2k}. Let

$$\pi_{2,k} = (1_{S2 \wr Sk})^{S2k}.$$

We will show that the irreducible constituents of the above permutation character are distinct. To do this we need a lemma.

Lemma 6.

$$(\pi_{2,k})_{S2k-1} = (\pi_{2,k-1})^{S2k-1}.$$

Proof. By Mckey's subgroup theorem (Curtis, Renier, 1981), the right side equals.

$$\sum_{t}(1_{S2\wr Sk)\,(S2k-1)t})^{S2k-1}.$$

the sum running over all representatives $(S_2 \wr S_k)tS_{2k-1}$ of double cosets. As $S_2 \wr S_k$ is transitive, there is exactly one such double coset, namely $(S_2 \wr S_k)\ S_{2k-1}$ and furthermore $S_2 \wr S_k \cap S_{2k-1} = S_2 \wr S_{k-1}$ since a one-point stabilizer will fix the block containing that point, and will act as $S_2 \wr S_{k-1}$ on the remaining 2k - 2 points. Thus we can proceed as follows.

$$(\pi_{2,k})_{S2k-1)} = (1_{S2\wr Sk-1)})^{S2k-1}.$$

By transitivity of induction,

$$(\pi_{2,k})_{S2k-1)} = ((1_{S2\wr Sk-1)})^{S2k-2})^{S2k-1}.$$

By definition of $\pi_{2,k-1}$, this gives the result

$$(\pi_{2,k})_{S2k-1)} = (\pi_{2,\,k-1)})^{S2k-1}. \square$$

Theorem 7. *If n is an integer, $n = 2k$, then the character $\pi_{2,k}$ decomposes as*

$$\pi_{2,k} = \sum_{\lambda|-k} \chi^{2\lambda},$$

where the sum runs over all partitions of the integer k.

Proof. We prove the theorem by induction on k. The case $k = 1$ is obvious. If $k > 1$, by the inductive hypothesis, $\pi_{2,k-1}$ is the sum of distinct irreducible characters of S_{2k-2} corresponding to even partitions of $2k - 2$, each occuring exactly once.

By the Branching Rule $(\pi_{2,\,k-1)})^{S2k-1}$ is the sum of all the irreducible characters of S_{2k-1} corresponding to partitions of $2k-1$ with exactly one odd part. So by the proceeding lemma, we get that $(\pi_{2,k})_{S2k-1)}$ is equal $\Sigma_\beta \chi^\beta$, where β runs through the partitions of $2k-1$ with exactly one odd part.

Another appliction of the Branching Rule shows that for these β, the following is true:

$$\sum_{\beta} \chi^{\beta} = \sum_{\lambda|-k} (\chi^{2\alpha})^{S2k-1}.$$

It therefore suffices to prove that $\pi_{2,k}$ cannot contain any connstituent χ^{γ} with odds parts γ_i. Since for each such γ, every constituents of $(\chi^{\alpha})_{S2k-1}$ has exactly one odd part, γ must be of the form $\gamma = (\gamma_1, \gamma_2)$, with γ_i odd. Hence it remains to show that no $\gamma = (\gamma_1, \gamma_2)$, γ_i odd, can be a constituent of $\pi_{2,k}$.

We notice that the induction hypothesis and the previous lemma yield that $\pi_{2,k}$ decomposes into distinct irreducible characters. Now $\chi^{(2k)}$ is a constituent of $\pi_{2,k}$ since $\pi_{2,k} = (1_{S2l Sk})^{S2k}$. We need to show that $\chi^{(2k-1,1)}$ cannot occur in the decomposition. As

$$(\chi^{(2k-1,1)})_{S2k-1} = \chi^{(2k-2,1)} + \chi^{(2k-1)},$$

the occurence of both $\chi^{(2k)}$ and $\chi^{(2k-1,1)}$ in $\pi_{2,k}$ would imply that $\chi^{(2k-1)}$occurs twice in $(\pi_{2,k-1})^{S2k-1}$ which is in fact a sum of distinct irreducibles. Thus, $\chi^{(2k)}$ is a contituent of $\pi_{2,k}$ while $\chi^{(2k-1,1)}$ is not.

In order to prove that $\chi^{(2k-2,2)}$ is a constituent of $\pi_{2,k}$ we consider $\chi^{(2k-2,1)}$, which is a constituent of $(\pi_{2,k-1})^{S2k-1}$. It satisfies

$$(\chi^{(2k-2,1)})^{S2k} = \chi^{(2k-1,1)} + \chi^{(2k-2,2)} + \chi^{(2k-2,1,1)}.$$

Hence $\chi^{(2k-2,1)}$ arises from the restriction of $+ \chi^{(2k-2,2)}$, for we have seen already that neither $\chi^{(2k-1,1)}$ nor $\chi^{(2k-2,1,1)}$ occur in $\pi_{2,k}$.

This suggests how we can proceed inductively. Let us assume that we have shown that $\chi^{(2k-2,l)}$, l even, is a constituent of $\pi_{2,k}$. We need to check that $\chi^{(2k-l-1,\, l+1)}$ does not occur, while $\chi^{(2k-l-2,\, l+2)}$ does. As

$$(\chi^{(2k-l-1,\, l+1)})_{S2k-1} = \chi^{(2k-l-2,\, l+1)} + \chi^{(2k-l-1,1)},$$

and

$$(\chi^{(2k-l,l)})_{S2k-1} = \chi^{(2k-l-1,\, l)} + \chi^{(2k-l,l-1)},$$

then $\chi^{(2k-l-1,\, l+1)}$ cannot occur, for otherwise, $(\pi_{2,k-1})^{S2k-1}$ would have repeated constituents. On the other hand, $\chi^{(2k-l-2,\, l+1)}$ is a contituent of $(\pi_{2,k-1})^{S2k-1}$ and

$$(\chi^{(2k-l-2,\, l+1)})^{S2k} = \chi^{(2k-l-1,\, l+1)} + \chi^{(2k-l-2,l+2)} + \chi^{(2k-l-2,l+1,1)},$$

so that $\chi^{(2k-l-2,\, l+1)}$ arises from the restriction of $\chi^{(2k-l-2,\, l+2)}$ which therefore must occur in $\pi_{2,k}$. This completes the proof.

Corollary 8.

$(S_{2k}, S_2 \wr S_k)$ *is a Gelfand pair.*

Remarks.

If we consider the analogous situation for the alternating group, we find that the pair $(A_{2k}, (S_2 \wr S_k) \cap A_{2k})$ does not always give a Gelfand pair. to see this, we first note that by Mackey's theorem and the preceding theorem, the corresponding permutation character is given by

$$(\pi_{2,k})_{A2k} = \sum_{\lambda|-k} (\chi^{2\lambda})^{A2k}.$$

If $k \equiv 0 (mod 4)$, say $k = 4n$ for some positive integer, n, then the partitions $(2n, 2n, 2n, 2n)$ and (4^{2n}) are both even partitions of $2k$ and are included in the sum $\Sigma\chi^{2\lambda}$. since these two partitions are associates of each other, they restrict to the same irreducible character of A_{2k}. Hence the irreducible constituents in the above decomposition are not distinct.

We remark that various other pairs of groups and subgroups can be investigated by the considering the decomposition of the permutation characters involved. Some of these results will be included in a later paper.

4. BOSE-MESNER ALGEBRAS

Let us consider the matrices in the centralizer algebra of a transitive permutation group G acting on a set of points X. The action of G on X induces an action on the cartesian product $X \times X$ via $(x, y)^g = (x^g, y^g)$ for $x, y \in X$ and $g, \in G$. *Let* $O_0, O_2, \ldots, O_d$ be the orbits of G on $X \times X$, where $O_0 = \{(x,x) | x \in X\}$. With each set O_i, we associate a matrix A_i whose rows and columns are indexed by the elements of X and whose (x, y)-entry is $(A_i)_{x,y} = 1$ if $(x, y) \in O_i$ and $(A_i)_{x,y} = 0$, otherwise. In particular, A_0, is the identity matrix of size $|X|$. We call the A_i the adjacency matrices. These matrices span the centralizer algebra.

One can verify that the matrices in the centralizer algebra C satisfy the following:

1. C contains the identity matrix I and matrix J with all entries equal to 1,
2. C is closed under the Hadamard or entry-wise product,
3. C is closed under ordinary matrix product, which is commutative when restricted to C, and
4. C is closed under transposition.

Using the language of association schemes (Bannai, Ito, 1984), the centralizer algebra above is the Bose-Mesner algebra of a commutative association scheme coming from a transitive permutation group. Association schemes are combinatorial objects that are obtained not only from permutation groups but also arise in the context of algebraic codes, designs and graphs. Each scheme on a finite set X gives rise to a subalgebra of the full matrix algebra $M(X)$ of complex-valued

matrices whose entries are indexed by X (the Bose-Mester algebra) that satisfies the above conditions.

Conversely, it is easy to show that any vector subspace C of $M(X)$ satisfying the four properties above is the Bose-Mesner algebra of some associations scheme on X. Thus any such subspace will be called a Bose-Mesner algebra on X. One of the motivations for studying these structures is their recently established connection with spin models from statistical mechanics that yield invariants of knots and linkes (Bannai, Bannai, Jaege, 1997.)

5. CONCLUSION

The centralizer algebra is an interesting algebraic object that is valuable in representation theory and other areas of mathematics. One may study this object from the point of view of Hecke algebras, which are more general structures. Determination of the structure of the algebra, that includes computing its dimension and finding a basis, is an important first step. A bigger goal involves the determination of the character table of commutative Hecke algebras. The process is equivalent to knowing lal the spherical functions on the coset space $H \setminus G$ that could be done in future work. This problem is the finite analogue of the problem of determining the spherical functions of a compact symmetric space.

Abstracting the main properties of the algebras leads to the notion of Bose-Mesner algebras, that historically originated from combinatorial structures called association schemes. Bose-Mesner algebras are also studied today in the construction of spin models from statistical mechanics whose partition functions yield invariants of knots and links.

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ON HIERARCHICAL CIRCULANT NETWORKS

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ABSTRACT

A fundamental consideration in the design of massively parallel and distributed computer systems is the topology of the processors (or the vertices, in graph theory). Well accepted designs are those that can be recursively decomposed, providing a way for the implementation of recursive algorithms. Hierarchical networks are recursively decomposable. In this paper, we examined the conditions for a network to be hierarchical. We limited our study to undirected and connected circulant networks.

Keywords: *Circulant networks; hierarchical; decomposition; Recursively decomposed; graph isomorphism; subgraph; edge-preserving; connected; undirected.*

1. INTRODUCTION

A fundamental consideration in the design of massively parallel and distributed computer systems is the topology of the processors or vertices. A successful topological structure must have an efficient communication scheme and programming paradigms that facilitate the design of algorithms [3].

Today, a large number of parallel machines are based on the hypercube or the binary cube interconnection network or topology because it admits optimal information dissemination schemes as well as accepts divide and conquer algorithms easily.

A number of other architectures are also well-accepted especially those that can be recursively decomposed, providing a way for the implementation of recursive algorithms. These network architectures are called hierarchical Cayley graphs. These include the k-ary n-cube, n-star and the n-pancake networks. These networks have been well-studied in the literature of parallel computer systems. According to Akers and Krishnamurthy (1984) hierarchical Cayley graphs are maxi-

mally fault tolerant. This means that the network is still connected even if one processories removed from the total number of processors adjacent to a processor.

In this paper we examined another type of hierarchical Cayley graph. This is called the hierarchical circulant networks. As of the present, only a few studies have been made on this type of hierarchical Cayley graph. In 1976, J.P. Hayes introduced the *ring-connected networks*, but if we take a closer look at the definition of *recursive circulants* by J. -H. Park and K. -Y. Chwa (1994), the ring-connected networks is a subclass of the recursive circulants. Another class of hierarchical circulant networks is the *recursive Paley graph* which was studied by this author in 1994 and formally introduced in 1996.

2. ON CIRCULAR NETWORKS

A *graph* is defined to be a pair (V, E) where V is the set of vertices and E the set of edges joining a pair of distinct vertices. A graph is *simple* and *undirected* if a pair of distinct vertices is joined by at most one edge and no edge joins the same vertex to itself. If two vertices υ_1 and υ_2 are adjacent then the symbol $\upsilon_1\upsilon_2$ (or $\upsilon_2\upsilon_1$) denotes the edge joining the two vertices. In this paper, we studied a class of graph called the circulant network.

Circulant network, (circulant graph or loop network) is an interesting network design which has attracted a number of research in interconnection networks.

Definition 1. *Undirected circulant network*

An *undirected circulant network* G of order n and degree $2t$ consists of vertices labeled from 0 to $n - 1$ such that each vertex v is adjacent to the vertices

$$\upsilon \pm s_1,\ \upsilon \pm s_2,\ \ldots, \upsilon \pm s_t$$

where the number $s_1 < s_2 < \ldots <$ st are distinct nonzero elements of Z_n and together with their negative counterparts are called the jump sizes of G. We denote this circulant network by $G(n; s_1, s_2, \ldots, s_t)$.

Example 1. *The circulant network* $G(24; 1, 2, 4)$

The circular network $G(24; 1, 2, 4)$ has 6 jump sizes: namely, ± 1, ± 2, and ± 4. Vertex 3 is adjacent to 6 vertices. These are:

1. 4, since $4 \equiv 3 + 1 \pmod{12}$;
2. 5, since $5 \equiv 3 + 2 \pmod{12}$;
3. 7, since $7 \equiv 3 + 4 \pmod{12}$;
4. 2, since $2 \equiv 3 - 1 \pmod{12}$;
5. 1, since $1 \equiv 3 - 2 \pmod{12}$; and
6. 11, since $-1 + 3 - 4 \pmod{12}$. Note that under *addition modulo* 12, the numbers -1 and 11 are equivalent.

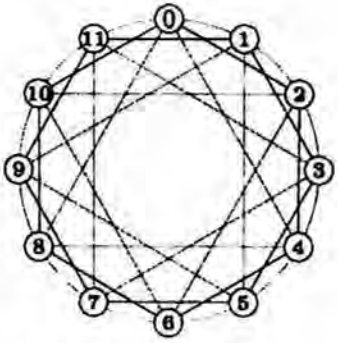


Figure 1. The Circulant Network $G(24; 1, 2, 4)$

3. ON SUBGRAPHS AND GRAPH ISOMORPHISM

Let us examine some of the properties of the graph which are important in this paper. In particular, let us define a subgraph of a graph, graph isomorphism, and hierarchical networks.

Definition 2. *Subgraph of a Graph*

A graph H is a *subgraph of a graph* G if the vertices of H are in G and the edges of H are in G.

Example 2. *A subgraph of* $G(24; 1, 2, 4)$.

The vertices 0, 2, 4, 6, 8, and 10 induced a subgraph of $G(24; 1, 2, 4)$ which we shall denote by $\Gamma(\langle 2 \rangle)$. The edges of this new graph are in $G(24; 1, 2, 4)$ where the jump sizes involved are ± 2 and ± 4.

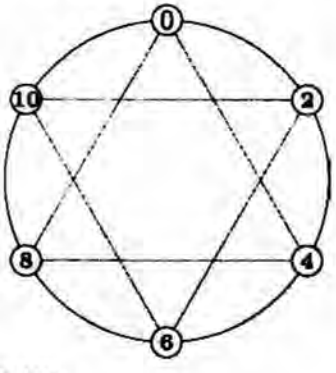


Figure 2. The subgraph $\Gamma(\langle 2 \rangle)$.

Definition 3. *Graph Isomorphism*

A graph G is isomorphic to another graph H if there exists an isomorphism ϕ from t he vertex set of G into the vertex set of H such t hat $\upsilon_1\upsilon_2$ is an edge in G if and only if $\phi(\upsilon_1)$ $\phi(\upsilon_2)$ is an edge in H. In order to show t hat two graphs are isomorphic, we have to find a one-to-one function and prove that this function preserves the edges (edge-preserving).

Example 3. *The graph* $G(6; 1, 2)$ *and* $\Gamma(\langle 2\rangle)$

Consider the graph $G(6; 1, 2)$ of order 6 and jump sizes ± 1 and ± 2 and the graph $\Gamma(\langle 2\rangle)$ as defined in the previous illustration.

The isomorphism ϕ. $V(G) \rightarrow V(H)$ where $V(G)$ is the vertex set of $G(6; 1, 2)$ and $V(H)$ is the vertex set of $\Gamma(\langle 2\rangle)$, is defined by

$$\phi(\upsilon) \equiv 2\cdot\upsilon \pmod{1}2 \qquad (1)$$

where the operation "$\cdot$" is the product taken under module 12.

The function is well-defined and a bijection.

- ϕ is one-to-one:

  $\phi(\upsilon_1) \equiv \phi(\upsilon_2) \pmod{12}$

  $2\cdot\upsilon_1 \equiv 2\cdot\upsilon_2 \pmod{12}$

  [redacted] $\upsilon_2 \pmod 6$

  Since $\upsilon_1, V_2 \in V(G)$, i.e. $0 \le \upsilon_1, \upsilon_2 \le 5$, it follows that $\upsilon_1 = \upsilon_2$. Hence, ϕ is one-to-one.

υ	$\phi(\upsilon)$
0	0
1	2
2	4
3	6
4	8
5	10

Table 1: Image under ϕ

- ϕ is onto:

  Let h be a vertex in $\Gamma(\langle 2\rangle)$. Then h is one of 0, 2, 4, 6, 8, 10. See Table 2.1. Hence, we can write $h = 2.h'$ where $h' \in \{0, 2, 3, 4, 5\}$. Take h'. Then $\phi(h') = 2\cdot h'$ (mod 12). Hence, ϕ is onto.

- ϕ is edge-preserving:

  Let $\upsilon_1, \upsilon_2 \in V(G)$.

  $\upsilon_1\upsilon_2 \in E(G)$ if and only $\upsilon_2 \equiv \upsilon_1 + s$ (mod 6) where $s = 1$ or $s = 2$. Thus, $\upsilon_2 = \upsilon + s + 6x$ for some integer x.

$$\begin{aligned}\phi(\upsilon_2) &\equiv 2\cdot\upsilon_2 \pmod{12}\\ &\equiv 2\cdot(\upsilon_1 + s + 6x) \pmod{12}\\ &\equiv 2\cdot\upsilon_1 + 2.s + 2\cdot 6x \pmod{12}\\ &\equiv 2\cdot\upsilon_1 + 2s + 12x \pmod{12}\\ &\equiv \phi(\upsilon_1) + 2s \pmod{12}\end{aligned}$$

Since 2s = 2 or 2s = 4, it follows that $\phi(\upsilon_1)\phi(\upsilon_2) \in E(H)$.
Now, if $\phi(\upsilon_1)\phi(\upsilon_2) \in E(H)$, then for s = 1 of s = 2,

$$\begin{aligned}\phi(\upsilon_2) &\equiv \phi(\upsilon_1) + 2s \pmod{12}\\ 2\cdot\upsilon_2 &\equiv 2\cdot\upsilon_1 + 2s \pmod{12}\\ \upsilon_2 &\equiv \upsilon_1 + s \bmod 6\end{aligned}$$

Since s = 1 or s = 2, it follows that $\upsilon_1\upsilon_2 \in E(G)$.
Hence, ϕ is edge-preserving.
Therefore, $G(6; 1, 2) \cong \Gamma(\langle 2\rangle)$.

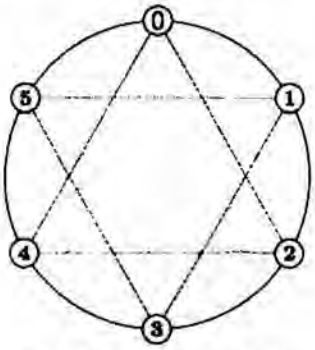


Figure 3(a): The graph G(6; 1, 2)

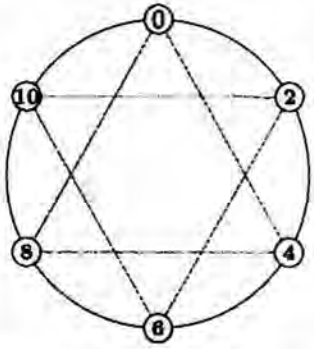


Figure 3(b): The graph $\Gamma(\langle 2\rangle)$

4. HIERARCHICAL CAYLEY GRAPH

Let G be a finite group with a set of generators S.

Definition 4. *Cayley Graph*

The Cayley Graph of G with respect to the generating set S is the graph with vertex set G, where g and gs are adjacent, for $g \in G$ and $s \in S$.

Lemma 1 *If S is closed under inverses then G is undirected or bidirectional.*

Proof. This is straightforward. If node a is adjacent to node b, where the direction is from a to b, then there exists $s \in S$ such that $b = as$. But, $bs^{-1} = a$. Since S is closed under inverses, $s^{-1} \in S$. Hence, node b is adjacent to node a. This gives the direction from b to a.

Therefore, the lemma follows.

Cayley graphs are popular in the design of parallel and distributed systems because of some of their properties, like vertex transitivity.

A circulant network is Cayley if the set of hops generate all the nodes of the circulant network. The configuration $G(12; 2, 4)$ is a circulant network but not Cayley since node 1 cannot be expressed as a linear combination of 2 and 4 under modulo 12. Also, this graph is not connected. See Fig. 4.

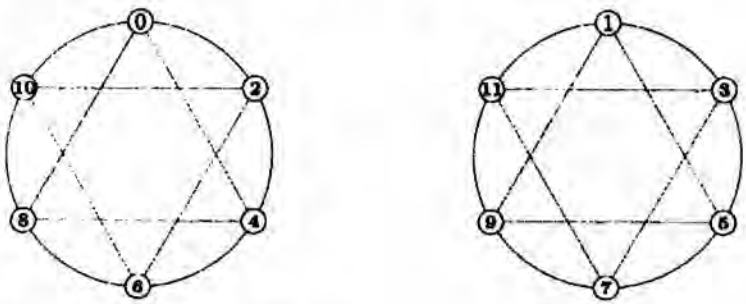


Figure 4. Circulant network $G(12; 2, 4)$

Let us have the following lemma to show the necessary and sufficient condition for a circulant network to be connected.

Lemma 2. *A circulant network is connected if and only if the greatest common divisor of* $a_1, a_2, \ldots, a_t$ *and* N *is equal to 1.*

Proof. Let us denote the greatest common divisor of a$a_1, a_2, \ldots, a_t$ and N by $gcd(a_1, a_2, \ldots, a_t, N)$.

Suppose that $gcd\ (a_1, a_2, \ldots, a_t, N) = 1$.

Then there exist integers $q_1, q_2, \ldots, q_t, r$ such that

$$a_1 q_1 + a_2 q_2 + \ldots + a_t q_t + Nr = 1.$$

This implies that $a_1 q_1 + a_2 q_2 + \ldots + a_t q_t + Nr \equiv 1 \pmod{N}$. Thus, node 1 and consequently all nodes can be reached from vertex 0. Hence, the circulant network is connected.

Suppose that $gcd\ (a_1, a_2, \ldots, a_t, N) = d > 1$. Then only nodes that are multiples of d are reachable from 0.

Therefore, the circulant network is not connected.

The following theorem that a connected circulant network is a Cayley graph.

Theorem 1. *A connected circulant network is Cayley.*

Proof. This is immediate from the previous lemma, since a node of a connected circulant network can be expressed as a linear combination of the different hops of the circulant network

Another property of a graph being considered is recursive decomposition which provides a way for the implementation of recursive algorithms (Berthome et al.) Hierarchical Cayley graphs can be recursively decomposed.

Definition 5. *Hierarchical Cayley Graph (Berthome et al.)*

A Cayley graph G is said to be *hierarchical* if it can be decomposed into a collection $\kappa(n)$ isomorphic subgraphs along with edges connecting them, where $\kappa(n) < | G |$. Each subgraph is a smaller Cayley graph from the same family as the original graph.

Among the hierarchical Cayley graphs are the k-ary n-cubes including the hypercubes, n-star graphs, recursive circulants and recursive Paley graphs.

Example 4. *The circulant network $G(12; 1, 2, 4)$ is hierarchical since we can decomposed it into a collection of 2 isomorphic subgraphs.*

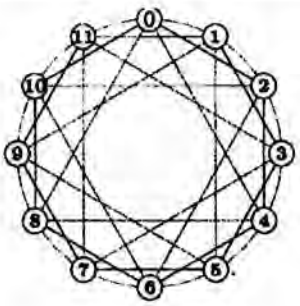
The subgraphs are:

1. $\Gamma(\langle 2 \rangle)$.
2. Induced subgraph of $\{1, 3, 5, 7, 9, 11\}$.

These subgraphs belong to the same family of $G(12; 1, 2, 4)$, the undirected and connected circulant networks.

Each of these subgraphs can be decomposed again into 3 subgraphs which are isomorphic to $G(3; 1)$. See Fig. 5.

The circulant network G(12; 1, 2, 4)



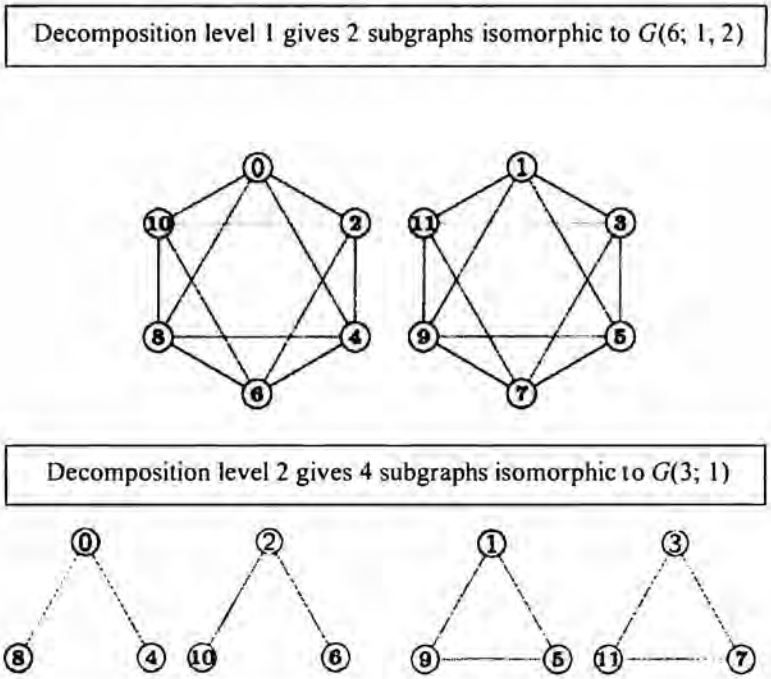


Figure 5. Recursive Decomposition of $G(12; 1, 2, 4)$

Definition 6. *Decomposition Level and Recursion Depth of the Hierarchical Cayley Graph*

We denote *decomposition level 1* as the first decomposition of the original graph into isomorphic Cayley subgraphs which belong to the same family of the original graph. If we can decompose these Cayley subgraph into isomorphic Cayley subgraphs belonging to the same family of the original graph, then this is *decomposition level 2*. If we can do this type of decomposition n times, then we reach *decomposition level n*. If after decomposition level *n*, we can not decompose the resulting subgraphs anymore, then the length of the decomposition is *n*.

The *recursion depth* of the hierarchical Cayley graph is the maximum length of all possible decomposition of the graph.

Example 5. *The recursion depth of* $G(12; 1, 2, 4)$

As shown in Fig. 5, the recursion depth of the hierarchical circulant network $G(12; 1, 2, 4)$ is 2.

Example 6. *The recursion depth of* $G(24; 1, 2, 3, 4, 6, 8)$

To find all the decompositions of the hierarchical circulant networks that give the maximum length, we consider all its maximal circulant subgraphs.

A *maximal circulant subgraph* of a circulant network G is a circulant subgraph of G which is not contained in any other circulant subgraph of G.

The two maximal circulant subgraphs of $G(24; 1, 2, 3, 4, 6, 8)$ are the subgraphs.

1. $\Gamma(\langle 2 \rangle)$ induced by $< 2 > = \{2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 0\}$. See Fig. 6(a); and
2. $\Gamma(\langle 3 \rangle)$. induced by $< 3 > = \{3, 6, 9, 12, 15, 18, 21, 0\}$ See Fig. 6(b)

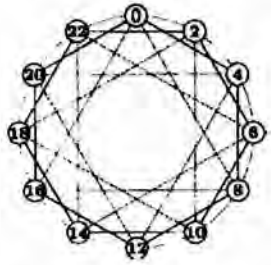


Figure 6(a). The Sugraph $\Gamma(\langle 2 \rangle)$

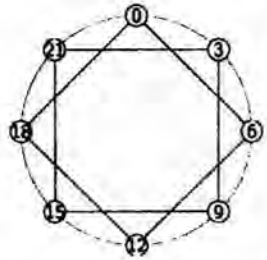


Figure 6(b). The Sugraph $\Gamma(\langle 3 \rangle)$

The subgraph $\Gamma(\langle 2 \rangle)$ is isomorphic to $G(12; 1, 2, 4)$ which his hierarchical of recursion depth equal to 2. Since we can decompose $G(24; 1, 2, 3, 4, 6, 8)$ into two isomorphic circulant subgraphs; namely.

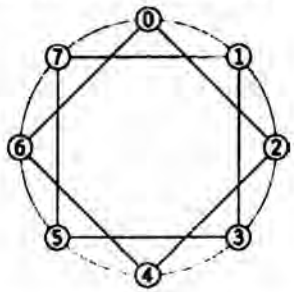
1. $\Gamma(\langle 2 \rangle)$ and
2. The subgraph induced by $\{1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23\}$, the length of this decomposition is 3.

The subgraph $\Gamma(\langle 3 \rangle)$ is isomorphic to $G(8; 1, 2)$ which can be decomposed into two isomorphic subgraphs; namely, the subgraph induced by $\{0, 2, 4, 6\}$ and the subgraph induced by $\{1, 3, 5, 7\}$. This induced subgraph is isomorphic to $G(4; 1)$, which is a cycle. The decomposition stops here. The length of this decomposition is 2. See Fig. 7

Since the maximum of the two lengths of decomposition is 3, it follows that the recursion depth of $G(24; 1, 2, 3, 4, 6, 8)$ is 3.

It is the aim of this paper to find the conditions for which undirected and connected circulant networks are hierarchical. The project is motivated by Akers and Krishnamurthy (1984) who showed that hierarchical Cayley graphs are *maximally fault tolerant.*

The circulant network G(8; 1, 2)



Decomposition level 1 gives 2 subgraphs isomorphic to $G(6; 1, 2)$

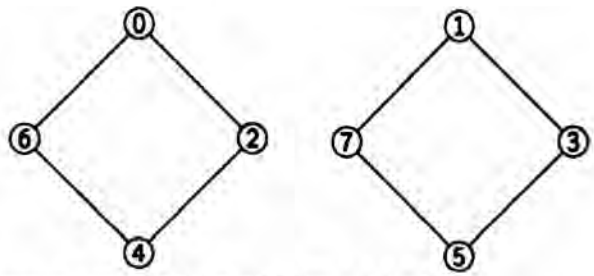


Figure 7. Recursive decomposition of G(24; 1,2,3,4,6,8)

5. CONDITIONS FOR HIERARCHY

Let us now discuss the conditions for an undirected and connected circulant graph to be hierarchical.

Theorem 2. *Let $n = ms^e$ for some positive integers m, e, s and s, $r > 1$. Then the circulant network $G(n; u, s^{t_1}, s^{t_2}, \ldots, s^{t_k})$ is hierarchical of recursion depth k where $gcd(u,n) = 1$, $t_1 < t_2 < \ldots < t_k \leq e$ and $t_{j+1} - t_j = d$ for $j = 1, 2, \ldots, k-1$.*

Proof. Let $G = G(n; u, s^{t_1}, s^{t_2}, \ldots, s^{t_k})$ and let $S(i,j) = \{j, s^{t_i} + j, 2s^{t_i} + j, \ldots, (ms^{e-t_i} - 1)s^{t_i} + j\}$ *or* $i = 1, 2, \ldots, k$ and $j = 0, 1, \ldots, s^{t_1} - 1$.

If $gcd(n,u) = 1$, then $gcd(n,u, s^{t_1}, s^{t_2}, \ldots, s^{t_k}$. Thus, G is connected.

Since $t_{j+1} - t_j = d$,

$$t_{i+r} - t_i = (t_{i+r} - t_{i+r-1}) + (t_{i+r-1} - t_{i+r-2}) + \ldots + (t_{i+2} - t_{i+1}) + (t_{i+1} - t_i)$$

$$= \overbrace{d + d + \ldots + d + d}^{r \text{ terms}}$$

$$= rd$$

Let us consider the largest possile value of j which is $s^{t_i} - 1$. Then the largest possible element of $S(i, j)$ where j is the maximum is

$$\begin{aligned}(ms^{e-t_i} - 1)\, s^{t_i} + s^{t_i} - 1 &= (ms^{e-t_i} - 1)\, s^{t_i} - 1 \\ &= ms^e - s^{t_i} + s^{t_i} - 1 \\ &= ms^e - 1\end{aligned}$$

Since $j < s^{t_i} + j < 2s^{t_i} + j < \ldots < (ms^e - s^{t_i} + j \leq ms^e - 1$, the addition and multiplication are taken under the entire set of integers and not under modulo n.

Claim 1: For fixed i and j, $|S(i,j)| = ms^{(k-i)d}$.

This is clear from the definition of $S(i, j)$.

Claim 2: For fixed i and j, the elements in $S(i, j)$ are distinct.

Suppose that $as^{t_i} + j = bs^{t_i} + j$. Then

$$\begin{aligned}as^{t_i} + j &= bs^{t_i} + j \\ as^{t_i} &= bs^{t_i} \\ a &= b.\end{aligned}$$

Therefore, for fixed i and j, the elements in $S(i, j)$ are distinct.

Claim 3: For each fixed i, $S(i, j_1, \cap S(i, j_2) = \emptyset$

Suppose that $\upsilon \in S(i, j_1) \cap S(i, j_2)$. Then $\upsilon \equiv as^{t_i} + j_1 \pmod{n}$ and $\upsilon \equiv bs^{t_i} + j_2 \pmod{n}$.

Without loss of generality, let us assume that $b \geq a$. Then

$$\begin{aligned}as^{t_i} + j_1 &= bs^{t_i} + j_2 \\ j_1 - j_2 &= (b - a)s^{t_i}\end{aligned}$$

Since $b > a$, it follows that $j_1 > j_2$ and $j_2 - j_1$ is an integral multiple of s^{t_i} which implies that $j_2 - j_1 > s^{t_i}$. But $0 \geq j_1, j_2 < s^{t_i}$ implies that $j_1 - j_2 < s^{t_i}$. Hence, we have a contradiction. Thefore, $\upsilon \notin S(i, j_1) \cap S(i, j_2)$ for any υ, i.e. $S(i, j_1) \cap S(i, j_2) = \emptyset$.

Claim 4: For each fixed i, $S(i,j)$ partitions the vertex set of G into s^{t_i} partitions.

For each fixed i, we have

$$\begin{aligned}\sum_{j=0}^{s^{t_i}-1} |S(i, j)| &= \sum_{j=1}^{s^{t_i}} |S(i, j)| \\ &= \sum_{j=1}^{s^{t_i}} ms^{e-t_i} \\ &= s^{t_i} \cdot ms^{e-t_i} \\ &= ms^e \\ &= |V(G)|\end{aligned}$$

Thefore, from Claims 1, 2, 3 and 4, for each fixed i, $S(i, j)$ partitions the vertex set of G into s^{t_i} partitions where each partition contains ms^{e-t_i} elements.

Consider the subgraph induced by $S(i, j)$ where i and j are fixed. We denote this by $\Gamma(S(i, j))$.

Let $as^{t_i} + j$ and $bs^{t_i} + j$ be two vertices in $\Gamma(S(i, j))$ that are adjacent. Then for some positive integer $l \geq k$,

$$\begin{aligned} bs^{t_i} + j &\equiv (as^{t_i} + j) + s^{t_i} + j \pmod{n} \\ bs^{t_i} &\equiv as^{t_i} + s^{t_i} \pmod{n} \\ b &\equiv a + s^{t_l - t_i} \pmod{ms^{e-t_i}} \end{aligned}$$

Hence $t_l \geq t_i$. This implies that $l \geq i$. Since s^l is a jump size, it follows that $i \leq l \leq k$. Thereore, the jump sizes in $\Gamma(S(i, j))$ are $\pm s^{t_i}, \pm s^{t_i}, \ldots, \pm s^{t_k}$.

Claim 5: $G(ms^{(k-i)d}; 1, s^d, s2^d, \ldots, s^{(k-i)d}) \cong \Gamma(S(i, j))$ such that $\Gamma(S(i, j))$ is the subgraph induced by $S(i, j)$ where i and j are fixed.

Let $G(ms^{e-t_i}; 1, s^d, s2^d, \ldots, s^{(k-i)d})$.

Define a function ϕ: $V(G_i) \rightarrow V(\Gamma(S(i, j)))$ by

$$\upsilon = \upsilon s^{t_i} + j \qquad (2)$$

Since i and j are fixed, ϕ is well-defined

To show that ϕ is one-to-one:

Suppose that $\phi(\upsilon_1) = \phi(\upsilon_2)$ for $\upsilon_1, \upsilon_2 \in V(G_i)$. Then

$$\begin{aligned} \phi(\upsilon_1) &= \phi(\upsilon_2) \\ \upsilon_1 s^{t_i} + j &= \upsilon_2 s^{t_i} + j \\ \upsilon_1 s^{t_i} &= \upsilon_2 s^{t_i} \\ \upsilon_1 &= \upsilon_2 \end{aligned}$$

Hence, ϕ is one-to-one.

To show that ϕ is onto:

Let $w \in V(\Gamma(S(i, j)))$. Then $w = \upsilon s^{t_i} + j$. *Thus, take* $\upsilon \in V(G_i)$. Hence, ϕ is onto.

To show that ϕ is edge-preserving:

Let $\upsilon_1, \upsilon_2 \in V(G_i)$.

$\upsilon_1\upsilon_2 \in E(G_i) \Leftrightarrow \upsilon_2 \equiv \upsilon_1 + s^{rd} \pmod{ms^{e-t_i}}$ where $r = 0, 1, \ldots, k - i$

$\Leftrightarrow \upsilon_2 \equiv \upsilon_1 + s^{rd}\, xms^{e-t_i}$ for some integer x

$$\begin{aligned}
\phi(\upsilon_2) &= \upsilon_2 s^{ti} + j \\
&= (\upsilon_2 + s^{rd} + xms^{e-ti})\, s^{ti} + j \\
&= (\upsilon_1 s^{ti} + j) + s^{rd+ti} + xms^{e-ti+ti} \\
&= \phi(\upsilon_1) + s^{ti+r-ti+ti} + xms^e \\
&= \phi(\upsilon_1) + s^{ti+r} + xms^e \\
&\equiv \phi(\upsilon_1) + s^{ti+r} \pmod{ms^e} \\
&\equiv \phi(\upsilon_1) + s^{ti+r} \pmod{n}
\end{aligned}$$

Since $r = 0, 1, \ldots, k - i$, s^{ti+r} is a jump size of $\Gamma(S(i, j))$.

Hence, $\phi(\upsilon_1)\phi(\upsilon_2) \in V(\Gamma(S(i, j)))$.

Suppose that $\phi(\upsilon_1)\phi(\upsilon_2) \in E(\Gamma(S(i, j)))$.

Then for some $r = 0, 1, \ldots, k - i$,

$$\begin{aligned}
\phi(\upsilon_1) &\equiv \phi(\upsilon_1) + s^{ti+r} \pmod{n} \\
\upsilon_2 s^{ti} + j &\equiv (\upsilon_1 s^{ti} + j) + s^{ti+r} \pmod{n} \\
\upsilon_2 s^{ti} &\equiv \upsilon_1 s^{ti} + s^{ti+r} \pmod{ms^e} \\
\upsilon_2 &\equiv \upsilon_1 + s^{ti+r-ti} \pmod{ms^{e-ti}} \\
\upsilon_2 &\equiv \upsilon_1 + s^{rd} \pmod{ms^{e-ti}}
\end{aligned}$$

Since s^{rd}, for $r = 0, 1, \ldots, k - i$, is a jump size in G_i, it follows that $\upsilon_1\upsilon_2 \in E(G_i)$.

Hence, for a fixed i and j, $G_i \cong (\Gamma(S(i, j)))$.

This implies also that for a fixed i, $(\Gamma(S(i, j_1))) \cong (\Gamma(S(i, j_2)))$, for any pair $j_1, j_2 \in \{0, 1, \ldots, s^{ti} - 1\}$.

Thus, the circulant network $G(n; u, s^{t1}, s^{t2}, \ldots, s^{tk})$ can be decomposed into s^{ti} isomorphic subgraphs that are isomorphic to the circulant network $G(ms^{e-ti}; 1, s^d, s2^d, \ldots, s^{(k-i)d})$.

Since i varies from 1 to k, the decomposition length is k. Since there are $k + 1$ distinct jump sizes (up to magnitude), k is the maximum, and hence, the recursion depth of $G(n; u, s^{t1}, s^{t2}, \ldots, s^{tk})$ is k.

Therefore, $G(n; u, s^{t1}, s^{t2}, \ldots, s^{tk})$ is a hierarchical circulant network of recursion depth k.

Example 7. *The circulant network* $G(192; 1, 2, 8, 32)$

$$
\begin{array}{ccc}
 & & G(192;\,1,\,2,\,8,\,32) \\
 & & \downarrow \\
G(96;\,1,\,4,\,16) & \cong & \Gamma(\langle 2\rangle). \\
 & & \downarrow \\
G(24;\,1,\,4) & \cong & \Gamma(\langle 8\rangle). \\
 & & \downarrow \\
G(6;\,1) & \cong & \Gamma(\langle 16\rangle).
\end{array}
$$

where

$\Gamma(\langle 2\rangle)$ is the induced subgraph of $\{0, 2, 4, \ldots, 190\}$

$\Gamma(\langle 8\rangle)$ is the induced subgraph of $\{0, 8, 16, \ldots, 184\}$

$\Gamma(\langle 16\rangle)$ is the induced subgraph of $\{0, 32, 64, 96, 128, 160\}$

Since $\Gamma(\langle 2\rangle) \cong \Gamma(\{1, 3, 5, \ldots, 191\})$, we can decompose $G(192, 1, 2, 8, 32)$ into 2 circulant isomorphic subgraphs.

In the next level of decomposition, we have 8 isomorphic subgraphs induced by $(\{j, 8+j, 16+j, 184+j\}$ where $j = 0, 1, 2, 3, 4, 5, 6, 7$. These subgraphs are isomorphic to $G(24; 1, 4)$.

In the last level of decomposition, we have 32 isomorphic subgraphs induced by $\{j, 32+j, \ldots, 64+j, 96+j, 128+160+j\}$ where $j = 0, 1, 2, \ldots, 30, 31$.

The number of jump sizes of $G(192, 1, 2, 8, 32)$ (up to magnitude) is 4, hence 3 is the maximum of the length of all decompositions of $G(192, 1, 2, 8, 32)$.

Therefore, $G(192; 1, 2, 8, 32)$ is a hierarchical circulant network of recursion depth 3.

Let $n = ms^e$ for some positive integers m, e, s and $s, r > 1$.

Consider $G(n; u, ws^{t_1}, ws^{t_2}, \ldots, ws^{t_k})$ where $gcd(u, n) = 1$, $gcd(w, n) = 1$, $t_1 < t_2 < \ldots < t^k \leq e$ and $t_{j+1} - t_j = d$ for $j = 1, 2, \ldots, k-1$.

Theorem 3 *The circulant network* $G(ms^{e-t_i}; 1, s^d, s^{2d}, \ldots, s^{(k-i)d})$ *is isomorphic to a subgraph of* G.

Also, G *is a hierarchical circulant network of recursion depth* k.

Proof. Let $S(i, j) = \{j, ws^{t_i} + j, 2ws^{t_i} + j, \ldots, (ms^{e-t_i} - 1)ws^{t_i} + j\}$ for $i = 1, 2, \ldots, k$ and $j = 0, 1, \ldots, s^{t_i} - 1$. Using the proof in the previous theorem, we can easily show that for fixed i, the set $S(i, j)$ partitions $V(G)$ into ms^{e-t_i} subsets with equal number of elements.

Consider the induced subgraph $\Gamma S(i, j))$ for a fixed i and j. We will show that this isomorphic to the circulant network $G(ms^{e-ti}; 1, s^d, s^{2d}, \ldots, s^{(k-i)d})$ which we shall denote by G_i.

Define a function $\phi: V(G_i) \rightarrow V(\Gamma(S(i, j)))$ by

$$\upsilon \equiv \upsilon w s^{ti} + j \pmod{n}. \tag{3}$$

Since i and j are fixed and w is constant, ϕ is well-defined.

To show that ϕ is one-to-one:

Suppose that $\phi(\upsilon_1) = \phi(\upsilon_2)$ for $\upsilon_1, \upsilon_2 \in V(G_i)$. Then

$$\begin{aligned} \phi(\upsilon_1) &= \phi(\upsilon_2) \\ \upsilon_1 w s^{ti} + j &\equiv \upsilon_2 w s^{ti} + j \pmod{n} \\ \upsilon_1 w &\equiv \upsilon_2 w \pmod{ms^{e-ti}} \\ \upsilon_1 &\equiv \upsilon_2 \pmod{ms^{e-ti}} \text{ since } gcd(w, n) = 1. \end{aligned}$$

Since $0 \leq \upsilon_1, \upsilon_2 < ms^{e-ti}$, it follows that $\upsilon_1 = \upsilon_2$.

Hence, ϕ is one-to-one.

To show that ϕ is onto:

This is similar to the proof in the previous theorem.

To show that ϕ is edge-preserving:

Again the proof is similar to that of the previous theorem.

Hence, $G(ms^{e-ti}; 1, s^d, s^{2d}, \ldots, s^{(k-i)d}) \cong \Gamma(S(i, j))$.

Thus, we can decompose G into ms^{e-ti} isomorphic circulant subgraphs. Thus, G is hierarchical.

From the pevious theorem, $G(ms^{e-ti}; 1, s^d, s^{2d}, \ldots, s^{(k-i)d})$ can be decomposed recursively $k - 1$ times.

Therefore, the recursion depth of the hierarchical network G is k.

Example 8. *The circulant network* $G(320; 1, 6, 24, 96)$.

$$\begin{array}{ccc} & & G(G(320; 1, 6, 24, 96)) \\ & & \downarrow \\ G(160, 1, 4, 16) & \cong & \Gamma(\langle 6 \rangle) \\ & & \downarrow \\ G(40, 1, 4) & \cong & \Gamma(\langle 24 \rangle) \\ & & \downarrow \\ G(10, 1,) & \cong & \Gamma(\langle 96 \rangle) \end{array}$$

Like the previous example, we can easily show that $G(320, 1, 6, 24, 96)$ is a hierarchical circulant network of recursion depth 3.

Theorem 4. *$G(n; u, s_1, s_2, \ldots, s_k)$ is a hierarchical circulant network if the following conditions are satisfied:*

1. *$gcd(n, u) = 1$; and*
2. *$gcd(n, s_i) = r_i$ and $r_i | r_1$ where $i = 1, 2, \ldots, t$*

Proof. *Let $G' = G(m_1; s_1', s_2', \ldots, s_t')$ where $n = m_1 r_1$, $r_i = s_i' r_i$ for $i = 1,2,\ldots, t$.*

Consider the set $S(j) = \{j, s_1 + j, 2s_1 + j, \ldots, (m_1 - 1)s_1 + j\}$ for $j = 0,1,2,\ldots, r_1 - 1$.

Clearly, the elements of $S(j)$ are distinct and $S(j)$ for $j = 0,1,2,\ldots, r_1 - 1$ partitions $V(G)$, the vertex set of $G(n; u, s_1, s_2, \ldots, s_t)$.

Consider the induced subgraph $\Gamma(S(i, j))$. We show that $G' \cong \Gamma(S(i, j))$.

For a fixed j, define a function $\phi: V(G') \to V(\Gamma(S(i, j)))$ by

$$\upsilon \equiv \upsilon c_1 r_1 + j \pmod{n}. \tag{4}$$

where $c_1 r_1 = s_1$ and $gcd(c_1, m_1) = 1$.

Since j is fixed and r_1 is constant, ϕ is well-defined.

To show that ϕ is one-to-one:

Suppose that $\phi(\upsilon_1) = \phi(\upsilon_2)$ for $\upsilon_1, \upsilon_2 \in V(G')$. Then

$$\begin{aligned}
\phi(\upsilon_1) &= \phi(\upsilon_2) \\
\upsilon_1 c_1 r_i + j &\equiv \upsilon_2 c_1 r_i + j \pmod{n} \\
\upsilon_1 c_1 r_i &\equiv \upsilon_2 c_1 r_i \pmod{n} \\
\upsilon_1 c_1 &\equiv \upsilon_2 c_1 \pmod{m_1} \\
\upsilon_1 &\equiv \upsilon_2 \pmod{m_1} \text{ since } gcd(m_1, c_1) = 1.
\end{aligned}$$

Since $0 \le \upsilon_1, \upsilon_2 < m_1$, it follows that $\upsilon_1 = \upsilon_2$. Hence, ϕ is one-to-one.

To show that ϕ is onto: Since any $w \in V(\Gamma(S(i, j)))$, $w = \upsilon s_1 + j$.

Thus, we take $\upsilon \in V(G')$.

To show that is ϕ edge-preserving:

Let $\upsilon_1, \upsilon_2 \in V(G')$.

$$\begin{aligned}
\upsilon_1 \upsilon_2 \in V(G') &\Longleftrightarrow \upsilon_2 \equiv \upsilon_1 + s_k' \pmod{m_1} \\
&\Longleftrightarrow \upsilon_2 \equiv \upsilon_1 + s_k' \pmod{m_1} \\
&\Longleftrightarrow \upsilon_2 \equiv \upsilon_1 + s_k' + x m_1 \text{ for some integer } x
\end{aligned}$$

Thus,

$$\begin{aligned}
\phi(\upsilon_2) &: \upsilon_2 r_1 + j \\
&: (\upsilon_1 + s_k' + xm_1) + j \\
&: \upsilon_1 s_1 + s_k' + xm_1 r_1 + j \\
&: (\upsilon_1 r_1 + j) + r_k + xm_1 r_1 \\
&: \phi(\upsilon_1) + r_k + xm \\
&\equiv \phi(\upsilon_1) + r_k \pmod{n}
\end{aligned}$$

Since r_k is a jump size of G and since $\phi(\upsilon_1)$, $\phi(\upsilon_2) \in V(\Gamma(S(i, j)))$, it follows that $\phi(\upsilon_1)\phi(\upsilon_2) \in V(\Gamma(S(i, j)))$.

Suppose that $\phi(\upsilon_1)\phi(\upsilon_2) \in E(\Gamma(S(i, j)))$.

Then

$$\begin{aligned}
\phi(\upsilon_2) &- \phi(\upsilon_1) + r_k \pmod{n} \\
\upsilon_2 r_1 + j &- (\upsilon_1 r_1 + j) + r_k \pmod{n} \\
\upsilon_2 r_1 &- \upsilon_1 r_1 + r_k \pmod{m_1 r_1} \\
\upsilon_2 &\quad \upsilon_1 + r_k' \pmod{m_1}
\end{aligned}$$

Since $r_k' r_1 = r_k$, it follows that r_k' is a jump size of G'. Now, $\upsilon_1, \upsilon_2 \in V(G')$. Hence, $\upsilon_1\upsilon_2 \in V(G')$.

Hence, ϕ is edge-preserving.

This implies that $G' \cong (\Gamma(S(j))$.

Since $S(j)$ for $j = 0, 1, 2, \ldots, r_1 - 1$ partitions $V(G)$ and since $G' \cong (\Gamma(S(j))$, it follows that G is decomposed into r_1 isomorphic circulant subgraphs.

Thefore, $G(n; u, s_1, s_2, \ldots, s_t)$ is a hierarchical circulant network

Let S be the set of all jump sizes of the circulant network G(up to magnitude). Suppose that the order of G is n. We denote the circulant network G by $G(n; S)$.

Theorem 5 *Let $G(n; S)$ be a circulant network.*

Suppose that $s_{i1}, s_{i2}, \ldots, s_{it} \in S$. $gcd(n; s_{ij}) = r_{ij}$ and $r_{ij} | r_{i1}$, for $j = 1, 2, \ldots, t$. Then $G(n; S)$ is hierarchical.

Proof. *Let $m_{i1} r_{i1} = n$.* Consider the circulant network $G(m_{i1}; r_1, r_2, \ldots, r_t)$ *where $r_i r_{i1} = r_{ij}$ for $j = 1, 2, \ldots, t$.*

Let $S(l) = \{l, s_{i1} + l, 2s_{i1} + l, \ldots, (m_{i1} - 1)s_{i1} + l\}$ for $l = 0, 1, \ldots, s_{i1} - 1$.

Again, for fixed l, the elements of $S(l)$ are distinct and $S(l)$ partitions $V(G(n; S)$ for $l = 0, 1, \ldots, s_{i1} - 1$.

Consider the induced subgraph $\Gamma(S(l))$ of $S(l)$. Then two vertices ω_1, ω_2, are adjacent in $\Gamma(S(l)$ if $\omega_2 \cong \omega_1 + s \pmod{n}$, where s is a jump size in $G(n; S)$.

We shall show that $G(m_{i1}; r_1, r_2, \ldots, r_l) \cong \Gamma(S(l))$.

Fr fixed l, define a function ϕ: $V(G') \rightarrow V(\Gamma(S(l)))$ by

$$\upsilon = \upsilon c_1 r_{i1} + l \tag{5}$$

where $c_1 r_{i1} = s_{i1}$ and $gcd(m_{i1}, c_1) = 1$.

Since l is fixed and s_1 is constant ϕ is well-defined.

To show that ϕ is one-to-one, onto and edge-preserving:

Actually, the solution is similar to the previous theorem.

Hence, $G(m_{i1}; r_1, r_2, \ldots, r_l) \cong \Gamma(S(l))$.

Since $S(l)$ for $l = 0, 1, 2, \ldots, r_{i1} - 1$ partitions $V(G)$ and since $G' \cong \Gamma(S(j))$, it follows that $G(n; S)$ is composed into r_{i1} isomorphic circulant subgraphs.

Therefore, $G(n; S)$ is hierarchical.

The theorems we have proved do not exchaust all the possible conditions for circulant networks to be hierarchical. It is recommended that future research be conducted to complete the search for all the possible conditions when a circulant network is hierarchical.

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A NOVEL 8 KD SULFUR-RICH PROTEIN IN SOYBEAN (GLYCINE MAX) COTYLEDON: PURIFICATION AND GENE CLONING

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ABSTRACT

An 8 kD methionine-rich protein (MRP) (2D-1) was purified through a combination of selective enrichment of the albumin fraction through heparin-Sepharose affinity chromatography, 2D-SDS-PAGE and radiolabeling of methionine-containing proteins with [1-14C] iodoacetate. The MRP contains 8.6% methionine, 8.6% cysteine, 11.4% lysine and high amounts of glutamic and aspartic acids. The N-terminus sequence facilitated the synthesis of degenerate oligonucleotide primers for use in Polymerase Chain Reaction (PCR) to amplify cDNA fragments specifically coding for the gene. The PCR product was then used to screen a cDNA library and analyze the DNA sequence which confirmed the high sulfur amino content of 17.2% Southern analysis revealed the presence of two copies of the gene in the genome. This work lays down the groundwork for cloning of the gene which actually encodes the 8 kD MRP and a methionine poor 2.5 kD peptide.

Keywords: Methionine-rich proteins (MRP); soybean *(Glycine max);* Heparin-Sepharose chromatography; Polymerase Chain Reaction (PCR); N-terminus; cDNA library, Southern analysis

INTRODUCTION

Legumes have become important sources of proteins with the increase in the demand for high fiber, low cholesterol food. Their full utilization is however limited by the deficiency in the sulfur amino acids, methionine, an essential amino acid and cysteine, although a non-essential amino acid, imparts a sparing effect on the former. Soybean, the world's single largest cash crop in the US (Soya Bluebook, 1982) contains only 56% as much sulfur amino acids as the nutritionally complete Food and Agriculture (FAO) reference protein (Kelley, 1973).

Several studies have already been conducted on a number of plant crops with the objective of cloning a gene coding for a sulfur-rich protein. More often a heterologous gene was used to increase the methionine content. Unfortunately, none had been applied successfully to soybean or any legume. More disturbing is the result of a recent study indicating the allergenicity of the Brazil nut MRP gene cloned in soybean. We propose that increasing the biosynthesis of naturally occurring MRP found in very small amounts in soybean is the most feasible genetic engineering strategy to address the deficiency. An endogenous gene coming from soybean itself with a long history of non-allerginicity would be the most likely candidate for cloning. This work lays down the foundation to cloning the soybean MRP gene, thereby enhancing its expression and elucidating the possible biological role in the seed.

This paper presents the purification of a sulfur-rich protein from soybean mature cotyledon using a combination of affinity chromatography and two dimensional SDS-PAGE. The MRP was earlier identified as an 8 kD albumin (Revilleza et al., 1996). In addition, the N-terminal sequence of the protein was determined which made possible the synthesis of oligonucleotide which were used in a polymerase chain reaction (PCR) to amplify DNA fragments coding for the MRP. The PCR product was later used as probe in cDNA library screening and Southern analysis.

MRP DETECTION AND PURIFICATION

Methionine-rich proteins resolved electrophoretically and blotted on Immobilon membrane, were identified based on the selective tagging of methionine residues as reported by Kho and de Lumen (1987). MRPs were identified to be in the low molecular weight albumin fraction of mature soy coteledon. The focus then centered on this fraction which was subjected to a number of chromatographic separations. This resulted in the identification on of heparin-sepharose (affinity) chromatography which facilitated the enrichment of the 8 kD MRP prior to two-dimensional electrophoresis. The single 8 kD band on one dimensional SDS-PAGE was in fact a mixture of three 8 kD proteins where the major component was referred as 2D-1 (Fig. 1). The 8 kD MRP was submitted for N-terminal microsequencing.

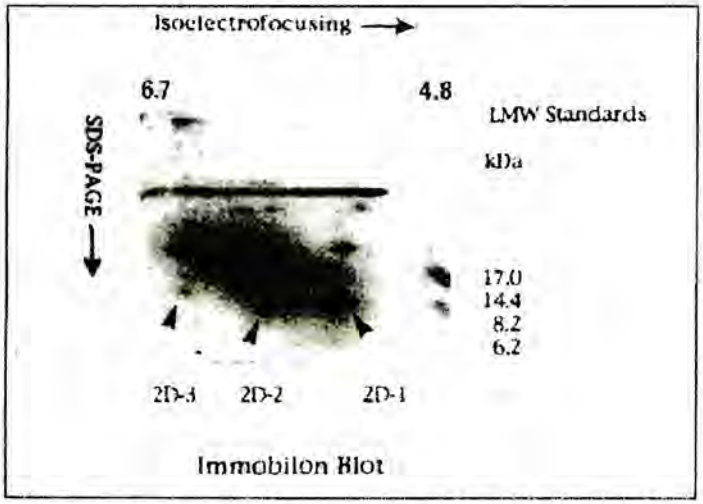

Figure. 1 2D-SDS-PAGE of soybean seed albumin methionine-rich protein enriched fraction eluted from heparin-sepharose affinity column. (Arrow points to the position of 2D-1, the major acidic protein).

cDNA LIBRARY SCREENING AND SEQUENCE ANALYSIS

Degenerate oligonucleotides deduced from the N-terminal sequence were synthesized and used as the gene specific primer for amplifying MRP gene encoding, with a mid maturation seed cDNA pool as template. PCR produced a truncated fragment which encoded the MRP. This was used as probe for Southern analysis and to fish out the cDNA clone harboring the MRP. The cDNA clone was sequenced and characterized (Galvez et al, 1997; de Lumen et al, 1999).

DISCUSSION

Purification of the MRP

The 8 kD MRP is distinct from the first MRP reported in soybean with a size of 10.8 kD and 12% methionine (George and de Lumen, 1991), although it is possible that they belong to a family of MRP. MRP has been reported in cereals and oil seeds such as Brazil nut, sunflower seed, corn and rice, but this is the second time only that a report of another MRP was found in legumes. The initial attempt to clone the 10.8 kD MRP was not successful due to the insufficient amount of protein for further analyses. In the absence of conserved regions from other known MRPs, gene cloning which encoded the soy MRP using heterologous sequences, was not feasible. Achieving the objective through the protein route, although more difficult, was a more logical solution.

Amino Acid and N-Terminal Sequence Analyses

The MRP (2D-1) contained high amounts of acidic amino acids aspartic and glutamic acids which then explained the acidic lpH on 2D-SDS-PAGE. The amino acid profile also yielded high levels of sulfur-rich proteins with 8.6% methionine and 8.6% cysteine. Lysine content was also high at 11.4% These represent the limiting amino acids in legumes and cereals respectively and are of great importance since MRPs from other sources were found to be limiting in lysine (Altenbach, 1987; Kortt et al., 1991). Table 1 gives the amino acid composition of 2D-1, defatted soy meal, Brazil nut MRP and FAO reference protein. If the Brazil nut

Table 1. Amino Acid Composition[a] of 8 kDa Soybean MRP,[b] Defatted Soymeal,[c] Brazil Nut MRP,[d] and FAO Reference Protein[e]

amino acid	8kDa-1D MRP	2D-1 MRP	Brazil nut MRP	defatted soymeal	FAO ref protein
methionine	7.7	8.6	18.8	1.4	3.5 (met + cys)
cysteine	5.0	1.5	7.9	1.3	
lysine	12.7	1.4	0.0	6.0	5.5
tryptophan	nd[f]	nd	0.0	1.2	
threonine	2.3	3.1	0.0	3.7	4.0
isoleucine	4.0	4.0	1.0	4.4	4.0
leucine	7.7	7.7	5.0	6.7	7.0
phenylalanine	1.6	0.3	0.0	4.5	6.0 (phe + tyr)
tyrosine	nd	nd	0.0	4.6	5.0
asparagine	nd	nd	2.0	nd	
aspartic acid	10.3	11.2	1.0	10.4	
serine	4.8	5.6	6.9	4.6	
glutamine	nd	nd	11.9	nd	
glutamic acid	28.8	35.1	14.9	18.4	
proline	2.9	2.8	5.9	5.3	
glycine	2.1	3.1	5.9	3.4	
alanine	2.0	2.1	1.0	3.6	
histidine	1.7	0.0	2.0	2.2	2.2
arginine	6.0	3.4	14.9	7.6	

[a]Values are in g/100 g of protein as determined by amino acid analysis. [b]Amino acid analysis was carried out on 8 kDa protein band on Immobilon membrane after 1D SDS-PAGE (8 kDa-1D) and on 2D-1 after 2D SDS-PAGE of fraction enriched for LMW proteins by heparin-Sepharose chromatography. [c]Rackis (1961). [d]Determined from cloned cDNA encoding mature protein, % of total (Altenbach and Simpson, 1990). [e]FAO/WHO Food and Agriculture Organization/World Health Organization of the United Nations (1973). f_{nd}, not detected.

MRP is to be overexpressed in a host plant, there is a possibility of the danger in causing an imbalance in essential amino acids. This is so since evidence showed that transgenic MRP increases at the expense of other proteins (Nordlee, 1994).

Protein microsequencing of the 8 kD MRP revealed the following 20 amino acid N-terminal sequence.

E G K D E D E E E E G H M Q K C A T E M

A database search using Blast showed that the N-terminal sequence yielded partial homology to glycinin in soybean and to a sucrose binding protein.

The middle N-terminal sequence of seven amino acids with the least codon degeneracy was used to design oligonucleotide primers for PCR amplification of the DNA coding for the 8 kD MRP.

PCR and Cloning of PCR Product

The PCR reactions using modified RACE protocols, yielded a 500 bp product which was cloned to GEM-T vector. The recombinant plasmid was used to transform an *E. coli* bacterial strain JM 109.

The deduced protein sequence of the 2D-1 PCR fragment which started from residue 9 of the N-terminus sequence, codes for an 8 kD protein containing 8.6 methionine and 8.6% cystein for a total sulfur content of 17.2% (Fig. 2). A stop codon was present after 211 bp. This cut short the open reading frame after 70 amino acids, leaving a large 3' untranslated region.

Southern Analysis and Library Screening

Southern hybridizations using 2D-1 PCR amplified product as probes revealed at least two bands in the autoradiograph. This implied that there could be at least two copies in the genome. Most of the genomic digests showed 2 restriction bands on samples digested with 6 base cutters and a single band on a 4 base cutter, Hinf I (Fig. 3).

A mid maturation seed cDNA library was probed with 2D-1 Ten positive clones were plaque purified after screening 6 x 10^5 cDNA clones

Characterization of the cDNA Clone Encoding the MRP

The cDNA encoding 2D-1, renamed Gm2S-1, has been completely sequenced and characterized (de Lumen, 1999). The number and position of the cysteine residues are conserved so they play a role in the formation of disulfide bridges, one of the determinants in the hierarchy of protein structure. Studies from MRP of other oilseeds suggested that the protein is synthesized as a large single-chain precursor which undergoes post-translational processing to yield 2 chain structures of the mature protein. In the case of Gm2S-1 cDNA, another low molecular weight protein (2.5 kD), was coded by the cDNA clone. Although this was

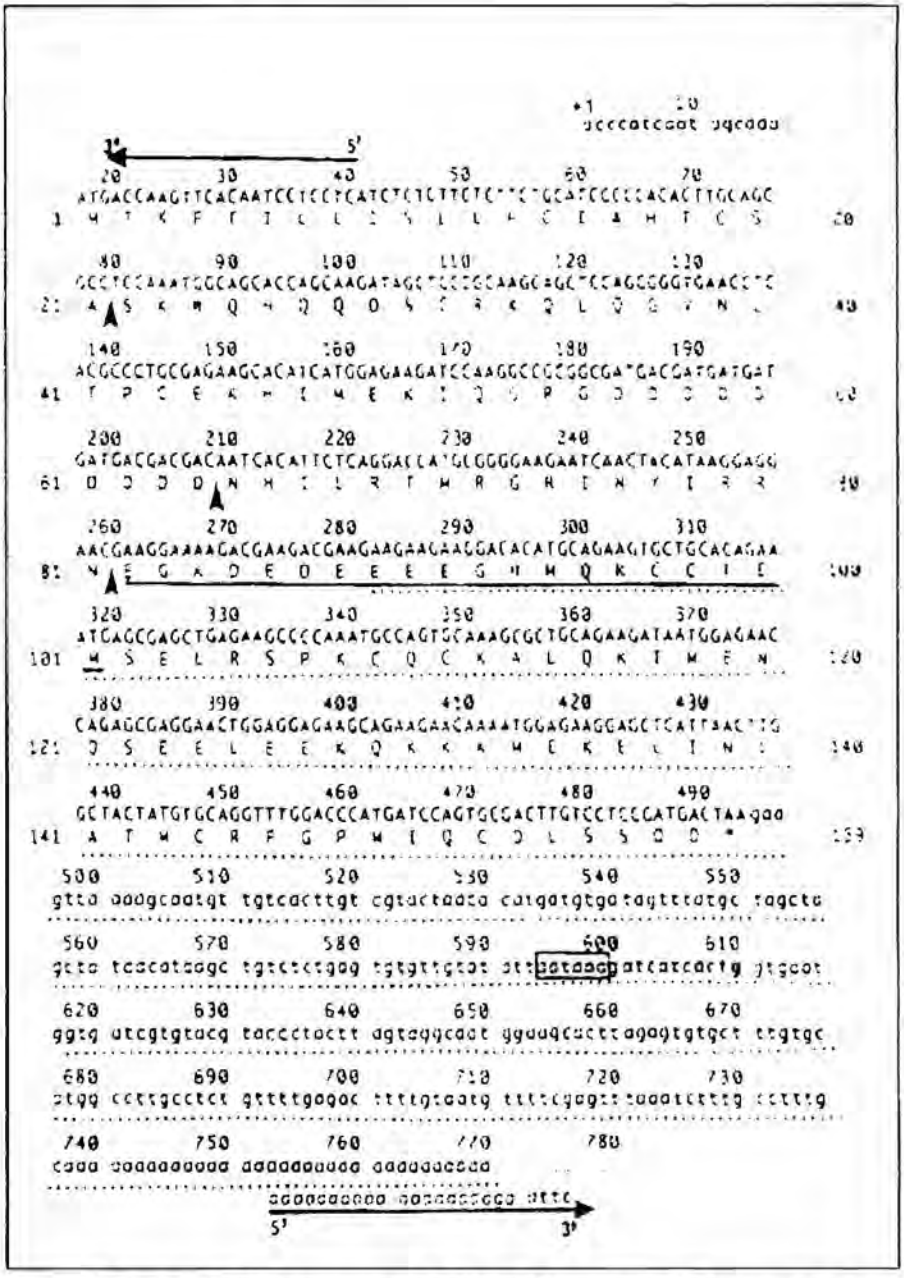

Figure. 2 Deduced DNA sequence of 2D-1 PCR generated fragment (Arrows flank coding region of 2D-1 which terminates after 211bb).

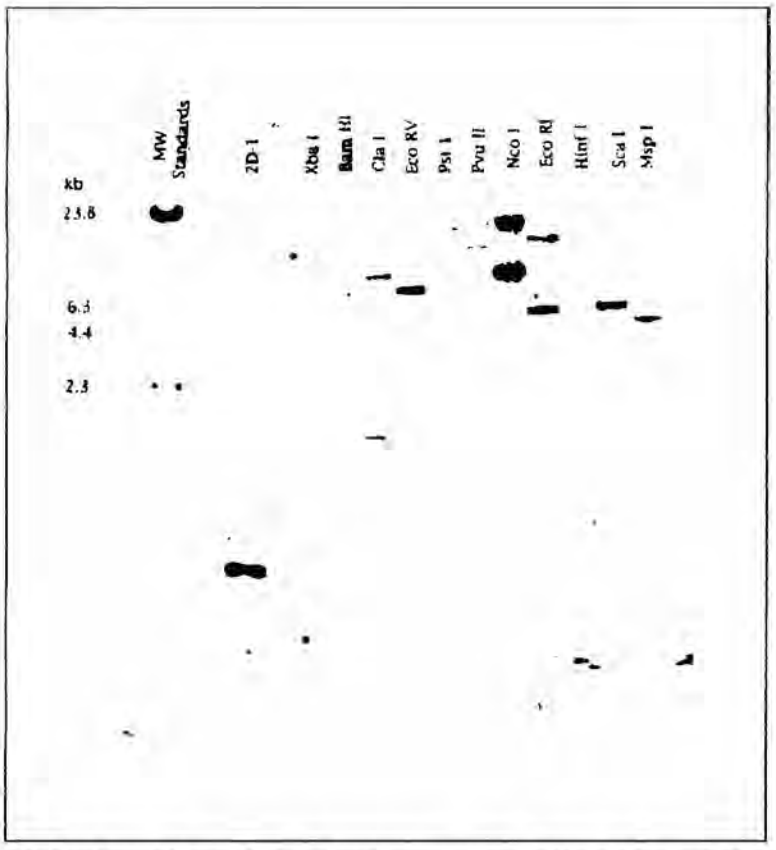

Figure. 3 Southern blot analysis of soybean genomic DNA with the PCR generated fragment 2D-1 as probe. (Ten micrograms of DNA was digested separately by 11 enzymes as labeled in each lane. The probe hybridized to at least two fragments in the genome as indicated by the number of bands the autoradiogram).

methionine- "poor", initial studies suggested that it possesses anti-mitotic property, a potential application in cancer research (Galvez and de Lumen, 1999).

The full length sequence of the 2.5 kb clone revealed a chimera of different transcripts, a glycinin subunit, a fragment homologous to a serine-rich protein from rice callus, a soybean oleosin gene, the complete coding region of Gm2S-1, and an 18 rRNA cDNA. The coding region of Gm2S-1 is within the 1.5 kb EcoR1-bAMH1 fragment between the oleosin fragment and the 18 S rNA cDNA (Fig. 4).

The open reading frame of Gm2S-1 encodes for 158 amino acids where 69 of the amino acids revealed by PCR generated a fragment located near the 3' end

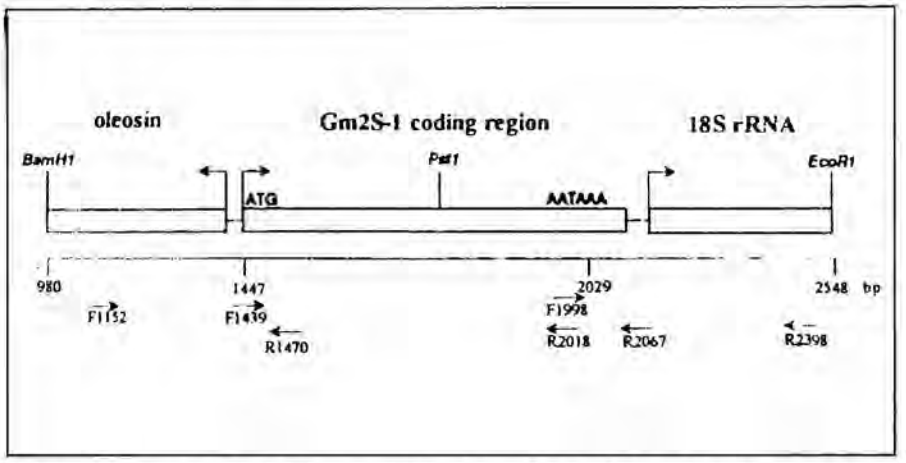

Figure. 4 BamH1 -EcoR1 Fragment containing the Gm2S-1 coding region

suggesting a post translational processing as verified by N-terminal sequence which did not start with a methionine residue. The preproprotein consists of a signal peptide (21 amino acids), a small subunit (43 amino acids), a linker polypeptide (17 amino acids) and a large subunit which is the 8 kD MRP (77 amino acids) (Fig. 5). The MRP contains 8% methionine, 13% lysine and 8% cysteine. The prepotein is highly hydrophilic with a predicted IpH of 5.6 while the mature MRP had an IpH of 4.9 (de Lumen et al, 1999).

Possible Biological Roles of Sulfur-Rich Proteins

The methionine and cysteine-rich proteins represent storage spaces for sulfur in the seed. During germination, the sulfur is mobilized and used not only for amino acid and protein structure, but also for the synthesis of cofactors and coenzymes. In addition the methionine is important in the synthesis of S-adenosyl methionine, a good alkylating agent and polyamines for DNA packaging (Lehninger et al., 1999).

Comparison of the 2D1-PCR DNA sequence with the Genbank data base showed 53% homology with a DNA binding protein from *Arabidopsis thaliana* (Khun et al., 1993) and 60% homology with a cysteine-rich protein from lupine (Gayler et al., 1990). Interestingly, the region of homology with the Gt-2 protein was in the acidic region near the carboxyl terminus. Acidic regions of many NA-binding proteins have been shown to function as transcriptional activators (Saha et al., 1993).

A survey of protein data bases on proteins with high levels of sulfur-rich proteins and enzymes with sulfur-rich motifs revealed some exciting results. Certain cysteine-rich proteins in animal cells have regulatory roles, including differ-

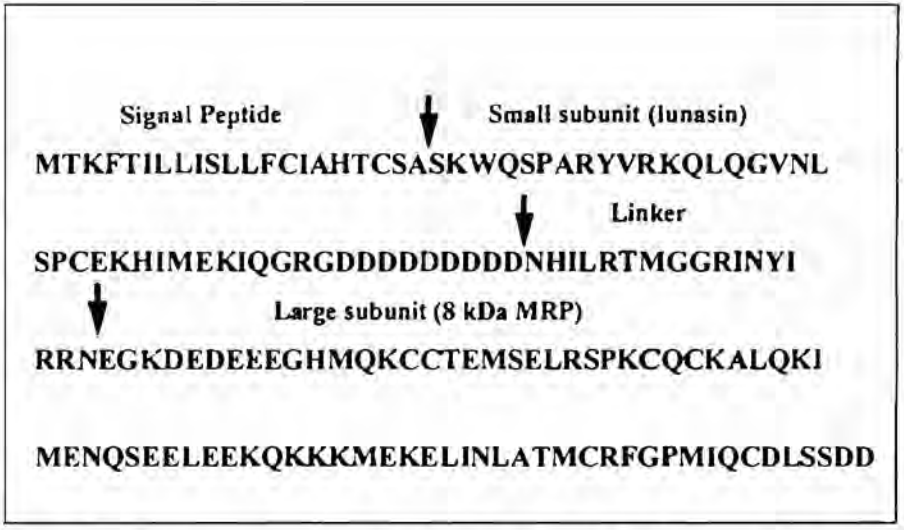

Figure 5. Complete protein encoded by the GM2S-1 cDNA clone. (The protein consists of a hydrophobic signal peptide, a small subunit (SSU) now called lunasin, a linker peptide and the large subunit (LSU) 8 kDa MRP. The arrows indicate proposed posttranslational proteolytic cleavate sites).

entiation, which makes one wonder if these plant protein counterparts play any regulatory role in seed development. Animal metallothioneins (MTs) are cysteine-rich, low-molecular-weight proteins that bind to heavy metals and are believe to play a role in metabolism and detoxification. Genes encoding MT-like proteins have been isolated in a number of plants although their function remains to be educated (Foley and Singh, 1994).

An important new family of proteins has recently been described which carries a novel cysteine-rich zinc-binding domain called the LIM domain. This protein family is present in mammals, amphibians, flies, worms and plants and its main function is in developmental regulation. Although a role in protein-protein interaction seems likely, intriguing similarities to GATA zinc fingers imply that are LIM domain may also be involved in binding to specific nucleic acids (Sanchez-Garcia and Rabbitts, 1994).

One role of MRP is suggested by the hypothesis that the conserved methionine-rich region of a nuclear protein from *Drosophila* and rat is proposed to play an important indirect role in its DNA-binding property as a transcription factor that controls terminal differentiation in *Drosophila* embryo (Weigel and Jackle, 1990). The 10.2 kD soybean protein (4.7% cysteine and 4.7% methionine) that binds to the regulatory sequence in leghemoglobin C^3 gene is proposed to have a regulatory role during soybean nodule differentiation (Bergmann et al, 1991). Interest-

ingly, the N-terminus sequence of one of the two methionine-cysteine-rich proteins identified in Acha, an African underutilized cereal is at least 46% homologous with 3 eukaryotic transcription factors (de Lumen et al, 1993). Two properties of the 8kD soybean albumin that makes it a transcript factor role: it is non-abundant, was detected early in seed development and maintained up to maturity. The length of the 3' untranslated region of the 2D-1 PCR product might be of significance concerning the stability of the transcript which explains its presence in the seed at a very low concentration, 3 μg/g. The long tail could have prevented the digestion of the coding region, thereby conferring stability. It is also possible that 2D-1 is not a typical seed storage protein and may have some biological function in seed development. In addition, 2D-1 may function as a trans-acting factor involved in activating the desiccation machinery in maturing seeds.

Since sources of the sulfur-rich proteins reported so far are oilseeds, it is possible that they play a role in lipid biosynthesis. It is noteworthy that the N-terminus sequence to each of the proteins isolated indicate have been post translationally processed.

SUMMARY AND CONCLUSION

With minimum number of steps, this study permitted the identification and purification of LMW sulfur-rich proteins from soybean albumin. These steps are central in the cloning and characterization of the gene encoding the MRP since there are no continuous conserved regions among reported MRPs from several sources. The use of heterologous probes for gene identification was therefore ruled out. Thus, the determination of the N-terminal sequence facilitated the design of primers for use in PCR which generated a truncated DNA fragment partially encoding the MRP. This served as probe for initial characterization studies and cDNA clone identification.

The genes encoding methionine-rich proteins (MRP) in seeds are candidates for overexpression to enhance the nutritional quality of legume proteins, which are relatively deficient in methionine. This is significant because of the findings in transgenic soybean. The transformation of soybean with Brazil nut MRP cDNA driven by a β-phaseolin promoter leads to the accumulation of the Brazil nut MRP up to 8% of the total protein, which is equivalent to a 26% increase in methionine content (Nordlee et al., 1994; Townsend et al., 1992). It remains to be seen if this increase is nutritionally significant. Using radioimmune allergosorbant test (RAST), the Brazil nut MRP in the transgenic soybean binds human IgE from sera of individuals allergic to Brazil nut whereas no binding is observed with comparable amounts of protein extracted from a genetically equivalent line of non-transformed soybean (Nordlee, 1994). Further, there are no extremely new cases of allergic responses to soy proteins inspite of their long history and widespread use as human food.

Overexpression of these sulfur-rich genes from soybean provides a viable means of increasing the methionine content of seed proteins in soybean and other legumems through genetic engineering, resulting in the enhancement of their nutritional and economic values as human food and animal feed, and in elucidating their biological roles.

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Research Note:

BIOLOGICALLY ACTIVE CONSTITUENTS FROM *MENTHA Cordifolia* OPIZ LEAVES

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ABSTRACT:

Isolates from yerba buena leaves were tested for their analgesic, antimutagenic, and anthelminthic activities. The acetic acid-induced writhing test showed that isolates β-sitosterol, β-sitosteryl-β-D-glucoside, and cis-8-pentadecenyllactone, at a dosage of 100 mg / kg mouse, each decreased the number of squirms induced by acetic acid by 70.0%, 73.0%, and 67.3%, respectively. β-sitosterol and an unsaturated carboxylic acid derivative are antimutagenic because they inhibited the mutagenicity of tetracycline by 65.3% and 68.7%, respectively, at a dosage of 0/01 mg / 20 g mouse, using the *in vivo* Micronucleus Test. *In vitro* tests using *Ascaris suum* showed that β-sitosterol is also anthelminthic as the behavior of worms approximate that of the positive controls, Combantrin and Antiox.

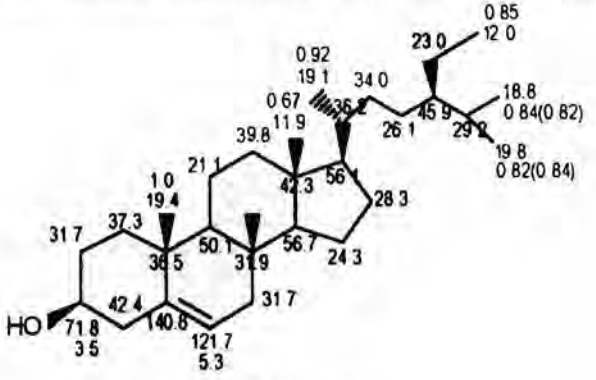
Keywords: *Mentha cordifolia* Opiz., yerba buena, analgesic, anthelminthic, antimutagenic, β-sitosterol, β-sitosteryl-β-D-glucoside

Mentha cordifolia Opiz., commonly known as Philippine mint, marshmint, or yerba buena, is listed as one of the priority plants under the Department of Science and Technology (DOST)-Philippine Council for Health Research and Development (PCHRD)- National Integrated Research Program on Medicinal Plants (NIRPROMP). The unextracted and unpurified leaves are presently being produced in tablet form, including pediatric tablets, and have been proven as an analgesic in clinical trials phases I, II, and III (DOST Technical Report Series No. 12, 1991).

This paper is on the bioassay-directed isolation and structure elucidation of the bioactive constituents from yerba buena leaves. The leaves were extracted by immersing them in methanol. The methanolic extract was then solvent-partitioned into hexane, $CHCl_3$, and EtOAc extracts. Subsequent bioassay using the acetic acid-induced writhing test showed that the hexane extract is analgesic. *In-vitro* tests using live *Ascaris suum* as test animals showed that the hexane extract is also anthelminthic. An *in-vivo* Micronucleus Test showed that the $CHCl_3$ extract is

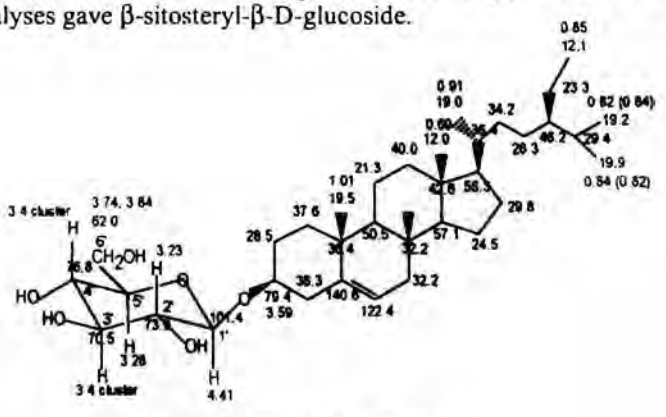
antimutagenic. The bioactive extract was then purified by sequential and repeated normal phase vacuum liquid chromatography using gradient elution. The structures of the bioactive isolates were elucidated by spectral analysis including gc-msd, ir, ^{1}H- and ^{13}C-nmr, COSY, HMQC, and HMBC.

The hexane fraction on sequential and repeated vacuum liquid chromatography (vlc)) over silica gel afforded the analgesic fractions labeled FB2 and FB10, eluted out at 20% EtOAc/ hexane and 30% EtOH/EtOAc, respectively. Vlc over silica gel of fraction FB2 yielded a greenish crystalline analgesic fraction which on recrystallization using ether/MeOH afforded white needle-like crystals 1 with an Rf 0.76, tlc (silica gel), EtOAc/hexane, 30:70, fuschia spot with vanillin-H_2SO_4. Spectral analyses gave β-sitosterol.



β-sitosterol

Fraction FB10 on vlc over silica gel 60G afforded 7 fractions with fraction FB10E, eluted from 8% MeOH/$CHCl_3$, exhibiting the highest analgesic activity. Vlc of FB10E yielded white crystals 2 upon recrystallization in ether/MeOH gave with an Rf 0.30, tlc (silica gel), MeOH/$CHCl_3$, 10:90, fuschia spot with vanillin-H_2SO_4. Spectral analyses gave β-sitosteryl-β-D-glucoside.



β-sitosteryl -β-D-glucoside

Confirmatory bioassay showed that β-sitosterol is analgesic, anthelminthic, and antimutagenic (Fig. 1, 2, 3). Its glucoside is also analgesic.

The analgesic cis-8-pentadecenyllactone is a white crystalline solid which degrades at 135.1°C. The antimutagenic unsaturated carboxylic acid is a yellowish crystalline solid which degrades at 210.8°C.

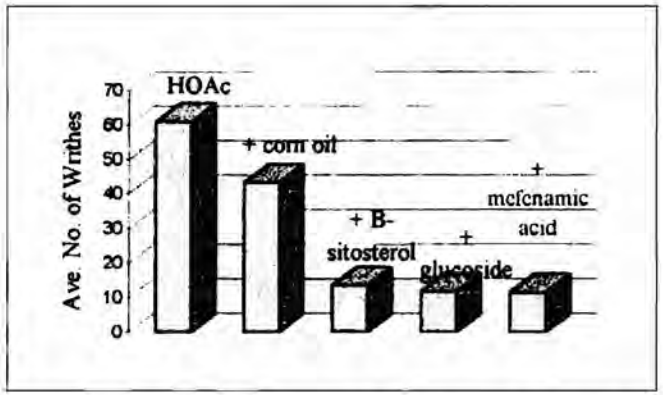


Figure 1. Analgesic activity using the acetic acid – induced writhing test

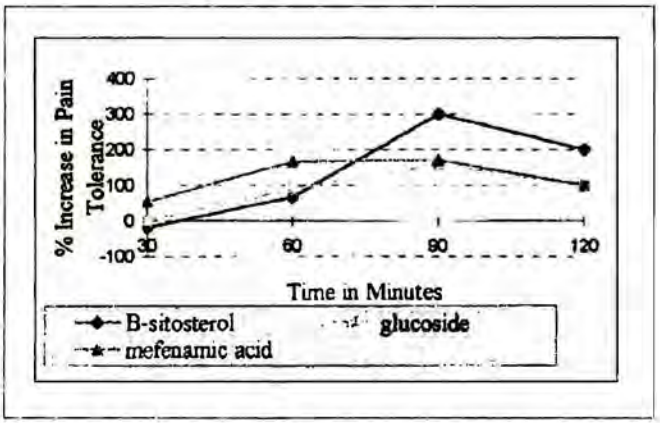


Figure 2. Analgesic activity using the hot plate method

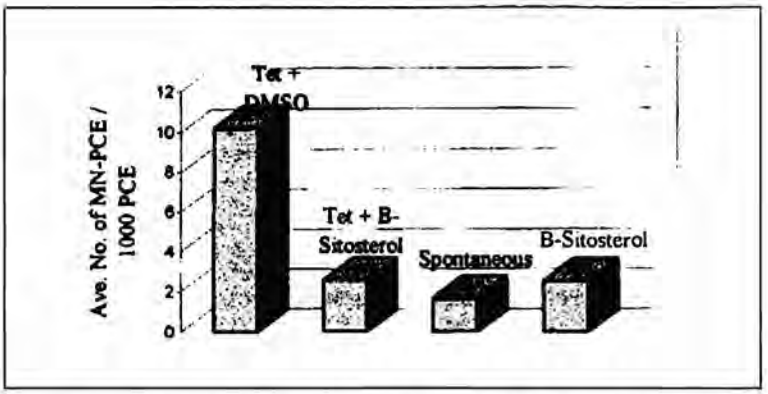


Figure 3. Antimutagenicity and mutagenicity using the micronucleus test

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METALS AND TOTAL ORGANIC LEVELS IN THE SAN MATEO LANDFILL LEACHATE AND ITS RECEIVING WATER SYSTEMS

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ABSTRACT

The San Mateo landfill has been discharging leachate into the Bosoboso River since it started operating in 1991. The effluent being discharged may potentially deteriorate the receiving water systems in the area. This study, which was conducted from August to December 1998, was aimed at assessing the impact of the San Mateo landfill on its receiving water systems as well as the receiving waters of the Bosoboso rivers using the following parameters: volume of discharge, conductivity, pH, turbidity, dissolved oxygen (DO), Chemical Oxygen Demand (COD), iron, copper and zinc in the landfill leachate.

The results of the study revealed the following. High conductivity readings were obtained in the landfill (highest value of 8.32 mS/cm) in comparison with other sites (0.291 mS/cm) indicating a high ion content in the leachate. The pH of all samples taken from the sites (pH range = 7.69 - 8.39) met the standard pH range of 6.00 - 9.00 for effluent waters as specified in the DENR Administrative Order 35. No significant differences in turbidity were observed among the sampling sites throughout the study. However, DO measurements showed very low values for the landfill site in comparison to the other sites, which is indicative of the presence of very high levels of organic matter. Very high values were observed for COD (3,910 ppm) in the landfill leachate. On the other hand, copper (0.252 ppm) and zinc (1.147 ppm) were found to be within the effluent limits.

The high COD level was found to be one major cause for the deteriorating water quality of the Bosoboso River. In the light of proposals to tap this river system as a source of drinking water for Metro Manila, these results highlight the need to implement a Water Quality Management Program to prevent further degradation of the river.

Keywords: San Mateo Landfill, Bosoboso River, Leachate, River Pollution, River Sampling, Chemical Oxygen Demand (COD), Iron (Fe), Copper (Cu), Zinc (Zn), Pollution load.

INTRODUCTION

The San Mateo Landfill (SML) was constructed in February 1991 within the 279.3-hectare Marikina Watershed, and presently covers a total surface area of about 106 hectares (Culibao, 1998). It currently receives an estimated 5,500 to 6,000 cubic meters of garbage daily from Metro Manila and four Rizal towns. Its leachate goes through a series of seven lagooning ponds that process its wastewater before being discharged onto a leachate stream which eventually merges with the Bosoboso River.

The Bosoboso River is connected to the Wawa River, which functioned as the primary source of Metro Manila's drinking water supply during 1909 - 1968. The Wawa Dam was closed by the Philippine Inter-Agency Committee in 1968 ue to structural instability (Ubac, 1997). After three decades of the Wawa Dam's closure, water shortage problems have risen sharply within Metro Manila; thus, proposals to reopen the Wawa Dam have been made (Ubac, 1997). However, concerns had been raised on the possible effect of landfill leachate on the quality of the water. Hence, an immediate need to closely monitor the water quality of the Bosoboso-Wawa River system arises. This was carried out in this study through the assessment of the following parameters: discharge and the physico-chemical parameters of temperature, conductivity, pH, turbidity, dissolved oxygen (DO), COD, Iron (Fe), Copper (Cu), and Zinc (Zn) in four selected sampling sites from August to December 1998 (Figure 1).

Figure 1. Topographical map of the study area. Letters indicate the sampling sites. Arrows show the direction of flow of the rivers and streams.

MATERIALS AND METHODS

Description of Sampling Sites

Site A: San Mateo Landfill (SML) -

The San Mateo Landfill is located on top of Mount Baytangan at an altitude of about 455 m above sea level. The linear distance from Site A to the Bosoboso River junction is about 1.1 km. However, the leachate stream takes the path of an arc to get to the junction; thus, the estimated distance to the junction along stream path is about 1.5 km.

Site B: Leachate Stream - 20 meters before merging with the Bosoboso River -

Site B is situated on an unnamed creek that receives the effluent discharge from the sanitary landfill. Its riverbanks were mostly vegetated with tall grass, and its substrate was composed of either soft mud, or mud with rocks.

Site C: 400 meters upstream of the Bosoboso River -

Site C had riverbanks with a muddy substrate and heavy vegetation.

Site D: 300 m downstream of the Bosoboso River -

The area surrounding Site D had varying physical characteristics along its river banks. The first 250 m stretch from the junction was muddy and had a lot of vegetation. However, the next 50 m span had very little vegetation and the substrate was mostly rock.

Sample Collection and Preservation

COD, Fe, Cu, and Zn were the major chemical parameters chosen in this study. Preliminary studies conducted revealed the presence of relatively high concentrations of organics. Furthermore, among the metals analyzed, detectable levels of Fe, Cu, and Zn were obtained from the samples of the San Mateo Landfill leachate.

Water samples for COD, Fe, Cu, and Zn analyses were obtained at mid-depth. Polyethylene bottles were used for storage. For COD analysis, two 500-ml bottles were used to make two replicates. These samples were then preserved by adding of concentrated sulfuric acid until the pH was below 2. For metal analysis, two 1000-ml replicates were collected per site. The samples were preserved by adding concentrated nitric acid until the pH was below 2.

Major Parameters

The COD levels for all samples were determined using the Open Reflux Method as described in *Standard Methods* (Eaton et al, 1995). However, a deviation from standard procedure was made for Site C whose COD values were all under 50 ppm. This was corrected by analyzing additional pairs of Site C samples spiked with a KHP equivalent of 10.0 mg O_2/L.

Iron (Fe), Copper (Cu), and Zinc (Zn) were analyzed using the Direct Air-Acetylene Flame Atomic Absorption Spectroscopy Method as described in *Standard Methods* (Eaton, Clesceri and Greenberg, 1995). However, Zn analysis entailed the use of a third degree calibration curve due to inadequate sample volume.

Minor Parameters and River Dynamics

Minor parameters such as temperature, conductivity, pH, turbidity, and DO were measured on site using the Horiba U-10 water quality checker. River dynamics involved determining of the water body's surface velocity, width and depth. Surface velocity was obtained through the use of a styrofoam ball float, a stopwatch, and a calibrated nylon string. Depth and width were measured using a calibrated pole and string.

Data Analysis

Discharge was calculated using the equation of Gordon, McMahon and Finlayson (1992) shown as follows:

$$Q = V_1A_1 + V_2A_2 + \ldots + V_nA_n$$

where: Q = discharge
V_i = velocity
A_i = area

Analysis of Variance (ANOVA, p = 0.95) was used to determine significant differences due to monthly and geographic variation. Pollution load (P), expressed as tons of O_2/day for COD, and as kg/day for metals, was computed using the following equation:

$$P = kQC$$

where: k = the unit conversion factor equivalent to 0.0864 for COD and 86.4 for metals
Q = discharge (m^3/s)
C = concentration (ppm).

The results were then subjected to Paired Sample t-Tests, p=0.95, (Pena-Muralla, 1995) to determine whether significant differences in pollution load levels exist between the final landfill leachate (Site A) and the water in the leachate

stream (Site B). Moreover, linear regression was performed to determine the pollution load contributions at Site B in cases where the alternative hypothesis was accepted. The same technique was also adopted to determine if there exists significant differences between the sites upstream (Site C) and downstream (Site D) of the Bosoboso River.

RESULTS AND DISCUSSION

Minor Parameters and River Dynamics

Sites C and D had significantly higher discharge values compared to Sites A and B. This finding was expected since Sites C and D were located along the Bosoboso River, while Sites A and B were situated at the outlet of the leachate pond and the leachate stream respectively. Values ranged from a minimum of 0.005 m^3/s during the months of September, November, and December at Site A, to a maximum of 7.74 m^3/s during December at Site C (Table 1).

As expected, the temperature in December was significantly lower than the other months as cool Siberian winds enter the country during this time. Values ranged from a minimum of 25.0°C at Site A during December, to a maximum of 31.6°C at Site D during August (Figure 2).

Significant differences in conductivity were observed between sampling sites. The values ranged from a minimum of 0.291 mS/cm in Site C during December to a maximum of 8.32 mS/cm at Site A of the same month (Figure 2). Data is indicative of high ion content within the landfill's wastewater (Site A) and the leachate stream (Site B), since the reference value of 0.05% sodium chloride is already 1.000 mS/cm.

pH on site ranged from a minimum of 7.69 during December at Site A, to a maximum of 8.39 at Site B of the same month (Figure 2). All samples passed the allowable pH range of 6.00 to 9.00 for effluent, and 6.5 to 8.5 for ambient waters as specified by Department of Environment and Natural Resources Administrative Order 35 and 34 (DAO 35 and 34 (1990)), respectively. The ANOVA results show that there exist significant pH variation for both month and geographic location. The month of October had pH levels significantly more acidic than September. However, samples taken in September were significantly more acidic

Table 1. Discharge values measured from August to December 1998 (m^3/s). Indicates the volume of water that is released per unit time.

	August	September	October	November	December
Site A	-	0.005	0.01	0.005	0.005
Site B	0.03	0.01	0.21	0.08	0.13
Site C	1.40	2.72	5.53	3.71	7.74
Site D	1.13	2.61	6.00	3.57	-

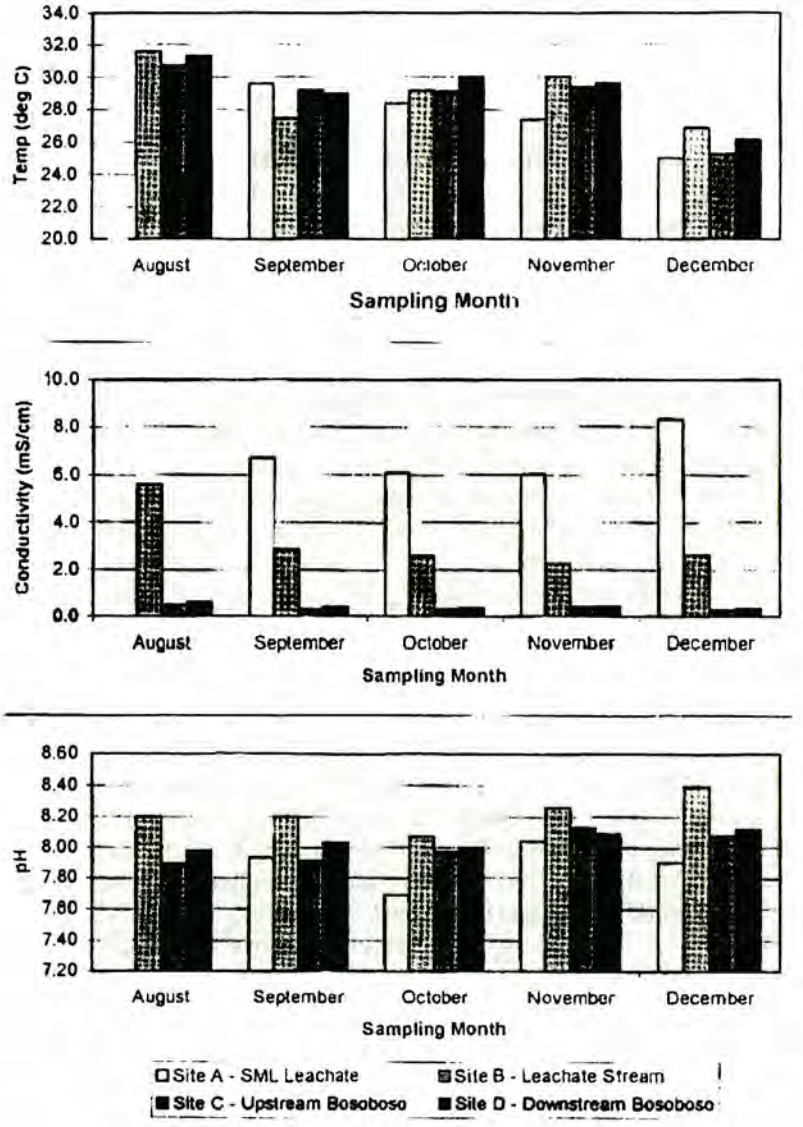

Figure 2. Temperature, conductivity, and pH variation from August to December 1998.

*no measurement available.

than those taken in November and December. All sampling sites had significantly different pH levels. The order of magnitude in decreasing acidity were as follows: Site A> C> D> B. Significantly low pH at Site A is indicative of anaerobic microbiological activity within the leachate ponds. This is because anaerobic decomposition of organic matter produces byproducts such as organic acids and carbon dioxide (Qasim and Chiang, 1994).

Turbidity on site ranged from a low of 26 NTU during November at Site D, to a high of 480 NTU during September at Site B (Figure 3). No significant

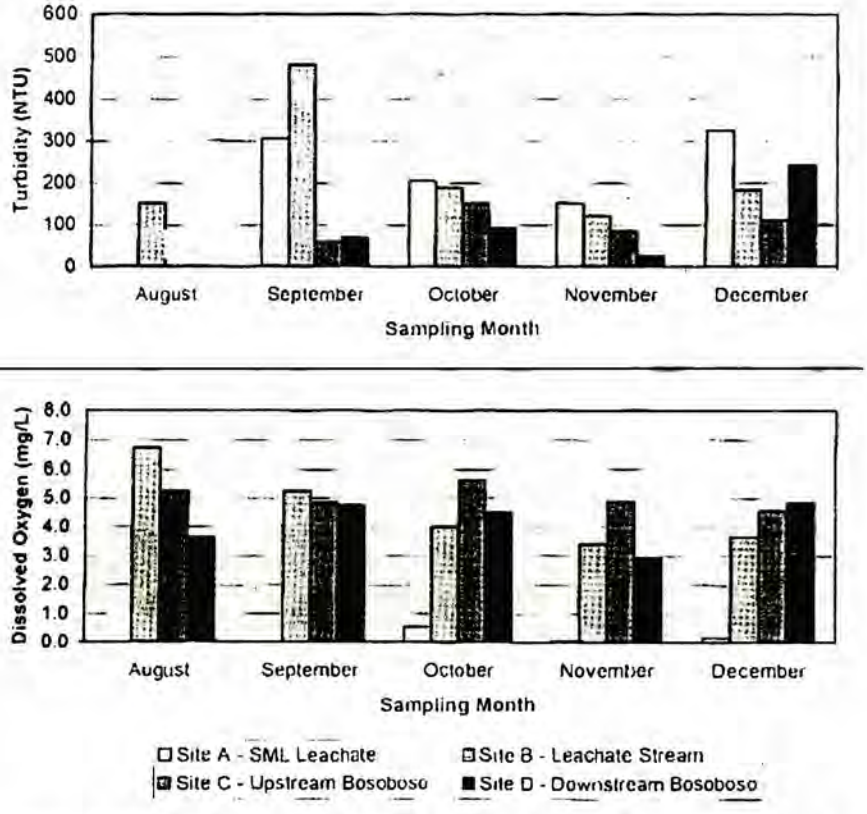

Figure 3. Turbidity and Dissolved Oxygen variation from August to December 1998.
*no measurement available.

variation in turbidity among the different sampling months and sites were observed.

Significant differences in DO levels were found between sampling sites. The values ranged from a minimum of 0.00 ppm during September at Site A, to a maximum of 6.74 during August at Site B (Figure 3). Significantly low DO values at Site A are indicative of the anaerobic decomposition of very high levels of organic matter within the leachate ponds.

Major Parameters

Chemical Oxygen demand (COD) –

Mean COD values ranged from a low of 2.83 ppm during October at Site C, to a high of 3910 ppm during August at Site B. Site A exceeded the maximum allowable COD level for effluent discharge of 100 ppm in the months of September to December (DAO 35, 1990) (Figure 4). All sampling sites were significantly different from each other. The order of magnitude in decreasing order were as follows: Sites B> A> D> C.

Mean pollution load levels ranged from a low of 0.5289 tons of Oxygen needed to oxidize organic matter per day during November at Site A, to a high of 38.84 tons of Oxygen per day during October at Site B (Table 2). Site B yielded a significantly higher COD pollution load than Site A as determined from the Paired Sample t-Test. At the Bosoboso River, Site D had a significantly higher COD pollution load when to Site C.

Iron (Fe) -

Mean Fe values ranged from a low of 0.067 ppm during November at Site C, to a high of 9.41 ppm during September at Site B. During the months of September to December, Site A discharged Fe above the 2.0 ppm standard set by the U.S. Public Health Service (USPHS) (Lund, 1971) (Figure 5). Site B possessed a significantly higher Fe concentration when compared to Site A. However, Site A was significantly higher than Sites C and D.

Mean pollution load levels ranged from a low of 0.880 kg of Fe per day during November at Site A, to a high of 1927 kg of Fe per day during December at Site C (Table 3). Site B carried a significantly higher Fe pollution load level compared to Site A as determined from the Paired Sample t-Test. At the Bosoboso River, there was no significant difference in Fe pollution load levels between Sites C and D.

Copper (Cu) -

Mean Cu values ranged from a low of 0.002 ppm during November at Sites C and D, to a high of 0.252 ppm during September at Site D. During the months of September to December, Site A was discharging Cu within the standard set by the USPHS at 0.5 ppm (Lund, 1971). On the other hand, mean Cu levels in the ambient waters of the Bosoboso River were within the allowable standard of 0.05

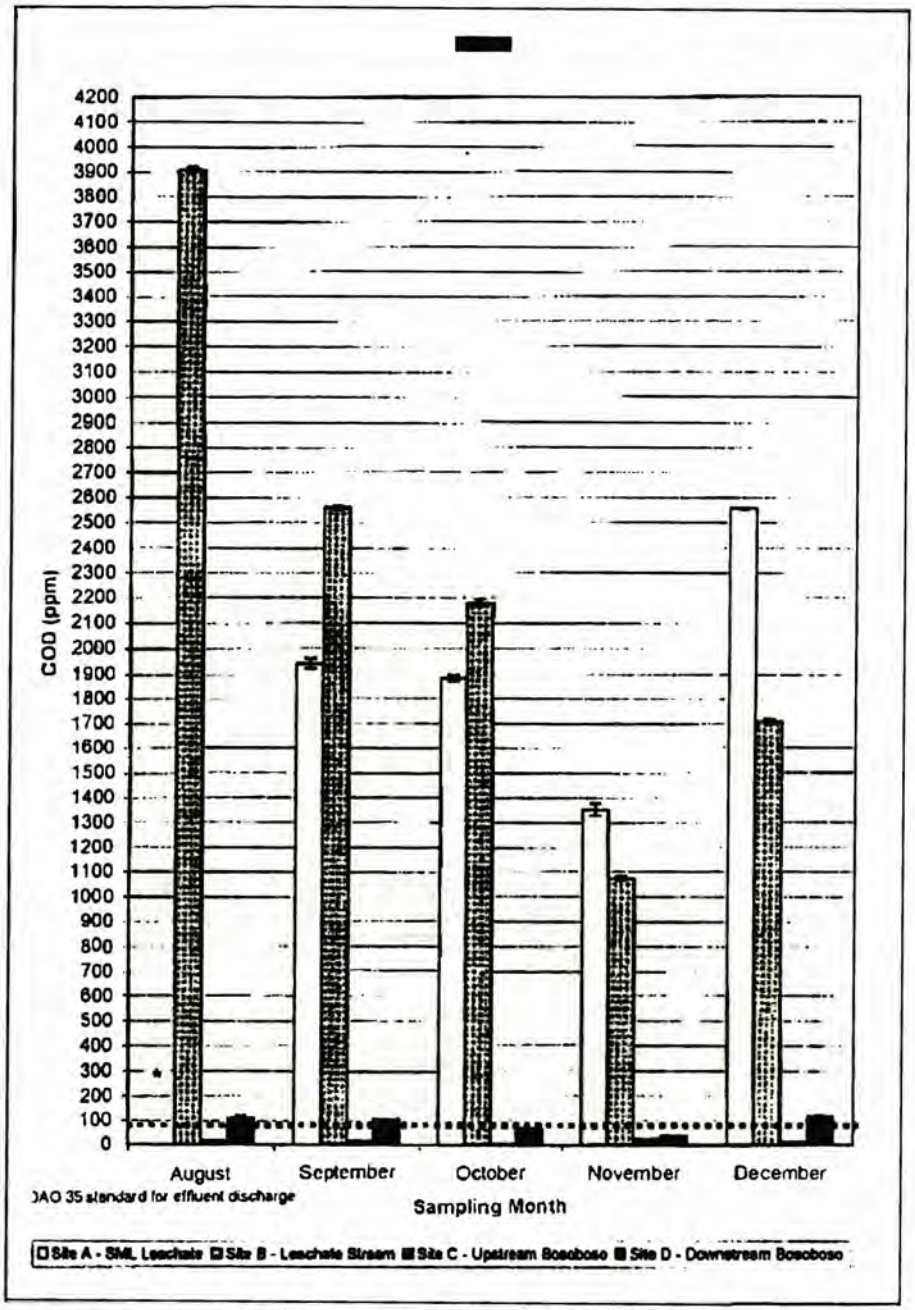

Figure 4. Average COD from August to December 1998 (n = 2)
*no measurement available.

Table 2. COD pollution load from September to December.
Data Expressed as Mean ± S.D. (tons O_2/day); n = 2

	Site A	Site B	Site C	Site D
Aug	–	11.04 ± 0.04	2.10 ± 0.13	10.78 ± 1.61
Sept	0.81 ± 0.02	2.35 ± 0.01	3.52 ± 0.56	23.96 ± 0.04
Oct	2.08 ± 0.03	38.84 ± 0.47	1.35 ± 0.08	37.18 ± 0.54
Nov	0.53 ± 0.02	7.27 ± 0.09	7.75 ± 0.25	12.16 ± 0.54
Dec	1.12 ± 0.00	18.61 ± 0.22	8.65 ± 2.88	–

Table 3. Fe pollution load from September to December.
Data Expressed as Mean ± S.D. (kg Fe/day); n = 2

	Site A	Site B	Site C	Site D
Sept	1.68 ± 0.23	8.64 ± 0.20	210.49 ± .55	432.42 ± 34.29
Oct	2.48 ± 0.35	154.13 ± 0.78	581.47 ± 5.18	619.84 ± 67.57
Nov	0.88 ± 0.12	58.58 ± 0.29	390.36 ± 3.48	368.61 ± 40.18
Dec	1.39 ± 0.00	80.01 ± 2.25	1928 ± 58.00	–

ppm (DAO 34, 1990), except during September at Site D (0.252 ppm) (Figure 5). The month of September showed a significantly higher Cu concentration compared to December. However, December was significantly higher than the months of October and November.

Mean pollution load levels ranged from a low of 0.0039 kg of Cu per day during November at Site A, to a high of 56.9 kg of Cu per day during September at Site B (Table 4). Site B had significantly higher Cu pollution load compared to Site A as determined from the Paired Sample t-Test. No significant difference in Cu pollution load was obtained between Sites C and D.

Zinc (Zn) –

Mean Zn values ranged from a low of 0.119 ppm during August at Site D, to a high of 1.147 ppm during September at Site B. From the months of September to December, Site A discharged Zn below the 1.0 ppm critical level established by the USPHS (Lund, 1971) (Figure 5). All sampling months were significantly

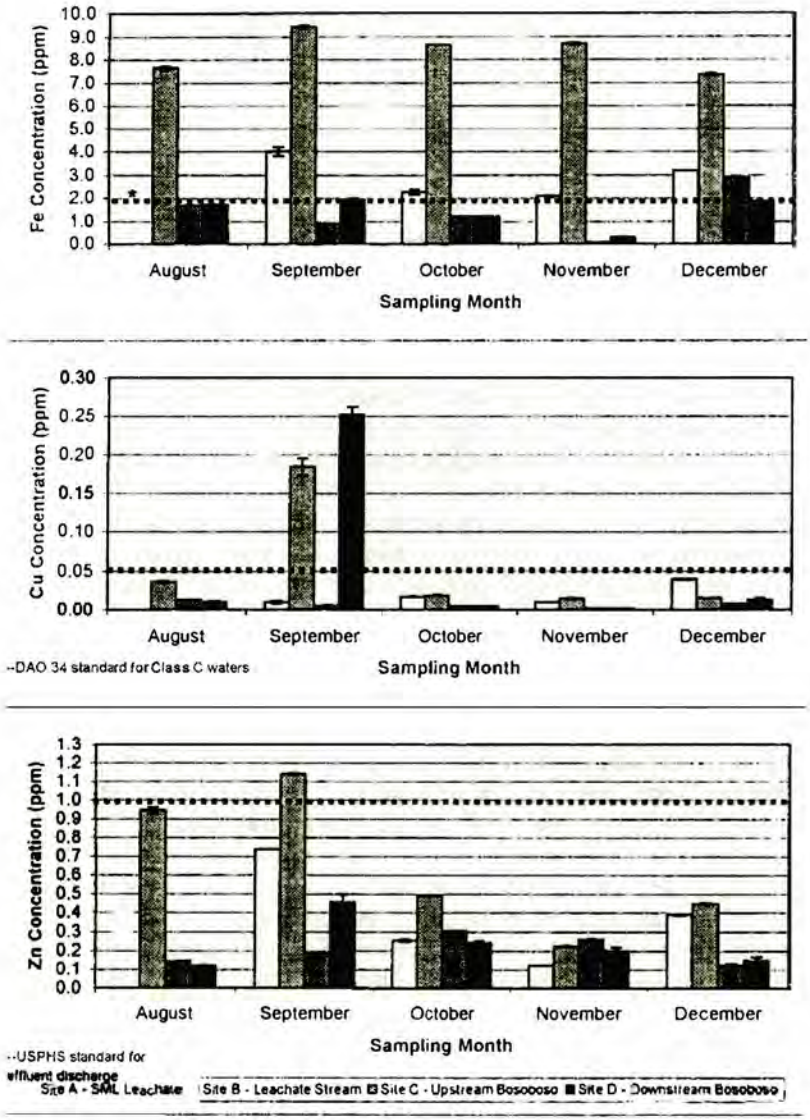

Figure 5. Mean Fe, Cu and Zn concentration from August to December 1998 (n-2).
*no measurement available.

Table 4. Cu pollution load from September to December.
Data Expressed as Mean ± S.D. (kg Cu/day); n = 2

	Site A	Site B	Site C	Site D
Aug	-	0.100 ± 0.014	1.63 ± 0.00	1.12 ± 0.06
Sept	0.005 + 0.002	0.169 ± 0.028	1.40 ± 0.13	56.90 ± 6.44
Oct	0.018 + 0.001	0.332 ± 0.010	2.46 ± 0.27	2.67 ± 0.29
Nov	0.004 + 0.000	0.102 ± 0.023	0.76 ± 0.00	0.73 ± 0.00
Dec	0.017 + 0.000	0.173 ± 0.000	5.30 ± 0.00	-

different from each other. The order of magnitude in decreasing order were as follows: September, October, December, and November. Furthermore, all sampling sites were also significantly different from each other. The order of magnitude in decreasing order were as such: Sites B, A, D, and C.

Mean pollution load values ranged from a low of 0.0476 kg of Zn per day during November at Site A, to a high of 146 kg of Zn per day during October at Site C (Table 5). Using Paired Sample t-Tests, it was shown that Site B possessed a significantly higher Zn pollution load when compared to Site A. No significant difference in Zn pollution load was recognized between Sites C and D.

Pollution Sources

All major parameters demonstrate that there exist significant pollution load differences between Sites A and B, with the latter having a greater value than Site A. This finding shows that other sources of pollution are present.

The first step in the analysis was to determine the percentage of pollutants contributed by the leachate ponds on the leachate stream. This was done by applying linear regression on the pollution load results of Sites A and B. Table 6 summarizes the results. It shows that among the four major parameters, only organic from the leachate ponds are consistently related to the organic load at the leachate stream. The COD pollution load contributed by Site A on Site B ranges from a low of 5.3% in September, to a high of 34% in October.

The next step was to determine the percentage of pollutants contributed by the unknown pollution sources at Site B. Pollution load of the unknown sources was obtained by subtracting the pollution load of Site A from Site B. Subsequently, linear regression was used to correlate the pollution load of the unknown sources with that of the leachate stream. The percent contribution made by the unknown sources on the leachate stream was then calculated by dividing the pollution load of the unknown sources by Site B. Table 7 summarizes the results. It shows that a

Table 5. Zn pollution load from September to December. Data Expressed as Mean + S.D. (kg Zn/day); n = 2

	Site A	Site B	Site C	Site D
Aug	-	2.668 + 0.131	17.35 + 0.32	11.62 + 0.92
Sept	0.307 + 0.000	1.048 + 0.007	44.68 + 0.63	102.80 + 27.17
Oct	0.279 + 0.022	8.750 + 0.020	145.86 + 0.30	124.45 + 18.74
Nov	0.048 + 0.002	1.515 + 0.081	83.14 + 3.06	61.95 + 5.42
Dec	0.170 + 0.003	4.908 + 0.021	82.00 + 8.67	-

Table 6. Pollution Load Contribution of Site A at Site B from September to December 1998.

Parameter	r^2	Range of mean pollution load contribution of site A at site B
COD	0.9076	low of 5.3% in Oct to a high of 34% in Sept
Fe	0.3527	
Cu	0.5596	
Zn	0.1043	

Table 7. Pollution load contribution of the unknown sources at site B from Sept to Dec 1998

Parameter	r^2	Percent contribution made by unknown sources on Site B	Standard Deviation
COD	0.9998	87%	13%
Fe	0.9999	94%	8.4%
Cu	0.9969	94%	2.9%
Zn	0.9990	90%	12.1%

very strong linear relationship exists for all major parameters between the unknown pollution sources and Site B. This implies that the pollution load at the leachate stream is highly dependent on the pollution load at the unknown sources. Furthermore, the amounts of organic, Fe, Cu, and Zn at Site B were mostly derived from the unknown pollution sources.

However, it must be mentioned that the pollution load percentages contributed by the unknown sources at Site B are estimates. The computations did not take into account certain pollution dynamics such as the deposition of pollutants unto the sediment, and the oxidation of organic matter within the leachate stream. Nevertheless, the deviations brought about by these pollution dynamics are most probably not very significant since all major parameters support the conclusion that the unknown pollution sources are the main contributors of pollution within the leachate stream.

CONCLUSIONS

In comparing Sites A and B for all major parameters, a significantly higher pollution load was found for Site B. This is strong evidence that the leachate ponds are not the only source of pollution for the leachate stream. Linear regression analysis of pollution loads, show that only COD had a strong correlation between the leachate pond discharge (Site A) and the leachate stream (Site B). However, the pollution load at Site B and the total load for the unknown pollution sources displayed strong correlation for all major parameters. Furthermore, pollutants found at the leachate stream were derived mostly from unknown sources. This implies that the quality of water at leachate stream is more dependent on the unknown pollution sources than on the leachate ponds. These unknown sources are most probably leaks within the landfill itself since there are no other known sources of pollution within the area.

As for the Bosoboso River, organic levels were significantly higher downstream (Site D) than upstream (Site C). This demonstrates that organic from the landfill and the unknown pollution sources significantly deteriorate the waters of the Bosoboso River.

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VARIETAL DIFFERENCES IN GROWTH, SODIUM UPTAKE AND ANTIOXIDANT RESPONSES OF RICE SEEDLING TO SALINITY STRESS

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ABSTRACT

The growth rate, leaf sodium uptake and possible involvement of activated oxygen species in the mechanism of damage by NaCl stress was studied in four varieties of rice *(Oryza sativa L.)* exhibiting different sensitivities to NaCl. The 3-week-old rice seedlings were subjected to 0, 6 and 12 dS m^{-1} (equivalent to 0, 60 and 120 mM NaCl) salinity levels for 1-week after which antioxidant capacities, growth rate and Na^+ uptake of the leaves were analyzed. High salinity treatment caused a decrease in growth rate in all the varieties tested except Pokkali. Varieties that are considered to be salt-sensitive, Hitomebore, IR28 and Bankat, exhibited a decrease in superoxide dismutase activity and an increase in peroxidase activity under high salinization. These varieties also exhibited increase in lipid peroxidation and electrolyte leakage as well as higher Na^+ accumulation in the leaves under salt stress. The salt-tolerant variety Pokkali, however, showed only slight increase and decrease in superoxide dismutase and peroxidase activity, respectively, and virtually unchanged lipid peroxidation, electrolyte leakage and Na^+ accumulation upon salinization. These results indicate that free radical-mediated damage of membrane may play an important role in the cellular toxicity of NaCl in rice seedlings and that salt-tolerant varieties exhibit protection mechanism against increased radical production by maintaining the specific activity of antioxidant enzymes.

Keywords: antioxidant responses, electrolyte leakage, growth rate, lipid peroxidation, *Oryza sativa* L., oxidative stress, peroxidase, salt stress, sodium chloride stress, superoxide dismutase

INTRODUCTION

Soil salinity primarily due to Na salts, particularly NaCl, is a major stress factor reducing plant growth and productivity throughout the world. The rapid expansion in the use of irrigation to meet the increasing demand for food crops and plant products has led to a decrease in crop productivity as a result of salinity stress.

Although most cereal crops exhibit high tolerance to soil salinity, a notable exception, however, is rice, the staple food of the majority of the world's population. Due to this, great interest focused on screening and developing rice varieties that are tolerant to salinity stress. Defining salt tolerance, however, is quite difficult because of the complex nature of salt stress and the wide range of plant responses. Thus, a better understanding of the mechanism that enable plants to adapt to salinity stress and maintain growth, development, and productivity during stress periods could help in breeding and genetic engineering of crop plants for salinity resistance.

Present evidences suggest that many damaging environmental stresses have their effects directly or indirectly through the formation of activated oxygen species following impairment of electron transport systems. These activated oxygen species, such as superoxide ($O_2\bullet-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$) and singlet oxygen ($^1O_2$) have the potential to inactivate enzymes and damage important cellular components such as lipids, proteins and nucleic acids (Davies 1987, Fridovich 1986). Fortunately, plants have evolved various enzymatic and non-enzymatic mechanisms that can minimize the damaging effects of activated oxygen. Among the enzymatic antioxidants are superoxide dismutase (SOD), catalase, peroxidase and enzymes of the ascorbate-glutathione cycle.

This study was designed to determine the effect of salt stress on growth, Na^+ content, antioxidant enzyme activities, lipid membrane peroxidation and electrolyte leakage of leaves of rice varieties exhibiting differences in salinity tolerance. Comparison of these responses could be useful in identifying differences related to the relative ability of each cultivar to cope with salinity. Result from this study can supply information on the involvement of activated oxygen species in the mechanism of damage by NaCl stress in rice plant, and also could allow deeper insights into the molecular mechanisms of tolerance to salt-induced oxidative stress.

MATERIALS AND METHODS

Plant materials and salinity treatments

Four rice varieties differing in salt tolerance and categorized into two ecogeographic landraces were used. The japonica landraces were Hitomebore (salt-sensitive) and Bankat (salt-sensitive) while the indica landraces comprised of IR28 (salt-sensitive) and Pokkali (salt-tolerant). Seeds of each variety were sown on styrofoam boards floating in deionized water and placed in a glasshouse under natural day and night light temperature maintained at 28°C. After one week,

deionized water was replaced with one-fourth strength modified Yoshida nutrient solution (Mae 1993) adjusted to pH 5.8 with 1 N KOH. After another week, the nutrient solution was replaced with one half strength nutrient solution and renewed twice a week. Water lost by evapotranspiration was compensated for by the daily addition of deionized water. Three weeks after sowing salinization was induced by adding NaCl to the one-half strength modified Yoshida solution to obtain electrical conductivities of 6 and 12 dS m^{-1}, which are equivalent to about 60 and 120 mM NaCl, respectively. Nutrient solution without NaCl addition (0 mM NaCl or 0 dS m^{-1}) served as the control.

Measurements were taken one week after salinity treatments on a completely randomized design with four replicates.

Growth measurements

Plant growth was estimated through relative growth rate (RGR) measurement. This was calculated from the increase in dry weight of plant shoots at the beginning and at the end of the salt treatment, using the equation $RGR = (\ln W_7 - \ln W_0)/(t_7 - t_0)$ where W is the shoot dry weight, t is the time and subscripts denote sampling 0 and 7 days after salinity treatment.

Sodium determination

The sodium content of the leaf, which represents plant sodium uptake, was determined by atomic absorption spectrophotometry on cooled extract of dried cut material in distilled deionized water, heated in a boiling water bath for one hour, and then autoclaved at 121°C for 20 minutes.

Determination of enzyme activities and lipid peroxidation

Assay of enzyme activities and estimation of lipid peroxidation were done on 50 mM phosphate buffer extracts (pH 7.0) of the frozen leaf samples. Total SOD activity, the basis of which is its ability to inhibit the photochemical reduction of nitro blue tetrazolium, and peroxidase activity using the guaiacol oxidation method were determined as described previously (Dionisio-Sese and Tobita 1998). Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) formation using the thiobarbituric acid method described by Stewart and Bewley (1980).

Electrolyte leakage measurement

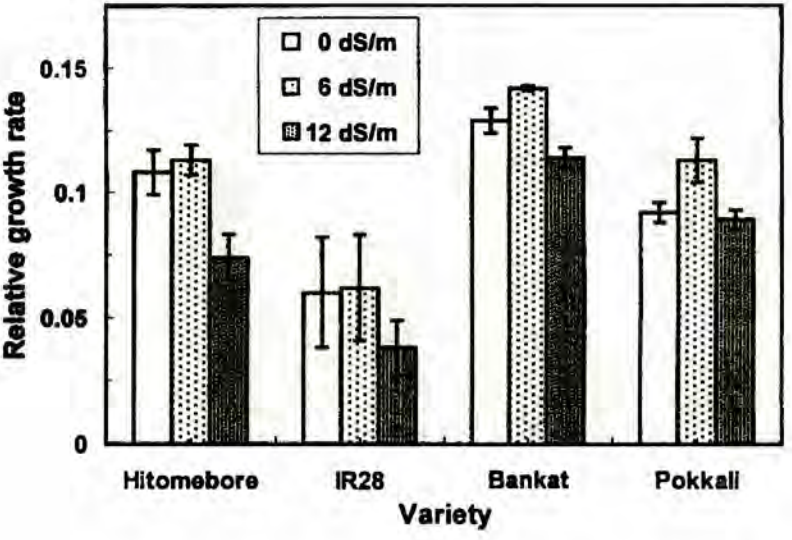
The electrolyte leakage rate (EL) was expressed following the formula $EL = EC_1/EC_2 \times 100$ where EC_1 and EC_2, respectively, are the electrical conductivity measurements of fresh leaf samples placed in water bath at 32°C for two hours and after autoclaving at 121°C for 20 minutes.

RESULTS AND DISCUSSION

There has been considerable effort directed at selection and development of rice varieties resistant to salinity stress. Progress, however, seems slow primarily due to an inadequate knowledge of salt tolerance mechanism.

In rice, as well as in any other crops, agronomic characters, such as yield, survival, leaf damage and plant height have been the most commonly used criteria for identifying tolerance (Lutts *et al.*, 1995, Yeo *et al.*, 1990). This is largely due to measurement ease and yield under saline conditions which is usually the ultimate requirement. In the present experiment, on the basis of the shoot's dry weight measurement during one-week salt treatment at the seedling stage, varieties Hitomebore, IR28 and Bankat, which are considered salt-sensitive, showed growth retardation at 12 dS m^{-1} (Fig. 1). In contrast, the salt-tolerant variety, Pokkali, did not show any growth inhibition at the same salinity level. Salinization was induced at the seedling stage since previous studies showed that during the vegetative growth of rice, this stage is the most sensitive to NaCl compared to germination and tillering stages (Lutts *et al.*, 1995).

It has been proposed, however, that selection and breeding to increase salt tolerance might be more successful if based directly, not on agronomic characters, but on the physiological mechanisms conferring tolerance (Noble and Rogers,



1992). Since salinity has both osmotic and ion effects on plant growth, ion exclusion is being considered as a selection criterion for improving salt tolerance in crops (Noble and Rogers, 1992). Rice plant studies revealed that the cause of injury from salinity stress is more likely to be from excessive sodium and not chloride ion. This is because Cl^- is tolerated over a wide range of concentrations (Clarkson and Hanson, 1980), and the disruptive effect of Na^+ in the conformation of macromolecular structure and its interference with the roles of cytoplasmic K^+ will preempt Cl^- toxicity (Gregorio and Senadhira, 1990).

Results of the analysis of Na^+ content of leaves of rice varieties differing in salt tolerance agree with the view that there is an inverse relationship between shoot Na concentration and salt tolerance (Yeo and Flowers, 1983). The salt-tolerant variety Pokkali did not exhibit a significant increase in Na^+ accumulation in the leaves even at high salinity level whereas the salt-sensitive varieties Hitomebore, IR28 and Bankat showed pronounced accumulation (Fig. 2).

It has been observed that when plants are subjected to environmental stress such as drought (Dhindsa and Matowe 1981), chilling (Wise and Naylor 1987), high light intensity and mineral deficiency (Cakmak and Marschner, 1992), ultraviolet radiation (Rao and Omrod, 1995), and herbicide treatment (Harper and Harvey 1978), the balance between the production of activated oxygen species and the quenching activity of antioxidants is upset, often resulting in oxidative damage. A greater resistance to this oxidative damage was observed in plants

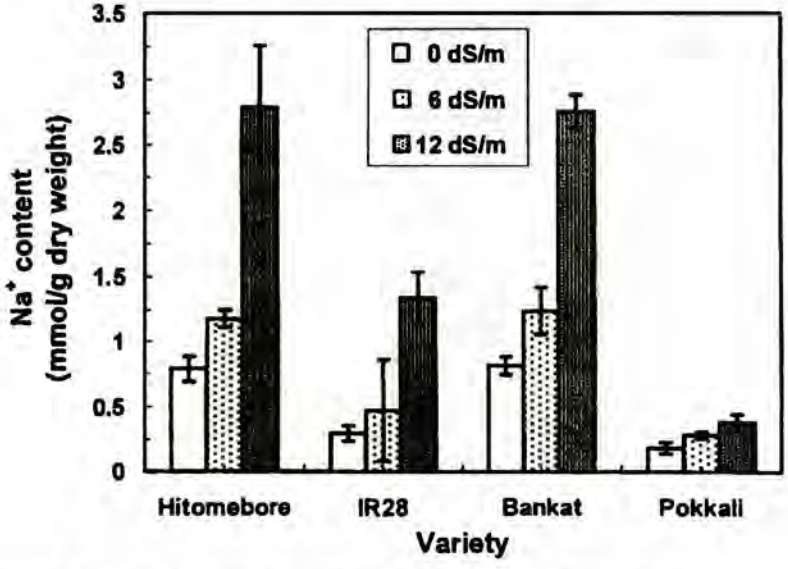


Figure 2. Effect of increasing salinity on Na^+ accumulation in the leaves of four rice varieties.

having high levels of antioxidants (Harper and Harvey 1978, Dhindsa and Matowe 1981, Wise and Naylor 1987).

The present study indicates different responses in the activities of antioxidant enzymes involved in the oxygen metabolism, and that these responses might be related to the distinct susceptibilities of the rice varieties to NaCl. Increasing magnitude of salinity stress brought about a significant decrease in SOD activity in the salt-sensitive varieties whereas the salt-tolerant variety Pokkali did not show any decline in SOD activity at all – in fact a slight increase in SOD activity was observed in Pokkali with increasing salinization (Fig. 3). Singha and Choudhuri (1990) also observed that NaCl decreased SOD activity in rice seedlings. Since no comparison was done among varieties differing in salt tolerance, it is assumed that the variety they have used for analysis was of the salt-sensitive type.

Aside from rice, salinity induced the decrease in SOD activity in the leaves, chloroplasts and mitochondria of pea plants (Hernandez *et al.* 1993; 1995). This decrease in SOD activity may be due to either decrease of enzyme synthesis, increase of enzyme degradation, or alternatively to conformational changes and reduced structural stability of the enzyme molecule. Hernandez *et al.* (1994) demonstrated in cowpea plants that the catalytic activity of SOD isozymes decreased as a function of salt concentration *in vitro*. Although the compartmentation of Na^+ within leaves and inside the cells was not determined in the present study, the high ion content and the parallel decrease in SOD activities found in the leaves of

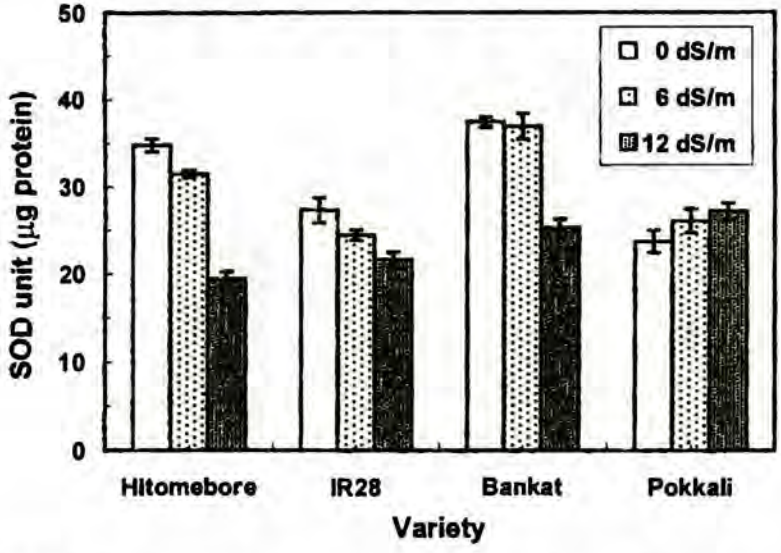


Figure 3. Effect of increasing salinity on the activity of SOD in the leaves of four rice varieties.

salinized rice plants is consistent with the possibility that salt directly inhibits the catalysis in vivo. However, the other possibility that the inhibition of SOD activity under salt stress is a consequence of an altered synthesis and accumulation of less active enzyme in salt-treated plants cannot be ruled out. Thus, there is a need for more data concerning how salinity stress may affect SOD activity, either directly on catalysis or through total enzyme capacity.

Since SOD is a major scavenger of $O_2\bullet-$, the observed decrease in SOD activity in salinized salt-sensitive rice varieties, in turn, could favour an accumulation of $O_2\bullet-$ radicals causing membrane damage. The extent of damage to the membrane can be monitored by measuring the amount of MDA produced when polyunsaturated fatty acids in the membrane undergo peroxidation. Unlike the salt-tolerant Pokkali, there was an observed increase of MDA in all the varieties tested when exposed to NaCl stress (Fig. 4). By generating changes in unsaturated fatty acids that affect membrane structure and properties, this enhanced free radical formation and lipid peroxidation under salt stress may also bring about an increase in membrane permeability or loss of membrane integrity. This is proven by the increase in electrolyte leakage in salt-sensitive varieties with increasing magnitude of salinity stress whereas the rate of electrolyte leakage was almost unchanged in Pokkali (Fig. 5). Salt stress-induced electrolyte leakage has also been previously observed in tomatoes (Tal and Shannon, 1983) and melons (Borochov-Neori *et al.*, 1991).

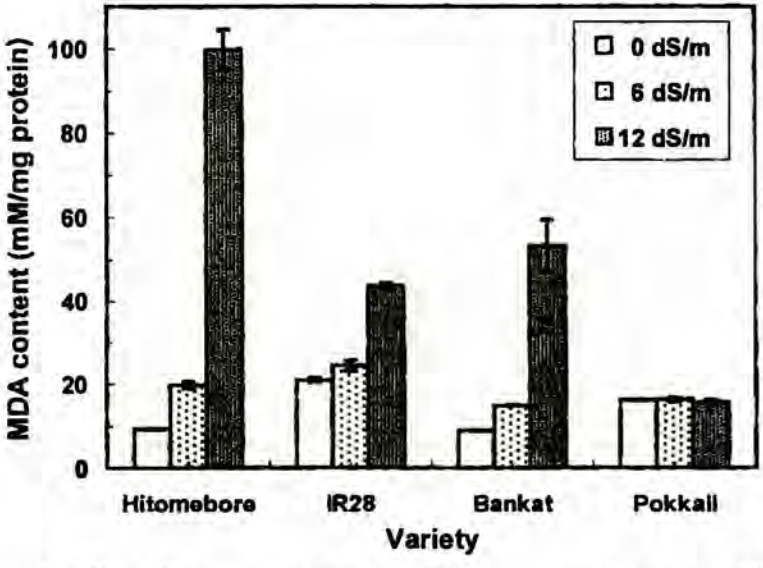


Figure 4. Effect of increasing salinity on MDA content in the leaves of four rice varieties.

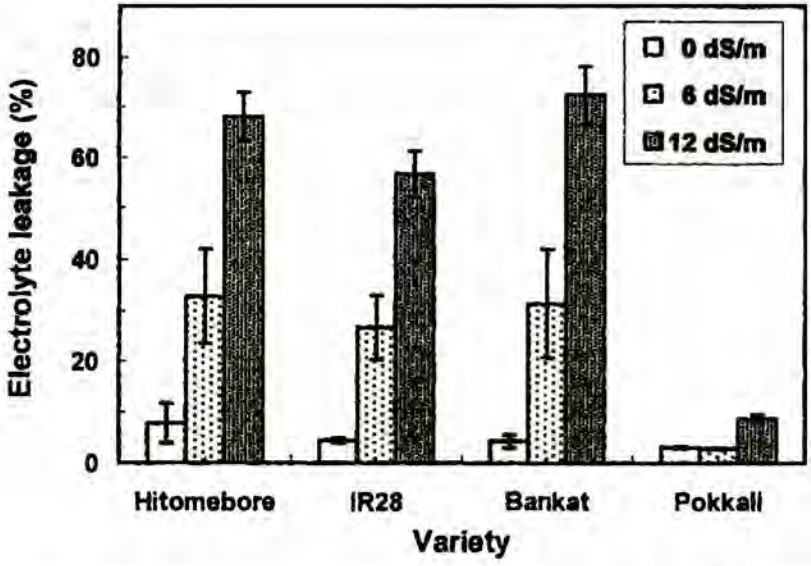


Figure 5. Effect of increasing salinity on electrolyte leakage rate of the leaves of four rice varieties.

Unlike SOD, peroxidase activity increased with increasing salinity level in Hitomebore, IR28 and Bankat whereas a slight decrease was observed in the tolerant Pokkali upon increasing exposure to salinity stress (Fig. 6). Various researchers dealing with rice (Mittal and Dubey, 1991) and other plants (Sheoran and Garg, 1979; Kalir *et al.*, 1984) have also reported increase in peroxidase activity under salt-stress. It is not clear whether the observed increase in peroxidase activity under salt stress was due to increased activity of peroxidase encoding genes or an increased activation of already existing enzymes. Mittal and Dubey (1991) suggested that salinity affects mainly the *de novo* synthesis of the enzyme since inhibition under *in vitro* conditions and activation under *in vivo* conditions was observed in salt-sensitive cultivars. Lopez *et al.* (1996), however, have shown that the salt-induced increase in ascorbate peroxidase activity in radish plants was not accompanied by a corresponding increase in mRNA level, suggesting that the salt-induced ascorbate peroxidase expression is probably the consequence of post-transcriptional events. In *Halimione portulacoides*, a halophyte which survives harsh saline conditions in salt marshes, it was suggested that the increase in peroxidase activity induced by exposure to salinity was due to conformational changes occurring in the protein molecule rather than an increase in protein synthesis or proteolysis (Kalir *et al.*, 1984).

Aside from their function in the metabolism of active oxygen, peroxidases in plants are also involved in the biosynthesis of cell wall (Negrel and Lherminier

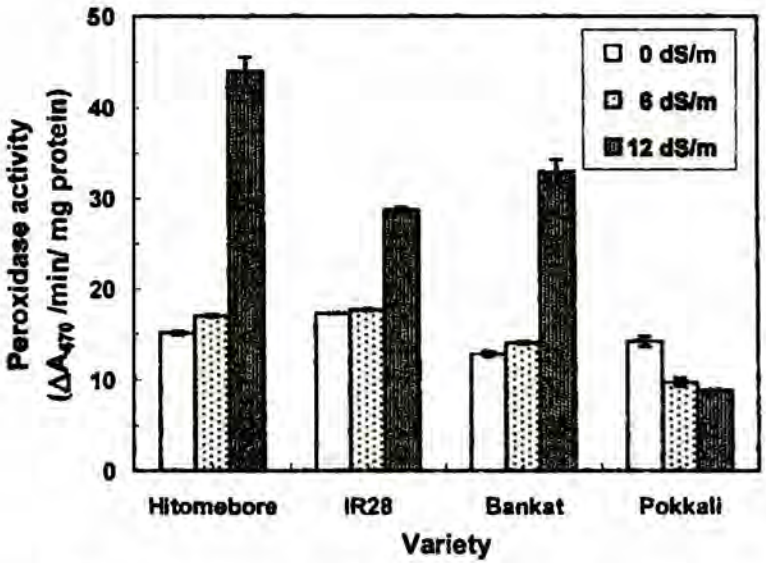


Figure 6. Effect of increasing salinity on the activity of peroxidase in the leaves of four rice varieties.

1987) including lignification and suberization (Espelie *et al.* 1986; Polle *et al.* 1994). Considerable evidence shows that high peroxidase activity is correlated with the reduction of plant growth (MacAdam *et al.* 1992; Zheng and Van Huystee, 1992, Lee and Lin, 1995). This might be attributed to peroxidase catalysis of ferulic acid conversion to diferulic acid on polysaccharides, the feruloylation of hemicelluloses, or the insolubilization of hydroxyproline-rich glycoprotein causing cell wall stiffening (Fry 1986; Waffenschmidt *et al.*, 1993). Morphologically, the most typical symptom of saline injury to a plant is retarded growth due to inhibition of cell elongation (Nieman, 1965), resulting in a stunted plant. Notwitstanding the other physiological and biochemical mechanisms involved, the observed decrease in rice growth of salt-sensitive varieties with increasing salinization might then be partly due to salt-induced increases in peroxidase activity.

The results of this study show that there were substantial differences between the growth and antioxidant responses of the four rice varieties to salinity treatment. During salt stress the salt-sensitive varieties, Hitomebore, IR28 and Bankat, exhibited high leaf Na^+ accumulation resulting in symptoms of oxidative damage such as decrease in SOD activity, increase in lipid peroxidation and electrolyte leakage, increase in peroxidase activity and decrease in growth rate. Salinity, however, only had a minimal effect on growth rate, leaf Na^+ accumulation and antioxidant metabolism in the salt-tolerant variety, Pokkali. Thus under salt stress,

the lower Na^+ accumulation and relatively unchanged SOD and peroxidase activities, by bringing about an unchanged capacity for oxygen radical scavenging and maintenance of cellular membranes as well as cell wall function, could explain the NaCl tolerance of tolerant rice varieties over the sensitive ones.

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THE CIGUATERIC POTENTIAL OF SOME PHILIPPINE RABBITFISHES (FAMILY SIGANIDAE)

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ABSTRACT

Extraction and bioassay for polar lipid toxins in *Siganus spinus* (Linnaeus), implicated in a human poisoning case in La Union, and other members of Family Siganidae namely *S. canaliculatus* (Mungo Park), *S. virgatus* (Cuvier & Valenciennes), *S. guttatus* (Bloch), and *S. punctatissimus* Fowler & Bean from several locales found toxins in the fish viscera that could amount to 10 MU/100g. One Mouse Unit (MU) was defined as the amount of toxin that can kill a 20g mouse within 24 hours. Histopathological analysis in experimental mice revealed damages in the ventricular myocardium and other tissues which could be caused by maitotoxin most possibly present in the extracts. Kymograms of frog neuromuscular and heart activity suggested both ciguatoxin-like and maitotoxin-like effects. The gut contents of Siganid fish as well as the La Union reef included algae which are known preferred substrates of ciguateric poison-elaborating dinoflagellates.

Key Words: Ciguatera, ciguatoxin, maitotoxin, ichthyosarcotoxism, Siganidae, rabbitfish, fish poisoning, neurotoxin, cardioactive toxin, ion channel activator

INTRODUCTION

Ciguatera is a human illness chiefly characterized by gastrointestinal, neurological, and cardiovascular disorders that may result from the ingestion of seafood. Originally, the term ciguatera referred to poisoning caused by "cigua", the Caribbean marine snail *Livona pica*, but is now applied to the similar disorders following the intake of various normally edible fishes. Within two to thirty hours after consumption of toxic fish, the following symptoms appear: nausea, often

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followed by watery diarrhea, sometimes vomiting, sensitivity disturbances such as the reversal of temperature sensations where in cold feels hot and hot feels cold, intense itching, numbness with tingling in the limbs, slow or accelerated pulse rates, slightly modified heart beats, general weakness, joint pains, and dizziness (SPC Health Programme Staff, 1991). The illness is an established phenomenon, and is well-recognized for centuries (Randall, 1958). It is separate and distinct from other illnesses such as botulism and scombroid poisoning (Ragelis, 1984). It may have existed longer than the 500 years documented by historical records as proven by the fishes that are not affected by the toxins that they bear (Lewis, 1984). Mann (1978) wrote of mankind being aware of it for some 6,000 years. It is widespread within the geographical belt between 35°N and 35°S (Mann,1978). Particularly in many islands of the Pacific, ciguatera is a serious problem, gravely affecting health and economies (Glaziou *et al.* , 1995). It is reported that some 20,000 people are affected by ciguatera annually (Yasumoto and Satake, 1996).

The clinical signs of ciguatera are diverse. Prevailing symptoms are determined by the kind of toxins involved. Ciguatera poisons are polyether compounds of two principal classes: ciguatoxin and maitotoxin. The ciguatoxin family includes ciguatoxin (CTX) and several congeners. The major ciguatoxin, $C_{60}H_{86}O_{19}$ (Murata *et al.*, 1989; Murata *et al.*, 1990) is a Na^+-channel activator. Maitotoxin (MTX), $C_{164}H_{256}O_{68}S_2Na_2$ (Murata *et al.*, 1994) on the other hand could affect a receptor-mediated affect Ca^{++}-channel (Yasumoto and Murata, 1993). Understandably , because of their modes of action, these are very potent toxins. Maitotoxin is the most potent marine biotoxin (Ohizumi and Yasumoto, 1983; Ohizumi *et al.*, 1983; Takahashi *et al.*, 1983). The fact, however, that the ciguatera syndrome is only occasionally fatal in humans (SPC Health Programme, 1991) may perhaps be due to individual sensitivities and not very particularly high levels of toxins in fish.

The toxins are derived from a fish diet, primarily being produced by epiphytic toxic dinoflagellates that are ingested by the herbivore with the toxins then being passed to carnivores (Yasumoto *et al.*, 1979; Bagnis *et al.*, 1980). The most highly ciguateric fishes would be the larger carnivores that would have bioaccumulated and oxidized (Yasumoto and Murata,1993) the toxins to a greater extent within their lifetimes.

Almost any reef fish can become ciguatoxic, under the right conditions (SPC Health Programme Staff, 1991), hence, ciguatera was easy suspect for the poisoning episode in La Union in 1996 which incriminated *Siganus spinus* (Linnaeus). The investigation on the ichthyosarcotoxism case (Pocsidio and Cabrera, 1999) had established toxin levels believed to be of ciguatera poisons in the internal organs of *S. spinus*. An assessment was then made of the ciguateric potential of the other members of the Family Siganidae, especially those being vended in fish markets in La Union and elsewhere.

This present report embodies the results of the extraction, mouse bioassays and histopathological and physiological observations with partially purified toxins

from the Siganid fishes as well as gut content analysis and survey of algal community in the Lingsat Reef in La Union for the occurrences of known preferred algal substrates of ciguatera poison-producing dinoflagellates. Ciguatera toxins have been reported to affect such organs as the heart and stomach (Terao et al., 1988; Terao *et al.*, 1992) and the analysis of these particular effects could contribute to the identification of the toxins in the absence of complete chemical elucidation.

MATERIALS AND METHODS

Siganid Fish

Fish were obtained from various fish markets. The vendors provided the information about specific sources. *S. guttatus* and *S. virgatus* from Calatagan, Batangaas were definitely feral, i.e., obtained from the wild. The same were true for *S. punctatissimus* from Palawan, *S. canaliculatus* from the difference locales and *S. spinus* and *S. virgatus* from San Fernando City. Except for the *S. spinus* from La Union which were freshly caught and not kept in cold storage, all the fish had been previously packed in ice.

Visceral organs were taken from fresh and frozen fish, processed for ciguateric poisons. Samples also from fish flesh, with and without bones, as well as separate flesh and head parts were used in later extractions.

The fishes were of the following approximate total lengths: *S. spinus* (12 cm), *S. canaliculatus* (13.5 cm), *S. guttatus* (15 cm), *S. virgatus* (17 cm). The single large *S. punctatissimus* had total length of 21cm. Smaller *S. punctatissimus* were about 13.5 cm in total length.

The scientific, English , and local names of the fishes (Rau and Rau, 1980; Schroeder, 1980; Lieske and Myers, 1994) are as follows:

1. *Siganus spinus* (Linnaeus). English names: Black trevally, Scribbled rabbitfish. Local name: Danggit (Visayan)
2. *Siganus canaliculatus* (Mungo Park). English names: White-spotted rabbitfish, White-dotted rabbitfish. Local names: Danggit (Visayan), Samaral (Tagalog), Bararawan (Cuyonin)
3. *Siganus virgatus* (Cuvier & Valenciennes). English names: Barhead rabbitfish, Virgate rabbitfish. Local names: Tagbago (Visayan), Samaral (Tagalog), Mandalada (Cuyonin)
4. *Siganus guttatus* (Bloch). English names: Golden spinefoot, Golden rabbitfish. Local names: Ketong, Kitung (Visayan), Barangen (Cuyonin), Samaral (Tagalog)
5. *Siganus punctatissimus* Fowler & Bean. English names: Pearly-dotted rabbitfish, Peppered rabbitfish. Local names: Danggit (Visayan), Mandalada (Cuyonin), Samaral (Tagalog).

Extraction and Assay Methods for Toxicity

The procedure for extraction and bioassay according to Yasumoto *et al.* (1984) was adopted.

Primary extraction from the tissue samples was done through acetone homogenization (JTBaker, AR) using a Nikon blender. The homogenate was filtered by suctioning through with an Eyela Aspirator on a Buchner funnel. Afterwards, extraction from acetone-suspended sample was done with diethylether (JT Baker, AR). Subsequently, partitioning was done in Hexane (Ajax, AR) and aqueous methanol (Merck, AR). The methanol layer was dried in *vacuo* using a Buchi Rotary Evaporator. The residue was then suspended and, emulsified, in 1% Tween 60 (Sigma). I ml and 0.5 ml of diluted and undiluted suspension were injected intraperitoneally to laboratory white mice that weighed 20g on the average.

According to this procedure, one mouse unit (MU) was defined as an amount of toxin that can kill a 20g mouse within 24 hours. From this relation, a lethal 40g sample would yield toxin level of 2.5 MU/100g or from a 20g sample, 5.0 MU/100g. Lethality is established with the death of two mice out of three.

Histopathological Studies

Sublethal doses of 0.5 MU and 0.25 MU *S. guttatus* visceral toxin in three single doses 24 hours apart were injected intraperitoneally to laboratory white mice. On the fourth day the mice were killed. The heart, stomach, and intestine were excised, fixed in 10% neutral formalin (Chemline, Tech.), then processed by paraffin method. Tissues were sliced for light microscopy examination and stained in eosin (Fluka) and haematoxylin (BDH). The stomach, intestine, liver, lungs, kidney, muscle, and spinal cord of mice that died immediately upon injection of a lethal dose (4 MU) were also processed similarly and prepared for light microscopy. Examination was done on a Carl Zeiss microscope.

Physiological Studies

Cardiac actions in the frog of the visceral toxins were recorded on kymograph system (Phipps and Bird). Brain-pithed adult frogs *(Rana catesbiana)* averaging 12 cm in head-to-toe length were administered by gavage 2 single doses of 1 MU *S. canaliculatus* visceral toxin 10 minutes apart. Kymograph recordings of ventricular beats were done immediately prior, immediately after, 5 minutes after first treatment, and immediately after and 10 minutes after second treatment.

Actions on gastrocnemius muscle-sciatic nerve preparations from frogs were also studied. Kymograph recordings were made of the responses of the skeletal muscle to the following: (1) nerve stimulation with electrical stimulus (Phipps and Bird Student's Inductorium) immediately prior, 5 minutes after, and 10 minutes after topical application of 0.4 MU *S.canaliculatus* visceral toxin on nerve and 5 minutes after and 10 minutes after topical application of additional 0.4MU, (2) direct electrical stimulation immediately prior and 5 minutes after muscle was

injected with 0.4 MU, (3) direct electrical stimulation immediately prior and 10 minutes after immersion of muscle in 0.4 MU, and (4) direct electrical stimulation after washing previously toxin-immersed muscle with Ringer's solution.

Examination of Gut Contents

S. canaliculatus collected from Palawan were obtained through the Navotas Fishing Port. Guts were removed and preserved in 10% formalin until use. Contents of each gut were emptied into separate glass petri dishes and examined under stereozoom microscope (American Optical). Food items were scored for their occurrences in 10 fishes. Chlorophyll content of the gut contents was measured photometrically according to the method by MacKinney (1941).

Survey of Algal Community in La Union Reef

Macroalgae were collected from intertidal zone in Lingsat Coast, La Union. The algae preserved in 10% formalin were later identified using a local field guide as major reference (Calumpong and Menes, 1997; Trono, 1997).

RESULTS

The various Siganid fishes showed toxicity levels that ranged from non-detectable levels to 10 MU/100g (Table 1). Only three out of the 12 batches of the Siganid fish did not yield toxic viscera. These were *S. guttatus* and *S. virgatus* from Lucena, Quezon, and small-sized *S. punctatissimus* from Palawan.

Toxic manifestations in mice were motor ataxia, tremors, convulsion, respiratory distress, hindleg paralysis, and jumping before death.

Most significant histopathological effects of sublethal *S. guttatus* toxin were some swollen and disrupted myocardial fibers in mice (Figs.1 and 2) and erosions in gastric mucosa. Mice administered lethal *S. canaliculatus* toxin exhibited erosions in gastric mucosa (Fig. 3), disruptions in intestinal villi. (Fig. 4), intra-alveolar edema (Fig. 5), loss of nuclear bags inside the muscle spindle (Fig. 6), loss of interconnecting processes in the spinal cord (Figs. 7 and 8), disintegrating cells and increased sinusoidal spaces in the liver (Fig. 9), and hemorrhage in the kidney (Fig.10).

Topical application of 0.4 MU *S. caniculatus* toxin on the sciatic nerve initially increased the duration of the gastrocnemius muscle twitch. Afterwards, with additional 0.4 MU of toxin on nerve, skeletal muscle activity declined. Immersion of skeletal muscle in 0.4 MU toxin abolished contraction. The muscle recovered little with washing. Injection of 0.4 MU toxin into the muscle also abolished contractility. The kymograph record of these events are shown in Figures 11 and 12.

An initial positive inotropic effect followed by diminished activity of the heart was observed in the frog which was fed 2 single doses of 1 MU *S. canaliculatus* visceral toxin (Fig. 13).

Table 1. The toxicity of some Philippine Siganid fishes

Species	Place of collection	Date of collection	Date of mouse bioassay	Sample	Toxin level MU/100g
S. canaliculatus	Lucena, Quezon (Nepa-Q-Mart)	3-7-97	3-28-97	viscera	2.5
S. canaliculatus	Bicol (Navotas Fish Port)	3-13-98	3-20-98	viscera	10
S. canaliculatus	Canaoay, San Fernando City Mart	8-23-98	2-27-99	viscera body body plus head	10 10 10
S. canaliculatus	Pagdalagan, San Fernando City	7-16-98	2-27-99	viscera body body plus head	5 10 ND ND
S. guttatus	Lucena, Quezon (Nepa-Q-Mart)	3-7-97	3-28-97	viscera	ND
*S. guttatus**	Calatagan, Batangas	2-3-98	2-20-98	viscera	2.5
*S. punctatissimus***	Palawan (Malabon Fish Market)	3-3-98	3-22-98	viscera flesh	5.0 2.5
S. punctatissimus **	Palawan (Malabon Fish Market)	3-3-98	3-22-98	viscera flesh	ND ND
S. spinus	Poro Point, San Fermando City Mart	8-23-98	2-27-99	viscera flesh with bone deboned flesh body plus head	5 5 5 5
*S. virgatus**	Calatagan, Batangas (coastal market)	2-3-98	2-20-98	viscera	2.5
S. virgatus	Lucena, Quezon (Nepa-Q-Mart)	3-7-97	3-28-97	viscera	ND
S. virgatus	Canaaoay, San Fernando City Mart	7-16-98	2-27-99	viscera body body plus Head	2.5 5 10

*definitely feral **large-sized ***small-sized
ND-nondetectable

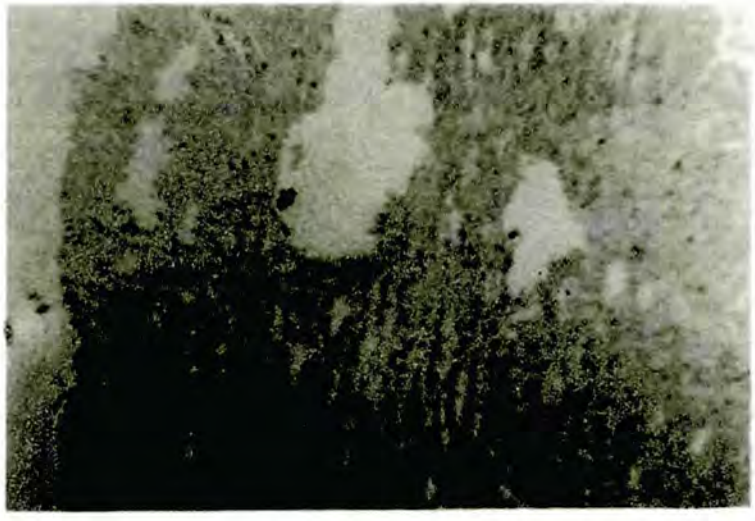

Figure 1. Photomicrograph of heart of albino Swiss mouse injected i.p. 0.5 MU *S. guttatus* visceral toxin. (c) Swollen ventricular myocardial fibers. Haematoxylin-eosin. X450.

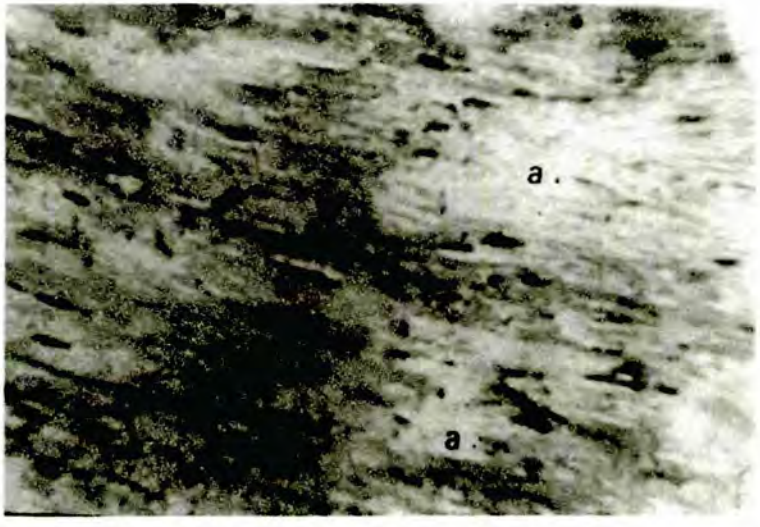

Figure 2. Photomicrograph of heart of albino Swiss mouse injected i.p. 0.5 MU *S. guttatus* visceral toxin. (a) Disrupted ventricular fibers. Haematoxylin-eosin. X1000.

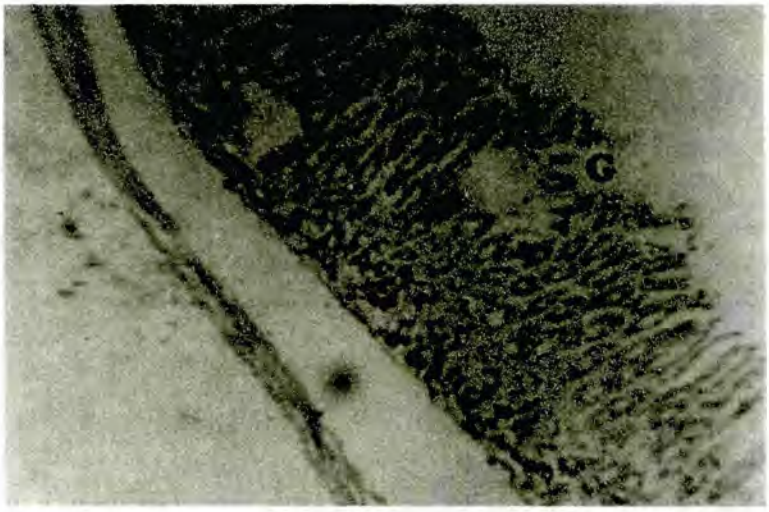

Figure 3. Photomicrograph of stomach of albino Swiss mouse injected i.p. 4.0 MU *S. canaliculatus* visceral toxin. (G) Erosion in gastric mucosa. Haematoxylin-eosin. X450.

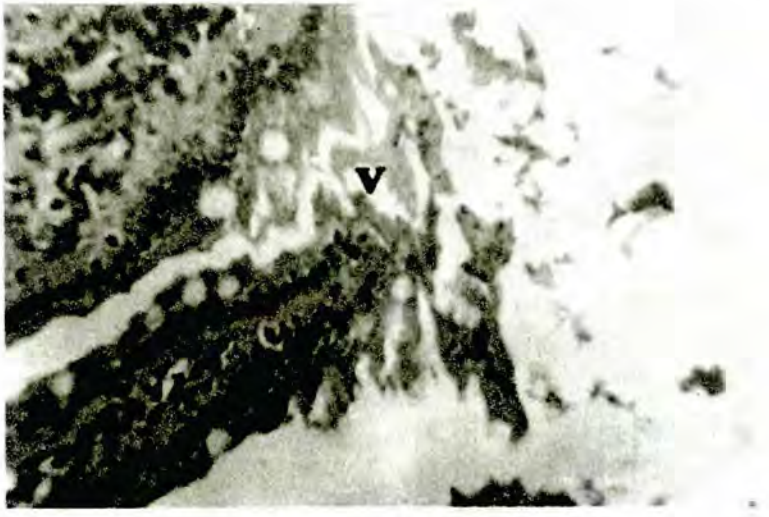

Figure 4. Photomicrograph of small intestine of albino Swiss mouse injected i.p. 4 MU *S. canaliculatus* visceral toxin. (V) Disruptions in intestinal mucosa. Haematoxylin-eosin. X450.

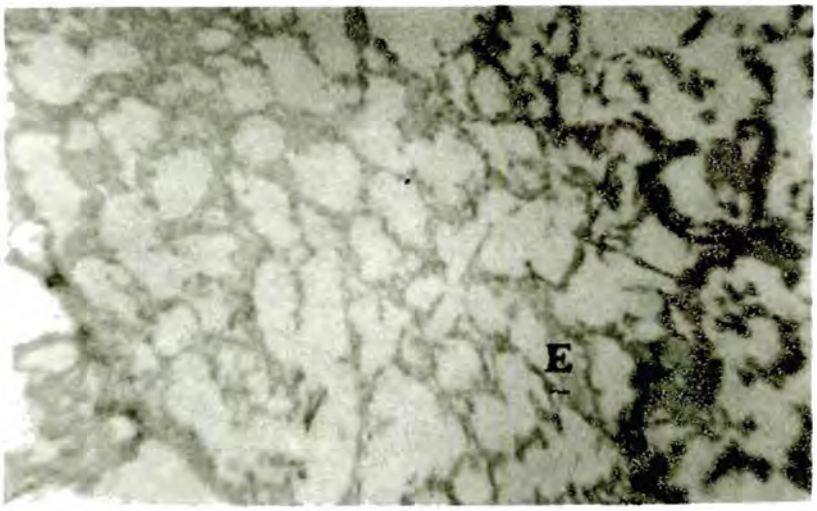

Figure 5. Photomicrograph of lung of albino Swiss mouse injected i.p. 4 MU *S. canaliculatus* visceral toxin. (E) Intra-alveolar edema. Haematoxylin-eosin. X450.

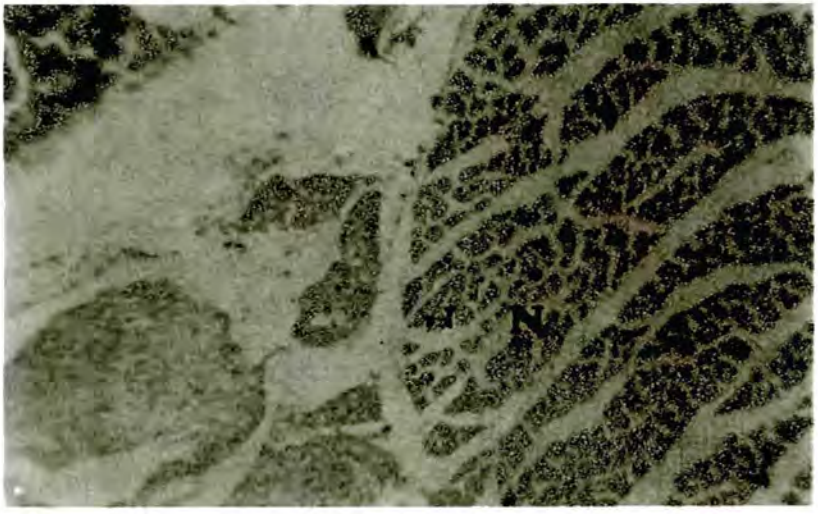

Figure 6. Photomicrograph of skeletal musle of albino Swiss mouse injected i.p. 4 MU *S. canaliculatus* visceral toxin. (N) Loss of nuclear bag inside the muscle spindle. Haematoxylin-eosin. X450.

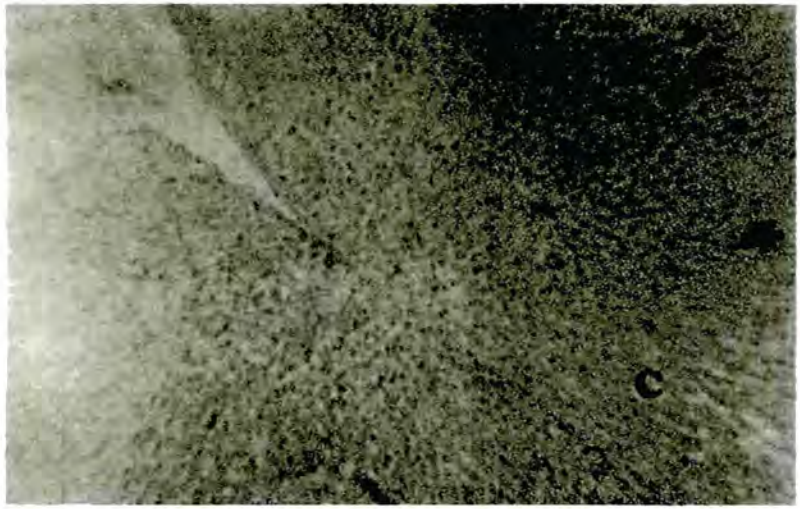

Figure 7. Photomicrograph of spinal cord of albino Swiss mouse injected i.p. 4 MU *S. canaliculatus* visceral toxin. (C) Loss of interconnecting processes. Haematoxylin-eosin. X100.

Figure 8. Photomicrograph of spinal cord of albino Swiss mouse injected i.p. 4 MU *S. canaliculatus* visceral toxin. (C) Loss of interconnecting processes. Haematoxylin-eosin. X450.

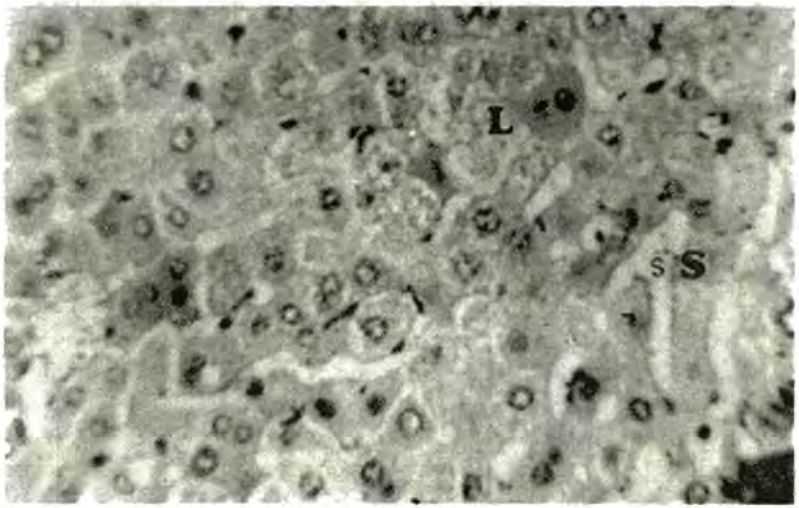

Figure 9. Photomicrograph of liver of albino Swiss mouse injected i.p. 4 MU *S. canaliculatus* visceral toxin. (L) Disintegrating cells. (S) Increased sinusoidal spaces. Haematoxylin-eosin. X450.

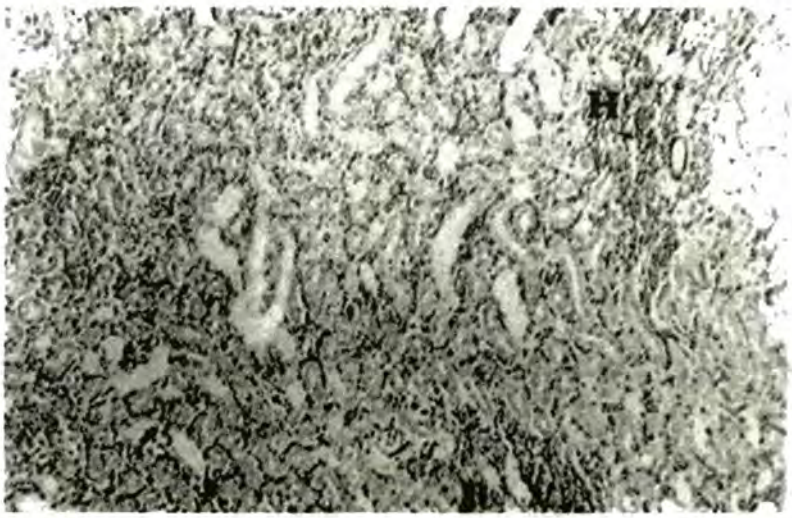

Figure 10. Photomicrograph of kidney of albino Swiss mouse injected i.p. 4 MU *S. canaliculatus* visceral toxin. (H) Hemorrhage. Haematoxylin-eosin. X450.

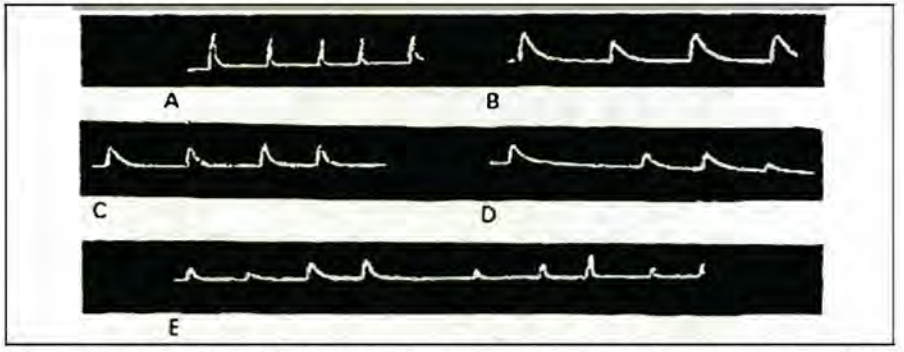

Figure 11. Responses of frog gastrocnemius muscle to electrical stimulus on sciatic nerve. Effects of topical application of 0.4 to 0.8 MU *S. canaliculatus* visceral toxin to sciatic nerve. Kymograph recording (A) immediately prior to application of 0.4 MU toxin, (B) 5 minutes after, (C) 10 minutes after, (D) 5 minutes after application of additional 0.4 MU toxin, (E) 10 minutes after.

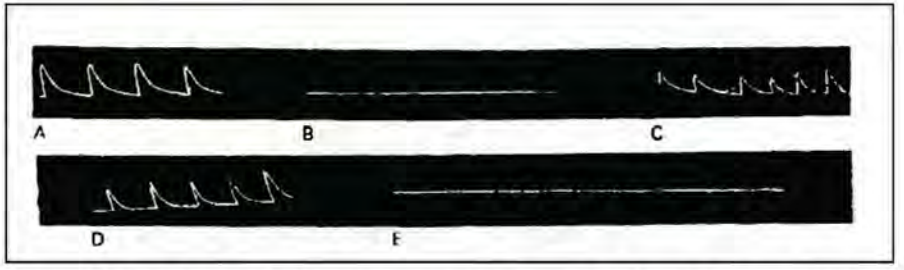

Figure 12. Responses of frog gastrocnemius muscle to direct electric stimulus. Kymograph recording (A) immediately prior to immersion of muscle in 0.4 MU S. canaliculatus visceral toxin, (B) after 10 minutes of immersion in the toxin, (C) after washing of toxin-immersed muscle with Ringer's solution, (D) immediately prior to injection of 0.4 MU toxin into muscle, (E) 5 minutes after injection.

Figure 13. Responses of frog ventricle to 1 MU S. canaliculatus visceral toxin in 2 single doses. Kymograph recording (A) immediately prior to feeding of 1 MU toxin, (B) immediately after feeding, (C) 5 minutes after feeding, (D) immediately after second feeding of 1 MU toxin, (E) 10 minutes after.

Examination of gut contents of *S. caniculatus* yielded 28.6% and 43.0% occurrences, respectively, of *Jania sp.*, and *Spyridia filamentosa* (Table 2). Both are known preferred algal substrates of the most important ciguateric poison - producing dinoflagellate, *Gambierdiscus toxicus* (Yasumoto *et al.*, 1979; Yasumoto *et al.*, 1980; Bagnis *et al.*, 1980; Shimizu *et al.*, 1992). The survey on the algal community in Lingsat Reef found the following macroalgae: Class Chlorophyceae- *Ulva sp., Halimeda macroloba, Chaetomorpha crassa, Caulerpa toxifolia, Caulerpa racemosa;* Class Phaeophyceae- *Sargassum sp, Padina sp, Hydroclathus clathratus; Class Rhodophyceae- Gracillaria verrucosa, Gracillaria salicornia, Galaxaura oblongata, Amphiroa sp., Hypnea boergesenii, Hypnea cervicornis, Jania sp, Laurencia papillosa, Acanthophora spicifera, and Mastophora rosea.*

Table 2. Gut contents of *S. canaliculatus*

Food Item*	Percent Occurrence**
Class Chlorophyceae	
Cladophora sp.	14.3
Caulerpa toxifolia	14.3
Halimeda sp.	28.6
Class Phaeophyceae	
Sphacelaria tribuloides	43.0
Dictyota cervicornish	28.6
Padina sp.	28.6
Hormophysa cuneiformes	14.3
Sargassum sp.	28.6
Dictyopteris sp.	14.3
Lobophora variegata	14.3
Class Rhodophyceae	
Grateloupia sp.	14.3
Amphiroa sp.	28.6
Cheilosporum sp.	14.3
Jania sp.	28.6
Hypnea sp.	43.0
Gracillaria sp.	43.0
Spyridia filamentosa	43.0

*The chlorophyll a content of gut contents by spectrophotometric analysis averaged 4.53 g/100g

**Percent Occurrence = No. of fishes in which food item occurs x 100 / total no. of fishes examined

Three out of ten fish examined had empty guts

DISCUSSION

The ciguateric potential of the Siganid fish, particularly those that were obtained directly from coral reefs, was evident as shown in the mouse bioassays done with several extracts from various fish samples. The histopathological and physiological analyses on some experimental animal models also revealed the potential danger of eating internal organs of the fish and even the flesh especially the large-sized members of the fish family. The toxic manifestations exhibited by the dying mice were similar in almost all instances to the lethality tests and suggested that the various Siganid fishes contain most probably the same kind of toxins. The presence of maitotoxin, one ciguateric poison, was indicated most specifically by the swelling and disruption of ventricular myocardial fibers and the erosion of gastric mucosa in the mice that were administered even sublethal doses of the Siganus visceral toxin. The kymograph recording results of the frog skeletal muscle and heart activity suggested both ciguatoxin and maitotoxin effects. Although no experiments were done to consider the possible role of commonly used fish poisons such as sodium cyanide or the local alkaloidal fruit or bark extracts of the local "bayating" or *Tinomiscium philippinensis* Diels (Menispermaceae), ciguatera, on the other hand, was indicated by the above results.

Although more selective for the fat soluble ciguatoxin, the extraction procedure used in this study could abstract the water-soluble maitotoxin with the use of the aqueous methanol in the later part of the extraction. Considering that maitotoxin is largely contained in ciguateric herbivorous fish (Yasumoto *et al.*, 1995), more of the substantial changes that were observed in the present study could perhaps be attributed to this poison.

Maitotoxin was demonstrated to target the heart and stomach (Terao *et al.*, 1988; Terao *et al.*, 1992). The destructive effects were attributed to the ability of the toxin to activate Ca^{++} channels. It has been suggested that maitotoxin could probably be binding to receptor-mediated Ca^{++} channels (Yasumoto and Murata, 1993), hence, there are the many possible pathways, metabolic or otherwise, which could ultimately cause cell death. Heart beat vigor depends much on the amount of calcium ions made available for the contractile mechanism (Guyton, 1986), so that the initial positive inotropic effect observed in this study could be explained by the increased inward conductance of calcium ions from the extracellular fluid . Nevertheless, the resultant accumulation of calcium would have likely caused the subsequent structural damages and diminution or even abolition of functional abilities such as what were observed in the skeletal muscles that were either injected or immersed in the Siganus toxin.

Ciguatoxin, though it may not be dominant in the extract, could also have affected the heart. Lewis (1988) reported that while low doses of ciguatoxin could induce positive inotropic response in the guinea-pig atria and papillary muscles by opening myocardial voltage-gated Na^{+} channels, high doses could cause negative inotropy associated with cell depolarization and signs of calcium overload.

In the initial experiment, whereby electrical stimulation was applied to the frog's sciatic nerve which was previously administered with the toxins, there was a resultant increase in the strength and duration of the gastrocnemius muscle twitch. This could be due to a prolonged presynaptic depolarization and subsequent increased Ca^{++} influx, increased amount of transmitter release, greater amplitude and duration of the muscle action potential, greater amount of Ca^{++} to effect the sliding of the filaments, and longer duration of the relaxation phase of the twitch. Capra (1992) reported that during electrical stimulation of the ventral coccygeal nerve in the rat tail, the administration of sulblethal dose of ciguatoxin increased the refractory period and extended the magnitude and duration of the supernormal period of the compound action potential. These changes were explained by the increased ease in the opening of the Na^+ channel and the time course of Na^+ channel opening that was caused by the toxin. Based on structure, ciguatoxin could undergo slow conformational change while binding to the voltage-sensitive sodium channel and alter the gating or inactivation mechanism of the channel (Yasumoto and Murata,1993). The physiologic activity of ciguatoxin , that of increasing sodium ion influx, has been described as similar to that of brevetoxin which binds to receptor site 5 on the α–subunit of voltage-gated sodium channel (Baden, 1995). Again, the paralysis that ensued after treatments by topical application on the nerve, immersion or injection into muscle would have to be attributed to the combined actions of both maitotoxin and ciguatoxin. Another possible action of ciguatera toxins that has been suggested is the antagonism of the nicotinic cholinergic receptor (Escalona de Motta *et al.*, 1992).

Furthermore, it may be worthy to note that macroalgae constitute the bulk of the diet of the Siganid fish. This was suggested by the high chlorophyll content in the gut contents. The occurrences of some algae that are known to harbor ciguatera poison-producing dinoflagellate in the food of the Siganid fish could be associated with the ciguateric potential. That the algae were also found in the algal community in the Lingsat Reef in La Union could also be significant. One important future study would be to actually obtain the toxic dinoflagellates and establish the reef as ciguateric.

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UPDATE: JAPANESE ENCEPHALITIS VIRUS ACTIVITY IN THE PHILIPPINES

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The virus, its transmission in nature, and the disease

Japanese encephalitis virus (JEV) causes acute encephalitis in humans and is principally rural in distribution. JEV is one of two members of the Flavivirus family that is active in the Philippines, second to the dengue viruses in prevalance. Recovery from JEV infection endows a lifelong immunity against JEV.

Transmission of JEV in nature is by means of a vector, the Culicine mosquitoes, that breed in the vast expanse of rice fields throughout the Philippines. This is a biologic transmission, meaning that the virus must first replicate in the gut of the mosquito before it can transmit the JEV while taking a blood meal. In nature, JEV is maintained in the amplifying host, hogs and domestic birds. Humans and vertebrates are incidental hosts. JEV is transovaially passed in the Culex mosquitoes.

The clinical features of encephilitis due to JEV are: 5 to 15 to 21 days of incubation period, 2 to 4 days of prodromal phase, 5 to 9 days of neurologic symptoms, that os prolonged seizures, respiratory dysfunction and finally death. The phatogenesis includes: transplacental infection leading to abortion, children, 3 to 15 years old, are primarily affected, and the ratio of apparent to inapparent infections ranges from 1:25 to 1000. In the brain, pathological features include neuronal degeneration, small hemorrhages with perivascular cuffing and monocytic infiltration. The disease is severe and life threathening with a case fatality rate of 25% and a rate of neurologic sequelae among surviving patients of 32 to 45%.

Japanese Encephalitis: a disease burden in Asia

Morbidity rates in China ranges from 5.5 to 24/10,000 population while in Taiwan and Thailand, it ranges from 1.8 to 2.5/10,000 population. In Indonesia,

Malaysia and the Philippines, 17 to 50% of hospitalized cases of viral encephilitis are due to JEV indicating high endemicity.

In Thailand, the case fatality rate among cases of Japanese encephalitis is 25%.

Japanese encephalitis among surviving patients suffer severe neurological sequelae. In Guam (1947) where the best documented follow-up of cases after ten years was reported, 40% continue to suffer disabilities, in Shanghai, China (1973-1997) 32% and in Thailand 45%.

Two-thirds of Asia's population live in rural areas, therefore 3 billion are at risk, chiefly children who are less than 15 years old. Based on the 1994 population estimates, 700 million are less than 15 years old and are at high risk. Thus, it is predicted that the annual incidence is 175,000 cases out of which 43,750 deaths and 78,750 disability cases occur as a result of Japanese encephalitis infections (assuming 2.5/25,000 children at risk, 25% of cases are fatal and 45% of surviving patients retain neurologic deficit).

Underreporting outweight overestimation of cases even if all cases of clinical encephilitis are reported without laboratory confirmation. Furthermore, atypical disease presentation contribute to underreporting (such as Guillain Barre syndrome, milder febrile illnesses without signs of enccphilitis, acute psychosis and deaths outside hospitals).

As early as 1958, Dr. William McD. Hammon, Professor of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennyslvania, USA and Commisioner of the US Army Epidemiological Board, reported serological evidence of JEV presence in the Philippines (Table 1 from Hammon et al, 1980). Neutralizing antibodies against JEV were significantly higher among rural (Negritos around the former Clark AIrbase and Sapang Bato in Pampanga) than among urban children consulting at the Philippine General Hospital representing urban areas. Dr. Hammon argued that these JEV neutralizing antibodies can not be cross-reacting antibodies due to dengue virus because the percentages of seropositively between two viruses in the same area have values counter to each other be it in the rural or urban areas. Others confirmed these findings (Basaca-Sevilla and Halstead, 1966, Macasaet et al, 1970, and Venson et al, 1970).

In 1977, JEV was first isolated in the Philippines from *Culex tritaneurynchus* caught by CDC traps from four barrios in Tagudin, Ilocos Sur (Table 2 Trosper et al, 1980) and from San Jose, Nueva Ecija (Table 3 from Ksiazek et al, 1980). *Culex vishnui, Culex bitaneurynchus* and *Anopheles annularis* yielded also JEV isolates, but their role in the transmission of Jev in nature has to be established. Table 4 shows the identification of two isolates: Ph Ar 281 and Ph Ar 382 by micro-neutralization test. Only the JEV antiserum gave a clear and specific neutralization of the presence of JEV in the Philippines.

Table 1. Group B viruses. Positive results of neutralization test

		JBE		Dengue*		MVE		WN		Ntaya		Zika		SLE		Ilheus		Uganda S	
Locality	Age (yrs)	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%
Negritos	6/12-4	7/18	39	4/14	29	1/14	7	2/12	17	1/11	9	0/11	0	0/10	0	1/10	10	0/4	0
	5-9	18/23	78	7/19	37	2/19	10	2/15	13	4/20	20	0/19	0	0/10	0	0/20	0	0/17	0
	10-14	5/6	83	4/6	67	3/6	50	2/4	50	2/6	33	1/6	17	1/6	17	1/6	17	0/4	0
	15-19	15/15	100	6/12	50	7/15	47	6/15	40	4/11	36	3/11	27	3/15	20	2/11	18	0/3	0
	20+	61/62	98	38/54	70	34/62	55	36/62	58	28/53	53	8/51	16	21/61	34	11/53	19	0/13	0
Sapang	10-14	12/52	23	22/40	55	8/52	15	6/52	11	8/29	28	3/24	12	5/31	16	0/26	0	0/16	0
Bato Fields	15+†	25/51	49	–		–		–		6/12	50	3/12	25	–		1/12	8	0/12	0
Manila	6/12-4	1/32	3	12/33	36	1/26	4	3/27	11	5/22	23	1/13	8	1/23	4	0/18	0	0/2	0
Urban	5-9	4/23	17	13/21	62	4/21	19	6/12	29	7/13	54	0/6	0	5/20	25	0/7	0	–	0
Total		148/282	52	106/199	53	60/215	28	63/208	30	65/177	37	19/153	12	36/176	20	16/163	10	0/71	0

*Combined results of HAI and neutralization tests with viruses of both types.

†Food handlers principally form this general area.

(From: Hammon et al, 1980)

Table 2. Mosquitoes collected near Tagudin, Ilocos Sur Province, Luzon, Republic of Philippines, July 1977

Species	No. of mosquitoes (%)	No. of pools	No. of positive pools
*Culex tritaeniorhynchus**	19,677 (59.1)	210	1
*C. vishnui**	4,895 (14.7)	63	1
C. fuscocephala	981 (2.9)	19	0
C. bitaeniorhynchus	109 (0.3)	5	0
C. gelidus	180 (0.5)	8	0
C. whitmorei	15 (<0.1)	1	0
C. fuscanus	16 (<0.1)	3	0
C. fatigans	16 (<0.1)	2	0
C. pseudovishnui	14 (<0.1)	1	0
Anopheles anuularis	2,851 (8.6)	35	0
A. vagus	1,210 (3.6)	17	0
A. peditaeniatus	108 (0.3)	4	0
A. indefinitus	431 (1.2)	5	0
A. tessellatus	20 (0.1)	1	0
Mansonia uniformis	142 (0.4)	6	0
Aedeomyia catastica	1,244 (3.7)	21	0
Aedes vexans	1,134 (3.4)	24	0
A. lineatopennis	215 (0.6)	8	0
A. albopictus	9 (<0.1)	1	0
Mimomyia luzonensis	7(<0.1)	1	0
Total	33,274(100.0	435	2

*Isolation: Ph Ar 281 C. tritaeniorhynchus pool of 100.
Ph Ar 384 C. vishnui pool of 70.
(From: Trosper et al, 1980)

To completely show on firm grounds that JEV present and actively cause encephalitis among humans in the Philippines, studies by V.F. Chan et al at the College of Public Health, UP Manila Virus Laboratory showed that viral encephalitis cases were due to several viruses, one of them JEV (Table 5 ; note Case No. 14130, female, 2 years and 10 months old with a sustained highly significant antibodies titer of 1:1280 in both the first and second blood samples (Chan et al, 1983). Based on the initial findings, we went on to test all cases of viral encephalitis cases who were referred to the Virus Laboratory during the period of 1981-1982. Indeed, more viral encephalitis cases were found to be due to JEV infection (Table 6, Chan et al, 1983). It was nessecary, however, to demonstrate that the antibodies against JEV were monotypic and specific and cross-reacting antidengue

Table 3. Mosquitoes collected near San Jose, Nueva Ecija Province, Luzon, Republic of the Philippines during August 1977 from which virus isolations were attempted

Species	No. of mosquitoes (%)	No. of pools	No. of positive pools
Culex vishnui	50,652 (63.8)	525	1*
Cx. tritaeniorhynchus	11,396 (14.4)	139	0
Cx, fuscocephala	4,183 (5.3)	57	0
Cx, annulirostris	2,801 (3.5)	33	0
Cx, bitaeniorhynchus	1,744 (2.2	29	0
Cx, whitmorei	1,734 (2.2)	28	0
Cx, gelidus	820 (1.0)	14	0
Cx, fuscanus	117 (0.2)	4	0
Cx, fatigans	36 (0.0)	1	0
Anopheles annularis	2,491 (3.2)	32	0
An. peditaeniatus	1,936 (2.5)	27	0
An. indefinitis	300 (0.4)	3	0
An. tessellatus	200 (0.3)	4	0
Aedes vexans	247 (0.4)	7	0
Ae. lineatopennis	164 (0.2)	3	0
Mansonia uniformis	307 (0.4)	7	0
Total	79,157 (100.0)	913	1

**GET virus isolate, pool of 80 Cx. vishnui.*
(From: Ksiazek et al, 1980)

antibodies because the serologic test used was hemmaglutination-inhabitation (HI) test. To our delight, the HI antibodies were found only against JEV and not against the dengue viruses. (Table 7).

Does JEV feature in outbreaks in the Philippines? YES, and we recall the 1982 epidemic in Nueve Ecija that we documented by showing significant antibody rise in HI antibodies against JEV in paired blood samples of cases.

Prevention and control

Vaccination of the population at risk, children 15 years old and younger, is only the effective option to prevent occurence of Japanese encephalitis in the population. The successful reduction of morbidity due to anti-JEV vaccination is clearly shown by the experience in Japan where inactivated vaccine (mouse brain-derived) has been used since many years back, and also that of China where atttenuated vaccine is being used. The only commercially available vaccine, how-

Table 4. Identification of isolates Ph Ar 281 and Ph Ar 384 by micro-neutralization tests using 1.5-2.5 Log10 virus dose against varying serum dilutions

Sera	JEV (NAK)	281	384	Virus WN (Eg 101)	MVE (Orig.)	SLE (Parton)
JEV*	160	120	320	<10	<10	<10
281**	12	24	12	<4	ND	ND
384**	4	8	12	<4	ND	ND
WN*	<10	<10	<10	640	ND	ND
MVE*	<10	<10	<10	ND***	160	ND
SLE*	<10	<10	<10	ND	ND	160

* Hyperimmune mouse ascitic fluid (HMAF).

** 2 dose mouse antisera.
In addition, the following HMAF were tested against Ph Ar 281 and Ph Ar 384 viruses and found to react at <1:10; CHIK (S-27), GET (MM2021), BEB (MM2354), SIN (AR339, WHA (M78), SAG (Orig.) EEE (Ten Broeck), WEE (Fleming), DEN-1 (Hawaii), DEN-2 (New Guinea-C), DEN-3 (H-87), Den-4 (H-241), TMU (MM1775), LGT (TP21, ZIKA (MR8766), YF (17D), SEP (Aus MK7148), KUN (MRM16), ING (India 633970), BUN (Orig.), BAT (MM2222), BAK (MM2325), UMB Ig 1424) and Normal Mouse Ascitic Fluid.

*** ND = Not Done

ever, is inactivated vaccine which is given three doses on days 0, 7, and 30 by intramuscular or subcutaneous injection and boosted every three years. Travelers to hyperendemic areas are also devised to have their complete vaccination (3 doses) prior to departure.

Vaccination of the amplifying hosts, hogs and domestic birds, is not cost-effective and is not practical, and can not be enforced because when the hogs are JEV infected, they recover. It is only when the pregnant sows that get infected wahen the disease manifests as abortion. Consequently, in countries where central hog farming is practiced the economic undertone of JEV infection is tremendously devastating.

Vector control is not a practical either, because of the bionomics of the Culicines. It will very costly to reduce the source of the Culicines considering their distribution in nature particularly in rice fields. Around one's immediate environment though, eliminate all possible receptacles of water, drain canals of stagnant water, and empty the garbage cans regularly to prevent them from breeding which is the best option to be JEV infection-free. Remember, the Culicines are the mosquitoes that bite you night-time and you hear them buzzing around your ears. Kill the mosquitoes before they kill you.

Table 5. Cytomegalo, Herpes simplex, and Japanese encephalitis viruses as etiologies of viral encephalitis among Filipinos, 1982

Case no.		Reciprocal antibody titers against viruses				Viral etiology
		Cytome-galo v.	Herpes simplex	Dengue 2	Japanese Encephalitis	
14075 Female	First	16	8	<20	<20	Cytomegalovirus
18/12 yr.	Second	16	8	<20	<20	
14076 Male	First	<8	64	<20	<20	Herpes simplex virus
15 yr.	Second	<8	128	<20	<20	
14117 Female	First	64	16	80	40	Cytomegalovirus
7/12 months	Second	64	<8	160	80	
14130 Female	First	8	8	<20	<1280	Japanese encepha-litis virus
2 10/12 yr.	Second	8	8	<20	<1280	
14134 Male	First	<8	<8	<20	<20	Negative; un-confirmed
6 6/12 yr.	Second	<8	8	<20		
14158 male	First	8	16	20	40	Herpes simplex virus
Age?	Second	8	16	20	40	
14517 Sex & Age	First	<8	<8	20	20	negative; un-confirmed
unknown	Second	<8	<8	20	40	
14708 Male	First	<8	16	40	160	Herpes simplex virus
19 yr.	Second	<8	16	40	160	
14727 Male	First	<8	<8	20	20	negative; un-confirmed
10 yr.	Second	<8	M8	20	20	

From: Chan et al, 1983.

Table 6. Serological responses of human viral encephalitis cases against japanese encephalitis virus, Metro Manila, 1982

A. Cases with paired blood samples (6)

Case No.	Age years	Reciprocal of HI antibody titer versus Japanese encephalitis virus		Interpretation
		First Blood	Second Blood	
14045	7	20	80	Jap. enceph. infection
14068	30	40	40	Not Jap. enceph. inf.
14075	18/12	<20	<20	Not Jap. enceph. inf.
14130	2	>1280	>1280	Jap. enceph. infection
14134	6 1/12	<20	20	Not Jap. enceph. inf.
14158	4	<20	<20	Not Jap. enceph. inf.

B. Cases with single blood sample (22)

Reciprocal of serum dilution	Number of viral encephalitis cases with HI titers	Percentage
< 20	9	
20	4	
40	4	81.8
80	1	
160	1	
320	0	
640	1	
1280	2	18.2
2560		
Total	22	100

From: Chan et al, 1983.

Table 7. Monotypic HI Antibody Titers Against Japanese Encephalitis Virus in cases of Human Viral Encephalitis, Metro Manila, 1982

Case No.	Serum	Reciprocal of HI Antibody Titers Against Viruses	
		Dengue 2	Japanese encephalitis
14130	First*	< 20	≥ 1280
	Second**	< 20	≥ 1290
14094	First	< 20	≥ 1280
14096	First	< 20	≥ 1280
14316	First	ND	640
14554	First	ND	≥ 2560

*CFT titers versys cytomegalovirus and Herpes simplex virus were consistently. 1:8.
From: Chan et al, 1993.

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DETECTION OF DENGUE VIRUS AND ANTIBODIES IN PATIENT SERA COLLECTED FROM 1995 TO 1999 IN THE PHILIPPINES

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ABSTRACT

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are persistent public health problems in the Philippines. In this study, two molecular techniques, reverse transcription-polymerase chain reaction (RT-PCR) and IgM-capture ELISA, are used to detect the presence of dengue virus and dengue antibodies in patient sera, respectively. Serum samples of clinically diagnosed DF and DHF patients from different hospitals in Metro Manila and from field collections of the Field Epidemiology Training Program of the Department of Health were used. From the samples collected from January 1995 to May 1999, a total of 1,429 sera were analyzed by RT-PCR and 149 (10.4%) samples were positive for dengue virus. Serotyping using serotype-specific primers showed that the predominant serotypes in the Philippines during this period are dengue serotypes 2 and 3. A total of 1,391 sera was tested for the presence of antibodies to dengue by IgM-capture ELISA using tetravalent dengue antigen. Seven hundred sixty two samples (57.8%) were found positive for anti-dengue IgM.

Key words: dengue virus, dengue fever, dengue hemorrhagic fever, molecular techniques, reverse transcription-polymerase chain reaction, IgM-capture ELISA, antibodies, serotype, tetravalent dengue antigen, anti-dengue IgM.

INTRODUCTION

Dengue virus causes millions of human infections each year. Illnesses resulting from dengue infections range from mild fever to severe hemorrhagic fever and sometimes fatal shock (Chan, 1987). The dengue virus is found in both tropical and subtropical regions of the world and its distribution overlaps with other pathogenic flaviviruses such as yellow fever and Japanese encephalitis virus. Hence, interpretation of both clinical and serological diagnosis can be complicated due to similar antigenic determinants that can elicit cross-reactive antibodies against these viruses (Bundo and Igarashi, 1985).

Hemagglutination-inhibition (HI) test and indirect immunofluorescent antibody tests (IFAT) have been used to detect and identify dengue viruses (Chan, 1987; Capeding *et al,* 1987; Hayes *et al* 1988; Henchal *et al*, 1982; Manoloto and Hayes, 1989). Virus serotype which infects is inferred by measuring a fourfold or greater increase in antibody titers to the particular infecting serotype. Specific diagnosis or identification may not be accurate due to the cross-reactivity of antibodies to different flaviviruses. In tests wherein paired serum samples are needed, this requirement causes delay in diagnosis. Virus isolation from patient serum has been achieved using cell culture methods as well as mosquito inoculation (Hayes *et al,* 1988). However, virus isolation takes from days to weeks and traditional virus identification is not always successful due to the small amount of viable virus in the sample. There is a need for an assay system that can be performed rapidly and is sufficiently sensitive and specific to be useful.

IgM antibodies have been reported to be more specific than total antibody titers in the detection of flaviviruses. Bundo and Igarashi (1985) developed an IgM capture ELISA that could differentiate Japanese Encephalitis infection from dengue infection. IgM has a distinct advantage of having multiple binding sites and is present during early stages of infection. Furthermore, polymerase chain reaction (PCR) development has greatly facilitated the development of several diagnostic protocols for detecting and identifying dengue viruses (Eldadah *et al,* 1991; Lanciotti *et al,* 1992; Morita, 1994; Seah *et al,* 1995; Thayan *et al,* 1995).

In this study, we have employed IgM-capture ELISA assay to detect anti-dengue antibodies in the serum samples collected by the different collaborating hospitals. RT-PCR was also used to detect and identify infecting serotype.

MATERIALS and METHODS

Serum Samples

Serum samples from patients suspected to have, or have been clinically diagnosed dengue fever or dengue hemorrhagic fever from the various collaborating hospitals were collected and sent to the Research and Biotechnology Division (RBD) of the St. Luke's Medical Center, Quezon City, Philippines. Serum samples were aliquoted in 1.5 ml cryovials and stored at -86°C until use.

Cell Culture and Virus Detection

Confluent cultures of *Aedes albopictus* C6/36 cells were inoculated with 20 µl of patient serum and the virus was allowed to adsorb for 2 hours at 28°C with agitation at 30 min. intervals. Cells were overlayed with 2 ml of Minimal Essential Medium containing 2% heat-inactivated fetal bovine serum and 0.2 mM each of nonessential amino acids and were kept at 28°C. Culture fluid and infected cells were harvested 7 to 10 days after inoculation. Dengue virus was detected from infected culture fluid and RNA extracts from infected cells by RT-PCR using dengue consensus primers. RT-PCR using dengue serotype specific primers was further performed on the culture fluid containing the virus.

RNA Extraction

The infected cell pellet was treated with 400 µl Trizol LS Reagent (Gibco, BRL). The cell lysate was passed several times through a pipette then transferred to a 1.5 ml eppendorf tube and kept for 5 min at room temp. Chloroform (150 µl) was added and the tubes shaken by hand vigorously for 15 sec. The tubes were then incubated at room temperature for 10 min and then centrifuged at 12,200 rpm for 15 min at 4°C. The aqueous phase was transferred to a sterile 1.5 ml tube and the RNA precipitated by adding 300 µl isopropyl alcohol. This was mixed by tube inversions and incubated overnight at –86°C. The samples were then centrifuged at 12,200 rpm for 10 min at 4°C. The supernatant was removed and the RNA pellet washed once with 500 µl 70% ethanol by vortexing. The pellet was then recovered by centrifuging for 5 min at 4°C. The supernatant was removed and the RNA pellet was vacuum-dried for 5 min. The RNA was then re-dissolved in 50 µl DEPC-treated water and incubated at 55°C for 10 min with little agitation. The RNA extract was stored at –86°C until use.

Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR)

For RT-PCR of infected culture fluid, 100 µl of ICF was heat-inactivated at 65°C for 10 min. 3 µl of detergent mix (1% NP-40 and RNAse inhibitor in 5:1 mixture) was mixed with 2.5 µl of the heat-inactivated ICF, and incubated at room temp. for 1 min. This was added to a master mix, previously prepared to give 50 µl final volume of each reaction, containing 200 nM each dNTPs, 25 pmol sense primer, 25 pmol antisense primer, 2.5 U Tth polymerase, and 5 U reverse tran-

scriptase. For RT-PCR of the RNA extract of infected cells, 2.5 µl of the RNA extract was added to the master mix to give 50 ul reaction mixture of the same composition as the above. The thermocyclers (Eppendorf Mastercycler 5330 and Perkin-Elmer Gene Amp PCR System 2400) were programmed to run the following reaction conditions in the order: reverse transcription at 53°C for 20 min, 35 cycles of denaturation at 94°C for 60 sec, annealing at 53°C for 60 sec, and extension at 72°C for 90 sec, then a final extension at 72°C for 10 min. RT-PCR products subjected to electrophoresis in 2% agarose gel were stained with ethidium bromide and visualized under UV illumination.

IgM-capture ELISA

The 96 well ELISA plate was coated with µ-chain specific anti-human IgM diluted in coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6 containing 0.02% NaN3) and incubated at 4°C overnight. Nonspecific binding sites were blocked with blockace for 1 hour at room temperature followed by washing with PBS-Tween (0.05% Tween-20 in PBS-). The ELISA plates were reacted with the test and standard positive sera at appropriate dilutions for one hour at room temperature. The plates were washed as above. Assay antigen (monovalent and tetravalent antigen), 100 µl, containing 25 ELISA units of each dengue serotype was added into each well and incubated as above. After washing, the plates were reacted with antiflavivirus IgG for 1 hour, which had been previously conjugated to horseradish peroxidase (HRPO). The plates were washed as above and HRPO reaction was carried out using 0.05% o-phenylenediamine dihydrochloride and 0.01% H_2O_2 in 0.05 M citrate-phosphate buffer, pH 5.0 in the dark. The reaction was terminated by adding of 1 N H_2SO_4. The ELISA OD was measured at 492 nm. The ELISA titer of the test serum samples was estimated by comparing its OD with those of the serially diluted positive standard. The test specimen scored positive when its P/N ratio is equal to or greater than 2.

RESULTS

A total of 1,429 serum samples was processed for virus detection by RT-PCR using flavivirus specific primers. Of these serum samples, 149 (10.4%) were RT-PCR positive (Table 1). Ninety (90) samples were serotyped by RT-PCR using serotype specific primers. There were 46 dengue serotype 2 (den 2) viruses detected followed by 31 den 3, 12 den 1 and 1 den 4 (Table 2). Figure 1 shows the RT-PCR products of 3 dengue positive isolates using serotype-specific primers.

A total of 1,391 serum samples were tested for the presence of IgM. Of these serum samples, 762 (57.8%) were IgM positive (Table 3). A comparative experiment using the individual 4 monovalent dengue antigens or combined (tetravalent) as assay antigen was done. Table 4 shows that out of 179 serum samples, 73 (41%) serum samples reacted with den 1, while 68 (38%) samples

Table 1. Detection of dengue virus by RT-PCR using dengue consensus primers.

	1995	1996	1997	1998	1999	Total
Number of samples tested	469	166	74	523	197	1,429
RT-PCR +	59	16	17	51	6	149
Percentage	12.6	9.6	23	9.8	3	10.4

Table 2. Serotyping of dengue virus by RT-PCR using type specific primers.

	1995	1996	1997	1998	1999	Total
Dengue 1	1	1	0	5	5	12
Dengue 2	21	8	8	8	1	46
Dengue 3	19	2	4	6	0	31
Dengue 4	0	0	1	0	0	1
Total	41	11	13	19	6	90

Figure 1. RT-PCR products of 3 dengue isolates using dengue serotype-specific primers. *Lane 1,* 99 St-12 is Den 1 (490 bp); *lane 2,* SLMC 70 is Den 2 (230 bp); *lane 3,* DOH 109 is Den 3 (320 bp). *M,* 100-bp marker.

Table 3. Detection of dengue virus by IgM Capture ELISA using tetravalent dengue antigen.

	1995	1996	1997	1998	1999	Total
Number of Samples Tested	164	67	23	821	244	1,391
IgM ELISA +	91	27	10	529	105	762
Percentage	55.5	40.3	43.5	64.4	43	57.8

Table 4. Comparison of the sensitivities of different monovalent antigens and tetravalent antigen used as an assay antigen in IgM capture ELISA.

n = 179	Monovalent Antigen				Tetravalent
	Den 1	Den 2	Den 3	Den 4	Antigent
1995	14	17	12	9	32
1996	2	2	2	2	2
1997	3	2	3	0	5
1998	54	47	45	26	78
Total	73	68	62	37	117

reacted with den 2 and 62 (35%) samples reacted with den 3. Only 37 (21%) serum samples were positive using den 4 as an assay antigen. On the other hand 117 (65%) serum samples gave a positive result when all 4 dengue serotypes were used as an assay antigen.

IgM and RT-PCR results were correlated with the collection day of the serum samples relative to the onset of fever (Table 5). There was an increase in the number of IgM positive cases if and when the serum was obtained several days after the onset of fever. On the other hand, the highest number of RT-PCR positive results was obtained 2-3 days after onset of fever. The number of positives declined thereafter.

DISCUSSION

The dengue virus of which there are 4 serotypes (den 1, den 2, den 3 and den 4) causes dengue fever and dengue hemorrhagic fever. In the Philippines, epidemic proportions of DHF have been occurring every 3 to 5 years (Chan, 1987). Serological diagnosis by HI have been performed to confirm initial clinical diagnosis and virus isolation using AP-61 cells and identification by

Table 5. Effect of the time of serum collection on the sensitivity of IgM capture ELISA and RT-PCR.

Age of Serum (Date of blood extraction-Date of onset of fever)	No. of IgM positive cases n = 762	No. of RT-PCR positive cases n = 149
0 - 1	18	11
2 -3	69	54
4 -5	170	36
6 -7	151	13
> 7	194	7
no data	160	28

immunofluorescent antibody tests using specific monoclonal antibodies was done at NAMRU-2 (Chan, 1987; Hayes *et al*, 1988). The predominant serotype from 1983 to 1984 as determined by Hayes *et al* (1988) was den 2 followed by den 1 and den 3. Den 4 was the least common. Den 3 was the most common serotype isolated from 1983 to 1986 (Manaloto and Hayes, 1989). Capeding *et al* (1997) isolated 20 dengue viruses from 143 serum samples submitted from 1992 to 1993. Of the 20 isolates, 16 belong to serotype 1 and 4 belong to serotype 2 complex. There was no reported isolation of den 3 or den 4. Capeding *et al* (1997) used the immunofluorescent test using type-specific monoclonal antibodies. In this study, we have used RT-PCR to detect and identify as well as specifically serotype the dengue virus. Similar to the NAMRU-2 study, we isolated and identified more den 2 viruses. On the other hand, we detected more den 3 than den 1 serotypes. Again, den 4 was the least detected.

The specificity of the RT-PCR assay depends on the ability of the type specific primers to recognize unique and specific nucleotide sequences in the virus genome (Lanciotti et al, 1992). Although false positive PCR results have been described in other PCR-based assays, this can be avoided by routinely performing several precautionary measures such as physical separation of pre- and post PCR procedures, use of positive displacement pipettes, careful handling and UV irradiation of reaction mixtures (Lanciotti *et al*, 1992; Morita, 1994). The use of appropriate positive and negative control also helps differentiate tube to tube contamination. The accuracy, sensitivity and speed of the RT-PCR assay makes it an effective method both for diagnosis and epidemiological surveillance (Eldadah *et al*, 1991; Seah *et al*, 1995; Thayan *et al* 1995). This assay method can be used to complement existing techniques such as the IgM capture ELISA method.

Furthermore, it can be used to amplify other regions of the virus genome for faster sequence analysis, which is very much useful for genetic and evolutionary studies.

Acute dengue cases have been diagnosed using IgM capture ELISA (Bundo and Igarashi, 1985). IgM antibodies are specific and indicative of early infection. Furthermore, IgM antibodies directed towards dengue antigen usually persist for only 2 to 3 months after an acute infection. Using IgM capture ELISA, it is possible to make a presumptive diagnosis using a single serum sample. In this study, 762 out of the 1,391 serum samples analyzed by IgM capture ELISA were positive suggesting early infection. More serum samples containing the anti-den IgM antibody were detected when all four dengue serotypes were used as the assay antigen. Using individual serotypes as assay antigen, den 1 seems to detect the highest number of sera containing IgM anti-den antibodies, followed by den 2 and den 3. Den 4 was the least reactive. While RT-PCR showed that den 2 and 3 are the predominating co-circulating serotypes, den 1 assay antigen is cross reactive to the other 4 serotypes. This probably explains why previous authors identified more den 1 serotypes using immunofluorescent antibody tests even with the use of monoclonal antibodies. Furthermore cross-reactivity to other flaviviruses always exists. It is therefore suggested that antigens from other flaviviruses endemic in that particular area be included in the future assay to make definitive diagnosis.

While it is tempting to compare the 2 diagnostic protocols (IgM-capture ELISA and RT-PCR), caution must be made in doing so. The former detects and measures antibodies whereas the latter detects virus genome. There are few cases which simultaneously have both anti-dengue IgM and the dengue virus or just the virus without anti-dengue IgM antibodies (Table 6). Most of the serum samples tested contained only anti-dengue IgM antibodies (Table 6). This is probably because most of the DHF cases would be secondary infection which shows shorter viremic period than the primary infection or most of the sera were collected at a

Table 6. Correlation between the presence of anti-dengue IgM antibodies and dengue virus.

	IgM+/RT-PCR+	IgM-/RT-PCR+	IgM+/RT-PCR-	IgM-/RT-PCR-
1995	4	11	84	57
1996	2	6	24	31
1997	3	10	5	3
1998	17	27	254	120
1999	2	4	82	106
Total	28	58	449	317

later part of the disease. Table 5 shows that if the serum samples are collected at a later time, the chances of detecting the virus by RT-PCR decreases whereas the chances of detecting anti-dengue IgM antibodies increases. It has been shown by previous authors that the highest detection rate of dengue virus infection by IgM capture ELISA was in the convalescent phase, while RT-PCR detection was more successful in the acute phase (Saat et al, 1994).

In conclusion, since one of the drawbacks of RT-PCR is that it is expensive, we recommend that serum specimens obtained from acute dengue cases first be tested by the IgM capture ELISA. Samples that are found to be negative should then be examined by RT-PCR to increase the diagnostic efficiency.

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DIAGNOSIS OF DIFFUSE HEPATOCELLULAR DISORDERS IN DAIRY CATTLE THROUGH ULTRASONOGRAPHY AND DIGITAL ANALYSIS

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ABSTRACT

Current diagnosis of liver disorders in cattle through clinical signs and biochemical analysis is not very accurate. In this study, evaluation of diffuse hepatocellular disorders in 226 Holstein-Friesian dairy cattle was conducted using ultrasonography and computerized analysis of ultrasonograms (digital analysis). Different echo patterns were distinguished, namely: bright pattern (BP), deep attenuation, vascular blurring and blurring of edges (BE) in fatty change of the liver; dark pattern and BE in hydropic degeneration; and BP in amyloidosis and hepatic dystrophy. In digital analysis of hepatic B-mode ultrasonograms, low echo means in hydropic degeneration, steep decline of echo means in fatty change, and high initial echo mean for hepatic dystrophy and amyloidosis were observed. The combination of ultrasonography and digital analysis provided a relatively more accurate method of diagnosis of diffuse hepatocellular disorders in dairy cattle.

Key words: amyloidosis, cattle, digital analysis, fatty change, hepatic dystrophy, hydropic degeneration, liver, ultrasonogram, ultrasound

INTRODUCTION

Diagnosis of hepatocellular disorders in dairy cattle is relatively difficult. The problem lies not only on the relative inaccessibility of the liver for examination, but most importantly on the multiple functions the liver performs. Thus, primary disorders of the liver can cause disorders in other regions of the body and disorders in other organs and tissues of the body can likewise cause secondary disorders in the liver.

Presently, diagnosis of hepatocellular disorders in dairy cattle has been made mainly on the bases of clinical signs, biochemical analysis and examination of biopsy tissue. Biopsy combined with examination of biopsy tissue, however, cannot be used routinely for diagnosis. In humans, although advanced diagnostic proce-

dures, including imaging techniques have been used, these methods, particularly, ultrasonography has not been applied widely in animals, especially in dairy cattle.

Diagnostic ultrasound or ultrasonography is one of the emerging imaging diagnostic tools that is currently being applied in both small and large animals. It is less invasive and has no danger of radiation, as compared with x-ray but with comparable resolution and accuracy. In many cases, the real-time capability of diagnostic ultrasound is better than that of x-ray. Ultrasonography is a technique used to locate or delineate deep tissues or structures by measuring the transmission or reflection of ultrasonic waves. It is a relatively safe, rapid, non-invasive procedure with high penetration and resolution used to image tissues and organs, in both animals and man. In addition, no special shields or building construction are required and many of the current instruments are portable (Cartee, 1981).

Computer analysis of ultrasonographic echoes, including digital analysis, has been used in humans in the diagnosis if diffuse disorders of the liver. Computerized ultrasound examination yields more information from the ultrasound image than normal observation, leading to increased diagnostic accuracy (Haberkorn *et al.*, 1989). Digital analysis can quantify the ultrasonogram images and assist in more objective analysis of lesions leading to more accurate diagnosis. Knowledge of this emerging technology is essential in diagnosis of diseases and disorders in both small and large animals.

The study was conducted to evaluate the usefulness of ultrasonography in combination with digital analysis in diagnosing hepatocellular diseases in dairy cattle.

MATERIALS AND METHODS

Animals

Two hundred and twenty-six (226) Holstein-Friesian dairy cattle, suffering from various disorders, aged 2-13 years old were used in the study. Of the 226 animals, 105 had locomotor disorders, 58 had reproductive disorders 32 had digestive disorders, 14 had nervous disorders, 8 had respiratory disorders, 7 had cardiac disorders and 2 had renal disorders.

Ultrasonography of liver of dairy cattle

The site for ultrasonography was prepared by shaving the upper and middle third regions of the last two intercostal spaces of the right flank of the animal. Aquasonic coupling gel (Echo Jelly®, Aloka Co. Ltd., Tokyo, Japan) was then applied and ultrasonography performed using a commercially available gray scale sonograph machine (Hitachi EUB-200V®, Hitachi Medical Corp., Tokyo, Japan) equipped with a 3.5 MHz transducer and a linear array electronic scanner.

The liver was imaged from the right side in the 11th and 12th intercostal spaces, just dorsal to the boundary between the upper and middle third of the rib. Constant imaging parameters (time gain compensation, gain, contrast, brightness

and dynamic range) were used. The machine power output and time gain compensation were fixed at main gain = 50 dB, near gain = 15 dB and far gain = 5 dB.

The B-mode ultrasonograms were recorded using a commercially-available still video (Still Video 1000MH®, Fujix, Fuji Photo Film Co. Ltd., Tokyo, Japan). A-mode ultrasonographic images of the liver were obtained using an image analysis software (Image Command 4198, TV Image Processor 4100®, Excel Application Software, Japan Avionics Co., Tokyo, Japan) for comparison with hepatic B-mode ultrasonograms.

After viewing all the ultrasonograms several times, the different ultrasonographic features were noted. Hepatic ultrasonograms were evaluated according to parenchymal echo characteristics, beam penetration, vascularity and hepatic edge visibility. The presence of the following were noted in individual ultrasonograms: a) hyperechoic areas scattered in the hepatic parenchyma (bright pattern); b) hypoechoic areas uniformly distributed in the parenchyma (dark pattern); c) hypoechoic areas in the distal region of the ultrasonogram (deep attenuation); d) diminished visibility of portal and hepatic veins (vascular blurring; and e) diminished visibility of the distal boundary of the hepatic parenchyma, i.e. the visceral surface of the caudate lobe (blurring of edges).

Hyperechocity and hypoechocity were determined subjectively based on whether the image of the liver was brighter or darker than the general average hepatic ultrasonogram. The evaluation of the ultrasonogram was made before histopathologic results were obtained.

Histopathological examination of liver samples

After ultrasonography, the animal was immediately slaughtered and liver and kidney samples were collected from the areas where the ultrasonograms were supposed to have been taken. The samples were immediately placed in 10% buffered formalin solution. Two specimens for the liver were prepared, one for staining with hematoxylin-eosin (H-E) and one for lipid staining. For H-E staining, the liver and kidney specimens were cut into small pieces (1.5×1.5×0.5 cm), and prepared according to standard histopathological procedures.

A portion of the liver histologically diagnosed as being characterized by fatty change was cut into a 1.0×1.0×0.2 cm piece and fixed in osmic acid-potassium dichromate solution for 72 hours and prepared histopathologically according to standard procedures. The specimens were then recorded by still video (Still Video 1000MH®, Fujix, Fuji Photo Film Co. Ltd., Tokyo, Japan) through a TV-microscopic camera (Camera Head HV-132®, Hitachi Denshi, Ltd., Tokyo, Japan) and the fatty occupying rate of the liver was calculated using a concentration measurement program of an image analysis software (Image Command 4198, TV Image Processor 4100®, Excel Application Software, Japan Avionics Co., Tokyo, Japan). The fatty occupying rate of the liver was determined histologically as the percentage of the area of the hepatic lobule stained with osmic acid-potassium dichromate solution. The area stained with osmic acid-potassium dichromate

solution was determined by calculating the means of five triangular areas within the specimen whose angles are formed by the central vein and two portal canals.

Digital analysis of ultrasonograms

Digital analysis, using gray scale histogram analysis, of the ultrasonograms taken during ultrasonography was conducted as follows. The B-mode hepatic ultrasonograms were recorded using a still video record/player (Still Video 1000MH®, Fujix, Fuji Photo Film Co. Ltd., Tokyo, Japan). Area samples (1×1 cm) from different regions of the ultrasonograms of the hepatic parenchyma were taken at a depth of 1-9 cm from the hepatic surface (Fig. 1).

The echoes within the area samples were quantified as echo mean (Emean) values of the histogram of the echo amplitudes (Fig. 2) using a Region of Interest (ROI) program of an image analysis software (Image Command 4198, TV Image Processor 4100®, Excel Application Software, Japan Avionics Co., Tokyo, Japan). The x axis represents the different shades of the gray scale while the y axis represents the number of echoes. The Emean represents the average of the echo amplitudes within the area sample. The histogram had 252 shades in the gray scale of the image, from 0 (black) to 251 (white).

Comparison of the Emeans of normal liver and liver with various hepatocellular disorders was made and the difference was analyzed using Student's t test for comparison of samples with unequal sizes and different standard deviations (Snedecor and Cohran, 1980).

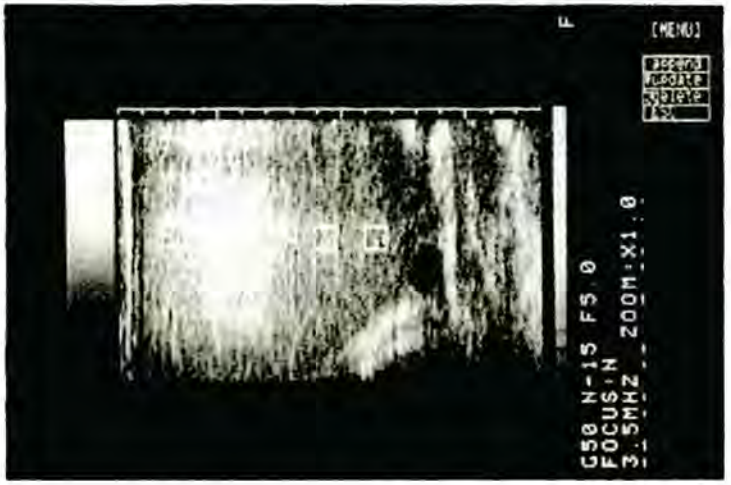

Figure 1. Area samples for hepatic histogram echo mean measurement.

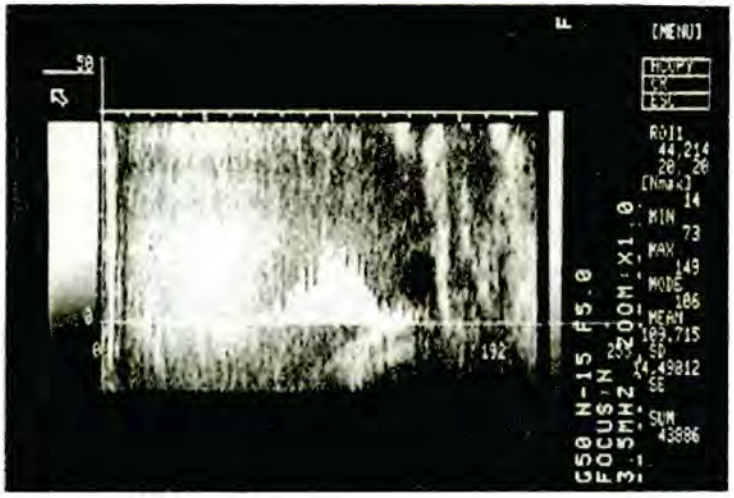

Figure 2. Echo histogram of hepatic ultrasonogram.

Diagnostic evaluation

The sensitivity, specificity, accuracy and positive and negative predictive values for the different diffuse hepatocellular disorders using ultrasonographic and digital analysis criteria were calculated according to the following formulas:

Calculation of diagnostic rates

Test	Histopathology	
	Positive	Negative
Positive	a	b
Negative	b	d

Sensitivity = $100 \times [\, a \div (a + c) \,]$
Specificity = $100 \times [\, d \div (b + d) \,]$
Accuracy = $100 \times [\, (a + d) \div (a + b + c + d) \,]$
Positive predictive value = $100 \times [\, a \div (a + b) \,]$
Negative predictive value = $100 \times [\, d \div (c + d) \,]$

Note: Test refers to the result of ultrasonographic findings or digital analysis while histopathology refers to the result of histopathological examination.

The test positive conditions for ultrasonographic findings were based on the following: a) for normal liver, ultrasonograms with no bright pattern, dark pattern, deep attenuation, vascular blurring and blurring of edges were considered test positive; b) for hydropic degeneration, ultrasonograms with dark pattern and blurring of edges were considered test positive; c) for fatty change, ultrasonograms with bright pattern, vascular blurring and deep attenuation were considered test positive; d) for hepatic dystrophy, ultrasonograms with bright pattern and blurring of edges were considered test positive; and e) for hepatic amyloidosis, ultrasonograms with bright pattern and blurring of edges were considered test positive.

The test positive conditions for digital analysis were based on the following: a) for normal liver, ultrasonograms with Emean values between 80-98, 75-93, 70-85, 63-78 and 55-70 at 1, 3, 5, 7 and 9 cm from the hepatic surface, respectively, were considered test positive; b) for hydropic degeneration, ultrasonograms with Emean values lower than 80 and 75 at 1 cm and 3 cm from the hepatic surface, respectively, were considered test positive; c) for fatty change, ultrasonograms with Emeans at 1 cm from the heaptic surface higher than 98 and Emeans lower than 63 and 55 at 7 cm from the hepatic surface, respectively, were considered test positive; d) for hepatic dystrophy, ultrasonograms with Emean at 3 cm from the hepatic surface greater than 93 were considered test positive; and e) for hepatic amyloidosis, ultrasonograms with Emean at 3 cm from the hepatic surface greater than 93 were considered test positive.

RESULTS

Of the 226 animals examined, 120 (53.1%) had a normal liver, 61 (27.0%) had hydropic degeneration of the liver, 41 (18.1%) had fatty change of the liver, 3 (1.3%) had hepatic dystrophy and 1 (0.5%) had hepatic amyloidosis. Hydropic degeneration was further classified into: a) mild, affecting only the centrilobular area (41 animals); b) moderate, affecting both centrilobular and midzonal regions (15 animals); and c) severe, affecting the whole hepatic lobule (5 animals). The fatty occupying rate (FOR) of the liver was classified as: a) mild, 1-15% FOR (21 animals); b) moderate, 15.1-30% FOR (10 animals); and c) severe, >30% FOR (10 animals).

The ultrasonogram of a normal liver shows slightly echogenic parenchyma with gradual attenuation of echoes. The parenchymal echoes are relatively uniform, the portal and hepatic vessels are visible and the hepatic edge is visible (Fig. 3).

In B-mode ultrasonograms of mild, moderate and severe hydropic degeneration, the parenchyma is more hypoechoic (dark pattern) and the edge is less distinct (blurring of edges) than that of the normal hepatic ultrasonogram (Fig. 4), especially in moderate and severe hydropic degeneration. In addition, the echoes in the amplitude mode (A-mode) are weaker compared to normal hepatic A-mode ultrasonogram.

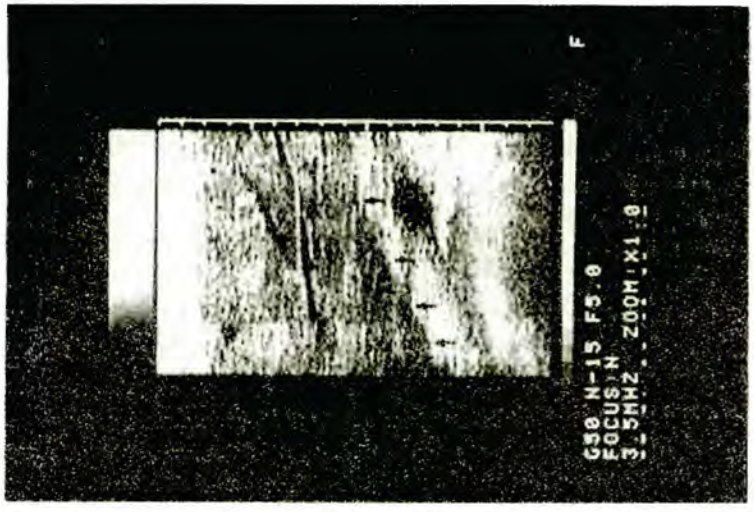

Figure 3. Ultrasonogram of a normal liver. PV: portal vein (with echogenic walls); HV: hepatic vein (without echogenic walls); black arrows show hepatic edge.

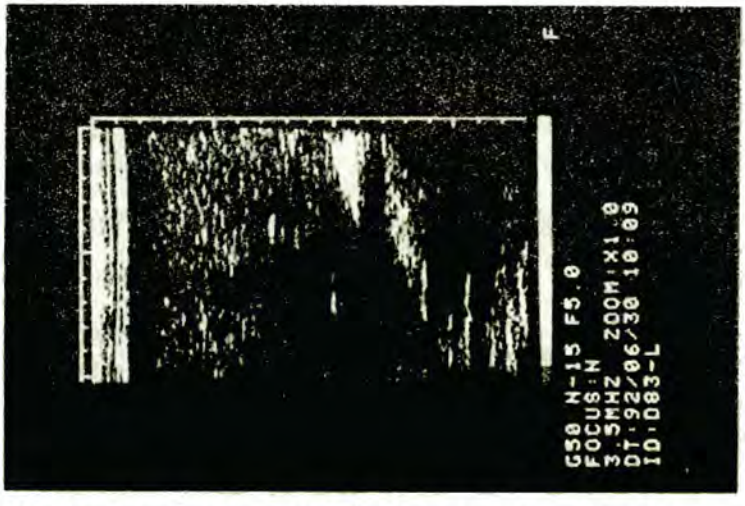

Figure 4. Ultrasonogram of severe hydropic degeneration of the liver.

Hyperechoic areas in the proximal region (bright pattern), hypoechoic areas in the distal region (deep attenuation), vascular blurring and blurring of edges can be seen in B-mode ultrasonograms of mild, moderate and severe fatty change of the liver (Fig. 5), especially in moderate and severe fatty change. In addition, the A-mode shows stronger echoes in the proximal region and more abrupt attenuation distally than in the normal hepatic A-mode ultrasonogram.

In B-mode ultrasonograms of hepatic amyloidosis and hepatic dystrophy (Figs. 6 and 7), the parenchyma of the liver in the ultrasonogram is more echogenic (bright pattern) than that of the normal liver and the parenchymal edges are barely visible (blurring of edges). The echoes in the A-mode, especially in the superficial portion, i.e. nearest the transducer-skin contact area, are stronger than the corresponding normal hepatic A-mode ultrasonogram.

Table 1 shows the echo mean (Emean) values of ultrasonograms of normal liver and diffuse hepatocellular disorders at different distances from the hepatic surface. The Emeans of normal liver decreased gradually from 1 cm to 9 cm from the hepatic surface, that of hydropic degeneration had a more rapid decline while that of fatty change decreased even more rapidly. The Emeans of hepatic dystrophy and hepatic amyloidosis both increased from 1 cm to 3 cm from the hepatic surface and decreased afterwards.

At 5 cm, 7 cm and 9 cm from the hepatic surface, the Emean values of hydropic degeneration were lower than the corresponding values in the normal liver. At 1 cm from the hepatic surface, the Emean value of fatty change was higher than that of normal liver. However, at 3 cm, 5 cm, 7 cm and 9 cm from the

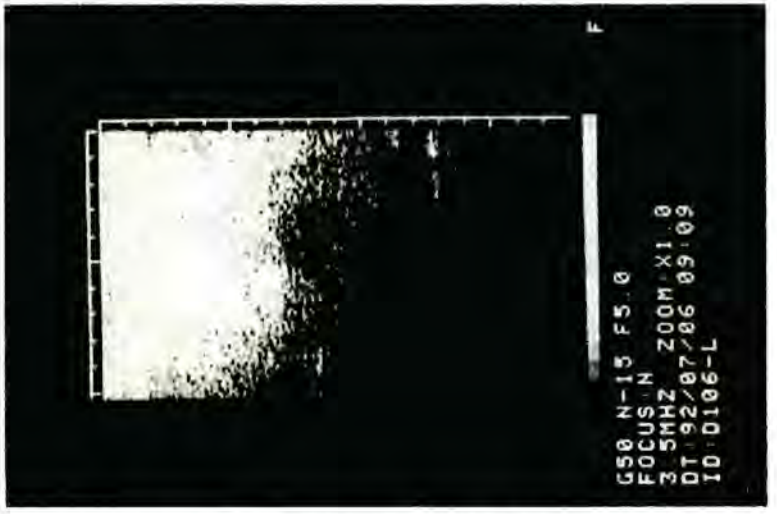

Figure 5. Ultrasonogram of severe fatty change if the liver.

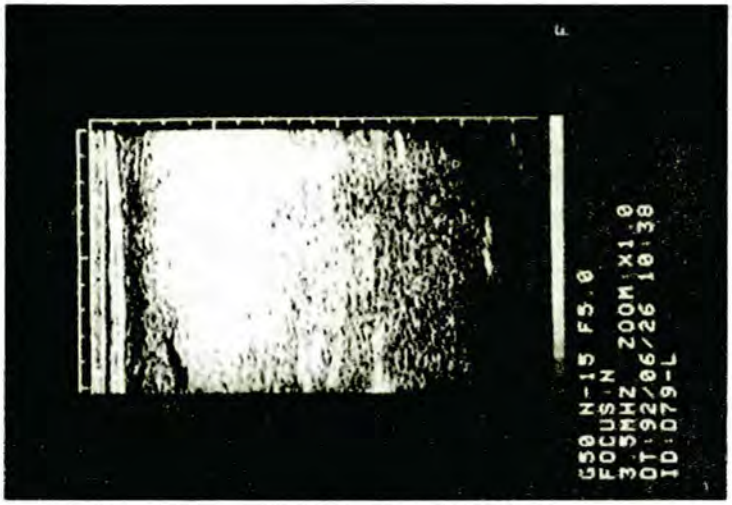

Figure 6. Ultrasonogram of hepatic dystrophy.

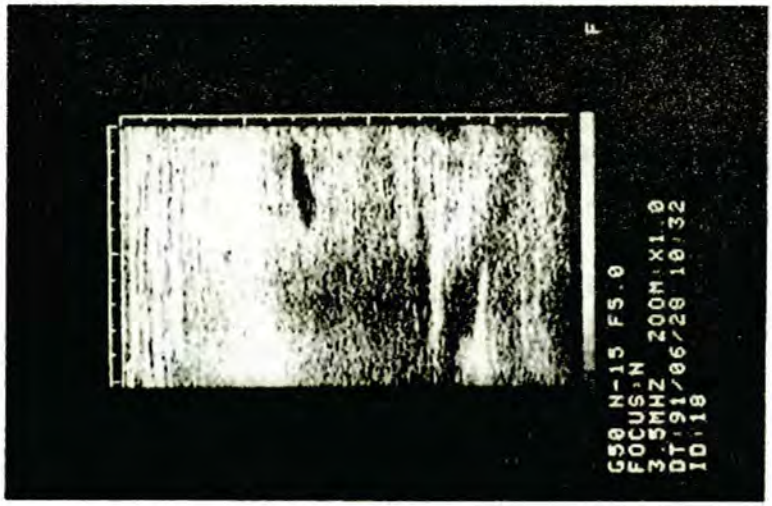

Figure 7. Ultrasonogram of hepatic amyloidosis.

Table 1. Echo mean values obtained from digital analysis of hepatic ultrasonograms of animals with a normal liver and diffuse hepatocellular disorders.

Disorder	Distance from hepatic surface				
	1 cm	3 cm	5 cm	7 cm	9 cm
Normal liver	90.3	87.8	77.9	73.2	64.3
Hydropic degeneration	91.7	86.7	74.7	69.1	60.6
Fatty change	97.7	81.0	68.2	61.8	56.7
Hepatic dystrophy	82.5	92.5	88.0	76.0	69.0
Hepatic amyloidosis	95.0	109.0	90.0	76.0	67.0

hepatic surface, the Emean values of normal liver were higher than the corresponding values in fatty change. At 3 cm, 5 cm, 7 cm and 9 cm from the hepatic surface, Emean values of hepatic dystrophy and amyloidosis were higher than the corresponding values in the normal liver.

Table 2 shows the echo mean (Emean) values of ultrasonograms of different degrees of hydropic degeneration compared to normal liver. Emean values in hydropic degeneration decreased with increasing severity of degeneration. At all distances, Emean values of severe hydropic degeneration were lower than those of normal liver ($p<0.05$). At 7 cm and 9 cm from the hepatic surface, the Emean values of moderate hydropic degeneration were lower than those of normal liver ($p<0.05$).

Table 3 shows the echo mean (Emean) values of ultrasonograms of different degrees of fatty change compared to normal liver. Emean values increased with increasing severity of fatty change. Severe fatty change had the steepest decline from 1 cm to 9 cm from the hepatic surface. At 1 cm, the Emean value of severe fatty change was higher than that of normal liver ($p<0.05$). At 7 cm and 9 cm from the hepatic surface, the Emean values of both moderate fatty change and severe fatty change were lower than that of normal liver ($p<0.05$).

Table 4 shows the diagnostic accuracy rates for normal liver and diffuse hepatocellular disorders using ultrasonography and digital analysis. Ultrasonography is highly specific and relatively accurate for diagnosis of hydropic degeneration and fatty change of the liver. The accuracy rates increased with increasing severity of disorder. For hepatic dystrophy and hepatic amyloidosis, high negative predictive values were obtained. Diagnostic accuracy rates obtained through digital analysis for hydropic degeneration and fatty change were higher than those obtained through ultrasonography.

DISCUSSION

The contrast that is seen when echo beams pass through different layers of tissues and organs is one of the basic principles used in diagnostic ultrasound. The

Table 2. Echo mean values obtained from digital analysis of hepatic ultrasonograms of animals with a normal liver and different degrees of hydropic degeneration.

	Distance from hepatic surface				
	1 cm	3 cm	5 cm	7 cm	9 cm
Normal liver	90.3	87.8	77.9	73.2	64.3
Hydropic degeneration					
Mild	93.0	89.0	76.0	70.0	61.0
Moderate	91.0	84.0	73.0	67.4*	60.0*
Severe	82.8*	75.6*	69.7*	66.8*	59.5*

*Significantly different from normal liver at $p<0.05$.

Table 3. Echo mean values obtained from digital analysis of hepatic ultrasonograms of animals with a normal liver and different degrees of fatty change.

	Distance from hepatic surface				
Disorder	1 cm	3 cm	5 cm	7 cm	9 cm
Normal liver	90.3	87.8	77.9	73.2	64.3
Fatty change					
Mild	91.5	81.4	72.8	69.1	61.3
Moderate	97.8	84.2	67.3	56.7*	53.6*
Severe	108.7*	77.2	60.5	54.8*	52.3*

*Significantly different from normal liver at $p<0.05$.

different shades of the gray-scale formed in the ultrasonogram can be attributed to the differences in acoustic impedance and absorption capacity between different tissues (Sanders and James, 1990). Basically, this contrast has been used to identify organs and structures within organs. It has also been used to identify changes within organs and changes in the relationship between organs. The increased presence of water, fat, amyloid, cells or connective tissue in the hepatic lobe causes changes in the parenchymal structure, giving rise to differing echo patterns.

In the evaluation of diffuse liver diseases in humans, different parameters have been used. Among them were parenchymal echogenicity, depth of beam penetration, visibility of hepatic portals veins, liver surface images (Arima *et al.*, 1990), hepatic micro- and macrotexture (Layer *et al.*, 1990), gall bladder wall

Table 4. Diagnostic accuracy rates for normal liver and diffuse hepatocellular disorders using ultrasonographic and digital analysis criteria.

Disorder	Sensitivity	Specificity	Accuracy	Positive Predictive value	Negative Predictive value
Ultrasonography					
Normal liver	53.0	71.6	61.9	66.7	58.6
Hydropic degeneration	41.0	81.2	70.4	44.6	78.8
Fatty change	61.0	83.8	79.6	45.5	90.6
Hepatic dystrophy	66.7	73.5	73.5	3.3	99.4
Hepatic amyloidosis	100.0	72.9	73.0	1.6	100.0
Digital analysis					
Normal liver	67.5	57.8	62.8	63.2	62.4
Hydropic degeneration	45.9	87.9	76.5	58.3	81.5
Fatty change	62.4	96.2	90.3	78.8	92.2
Hepatic dystrophy	66.7	72.2	72.1	3.1	99.4
Hepatic amyloidosis	100.0	72.0	72.1	1.6	100.0

echoes (Sato and Ogimoto, 1985), volume of the caudate lobe (Hess *et al.*, 1986), ultrasound velocity (Nishimura *et al.*, 1986), brightness scala values (Hoshino *et al.*, 1990), masking sign (Kojima *et al.*, 1989), stilette sign (Ingram and Joseph, 1989), fatty bandless sign (Mizuguchi *et al.*, 1986), coarsening (Hess *et al.*, 1986), homogeneity of hepatic echo patterns (Herdt *et al.*, 1983), elasticity, echo structure and sonic conductivity of the liver (Steinmaurer *et al.*, 1984).

In the normal hepatic ultrasonogram in dairy cattle, the parenchymal pattern consists of numerous weak echoes homogeneously distributed over the entire area of the liver (Braun, 1990). There is a gradual attenuation of the echo beam as it passes through the normal liver tissue. The hepatic and portal veins can be seen within the normal texture. In addition, the parenchymal edges are normally visible.

In animals with amyloidosis, the amyloid is deposited between the sinusoidal reticulum and hepatic cords (Kelly, 1985) of the peripheral parts of the lobules or intermediate zone (Cohrs, 1966). This deposition gives rise to increased echoes as seen in the ultrasonogram. Increased hepatic parenchymal echogenicity and small high echo spots scattered in the hepatic parenchyma were also observed in humans with amyloidosis (Otani *et al.*, 1986).

Two of the animals with hepatic dystrophy which had bright pattern had severe tissue necrosis, while the other one had only mild necrosis. The increased echogenicity seen in hepatic dystrophy, therefore, can be attributed to increased inflammatory cellular infiltration (Park *et al.*, 1981) and tissue necrosis (Sumino *et al.*, 1985). In this study, it was not possible to distinguish between amyloidosis and hepatic dystrophy by means of increased parenchymal echoes in the ultrasonogram alone.

The deposition of water in hydropic degeneration can be distinguished in the ultrasonogram. Tissues and organs with a physical density of 1.0 can be identified on gray-scale ultrasound scans by their specular and nonspecular echo patterns (Park *et al.*, 1981). The decreased echoes seen in hydropic degeneration can be attributed to the increased water content of the hepatocytes (Park *et al.*, 1981) since water has a lower absorption coefficient and higher acoustic impedance than normal hepatic tissue (Sanders and James, 1990). In animals where there is blurring of edges, the hepatic parenchyma tends to blend with the surrounding tissues so that the visceral surface of the liver cannot be accurately delineated.

In humans, bright pattern, deep attenuation, vascular blurring and blurring of edges were also observed in ultrasonograms of fatty change of the liver (Arima *et al.*, 1990). Increased parenchymal echogenicity in the proximal region in fatty change is caused by a higher absorption coefficient and lower acoustic impedance of fat as compared with normal liver tissue (Sanders and James, 1990). The increased echogenicity of the ultrasonogram seen in fatty change is due to the markedly echogenic mixture of fat and water in the hepatic parenchyma (Behan and Kazam, 1978). Due to immiscibility, multiple water-fat and fat-water interfaces form and cause increased echogenicity and attenuation of the ultrasound beam (Behan and Kazam, 1978).

Computer analysis of ultrasonographic echoes, including digital analysis, has been used in humans in the diagnosis of diffuse disorders of the liver. Computerized ultrasound examination yields for information from the ultrasound image than normal observation, which leads to increased diagnostic accuracy.

Digital analysis, in this study, was used to objectively quantify the echo amplitudes by calculating the echo mean of the histogram of the specific areas in the ultrasonogram, namely, the proximal hyperechogenicity or bright pattern, dark pattern and distal hypoechogenicity or deep attenuation observed in the hepatic ultrasonograms through ultrasonography. Since the degree of brightness of an ultrasonogram is subject to the observer's own evaluation, the use of digital analysis attempted to remove the observer's bias by presenting the results in terms of quantifiable figures. The high degree of accuracy obtained through digital analysis shows that this method can be more objective and accurate than ultrasonography alone.

Analysis of ultrasonograms, in this study, was able to identify different characteristics in the hepatocellular disorders, namely, hydropic degeneration, fatty change of the liver, hepatic dystrophy and amyloidosis. In humans, analysis of ultrasonograms have been used in the differential diagnosis of acute hepatitis (Schuster *et al.*, 1988), chronic hepatitis (Garra *et al.*, 1987), liver cirrhosis (Lerski *et al.*, 1982), fatty change (Lerski *et al.*, 1982), hepatic fibrosis (Lin *et al.*, 1987), hepatic tumors (Nicholas, 1979), chronic liver diseases (Naumov and Loukanov, 1987) and diffuse liver diseases (Cloostermans et al., 1987).

In this study, the higher Emean values at 1 cm from the hepatic surface in severe fatty change compared with normal liver represent the high echo ampli-

tudes or bright liver pattern. The lower Emeans at 5 cm, 7 cm and 9 cm seen in severe fatty change compared with normal liver, on the other hand, represent decreased penetration of echoes or deep attenuation. The lower Emeans at 3 cm, 5 cm, 7 cm and 9 cm from the hepatic surface seen in hydropic degeneration compared with normal liver, on the other hand, represent the dark pattern observed in ultrasonograms of hydropic degeneration.

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PHILIPPINE LAKES: STATUS AND STRATEGIES FOR SUSTAINABLE DEVELOPMENT

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ABSTRACT

The Philippines is blessed with more than 100 freshwater lakes ranging from a few to a thousand hectares in size. The lakes are either tectonic, kettle or maare in type. These bodies of water are important for fisheries because of their domestic, agricultural, industrial and recreational uses.

An assessment of 36 major Philippine lakes indicated that 55.5% are threatened, 41.7% are in good condition and 2.7% are in critical condition.

With the exception of Laguna de Bay which is managed by an authority, the management responsibility of the other lakes in the country falls on the local government units. Despite the existence of laws and ordinances for conservation and protection of inland waters, however, there is poor enforcement of such measures due to lack of capability and political will on the part of the implementors.

The strengthening of the LGUs in cooperation with the Fisheries and Aquatic Resources Management Councils mandated by law and the bolstering of the technical and political capability of LGUs to formulate and implement integrated lake management plans are recommended as the key strategies for the sustainable development of Philippine lakes.

Keywords: Lakes, lake management, inland waters, sustainable development, management strategies

INTRODUCTION

Lakes are natural inland bodies of standing water. From the Latin word *lacus* (meaning pond), the term "lake" refers to a depression in the ground that gets filled with water. There are three types of lakes according to how they were formed. Tectonic lakes come about as a result of "mountain-producing forces of

the earth". Kettle lakes on the other hand are formed by "craters of extinct volcanoes". Finally, maare lakes are small, circular, deep bodies produced by "explosions of volcanic gas chambers" (Ruttner, 1952).

The lakes in the Philippines with a total area of over 200,000 hectares are of the tectonic, kettle and maare types. These water bodies range from a few to a thousand hectares in size varying in depth locations. The largest lake in the country is Laguna de Bay (90,000 ha) while the deepest and highest lakes are Lake Taal (200 m) and Lake Venado (2,666 m), respectively.

Lakes are not only important for their water and fisheries but also for their varied domestic, agricultural, industrial and recreational uses. As much as 15% of the country's total fisheries production comes from lakes (Fellizar, 1995). Laguna de Bay is being tapped as a source of domestic water supply of Metro Manila as well as for hydroelectric power, irrigation and for cooling industrial plants. The hydroelectric power plants that is run by the Agus River which is supplied by Lanao Lake is expected to provide 70% of the power needs of Mindanao by the year 2000 (Anon., 1997).

With population growth, urbanization and industrial development, human impacts on the country's aquatic resources such as lakes have increased. Problems like lake sedimentation, overexploitation of fisheries and pollution have come about (Guerrero, 1996). There is an urgent need for effective management of these lakes to ensure their sustainability.

This paper will assess the status of the major Philippine lakes and recommend strategies for their sustainable development.

STATUS OF MAJOR PHILIPPINE LAKES

There are over 100 recorded lakes in the Philippines (Table 1). The region with the most number of lakes is Southern Tagalog (22) followed by the Cordillera Autonomous Region (21). Within the Southern Tagalog region, the province of Laguna has the most number of lakes (12).

Based on physico-chemical and biological criterias, the status of the 36 major lakes (with areas of 100 hectares and larger) in the Philippines was assessed. Lakes that are in good condition have water quality within acceptable standards, little or no sedimentation and no overfishing stress. Lakes that are threatened show moderate pollution, sedimentation and overfishing stresses. Lakes in the critical list are under heavy pollution, sedimentation and overfishing pressures.

The assessment which was based on reports and personal observations showed that 41.7% of the lakes are in good condition, 55.5% are threatened and 2.7% are in critical condition (Table 2).

Laguna de Bay is the most heavily stressed lake in the country. The forest cover of the lake's watershed has been reduced from 93,000 ha in 1963 to less than 18,000 ha in 1988 because of human activities (Valerio, 1990). Soil erosion from deforested areas is the main contributor of sediment to the lake. The sediment

Table 1. Distribution and number of Philippine lakes

Region	Provinnce	Number
I	Ilocos Norte	
	Ilocos Sur	
	Pangasinan	2
II	Cagayan	1
CAR	Abra	4
	Ifugao	2
	Kalinga	5
	Benguet	7
	Mt. Province	3
III	Tarlac	3
	Zambales	4
	Nueva Ecija	
IV	Laguna/Rizal	
	Laguna	
	Batangas	1
	Quezon	4
	Mindoro Oriental	3
	Palawan	1
V	Camarines Sur	8
	Sorsogon	1
VI	Iloilo	
VII	Negros Oriental	
	Cebu	1
VIII	Leyte	8
IX	Zamboanga del Sur	1
X	Bukidnon	3
XI	South Cotabato	5
XII	North Cotabato	5
XIII	Surigao del Norte–Agusan	
	Agusan	
ARMM	Lanao del Sur	4
	Maguindanao	
	Total	101

Table 2. Status of major Philippine lakes

Lake	Region/Province	Condition
Paoay	I, Ilocos Norte	T
Cabalangan	II, Cagayan	T
Alindayat	III, Zambales	T
Paitan	III, Nueva Ecija	T
Laguna de Bay	IV, Laguna and Rizal	C
Sampaloc	IV, Laguna	T
Taal	IV, Batangas	T
Naujan	IV, Oriental Mindoro	T
Caluangan	IV, Oriental Mindoro	T
Mangua	IV, Palawan	T
Bato	V, Camarines Sur	T
Buhi	V, Camarines Sur	T
Balinsasayao	VII, Negros Oriental	G
Mantohod	VII, Negros Oriental	G
Lanao	VII, Negros Oriental	T
Danao	VII, Cebu	G
Danao	VIII, Leyte	T
Bito	VIII, Leyte	T
Wood	IX, Zambaonga del Sur	T
Sebu	XI, South Cotabato	T
Manghan	XI, South Cotabato	T
Balut	XII, North Cotabato	G
Sultan	XII, North Cotabato	G
Malinao	XII, North Cotabato	G
Buranibud	XII, North Cotabato	G
Labas	XII, North Cotabato	G
Nunungan	XII, Lanao del Norte	G
Blingkong	XII, North Cotabato	G
Mainit	XIII, Surigao del Norte-Agusan	T
Pagusi	XIII, Agusan	G
Butig	ARMM, Lanao del Sur	G
Dapao	ARMM, Lanao del Sur	G
Putian	ARMM, Lanao del Sur	G
Lanao	ARMM, Lanao del Sur	T
Buluan	ARMM, Maguindanao	T

C- Critical condition
G- Good condition
T- Threatened

loading has been estimated at 1.5 million m^3/yr (SOGREAH, 1991). A decreasing trend in Secchi disk transparency from 46 to 59 cm in 1986 to 31 to 38 cm in 1992 was noted (Barril *et al.*, 1994).

Domestic sources account for 70% of the organic wastes discharged into the lake. The total nitrogen load originating from municipal wastes is estimated to be about 3,000 t/yr (Lopez, 1989). The nitrogen and phosphorus loadings into the lake from agricultural activities have been estimated to increase from 11,200 t/yr in 1975 to 29,000t/yr in 2000 (Santos-Borja, 1990).

The number of industrial plants (e.g. paper mills and manufacturing firms) around the lake increased from 117 in 1963 to 1,481 in 1995. The majority of these firms (68%) are considered to be pollutive and only about 50% of such industries have wastewater facilities (URSI, 1989).

In terms of water quality, the dissolved levels in Laguna de Bay have been found to be lower than the standard while pH, phosphates and coliform count are higher than the set standards (Juliano, 1998).

Because of overfishing and habitat degradation, the number of indigenous fishes in the lake has also been reduced. Of five species of *kanduli* described by Aldaba (1931), only two species exist at present (Vallejo, 1986).

A socio-economic survey of 3,055 fishermen in Laguna de Bay in 1995-1996 showed that their catch per unit effort was only 0.25 kg/h (Palma, 1998). Lake fisherfolk have low educational attainment and their income is one of the lowest in the country (Juliano, 1998).

The problems of overfishing and other human impacts have also adversely affected the other major lakes in the country. In Lake Lanao, only three endemic *cyprinids* remain out of the 15 species reported by Herre in 1933 because of the introduction of exotic indigenous fishes such as the *Glossogobius giurus and Hypseleotris agilis* (Escudero, 1995).

In Lake Taal, the exploitation rate for the *tawilis (Sardinella tawilis)* exceeds the optimum range indicating overfishing of the resource despite various existing fisheries laws (Villanueva *et al.*, 1996). A declining trend in the catch of migratory fishes like the mullet and milkfish has been observed in Lake Naujan (Pasumbal and Perez, 1997). The disappearance of the *sinarapan* fishery in Lake Buhi in 1978 has been attributed to human activities related to use of motorized push nets, agricultural operations, introduction of exotic fishes, pollution and other activities around the lake (Aypa *et al.*, 1995).

The introduction of aquaculture in many lakes has also caused negative impacts. Milkfish pens in Laguna de Bay limit small fishermen to access their traditional fishing areas. The pens also contribute to sedimentation of the lake by impeding the flow of the water (Guerrero, 1996). Cages used for tilapia culture in Lake Taal and Lake Sebu has increased pollution load due to feeding and has induced fishkills (Mercene, 1997; Mama-ay and Kiman, 1997).

STRATEGIES FOR SUSTAINABLE DEVELOPMENT OF PHILIPPINE LAKES

There are numerous administrative measures that regulate the conservation, protection and management of inland waters including lakes in the Philippines. As early as 1939, Fish and Game Administrative Order 12 imposed a five-year closed season in certain waters of Rizal, Laguna, Batangas and Mindanao for the conservation of aquatic resources which included rivers connected to lakes. Fisheries Administrative Order 106, Series of 1971, set rules and regulations governing fishing in lakes and inland waters within watershed reservations throughout the country including application for permits to fish, use of fishing gear and bag limits (Juliano, 1998).

In 1966, the Laguna Lake Development Authority (LLDA) was organized by virtue of Republic Act 4850 as a quasi-government agency with regulatory and proprietary functions. The mandate of the LLDA is "to lead, promote and accelerate the development and balanced growth of the Laguna de Bay basin within the context of national and regional plans and policies for social and economic development and to carry out the development of the basin with due regard and adequate provision for environmental management and control, preservation of the quality of human life and ecological systems, and the prevention of undue ecological disturbance, deterioration and pollution".

In 1973, the establishment of a 5,000-ha fish sanctuary in Laguna de Bay was promulgated by Fisheries Administrative Order 110. Moreover, rules and regulations governing fishpen operations in Laguna de Bay (FAO 114, Series of 1973) and the construction, establishment, or operation of fishpens and fish cages in Philippine waters were instituted (FAO 160, Series of 1986).

Apart from Laguna de Bay, the management of the other major lakes in the country has only been cursorily addressed. Lake Lanao, for instance, has no single authority or agency that looks after the conservation and protection of the lake's watershed, basin and fisheries as an integrated ecosystem. This is inspite of the Lake Lanao Watershed Protection Plan proposed by the Lake Lanao Watershed Protection and Development Council (Anon., 1997).

A Presidential Commission on Tagaytay-Taal Lake was formed in 1996 which came up with a zoning plan for the lake. According to the plan, four zones which are comprised of the open fishing zone, fishery reserve zone, aquaculture zone and tourism zone will be established. The plan also limits aquaculture activities in the lake by setting 10% of the total lake area for such activities (Villanueva *et al.*, 1996).

For Lake Naujan, a Naujan Lake Management Plan has been proposed (Anon., 1998). By virtue of a municipal ordinance, a *sinarapan* sanctuary was declared in 1982 (Soliman, 1994). In Lake Sebu, despite a municipal ordinance regulating fish cages which has been in effect since 1994, fish kills in the lake attributed to heavy organic loading from tilapia feeds, have worsened (Beniga, 1997).

In 1975, Presidential Decree (PD) 704 was issued which gave the Bureau of Fisheries and Aquatic Resources management, conservation, development, protection, utilization and disposition responsibilities over all fisheries and aquatic resources in the country except municipal waters which are under the municipal or city government concerned. The decree also provided for the establishment of fish refugees, sanctuaries and fishery reservation. Moreover, the decree declared a certain identification of fish species and prohibited all forms of illegal fishing.

Then in 1998, the Fisheries Code (RA 8550) was enacted superseding PD 704. The code affirmed the rights of the fisherfolk to be protected in the preferential use of municipal waters and in the application of integrated coastal area management within specific natural fishery management areas appropriately supported by research, technical services and guidance. Furthermore, the law institutionalized the Fisheries and Aquatic Resources Management Councils (FARMCs) with members stakeholders. These FARMCs shall assist local government units (LGUs) in the management, conservation, development, protection, utilization and disposition of all fisheries and aquatic resources within their jurisdiction by enacting ordinances in accordance with the National Fisheries Policy and enforce all fisheries laws, rules and regulations enacted by the municipal, city and provincial councils.

It is clear from the above legal framework that the "ball" for the conservation, protection and management of Philippine lakes is in the hands of LGUs in working closely with the FARMCs.

The rational use of lakes on a sustainable basis will require three essential elements: (a) sound planning based on scientific biophysical, ecological and socioeconomic studies; (b) strong political will to carry out the management plan; and (c) strict enforcement of regulatory measures necessary for sustainable development of lakes.

Integrated lake management planning should treat the lake and its watershed as one ecosystem. It must involve other sectors of society, using the lake as a strategy (Juliano, 1998). In the formulation of lake management plans, the holistic or systems approach should be adopted wherein the environmental and socioeconomic profiles of the lake basin are determined and all sectors/stakeholders concerned are consulted and involved in the process. The plans should also identify gaps in knowledge, clearly define issues that need to be addressed as well as lay out a prioritized course of action to be taken in resolving such issues within a timeframe and with necessary human and budgetary support.

An integrated lake management plan should include knowledge of the lake environment and the optimum balance of its various resources, hydraulic management to promote circulation of the water, pollution control, and socioeconomic activities of the adjacent areas (Fortes, 1995).

For the proper formulation and implementation of the integrated lake management plans, a multi-sectoral institutional mechanism such as the FARMC is needed. The proposed structure for a Lake Management Council suggested by Juliano (1998) is recommended.

Strong political will is had with competent leadership and the active support of the FARMCs. The capability of LGUs to implement integrated lake management plans needs enhancing through capability-building strategies like trainings along with the provisions for facilities and funding.

In the final analysis, however, the effectiveness of any lake management plan will depend on how well regulations are enforced to derive the expected benefits.

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MOLECULAR CLONING OF THE PHILIPPINE ISOLATE OF BANANA BUNCHY TOP VIRUS (BBTV)

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ABSTRACT

Coding sequences of three single-stranded DNA (ssDNA) components from the Philippine isolate of banana bunchy top virus (BBTV) were cloned. These three viral genomic components encode a replicase protein, a coat protein and movement protein. A polymerase chain reaction (PCR) method was utilized to amplify specific ss DNA sequences using total nucleic acid extracts from banana leaf samples. Using plant transformation vector pBI121, constructs were made containing each of these three viral genes.

Key words: Banana, Banana bunchy top virus (BBTV), molecular cloning, polymerase chain reaction (PCR)

INTRODUCTION

Banana bunchy top disease, caused by banana bunchy top virus (BBTV), is the most devastating viral disease of bananas in many banana producing areas, including Asia, Africa and the South Pacific (Dale, 1987). The virus causes stunting and significant reduction in yield. Bananas infected at the early stage of growth do not produce fruit. BBTV, an 18-20 nm isometric virus, has a multicomponent genome of at least six circular single stranded DNA (Harding et al., 1991; Thomas and Dietzgen, 1991; Wu and Su, 1990).

Attempts to control BBTV include use of disease-free planting materials and eradication of infected plants. There is no known genetic resistance to BBTV

among cultivated genotypes of banana. Most commercially important cultivars are sterile and parthenogenetic and not amenable to traditional breeding strategies. The use of coat protein and other mutated viral genes for development of resistant transgenic plants can be applied to banana-BBTV system.

Components of BBTV genome have been cloned in Australia, Taiwan and Hawaii (Burns et al., 1995; Harding et al., 1993; Wu et al., 1994; Xie and Hu, 1995; Yeh et al., 1994). In this paper, we report the cloning of coding regions of three components of the Philippines BBTV isolate. These constructs will be used for banana plant transformation.

MATERIALS AND METHODS

Primers were designed and synthesized based on published sequences of the coding regions of the three BBTV DNA components. (Burns et al., 1995). For the BBTV component 1 (BBTV-Rep), which encode the replicase protein, two oligonucleotide primers, RL and RR were designed. RL is located just downstream of the stem-loop region and upstream of the coding region. It extends from nucleotide 77 to 94 with seven extra nucleotides GGC GAA T added at the 5' end to produce an EcoRI site. RL is derived from the viral sense orientation. RR is located downstream of the coding region from nucleotide 1019 to 1002 in the complementary orientation.

RL: 5'-GGC GAA TTC TAT AAA TAG ACC TCC C-3'
RR: 5'-CGG AGC GTG CGC TGT AAA-3'

For the BBTV component 3 (BBTV-CP), which encode the coat protein, two oligonucleotide primers, CPL and CPR were designed. CPL is located upstream of the coding region. It extends from nucleotide 228 to 247 with eight extra nucleotides CAT CCGA CC added at the 5' end to produce an NcoI site. CPL is derived from the viral sense orientation. CPR is located downstream of the coding region from nucleotide 799 to 780 in the complementary orientation with nine extra nucleotides.

CPL: 5'-CAT CGA CCA TGG CTA CGT ATC CGA AGA A-3'
CPR: 5'-CTC TCC ATG GCG TGT TGT ATG TTA TTT GG-3'

For the BBTV component 4 (BBTV-MP), which encode the movement protein, two oligonucleotide primers, MPL and MPR were designed. MPL is located upstream of the coding region. It extends from nucleotide 279 to 298 with eight extra nucleotides CAT CGA CC added at the 5' end to produce an NcoI site. MPL is derived from the viral sense orientation. RR is located downstream of the coding region from nucleotide 640 to 621 in complementary orientation with ten extra nucleotides.

MPL: 5'-CAT CCG CCA TGG CAT TAA CAA CAG AGC G-3'
MPR: 5'-CTC TCC ATG GAC CGT GTA TTA GAA CAT AGG-3'

The above primers were used in PCR (polymerase chain reaction) to amplify coding sequences of the three BBTV components. Total nucleic acid extracted was used as templates. Samples of total nucleic acid were produced from Philippine isolate of BBTV-infected and healthy leaf lamina and midribs following procedures developed by Xie and Hu (1995). PCR was conducted under the following conditions: 94°C 5 min; 30 cycles of 94°C 1 min, 58°C 1 min, 72°C 3 min; and a final 72°C 10 min.

For BBTV-Rep, the amplified product was treated with Klenow fragment, digested by EcoRI and then ligated into pBluescript ks. To make constructs containing the replicase gene in plant transformation vector pBI121, the PCR fragment in pBluescript ks was subcloned into pBI 525 and then into pBI121.

For BBTV-CP and BBTV-MP, the amplified products were treated with Klenow fragment, digested by NcoI and then ligated into NcoI linearised pBI525. to make constructs containing the desired genes, the gene fragment in pBI525 was digested with HindIII and EcoRI and the ligated into pBI121. Cloning and transformation of Escherichia coli employed standard procedures (Sambrook et al., 1989).

RESULTS AND DISCUSSION

The result of typical amplification of BBTV components from extracts of infected plant is shown in Fig. 1 (BBTV component 1); Fig. 2 (BBTV component

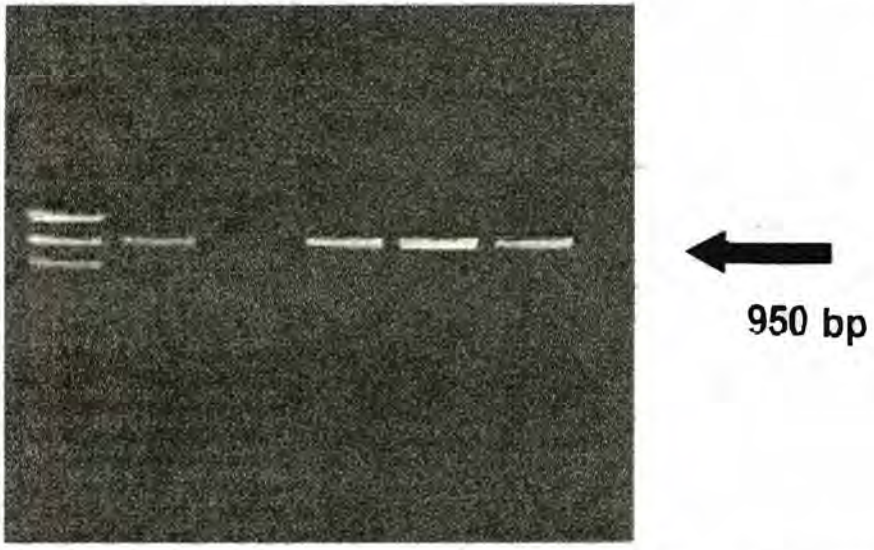

Figure 1. Agarose gel electrophoresis of PCR products derived from banana leaf extracts using primer RL and RR. Lane 1: DNA size marker Lane 3: healthy control. Lane 4-6: DNA extracted from BBTV-infected leaf.

Figure 2. Agarose gel electrophoresis of PCR products derived from banana leaf extracts using primer CPL and CPR. Lane 1: DNA size marker Lane 3: healthy control, Lane 4-7: DNA extracted from BBTV-infected leaf.

3); Fig. 3 (BBTV component 4). A band of approximately 950 bp was amplified using RL and RR primers, 586 bp for CPL and CPR primers and 380 bp for MPL and MPR primers. Bands were amplified in all reactions containing target DNA, primers and enzyme. No specific product was produced in the absence of either primers or target DNA, nor from the reactions which contained total nucleic acid extracted from healthy leaf.

The PCR products were cloned and transformants were analyzed by mini-alkaline lysis method. Inserts from potential recombinants were excised from the purified plasmids. The sizes of DNA inserts for the three BBTV components were obtained as expected. A total of six clones for BBTV-Rep, five for BBTV-CP, and three for BBTV-MP were chosen for further analysis and characterization. These gene constructs will be utilized for banana plant transformation work.

In this paper, the cloning experiments relied on primers designed based on published sequences of BBTV. PCR was shown to be a useful method in producing BBTV clones form infected tissue samples. The PCR method can be used for small circular DNA viruses provided that some sequence data is available.

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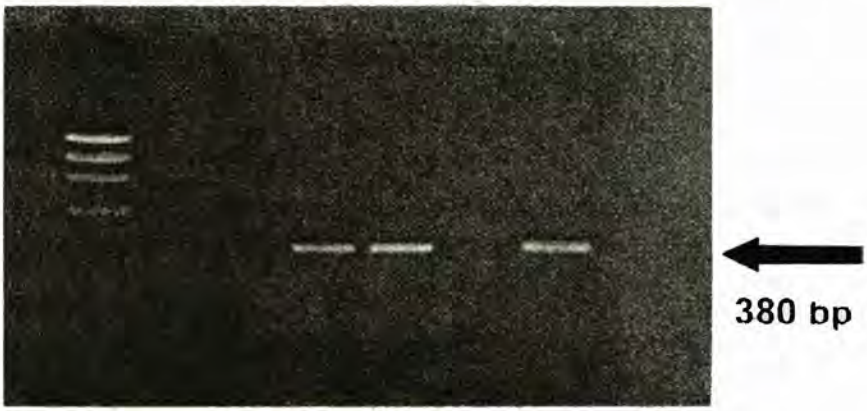

Figure 3. Agarose gel electrophoresis of PCR products derived from banana leaf extracts using primer MPL and MPR. Lane 1: DNA size marker Lane 3: healthy control, Lane 4-7: DNA extracted from BBTV-infected leaf.

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APPLICATIONS OF MOLECULAR MARKER TECHNOLOGY IN THE PHILIPPINE RICE BREEDING

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ABSTRACT

There is an urgent need to increase the genetic yield potential of rice since past yield increases have been negated by unabated population growth and reduction in rice hectarage in many countries including the Philippines. Molecular marker technology promises to speed up and improve the overall efficiency of plant breeding and, hence, the attainment of higher yield potential. This paper describes efforts at PhilRice to use molecular marker-aided approaches for breeding direct-seeded and hybrid rice varieties, considered 'new frontier' varietal types for the next millennium.

For hybrid rice breeding, the nuclear genome diversity of 22 cytoplasmic male-sterile (CMS) lines used as female parents for breeding hybrids in the Philippines were assayed at loci amplified by 20 microsatellite or simple-sequence repeat (SSR), 25 RAPD, and 10 amplified fragment length polymorphism (AFLP) primers or primer combinations. A high degree of polymorphism was detected in the CMS lines. Microsatellites and AFLPs appeared to be the most suited for DNA fingerprinting. Cluster analysis based on 222 molecular markers classified the CMS lines into nine groups. The groupings could guide hybrid rice breeders in developing genetically diverse and heterotic rice hybrids.

To determine the utility of markers for predicting heterosis or hybrid vigor, the relationship of SSR heterozygosity and heterotic potential was studied for eight traits in 48 rice hybrids derived from 5 CMS and 10 male parents. Based on 43 microsatellite loci, the CMS and male parents clustered into 2 and 8 groups, respectively, at 75% level of genetic similarity. Microsatellite heterozygosity (based on all the markers used) and heterotic performance were significantly correlated for the number of tillers per plant and LAI when all F_1's were used in the analysis. Significant correlation were

observed for maturity and number of tillers per plant between SSR polymorphism and heterosis relative to the male parent when only hybrids with positive heterosis for each trait were analyzed. Significant negative correlations were observed between heterozygosity and heterosis for maturity, harvest index, and grain yield, relative to the check varieties. Correlations for other traits were insignificant. The relationship of molecular marker heterozygosity and heterosis, therefore, appears to be complex and could vary with the traits and germplasm studied.

For breeding direct-seeded rice, 25 quantitative trait loci (QTL) underlying seedling vigor (SV), one most important traits in direct-seeded rice culture, have been mapped through interval and single-point analyses to different rice chromosomes using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers. To identify high-SV donors in local germplasm, 37 RAPDs flanking SV QTL were used as diagnostic probes to genotype 49 cultivars at 11 SV QTL. High-SV genotypes were identified in addition to known SV genetic donors and cluster analysis discriminated the 49 cultivars into eight major clusters. Newly identified high-SV genotypes such as Aus 257, Vandana, Dular WB, CG-14, CG-17, CG-20 and UG-20, that could possess alternative SV QTL alleles, have been used to develop crosses designed to concentrate favorable SV alleles in new populations that could be subjected to marker-aided selection.

Keywords: Molecular markers, breeding, direct seeding, hybrid, rice

INTRODUCTION

Molecular marker technology provides a very powerful tool for genetic analysis at the DNA level. DNA-based markers such as simple-sequence repeats (SSR) or microsatellites, amplified fragment length polymorphism (AFLP), and random amplified polymorphic DNA are randomly distributed across the rice genome (Panaud et al., 1996; Mackill et al, 1996; Redoña et al., 1996). Furthermore, all three types of markers are polymerase chain reaction (PCR)-based and does not require the use of radioisotopes, a limiting factor in developing country-laboratories lacking radioactive containment and waste disposal facilities.

Plant breeding programs have begun using molecular markers to addressing specific and applied breeding goals. Breeding areas where molecular markers have already been used include QTL analysis, marker-assisted selection, and genetic diversity analyses. In breeding direct-seeded rice varieties, QTLs have been mapped for seedling vigor (Redoña and Mackill, 1996a, b and c) and submergence tolerance (Xu and Mackill, 1996). In hybrid rice breeding, molecular markers were also useful in determining the relationship between genetic diversity and hybrid performance (Zhang et al., 1995; Xiao et al., 1996). Tagging economically-important genes with molecular markers will facilitate their transfer of gene(s) into elite breeding lines and cultivars and pave the way for map-based or positional cloning.

Table 1. Major features of different types of molecular markers.

Property	AFLPs	RAPDs	SSRs
Inheritance	Dominant	Dominant	Codominant
Genomic Distribution	Random	Random	Random
Technical ease	Difficult	Easy	Medium
Radioisotopes required	None	None	None
Fine mapping suitability	High	Low	Medium
Resolution	High	Medium	High
No. of loci per reaction	50+	8	5+
Clonability	Yes	Yes	Yes
No. of markers	Unlimited	~10000	~2000

MOLECULAR MARKER TECHNOLOGY AND HYBRID RICE VARIETAL DEVELOPMENT

Genetic Characterization of CMS Lines

Hybrid rice breeding in the Philippines is predominantly based on the cytoplasmic-genetic male sterility (CMS) or three-line system. However, concerns over the genetic vulnerability of the limited number of commercially usable CMS lines have been raised previously. Maintaining genetic diversity in CMS germplasm would reduce risks associated with genetic uniformity and could also facilitate the development of heterotic pools and combinations. Although various CMS sources have been used in developing CMS lines, there is limited information confirming the distinctness of each CMS source (Virmani and Banghui, 1988). Investigations based on agronomic traits, cross-pollinating ability, and restoration and maintaining relationships revealed differences in the CMS lines used for breeding rice hybrids in the Philippines (Xu et al., 1995). Molecular markers such as RAPD, microsatellites, and AFLP, however, allow for a more comprehensive characterization of genetic diversity in germplasm pool. These marker types were, therefore, used to assess the nuclear genome variation of CMS lines used in breeding F_1 hybrids at PhilRice and to determine the genetic relationships among the CMS lines based on molecular data.

Twenty-two CMS lines were studied including 16 developed at/or introduced through the International Rice Research Institute (IRRI) and provided by Dr. S. S. Virmani, four from Yunnan Agricultural University (YAU), China provided by Professor Li Zhengyou, and two developed at PhilRice (Figure 1). IRRI CMS lines mostly belong to the CMS-WA types while those from YAU are CMS-ZTB types. DNA extraction from 8-week old plants followed the CTAB procedure (Murray and Thompson, 1980). PCR reactions were run on a PTC-100 thermocycler

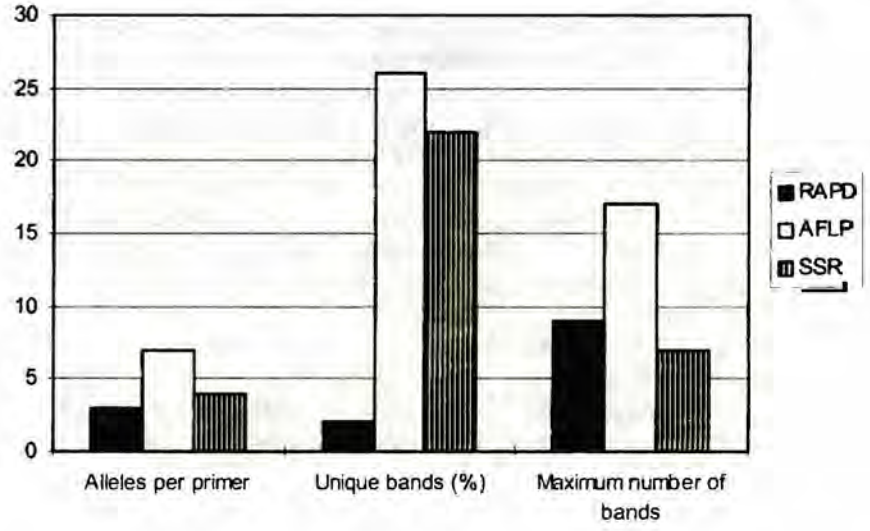


Figure 1. Molecular genetic variation detected by the three types of markers used in determining the genetic diversity of CMS lines at PhilRice.

using 22 SSR primers, 30 random 10-mers, and 10 +3/+3 AFLP primer combinations. To widen the genomic coverage of marker assays, RAPD primers, RM pairs and AFLP primer combinations that produced bands mapped to different rice chromosomes (Redoña and Mackill, 1996; Panaud et al., 1996; Mackill et al., 1996) were used. RAPD products were electrophoresed on 2% agarose gels and visualized under UV light after ethidium bromide staining. PCR products for microsatellite and AFLP analyses were ran on 6% (w/v) polyacrylamide denaturing gels and visualized using Silver Sequence^{TM} DNA staining reagents. Binary scoring was based on presence or absence of bands using only non-redundant information. Similarity coefficients were derived using the Dice method, and were utilized in cluster using the unweighted pair-group method (UPGMA) and the computer software NTSYS-Pc (Rohlf, 1990).

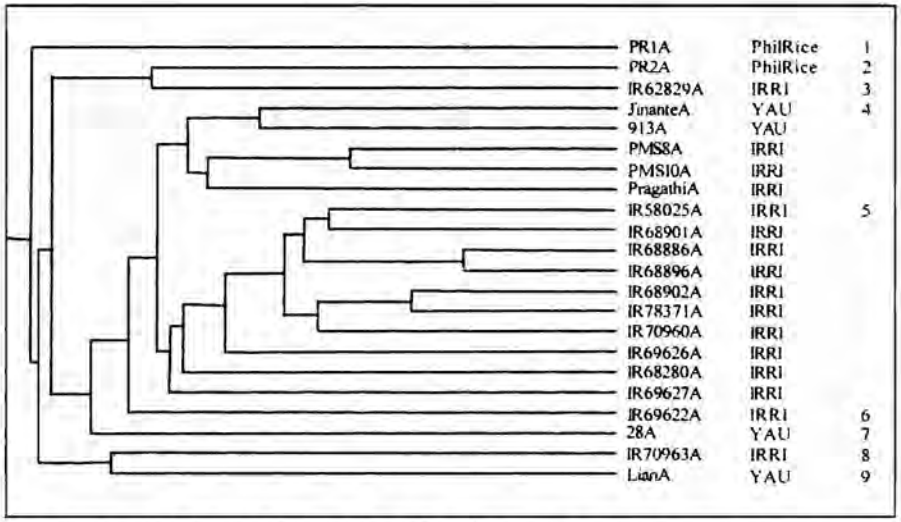
RAPDs, SSRs, and AFLPs effectively detected molecular genetic variation among the CMS lines. Seventy-seven of the 79 SSR alleles (97%) were polymorphic with the number of alleles ranging from 2 to 7 and averaging 3.8 per primer (Figure 1). The 30 RAPD primers amplified 83 polymorphic bands, ranging from 1 to 9 and averaging 2.7 per primer. For AFLPs, 72 bands showed polymorphism with the number of polymorphic bands ranging from 3 to 17 and averaging 7.2 per primer combination. Molecular genetic variation, therefore, was existent in the CMS germplasm assayed. SSRs and AFLPs were more effective in detecting unique alleles than RAPD with 17 SSR alleles (22%), 19 AFLPs (26%), and two

RAPDs (2%) amplified in only one CMS line. Comparing these markers, Powell et al. (1996) noted that SSRs had the highest expected heterozygosity and AFLPs had the highest number of loci simultaneously assayed per experiment. The unique AFLPs, SSRs and RAPDs could be useful as diagnostic tools for varietal identification or DNA fingerprinting of CMS lines.

Diversification of cytoplasm is being resorted to by hybrid rice breeding programs using the CMS system in order to reduce genetic vulnerability. Most of the IRRI lines used possess the WA cytoplasm derived from *Oryza sativa f. spontanea*. With the exception of IR69627A, these lines had at least 50% of their SSR alleles in common with IR58025A, a line that has been used extensively to develop hybrids in the tropics (Table 2). Cluster analysis based on the three types of markers separated the lines into 9 groups with a major group, consisting of 10 IRRI CMS lines, included IR58025A (Figure 2). CMS lines from China and PhilRice, aside from having a different cytoplasmic base, appeared to have a different nuclear genetic base than IRRI lines that mostly possess the wild-abortive cytoplasm derived from *Oryza sativa f. spontanea*. Results indicate, however, that CMS lines with the same cytoplasmic base could also possess nuclear genome diversity. Hence, molecular characterization of both cytoplasmic and nuclear genomes should be more effective in overall genetic diversity assessment of a hybrid rice germplasm pool.

Table 2. Percentage microsatellite alleles shared between IR58025A or IR62829A and the CMS lines.

CMS Line	% Allele shared w/ IR58025A	% Allele shared w/ IR62829A	CMS Line	% Allele shared w/ IR58025A	% Allele shared w/ IR62829A
28A	45.5	45.5	IR70960A	77.3	54.5
IR58025A	100.0	54.5	IR70963A	50.0	54.5
IR62829A	54.5	100.0	IR78371A	68.2	50.0
IR68280A	59.1	59.1	913A	50.0	50.0
IR68886A	81.0	54.5	Jinante A	50.0	40.9
IR68896A	77.3	54.5	Lian A	52.4	45.5
IR68901A	63.6	59.1	PMS8A	54.5	50.0
IR68902A	68.2	50.0	PMS10A	68.2	54.5
IR69622A	72.7	50.0	PR1A	27.3	31.8
IR69626A	59.1	50.0	PR2A	50.0	63.6
IR69627A	40.9	45.5	Pragathi A	59.1	40.9



Based on 77 SSRs, 83 RAPDs and 77 AFLPs. Numbers indicate groupings (top to bottom).

Figure 2. Molecular genetic variation detected by the three types of markers used in determining the genetic diversity of CMS lines at PhilRice.

Prediction of Heterosis using Microsatellite Markers

The power of molecular markers to assess the level of genetic diversity between two parents have generated considerable interest in their utility for predicting hybrid performance in crop breeding programs. Short tandem repeats or microsatellite DNA sequences offer a reliable and effective means of assessing genetic variation in many crops including rice (Xiao et al., 1996). Several studies have reported variable results on the relationship between marker distance and F_1 performance. However, Zhang et al. (1995) noted that despite the inconsistency in the correlations of molecular divergence and performance of the F_1 hybrid, genetic distances based on marker genotypes are in close agreement with pedigree information and can unambiguously resolve lines into their respective heterotic groups.

Fifteen rice varieties used in breeding hybrids in the Philippines, including five cytoplasmic-genetic male sterile (CMS) lines and ten restorer lines, were utilized to develop a partial diallel set of crosses. Eight of these were from the Philippines, five were from China and one each came from Vietnam and IRRI. The CMS lines were earlier shown to be genetically diverse based on 222 AFLP, SSR and RAPD markers (Redoña et al, 1998). The 48 F_1 hybrids generated and their male parents were planted in a field following a randomized complete block design with 2 replications. Two (2) check varieties, an inbred PSBRc28 and a hybrid PSBRc72H or Mestizo, were included to investigate the standard heterotic performance of the hybrids. Three to five plants were examined for eight vegetative

and reproductive characters namely plant height, maturity, leaf area index (LAI), root length, root weight, number of productive tillers, harvest index and grain yield per plant. DNA extraction, SSR assays, and cluster analysis were as described previously. Marker heterozygosity of the F_1's or the genetic distance between the parents was measured as the percentage difference of marker genotypes between the two parents of each cross combination.

Based on 43 microsatellite loci, the CMS and male parents clustered into 2 and 8 groups, respectively, at 75% level of genetic similarity. The hybrids derived from the crosses among these parents showed very low to intermediate heterosis (superiority over the male parent) for plant height, maturity, harvest index, and grain yield, whereas heterosis was high for LAI, root length, root weight, and number of productive tillers. Microsatellite allele heterozygosity (based on all the markers used) and heterotic performance were significantly correlated for the number of tillers per plant and LAI when all F_1's were used in the analysis. Significant correlation were observed for maturity and number of tillers per plant between SSR polymorphism and heterosis relative to the male parent when only hybrids with positive heterosis for each trait were analyzed. Significant negative correlations were observed between heterozygosity and heterosis for maturity, harvest index, and grain yield, relative to the check varieties. Correlations for other traits were insignificant.

The materials used in the study represents the breadth of genetic diversity currently being used to develop Philippine rice hybrids. Microsatellite DNA analysis effectively detected the presence of genetic variation in these materials. This diversity can be exploited to develop hybrids with wide genetic base thereby avoiding genetic uniformity. Breeding superior hybrids would be greatly expedited if heterotic performance can be predicted based on marker data. However, in this study, the relationship of parental genetic diversity based on all the markers used (general heterozygosity) with heterotic performance of the hybrids was generally low for the traits measured. Of the eight traits analyzed, marker-heterosis correlations were highest for LAI and the number of productive tillers. Negative and/or insignificant correlations were observed for grain yield and harvest index. Hence, it appears that for this set of germplasm, molecular marker data may not be useful for heterosis prediction. The relationship between molecular DNA divergence and heterosis appears to be complex and variable as has been reported in other studies using different germplasm. It has been suggested that the use of DNA markers closely linked to specific traits to determine specific heterozygosity may be more effective for predicting heterotic performance (Zhang et al., 1996). However, this would require prior knowledge of QTL for traits of interest in the germplasm being assayed, information that may not be readily available in many hybrid breeding programs in the tropics.

MOLECULAR MARKER TECHNOLOGY AND INBRED RICE VARIETAL DEVELOPMENT

Marker-aided Genetic Characterization of Cultivars for Direct Seeding

Direct seeding is the main cultural practice of farmers in at least three major rice-growing season in the Philippines. Farmers realize more profit using direct seeding due to higher yields and lower hired labor requirement (Francisco, 1997). Current Philippine rice varieties, however, have been bred and selected for under transplanted culture. Hence, there is a need to develop varieties and management practices specifically adapted to direct seeding to maximize productivity under this rice culture.

Seedling vigor (SV), or a plants ability to emerge rapidly from soil or water (Heydecker, 1960), is one of the most important traits under direct seeding. In rainfed areas, vigorous seedling growth provides the rice plant the competitive advantage against weeds (De Datta, 1986) and minimizes damage caused by poor water control (Nanda and Coffman, 1979). Long shoots, roots, mesocotyls, and coleoptiles have been associated with good SV in rice (Peterson et al., 1978; Redoña and Mackill, 1996a; Turner et al., 1982; Dilday et al, 1990). Genetic variation for these traits have been observed and molecular markers have been used to identify quantitative trait loci (QTL) for SV-related characters (Redoña and Mackill, 1996b; 1996c). However, there is a limited information on SV performance of germplasm used in many tropical rice breeding programs.

Forty-nine promising parental cultivars for direct-seeded rice breeding were aanalyzed for SV performance using controlled laboratory tests and and in molecular marker assays designed to identify high-SV donors. The three major rice varietal groups – indica, japonica, and javanica, were represented and two high-SV donors, Italica Livorno (IL) and Black Gora (BG), used in previous SV studies (Redoña and Mackill, 1996b; 1996c), were also included. SV-related traits- length of shoots, roots, coleoptile, and mesocotyl, were measured on 10-day old seedlings in a slant board test, a laboratory technique for SV screening (Jones and Peterson, 1976), using a constant temperature of 25°C. Three replications were used and SV traits were measured on 20 plants per cultivar per replication, DNA extraction from leaf tissue samples and PCR assays were as described earlier. A total of 59 random amplified polymorphic DNA (RAPD) primers were used. Thirty-nine of these produced bands that mapped to SV QTL positions in seven chromosomes (Redoña and Mackill, 1996c). The other 20 primers were randomly selected in order to provide a basis for comparison with mapped RAPDs. SV phenotypic data were analyzed using simple computer macros and SAS procedures (SAS Institute, 1989). Binary scoring for the 39 previously mapped RAPDs, identified based on DNA fragment size, and the 70 bands from the 20 randomly-selected primers and cluster analysis were as described earlier.

Highly significant differences were observed among the 49 cultivars for all four SV traits measured- length of shoots, roots, coleoptile and mesocotyl. All

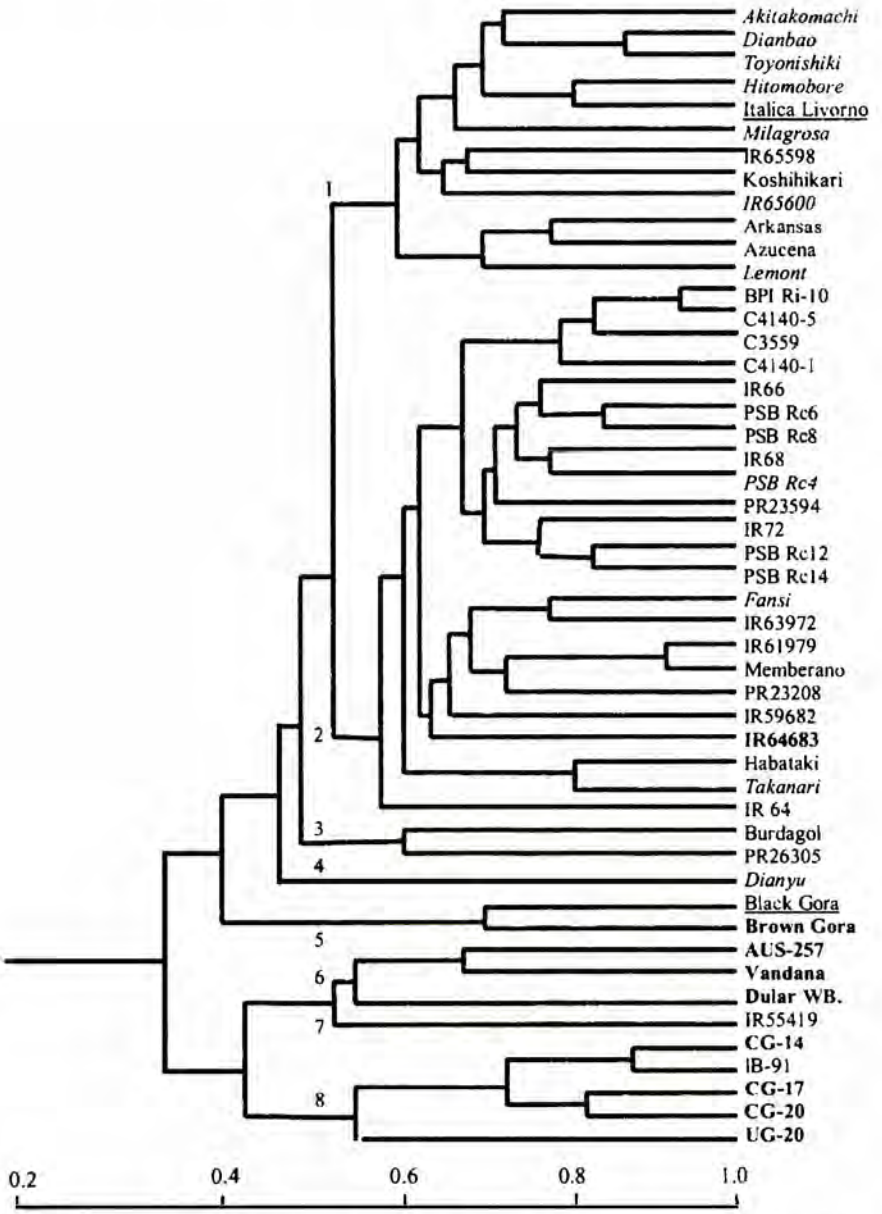


Figure 3. Grouping of cultivars based on genetic similarity coefficients derived using RAPDs bordering regions influencing seedling vigor. Main clusters are marked from I to 8, with high-SV cultivars indicated in bold, low-SV in italics, and known donors underlined.

Table 3. Correlation between parental genetic distance and heterosis for eight traits in the 48 F_1 hybrid combinations.

a) 1998 WS

Trait	*Male parent*		*PSBRc28*		*PSBRc72H*	
	I[a]	II[b]	I[a]	II[b]	I[a]	II[b]
Plant height	0.14	0.39	-0.18	-0.10	-0.18	0.14
Maturity	-0.05	-0.60*	-0.07	0.35	-0.07	0.45*
Leaf area index	0.34*	0.45**	-0.26	0.02	-0.26	-0.15
Root length	0.05	-0.07	0.13	0.07	0.13	0.14
Root weight	0.16	0.13	0.06	0.00	0.06	0.04
No. of tillers/plant	0.37*	0.43**	-0.05	-0.07	-0.05	-0.02
Harvest index	-0.31*	0.22	-0.38**	none	-0.38**	0.07
Yield	-0.12	0.09	-0.29*	-0.23	-0.29*	-0.23

b) 1998 DS

Trait	*Male parent*		*PSBRc28*		*PSBRc72H*	
	I[a]	II[b]	I[a]	II[b]	I[a]	II[b]
Plant height	-0.40**	-0.00	-0.04	0.26	-0.05	0.54
Maturity	-0.20	0.10	-0.40**	0.29	-0.40**	none
Leaf area index	0.10	0.41	-0.10	-0.20	-0.14	-0.20
Root length	-0.10	-0.30	-0.04	-0.22	-0.04	-0.04
Root weight	-0.10	0.06	-0.05	0.31	-0.05	0.34
No. of tillers/plant	0.17	-0.10	0.11	0.12	0.11	0.12
Harvest index	-0.10	0.46	-0.20	-0.10	-0.21	-0.11
Yield	-0.30	-0.30	-0.30	-0.20	-0.31	none

c) Average of 1998 WS & DS

Trait	*Male parent*		*PSBRc28*		*PSBRc72H*	
	I[a]	II[b]	I[a]	II[b]	I[a]	II[b]
Plant height	-0.15	0.01	-0.10	0.24	-0.10	-0.19
Maturity	-0.08	0.27	-0.15	-0.19	-0.15	none
Leaf area index	0.39**	0.45*	-0.17	0.11	-0.21	-0.12
Root length	0.10	-0.06	0.10	-0.12	0.10	0.02
Root weight	0.13	0.05	0.05	0.03	0.05	0.002
No. of tillers/plant	0.41**	0.40*	0.04	0.03	0.03	0.21
Harvest index	-0.26	0.77*	-0.26	none	-0.29*	-0.66
Yield	-0.30*	-0.03	-0.29*	-0.43	-0.30*	-0.81

[a] Correlations based on 48 F_1 hybrid combinations.
[b] Correlations based on F_1 hybrid combinations with positive heterosis.

traits exhibited continuous distribution indicating quantitative inheritance for SV in rice. Several cultivars gave consistently superior or inferior performance for most SV parameters. The aus cultivar Black Gora (BG) was among the 10 most vigorous for all four SV parameters. However, other high-SV donors were also identified including CG-17, CG-14, CG-20, AUS-257, UG-20, and Brown Gora, that were among the 10 most vigorous cultivars for three of four SV traits. Cluster analysis, based on genetic similarities at all 39 RAPD loci flanking SV QTL, discriminated the 49 cultivars into 8 major groups with one cluster composed of 23 or 46% of the test cultivars (Figure 3). The clustering patterns did not strictly follow traditional bases for grouping based on subspecific classification, pedigree, and/or geographical origin. For example, while japonicas formed cluster 1, indicas, japonicas and javanicas grouped together in cluster 2, and cultivars from Japan, USA, China, and the Philippines formed cluster 1.

The clustering of cultivars based on markers bordering SV QTL appeared to have some relationship with actual SV performance based on phenotypic data. For example, clusters 6 and 8 consisted mostly of cultivars with high SV, while cluster 1 was composed mainly of cultivars with low-SV cultivars. This suggests that classification based on marker genotypes at SV QTL may be reflective of actual SV phenotypic performance. High-SV cultivars belonging to different clusters may possess alternative SV alleles. Three major groups of SV donors could be discerned- the first group composed of Black Gora and Brown Gora, the second comprised of Aus 257, Vandana and Dular WB, and the third group composed of CG-14, CG-17, CG-20 AND UG-20. IL clustered differently from the other high SV cultivar BG. Redoña and Mackill (1996d) suggested that different sets of SV alleles may be present in these SV genetic donors. Crosses between high-SV cultivars from different clusters should provide broader genetic SV variability in the resulting breeding populations that could be exploited in breeding varieties for direct-seeded culture. Such crosses have, therefore, been generated at PhilRice's breeding program.

Genetic Diversity Analysis of Progenitor Cultivars of Modern Rice Varieties using AFLPs

In the Philippines, very few studies have been conducted on the genetic diversity of varieties approved for commercial release and their progenitor cultivars. Results from these few studies indicate that the genetic base of Philippine modern varieties seem to be relatively narrow (de Leon, 1994; Caldo, 1996). Information on the genetic relatedness or diversity of modern Philippine rice varieties can influence the direction of PhilRice's breeding program.

AFLP markers are among the most promising genetic markers for genetic diversity studies in rice (Mackill et al., 1996). Eighty land race progenitors of modern Philippine varieties used by Caldo (1996) and assayed with RAPD and microsatellite markers by Sebastian et al. (1998a) were used in this study. DNA extraction and AFLP procedures as well as cluster analysis were as earlier described.

Using one selective nucleotide in the first amplification and 14 Eco R1-Mse1primer combinations with three selective nucleotides, a total of 110 major AFLPs were detected among the 80 landrace progenitors evaluated (Table 4). The average number of polymorphic loci per primer combination was 8 loci and the range was from 4 to 13 loci. These results were comparable to those obtained by Mackill et al. (1995), also using Eco R1- Mse1 primer combinations, who reported an average of eight AFLPs per primer combination.

Cluster analysis produced two major clusters (Figure 4) at the highest fusion level, designated as Cluster 1 and Cluster 2, with the latter including most of the test materials. Cultivars in Cluster 1 included many cultivars from the southern US rice belt that have been previously classified by RAPD analysis as belonging to the tropical japonica or javanica subspecies (Mackill, 1995). Cluster 1 also includes traditional Philippine cultivars such as the premium-quality rices Milagrosa and Azucena. It is interesting to note that based on isozyme analysis (Mallik et al., 1995), most traditional Philippine varieties have also been classified as tropical japonicas. Results of this AFLP study lend support to this observation. Also in Cluster 1 are known tropical japonica cultivars from Africa such as OS4. Cluster 1, therefore, may represent the tropical japonica group among the cultivars assayed. Grouping patterns for cultivars in Cluster 2 based on geographical origin, on the other hand, cannot be easily discerned. However, cultivars originating from India, a known center of diversity for the indica subspecies, belonged to Cluster 2. Two major subclusters could be discerned at next highest fusion level. One subcluster included the wild species *Oryza nivarra* from India that, like cultivated rice, also possesses the AA genome. Clustering patterns of cultivars from the same country were not clear-cut. For example, the Chinese cultivars generally clustered close to each other while the Philippine traditional cultivars *Palawan* and *Kinampupoy* clustered far apart within Cluster 2.

Also evident in the dendogram were the close clustering of different accessions of the same cultivar. This implies that these accessions may actually be just one genotypic sample. AFLPs, therefore, appear to be an effective way for checking duplications of entries conserved in gene banks, thereby, saving a lot of resources that would otherwise be needed for germplasm storage and rejuvenation.

CONCLUSION

Molecular marker technology is beginning to be used in breeding both inbred and hybrid rice varieties in the Philippines. In addition to the above studies, other molecular marker applications are being conducted at PhilRice by different research groups. Among these are mapping studies for rice tungro virus resistance (Sebastian, 1996) and blast resistance (Tabien, 1998) and tagging of thermosensitive genetic male sterility (TGMS) genes using microsatellite markers. All these studies aim to facilitate the transfer of the target genes through marker-based selection into elite breeding lines both for inbred and hybrid rice breeding. Molecular markers

Table 4. AFLP primers used to screen the progenitor cultivars and the number of polymorphic loci detected per primer combination.

A. First Amplification
1. Eco +1 : 92R11 (A)
2. Mse +1 : 92H18 (A)

B. Second Amplification

	EcoRI +3	*Sequence*	*Mse +3*	*Sequence*	*# of bands*
1	92SO3 (A)	5' -GACTGCGTACCAATTCAAG-3'	92GO5 (A)	5' - GATGAGTCCTGAGTAAAGG -3'	7
2	92SO6 (A)	5' -GACTGCGTACCAATTCAGA -3'	92F38 (A)	5' - GATGAGTCCTGAGTAAACC - 3'	4
3	92SO5 (A)	5' -GACTGCGTACCAATTCACA -3'	92GO7 (A)	5' - GATGAGTCCTGAGTAAAGT -3'	6
4	92SO6 (A)	5' -GACTGCGTACCAATTCAGA -3'	92GO6 (A)	5' - GATGAGTCCTGAGTAAAGC -3'	5
5	93B11 (A)	5' -GACTGCGTACCAATTCACC -3'	92G10 (A)	5' - GATGAGTCCTGAGTAAATA -3'	9
6	93B11 (A)	5' -GACTGCGTACCAATTCACC -3'	92GO3 (A)	5' - GATGAGTCCTGAGTAAAAT -3'	8
7	92SO3 (A)	5' -GACTGCGTACCAATTCAAG-3'	92GO8 (A)	5' - GATGAGTCCTGAGTAAACG-3'	9
8	92SO6 (A)	5' -GACTGCGTACCAATTCAGA -3'	92GO5 (A)	5' - GATGAGTCCTGAGTAAAGG -3'	9
9	92SO3 (A)	5' -GACTGCGTACCAATTCAAG-3'	92GO3 (A)	5' - GATGAGTCCTGAGTAAAAT - 3'	13
10	92SO7 (A)	5' -GACTGCGTACCAATTCATA -3'	92G10 (A)	5' - GATGAGTCCTGAGTAAATA -3'	4
11	93B11 (A)	5' -GACTGCGTACCAATTCACC -3'	92G11 (A)	5' - GATGAGTCCTGAGTAAATG -3'	8
12	92SO6 (A)	5' -GACTGCGTACCAATTCAGA -3'	92GO8 (A)	5' - GATGAGTCCTGAGTAAACG-3'	4
13	92SO3 (A)	5' -GACTGCGTACCAATTCAAG-3'	92GO6 (A)	5' - GATGAGTCCTGAGTAAAGC -3'	12
14	92SO3 (A)	5' -GACTGCGTACCAATTCAAG-3'	92G11 (A)	5' - GATGAGTCCTGAGTAAATG -3'	12

Total number of AFLPs 110

Average number of AFLPs per gel 8

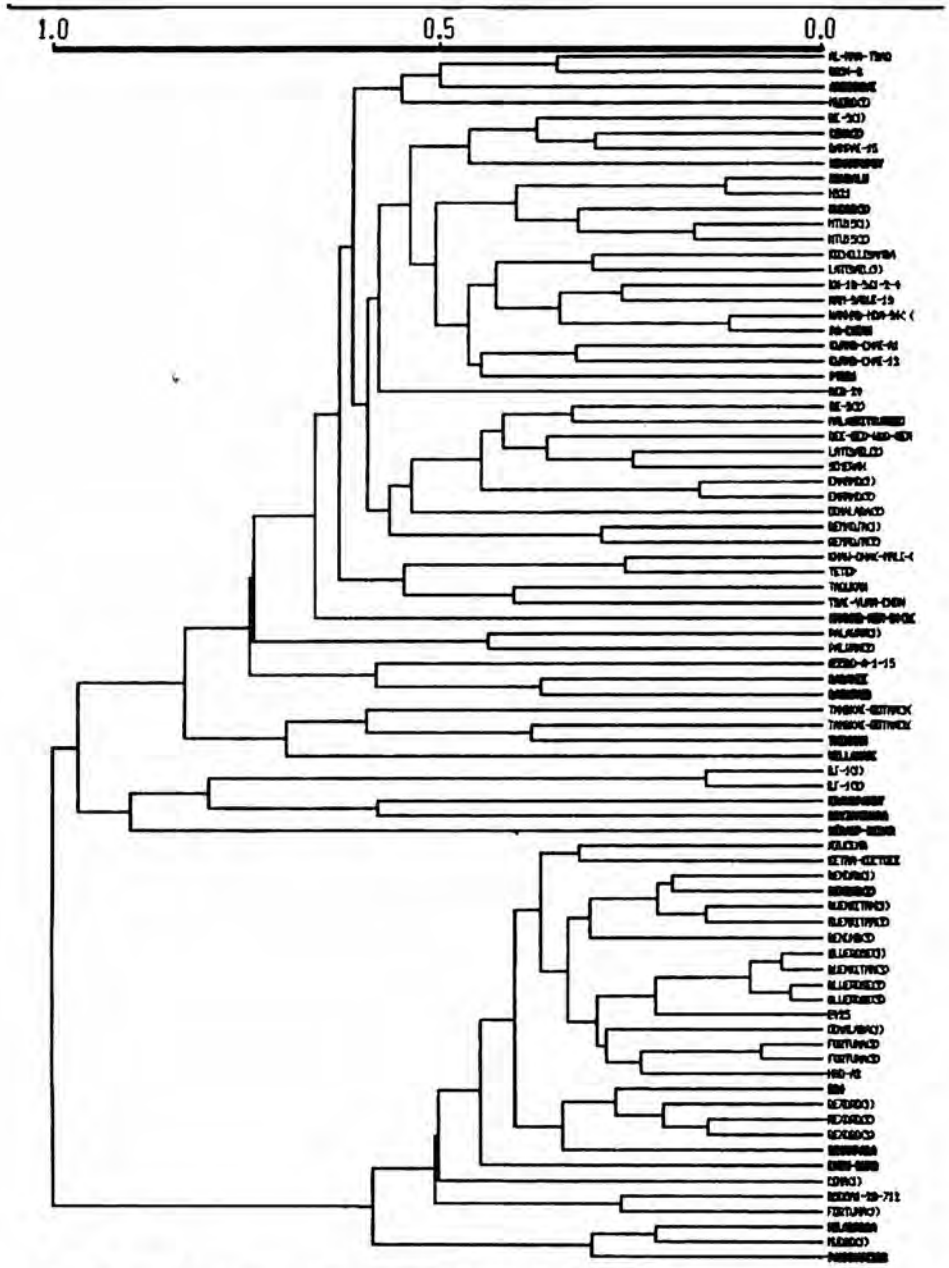


Figure 4. Molecular genetic variation detected by the three types of markers used in determining the genetic diversity of CMS lines at PhilRice.

associated with desired genes or QTL could be useful in various stages of varietal development: (a) markers may be utilized in screening parental materials and breeding lines for desired genotypes at marker loci in order to introgress and/or pyramid favorable genes/QTL alleles in new populations, (b) advanced breeding lines derived from mapping populations that are recombinant for the greatest number of favorable genes/alleles at QTL may be selected and used directly as cultivars or indirectly as parental materials, (c) recombinant lines may be used in backcrosses to other cultivars and selection of favorable genes/alleles at QTL based on marker genotypes may be undertaken (Redoña and Mackill, 1996c). However, cheaper, technically simpler, and readily available marker technologies may still need to be developed for markers to find large-scale breeding applications. This is especially true in the Philippines where the use of molecular markers are currently limited to a few well-equipped and adequately-financed research institutions with trained technical manpower.

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DETECTION AND GENOTYPING OF HUMAN PAPILLOMA VIRUS ASSOCIATED WITH CERVICAL CANCER AMONG FILIPINO WOMEN

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ABSTRACT

Infection of cervical epithelial cells with human papillomaviruses (HPV) is a major factor in cervical cancer development. Out of more than 70 HPV types identified, about 30 types have been found to infect the genital and cervical mucosa. This study aimed to detect HPV in cervical cancer patients and identify which HPV genotypes are prevalent among Filipino women. Cervical tissue biopsies were collected from patients with cervical malignancies from various hospitals. A broad spectrum of mucosal HPV types have detected by consensus primer-mediated polymerase chain reaction (PCR) using the degenerate form of the MY09/MY11 primer pair which amplifies a ~450 base pair segment of the HPV major capsid gene. Of the 162 patients in the study, 137 (84.6%) tested positive for HPV. PCR products generated are cloned into pUC18 and sequenced in ABI PRISM 310 Genetic Analyzer. Homology search and phylogenetic analysis of the resulting sequences from 11 samples show close sequence relation to HPV type 6.

Keywords: cervical cancer, human papillomavirus, oncogenic agent, polymerase chain reaction, degenerate consensus primer, major capsid gene, genotypes, cloning, DNA sequencing, phylogenetic analysis

INTRODUCTION

Epidemiological and molecular studies on cervical cancer, the second most common form of cancer in Filipino women, stimulated research on the role of sexually transmitted agents on the carcinogenesis of this neoplasia.

Human papillomaviruses (HPV) are the cause of common warts, plantar and genital warts. They are also associated with the majority of benign and malignant lesions of the anogenital tract. Out of more than 70 pathogenic human strains of HPVs identified so far, the HPV 6 and HPV 11 (low-risk types) have been predominantly associated with benign genital warts and low grades of cervical dysplasia, whereas HPVs 16, 18, 31, 33, 35, & 39 (high-risk types) have been found mainly in severe dysplasia and malignant cervical carcinoma (Choi, 1990; Garuti, *et al*, 1989). A number of them have been implicated as an infectious oncogenic agent, first because the HPV DNA has been detected in more than 90% of all cervical cancers (Campion, 1987; Crum, *et al*, 1984; Reid, *et al*, 1988), and second since they encode proteins (E5-E7) which modify responses and form complexes with products of tumour suppressor genes (p53 and Rb) (Dyson, *et al*, 1989; Scheffner, *et al*, 1990).

HPV infections have been diagnosed through established cytologic and histologic criteria as well by electron microscope search for viral particles (Syrjanen, *et al*, 1985) and immunologic detection of HPV group-specific capsid antigen (Woodruff, *et al*, 1980). Due to the lack of appropriate tissue culture systems for *in vitro* propagation of HPV, diagnosis is predominantly based on the detection of its DNA. Originally, DNA hybridization techniques such as Southern blotting and *in situ* hybridization have been used for HPV detection. Early detection of cervical cancer in the premalignant stages is the goal of routine cervical cancer prevention efforts. The advent of polymerase chain reaction (PCR) significantly improved the sensitivity of HPV DNA detection.

The aim of this study is to detect HPV associated with cervical cancer by PCR and subsequently determine the viral genotype by nucleic acid sequencing and phylogenetic analysis. This study will provide epidemiological data on the presence of HPV in cervical cancer in the Philippines.

METHODOLOGY

Sample Collection

Freshly excised cervical biopsies from female patients referred to the OB-GYNE clinic for suspected cervical cancer were collected in sterile saline solution and transported frozen to the lab for processing. Collaborating hospitals include Dr. Jose Fabella Memorial Hospital, Philippine General Hospital, Dr. Jose Delgado Memorial Hospital, East Avenue Medical Center and St. Luke's Medical Center.

DNA Isolation

Tissue specimens were incubated at 55°C overnight in a digestion buffer containing 10mM Tris-HCl, pH 8, 100mM NaCl, 25mM EDTA, 0.5% (w/v) SDS and 100ug/ml Proteinase K. Samples were deproteinized twice with phenol/ chloroform/isoamyl alcohol (25:24:1). The DNA was precipitated with 7.5M

ammonium acetate and absolute ethanol, and resuspended in 100 μL double distilled, deionized water.

Polymerase Chain Reaction

Four microliters (4 μL) of the DNA solution was supplemented with 5 μL 10X PCR buffer (500 mM KCl, $MgCl_2$, 100 mM Tris-HCl, pH 9), 50 pmoles of each primer (5'CGTCCMARRGGAWACTGATC3'/5'GCMCAGGGWCA-TAAYAATGG3'), 0.25 μL 20 mM dNTP mix, 1U of Taq DNA polymerase (Pharmacia), and autoclaved distilled water to a final volume of 50 μL. Samples were overlaid with mineral oil and subjected to 40 cycles in an Eppendorf Mastercycler. The temperature profile was as follows: 10 min initial denaturation at 94°C, 1 min denaturation at 94°C, 1.5 min primer annealing at 45°C, 1 min extension at 72°C, and a 10 min final extension at 72°C.

Cloning of the PCR Product

One hundred microliters (100 μL) of the PCR product mix was loaded onto a 1% low-melting temperature gel with large wells. The PCR product was recovered using the GFXä PCR DNA and Gel Band Purification Kit (Pharmacia) and resuspended in 50 μL sterile distilled water. Using the SureCloneä Kit (Pharmacia), the purified PCR product was blunted, phosphorylated and ligated unto blunt-ended pUC18 overnight at 16°C. Transformation was done by mixing 4 μL of the ligation mix with 50 μL competent *E.coli* XL-1 Blue cells. The transformation mix was incubated on ice for 30 minutes, heat-shocked at 42°C for 30 seconds, and was placed back on ice for 2 minutes. Four hundred fifty microliter (450 μL) SOC medium was then added and the mix was incubated at 37°C with gentle shaking in a Thermomixerä (Eppendorf, Germany) for an hour. Forty microliters (40 μL) of X-Gal solution (40 mg/mL) was spread on previously prepared LB agar plates containing 100 ug/mL ampicillin, and was allowed to soak for at least 30 minutes. Fifty μL and 200 μL of the transformation mix were spread on separate LB plates and grown overnight at 37°C. The plates were then shifted to 4°C for 2-3 hours for proper color development.

DNA Sequencing

The clones were sent to the Department of Virology at the Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan for DNA sequencing. Capillary gel sequencing using the ABI PRISM 310 Genetic Analyzer was utilized for this purpose.

RESULTS

One hundred sixty three (163) cervical specimens were collected from the collaborating hospitals. Genomic DNA was extracted from the tissue samples and

subjected to PCR using the consensus L1 MY09/MY11 primer pair. One hundred thirty seven (137) samples yielded the expected 450-bp PCR product (Figure 1).

PCR products of eleven samples were cloned. After screening the clones with the β-Gal colorimetric (blue/white colony) selection, plasmid preparations were checked further for inserts. The plasmid DNA from five clones from each sample were analyzed on a 0.9% agarose gel along with circular pUC18 as negative control. The size of the plasmid DNA from the clones should be predictively greater than the vector without the inserted PCR product. Plasmid DNA that exhibits a band which migrated slower than pUC18 was considered positive for the insert. Furthermore, these selected plasmids were double digested with EcoR1 and BamH1 to identify the ligated 450-bp PCR product fragment.

All DNA sequences were derived from at least five independent clones of each of the 11 samples and were done at the Institute for Tropical Medicine in Nagasaki University, Japan. Alignment of the DNA sequences with the corresponding region for all other HPV in data bases and computer-assisted homology search from the BLASTN programs showed a close sequence relation with HPV type 6. An aligned sequence (Figure 2) shows base differences with the prototype (type 6) and differences among samples.

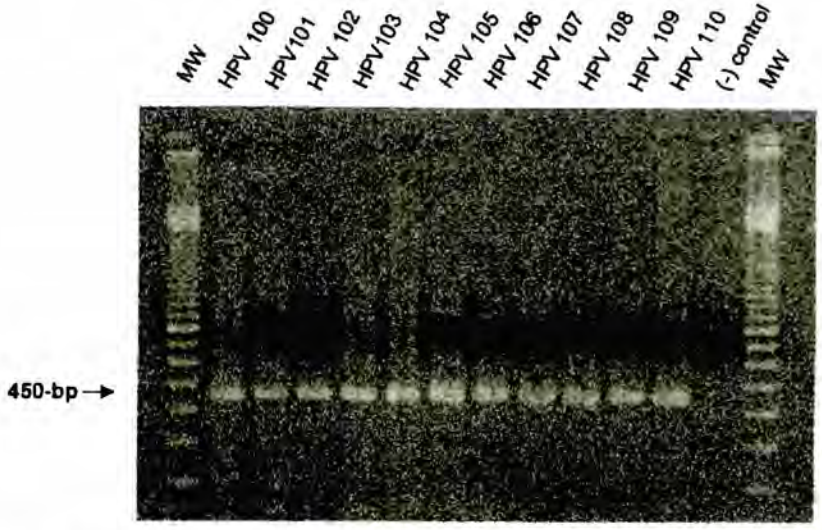

Figure 1. PCR products from genomic DNA isolated from cervical biopsies using MY09/MY11 primer pair after electrophoresis in a 2% agarose gel. Numbers above the lanes represent the isolate code. Negative control contains no DNA as template. Molecular weight (MW) marker used is a 100-base pair ladder (Pharmacia: 800-bp mark is the most intense band).

A phylogenetic tree (Figure 3) was constructed by distant matrix algorithms (DNADIST) and by UPGMA method of clustering using the PHYLIP 3.5 package.

DISCUSSION

Unlike other viruses whose members are classified into serotypes based on antigenic relatedness, papillomaviruses are designated as types based on the relatedness of their genomes. An HPV isolate is considered a new type if it meets certain criteria. For an isolate to be recognized as a new HPV type, the nucleotide sequences of the E6, E7 and L1 open reading frames (ORF) should demonstrate <90% identity with established types; isolates that differ from an established type by 2-10% are considered subtypes, and those that have <2% divergence are termed variants (Bernard *et al*, 1994).

Since comparing three ORFs is a laborious undertaking, an alternative method is to analyze only a segment of the HPV genome. The HPV L1 ORF contains both conserved and divergent regions. The regions that are flanked by the MY09 and the MY11 primers appear to be highly conserved between, if not all, genital HPV types (Peyton & Wheeler, 1994). It is therefore, reasonable to believe that isolated genital HPVs could be identified by comparing sequences of this region.

Sequence comparison of the 11 isolates to those of reference type HPVs identified these isolates as variants of the HPV type 6 for they all exhibited greater than or equal to 98% sequence similarity. Since the homologies are quite high, there seems to be no significant differences in the genetic distances. Although HPV 16 is the type highly related to a number of cervical neoplasia cases and prevalence of the type 6, a low-risk HPV, in these cases is unpopular, some papers indicated otherwise. Shen et al reported a prevalence rate of 0% type 16 in all their specimens for routine hysterectomy and cervical neoplasia; while Melkert et al reported a low but consistent 1-2% rate of type 16 in women 35-55 years of age. Both papers noted a significantly lower rate of type 16 infection in older women. The generalization about the specific types being associated with certain forms of precancerous and cancerous lesions of the cervix is not absolute. HPV 16 (Syrjanen *et al*, 1986; Shirasawa *et al*, 1986) has been detected in mild dysplasia and in clinically normal women (Gissman, 1986] while HPV 6-like sequences have been reported in CIN III and in invasive carcinoma (Hoepner & Loning, 1986).

HPV infection is a multicentric disease. The spectrum of HPV infection ranges from subclinical infection to exophytic condyloma to CIN and invasive cancer (Schiffman, 1992). Although an individual may exhibit one clinical symptom over another, multiple HPV types and clinical presentations can coexist. If one has to rule out the possibility of contamination with lower-risk HPVs in the lower genital tract during specimen collection, then one must consider the possibility of multiple-type infection (especially in immuno-compromised women) in one subject. LR-HPV DNA is usually found in episomal form and in high-copy number within

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HPV  6    CAGGGA-CATAAC ATGGTATTTGTTGGGGTAATCAACTGTTTGTTACTGTGGTAGATACCACACGCAGTACCAACATGACATTATGT89
HPV  003  — — — T — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — .
HPV  046  — — — T — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — .
HPV  047  — — — T — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — .
HPV  050  — — — T — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — .
HPV  053  — — — A — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — .
HPV  054  — — — T — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — .
HPV  056  — — — T — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — G — — —
HPV  093  — — — T — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — .
HPV  094  — — — T — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — .
HPV  095  — — — T — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — .
HPV  096  — — — T — — — T — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — .

HPV  6    GCATCCGTAACTACATCTTCCATACACCAATTCTGATTATAAAGAGTACATGCGTCATGTGGAAGAGTATGATTTACAATTTATTTTT180
HPV  003  — — — — — — — — — — — — — — C — — — — — — — — — — — — — — — — — — — — — — — — — — — — —
HPV  046  — — — — — — — — — — — — — — — C — — — — — — — — — — — — — — — — — — — — — — — — — — — —
HPV  047  — — — — — — — — — T C — — — — C — — — — — — — — — — — — — — — — — — — — — — — — — — — —
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HPV  096  — — — — — — — — — — — — — — — C — — — — — — — — — — — — — — — — — — — — — — — — — — — —

HPV  6    CAATTATGTAGCATTACATTGTCTGCTGAAGTAATGGCCTATATTCACACAATGAATCCCTCTGTTTTGGAAGACTGGAACTTTGGGTTA270
HPV  003  — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — —
HPV  046  — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — —
HPV  047  — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — —
HPV  050  — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — —
HPV  053  — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — —
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HPV  093  — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — —
HPV  094  — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — —
HPV  095  — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — —
HPV  096  — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — — —
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Figure 2. DNA sequence alignment of the 11 isolates with HPV type 6 generated using DNAsis; HPV 6 sequence was obtained from Genbank. For the 11 isolates, only the bases different from those of HPV type 6 are shown.

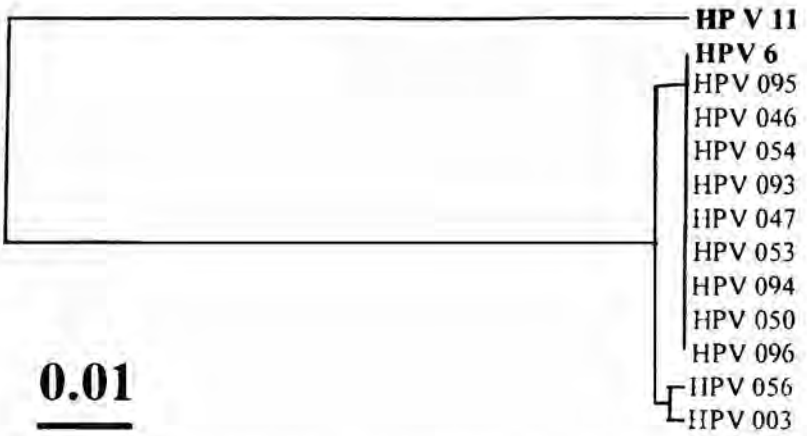

Figure 3. Dendogram showing relationship among the 11 isolates and two established HPV types (HPV 6 & 11). The dendogram was constructed by distant matrix algorithm (DNADIST) and by UPGMA method of clustering in the PHYPLIP 3.5 package.

the cells. In contrast, a marked reduction in HPV DNA replication occurs in high grade precursor lesions and cancers because of the integration of the viral DNA into the chromosome (Shen *et al*, 1995).

It has also been speculated that the currently used L1 consensus MY09/ MY11 primers has a lower stringency with the low-risk HPV (6/11) DNA which are distantly related to other mucosal HPVs [Galloway, 1994]. More recent reports on the modification of these primers into a degenerate form [Ong *et al*, 1994; Meyer *et al*, 1995] allowed a broader range of HPVs to be detected and amplified. HPV typing in genital specimens is a diagnostic challenge because of the numerous types that must be detected and distinguished. Our PCR-based procedures can still be modified to an extent to provide sensitivity and specificity in the identification of a still growing variety of HPV. Beyond this, RFLP analysis can be utilized (refer to Figure 6) to assess the established types [Meyer *et al.*, 1995] although DNA sequencing is still the one recommended to identify novel HPV types and for designing probes for these new types.

With a growing number of pathogenic agents that are yet to be documented and analyzed, rapid and sensitive methods are needed. The advent of PCR and DNA sequence analysis allows easy and accurate detection. Without an established excellent clinical and histopathological data, one can rely on these molecular techniques.

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RESEARCH NOTE:

ASSOCIATION BETWEEN CHROMOSOME FRAGILE SITE AND ONCOGENE LOCATION: A PROFILE OF FILIPINO HEAD AND NECK CANCER PATIENTS

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ABSTRACT

Chromosome instability is often associated with predisposition to cancer. Peripheral blood lymphocytes from 25 cancer pateients and 25 control individuals were cultured for 72 h. Chromatid and chromosome breaks were induced by treating lymphocytes with bleomycin, a known mutagen. Chromatids and chromosomes were scored in50 spreads for each individual. Frequency of breaks was tallied per chromosome number. A correlation was made between frequency of breaks and differential sensitivity of chromosomes to mutagen. Association between location of oncogene and fragile sites on chromosome was determined.

Keywords: mutagen, head and neck cancer, bleomycin, lymphocytes, chromatid and chromosome breaks, fragile site, oncogene

INTRODUCTION

A number of assays have been developed to assess the susceptibility of genetic material to mutagen-induced genetic lesions. Specifically, chromosomal aberrations are considered the most readily discernable manifestations of genetic

damage in cells. The bleomycin sensitivity test is one assay which induces mutagen-induced chromosomal lesion in cultured cells. The assay has been proven to play a significant role in predicting carcinogenesis in tissues that are in contact with the external environment (Hsu et al., 1989).

Bleomycin is a an antitumoral glycopeptide, described to be a radiomimetic agent because it can cause single and double strand breaks and chromosome damage in all phases of the cell cycle, similar to ionizing radiation.

Chromosome sensitivity to mutagen-induced chromosomal damage is positively associated with the incidence of upper aerodigestive tract cancers. These cancers are known to be caused by environmental carcinogens particularly found in tobacco and alcohol (Spitz *et al.*, 1989; Spitz and Hsu, 1994). The concept behind this method rests on the idea that chromosomes of high risk individuals when treated with bleomycin will likely accumulate more mutations than low risk individuals.

Fragile sites (FS) in human chromosomes are regions prone to breakage which result when cells are exposed to specific chemical agents or conditions of tissue culture (Sbrana and Musion, 1995). Some studies suggest a relationship between FS and cancer as indicated by the preferential clastogenic action on these sites by many mutagens and carcinogens known to act through different molecular mechanisms. It is believed that FS may be general targets of mutagenic action (Yunis *et al.*, 1987).

PATIENTS AND METHODS

Subjects

Thirty (30) patients who were diagnosed with head and neck cancer in a government hospital in Manila and who have had no prior treatment for cancer were included in the study. All patients signed an informed consent form and answered a basic questionnaire specifically prepared for the study. Ten milliliters (10 mL) of peripheral blood were extracted from each subject using a heparinized vacutainer tube.

Thirty (30) non-cancer individuals comprised the control group. As a rule blood samples from the control group were cultured simultaneously with the cancer specimen.

Culture Conditions

Microculture (whole blood culture) technique was performed in RPMI 1640 supplemented with 10 fetal bovine serum (FBS) with 2% phytohemagglutinin (PHA) for 72 hours at 37°C in 5% CO_2 atmosphere. For each subject, two culture conditions were carried out: one treated with bleomycin and the other without bleomycin. The mutagen was added during the last five hours of culture. Colcemid (0.2 μg/ml), an arresting agent was added to the cultures two hours before harvest. Chromosome preparations were obtained by standard techniques: through 0.075M KCl hypotonic shock, methanol: acetic acid fixation, and air-dry slide preparation.

Cytogenetic Analysis

Slides were allowed to age for 5-10 days prior to staining. G-banding was done by subjecting the chromosomes to a mild treatment of trypsin followed by Giemsa staining (5% solution). When adequate mitoses were obtained, 50 cells were screened per subject. Chromatid gap and breaks, as well as chromosome gap and breaks were scored for each subject and the average number of aberrations per cell was computed. Results were expressed as breaks per cell (b/c).

Results

The ages of the cancer group (A) ranged from 18 to 77 years old and the mean age was 48. There were 19 males and 11 females. Table 1 shows the profiles of sensitivity in all 30 cancer subjects and 30 controls. One thousand five hundred (1,500) cells were screened from the cancer patients and the total aberrations scored was 1,836. The mean value was 1.22 break per cell (b/c).

In the control group, ages ranged from 17 to 66 and the mean age was 28. There were 15 males and 15 females. A total of 980 chromosome breaks was recorded from among 1,500 metaphase cells analyzed. The mean number of break per cell was 0.653 (Table 1).

In the cancer group, a good number of aberrations was localized on chromosome 3. An abnormal metaphase cell from a cancer patient showing various structural aberrations like chromatid gaps (ctg), chromatid break (ctb), acentric fragments (ac).

DISCUSSION

Results of this study show a higher mean number of breaks per cell in the cancer group compared to the control subjects. The mean value of b/c in the cancer group was 1.22 while that in the control group was 0.653. The bleomycin assay is a mutagen-sensitivity assay, in which bleomycin, a mutagen, induces chromosome breaks. It may be used to identify individuals with less efficient DNA repair mechanisms, an indication of them having a much higher risk of developing tumors than the normal controls. This concept of chromosomal fragility has been linked to cancer development.

Analysis of G-banded chromosomes reveals that certain chromosomes or chromosome regions are more susceptible to damage than the others. These damage-prone areas of the chromosomes called fragile sites, are often implicated in chromosomal rearrangements present in malignant disease. Though quite controversial, this hypothesis is supported by the observation that there is an association between FS localization, cancer breakpoints and oncogenes (Sbrana and Musio, 1995). The findings of these fragile sites in cancer are further supported by studies which claim that FS may be general targets of mutation (Yunis and Soreng, 1987).

Table 1. Distribution profiles of bleomycin sensitivity in 30 head and neck cancer patients and 30 controls.

Breaks per cell (B/c)	Subjects	Controls
0.00-0.20	1	6
0.21-0.40	7	4
0.41-0.60	5	5
0.61-0.80	2	5
0.81-1.00	3	3
1.01-1.20	0	3
1.21-1.40	2	2
1.41-1.60	1	2
1.61-1.80	2	0
1.81-2.00	2	0
2.01-2.20	1	0
2.21-2.40	0	0
2.41-2.60	0	0
2.61-2.80	2	0
2.81-3.00	0	0
3.01-3.20	1	0
3.21-3.40	0	0
3.41-3.60	0	0
3.61-3.80	0	0
3.81-4.00	0	0
4.01-4.20	0	0
4.21-4.40	0	0
4.41-4.60	0	0
4.61-4.80	0	0
4.81-5.00	0	0
5.01-5.20	0	0
5.21-5.40	0	0
5.41-5.60	0	0
5.61-5.80	0	0
5.81-6.00	1	0
no. of individuals	30	30
mean b/c value	1.22	0.653
standard deviation	1.20	0.464
coefficient of variation	98.0%	71.1%
% > 0.80	46.7%	33.3%
% > 1.00	36.7%	23.3%

Our data provide substantial biological rationale to expand this study in terms of sample size as well as to include other high risk groups. These may include not only the patients with environmentally-induced cancers, but also those who are constantly exposed to harmful substances like industrial workers, pesticide handlers, drivers, painters, sidewalk vendors or even the ordinary commuter who walks the streets and breathe the polluted air.

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RESEARCH NOTE:

A NEW MEDIUM FOR ISOLATION, CULTURE AND METRONIDAZOLE-SENSITIVITY TESTING OF *Helicobacter pylori:* DIAGNOSTIC VALUE FOR EARLY ERADICATION OF *H.pylori* INFECTION

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ABSTRACT

In the Philippines resistance to metronidazole has become a problem in the eradication of *Helicobacter pylori*, a bacterium now known to be the cause for chronic gastritis and peptic ulcer disease. In this study, a new medium was designed to ensure optimum growth of the bacteria for antimicrobial susceptibility testing. This complex basal medium used Columbia Blood agar base, urea agar base plus supplements such as horse serum, polyvitex, and peptone. For culture, antibiotics such as vancomycin, polymixin B and trimethoprim lactate were added. Since the medium does not contain whole blood but has urea agar base in its composition it is suitable for isolating *H. pylori* as well as direct testing of urease activity. This medium was tested using the six local isolates of *H. pylori* (as previously reported by the RBD Microbiology Laboratory) plus *H. pylori* ATCC 49503 strain as reference and other gram negative bacteria such as *Proteus vulgaris* ATCC 13315 and *Klebsiella pneumoniae*. Agar plates which contained this medium was inoculated with the different isolates and bacterial strains then incubated at 37°C in a microaerophilic environment (5% O_2, 10 CO_2 and 85% N_2) for 7 days. Confirmation of *H. pylori* was done by Gram staining and biochemical tests for catalase, oxidase and urease.

Keywords: *Helicobacter pylori*, metronidazole-resistant, urease, catalase, oxidase, microaerophilic, urea agar base, vancomycin, polymixin B, trimethoprim lactate.

INTRODUCTION

Helicobacter pylori is now accepted as one of the most frequent bacterial infections in the world. Ninety-five percent of chronic gastritis is attributed to this bacterium. It is also strongly associated with peptic ulcers and gastric carcinoma.

Patients infected with this bacterium are treated with antibiotics such as amoxycillin, tetracycline and metronidazole. Metronidazole together with omeprazol is the most common antibiotic used in the treatment of *H. pylori* (Beex et al., 1990). However with the emergence of *H. pylori* strains resistant to metronidazole and other 5-nitroimidazoles (Borody et al., 1995), this has become a problem.

The Philippine College of Physicians in their annual convention discussed "the challenges posed by *H. pylori* infection eradication." They attributed the high recurrence rate of infection in the Philippines to metronidazole resistance (Cellini et al., 1992). Thus, it is important that antimicrobial susceptibilty testing be done before treatment to ensure that antibiotics would be effective.

Since culture of *H. pylori* is very difficult even with known selective and non-selective media, the RBD Microbiology Laboratory developed a medium that is useful for culture and antimicrobial susceptibility testing. This new medium is a modified version of the one developed by Cellini in 1992.

MATERIALS AND METHODS

Bacterial Isolates

Five isolates of *H. pylori* isolated by the RBD Microbiology Laboratory from 1995-1999 were used in developing the modified medium in this study. The five isolates namely Hpg-03, Hpg-05, Hp-40, Hp-42 and Hp-43 were stored at -80°C in BHI with 10% glycerol before use. The isolates came from gastric biopsies of patients diagnosed with duodenal ulcers or gastritis.

Reference and Control Strains

H. pylori ATCC 49503, *Proteus vulgaris* ATCC 13315 and *Klebsiella pneumoniae* acquired from the culture collection of the Institute of Tropical Medicine, Nagasaki University were used as reference and control organisms, respectively. These strains were also stored at -80°C in cryobanks.

Media

Four media were developed and compared (Table 1) :

(1) HpNM1 consists of 4.4% Columbia Blood Agar base, 2.9 % Urea Agar base, 2.3% peptone, 0.1% Dextrose, 1% (vol/vol) Polyvitex and 7%(vol/vol) Horse serum.

(2) HpNM2, 1.5% of Bacto agar replaced CBA. Polyvitex and dextrose were eliminated.

(3) HpNM3, 0.3% yeast extract, 0.5% Beef extract and 0.1% glucose were added.

(4) HpNM4 is the same as HpNM3 except that horse serum was not added to the medium.

Table 1. Composition of the four media developed for *H. pylori*

Ingredients	MEDIA			
	HpNM1	HpNM2	HpNM3	HpNM4
Urea agar base	2.9%	2.9%	2.9%	2.9%
Proteose peptone	2.3%	2.3%	2.3%	2.3%
Polyvitex	1.0%	-	-	-
Dextrose	0.1%	-	-	-
Horse Serum	7%	7.0%	7.0%	-
Columbia Blood Agar	4.4%	-	-	-
Yeast Extract	-		0.3%	0.3%
Beef Extract	-		0.5%	0.5%
Glucose	-		0.1%	0.1%
Bacto-agar	-	1.5%	1.5%	1.5%

- Antibiotics were added to the media for culture:
  Vancomycin – 2.5 mg/250 ml
  Polymixin B - 1.25 mg/250 ml
  Trimethoprim- 625 units/250 ml

For culture, the following antibiotics were added: vancomycin (2.5 mg/ 250ml), polymixin B (1.25 mg/250ml) and trimethoprim (2.5mg/250ml).

Antimicrobial Susceptibility Testing

Local isolate Hp-40, which was found to be metronidazole-resistant, was inoculated into the HpNM1 medium and CBA with 7% laked horse blood. A 5 μg metronidazole disc was placed on top of the agar.

RESULTS AND DISCUSSIONS

The frozen local *H. pylori* isolates were thawed and rapidly seeded onto HpNM1, HpNM2, HpNM3 and HpNM4 and incubated at 37°C in a microaerobic atmosphere for 3-4 days. At the same time, the reference and control strains were cultured under the same conditions as the local *H. pylori* isolates for comparative studies of urease activity.

Upon inoculation of the isolates and control strains into the different media, color change in the media was observed every 15 min, 1 h, 6 h and 24 h and on the 3rd and 4th day. Observation was made before and after the plates were placed in a microaerophilic atmosphere.

Rapid color change was observed after 15 min at room temperature in Hp-40, Hp-42 and Hp ATCC 49503 (Table 2). The remaining three isolates, Hpg-03,

Table 2. Qualitative growth of H. pylori isolates on the different media and color change after different hours and days.

	HPNM1			HPNM2			HPNM3			HPNM4		
	15 mins. / 1 hr	6 hrs. / 1 day	3 days / 4 days	15 mins. / 1 hr.	6 hrs. / 1 day	3 days / 4 days	15 mins. / 1 hr.	6 hrs. / 1 day	3 days / 4 days	15 mins. / 1 hr.	6 hrs. / 1 day	3 days / 4 days
HPG-03	−* / −*	+* / −*	− / +*	−* / −*	−* / −*	− / −*	−* / −*	−* / −*	− / −*	−* / −*	−* / −*	−* / −*
HPG-05	− / −*	−* / −*	+* / +*	− / −*	−* / −*	−* / −*	− / −*	−* / −*	−* / −*	− / −*	−* / −*	−* / −*
HP-40	−* / −*	−* / −*	+* / +*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*
HP-42	−* / −*	−* / −*	+* / +*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*
HP-43	− / −*	−* / −*	+* / +*	−* / −*	−* / −*	−* / −*	− / −*	−* / −*	−* / −*	− / −*	−* / −*	−* / −*
ATCC 49503	−* / −*	−* / −*	+* / +	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*	−* / −*
Klebsiella pneumonide	− / −	− / +*	+* / +*	− / −	− / −*	+* / −*	− / −	− / −*	+* / −*	− / −	− / −*	+* / −*
Proteus vulgaris	− / −	− / +*	+* / −*	− / −	− / −	+* / −*	− / −	− / +*	+* / +*	− / −	− / −*	+* / −*

LEGEND:

(+) Presence of growth

(--) Negative for growth

* color change from yellow to red

Hpg-05 and Hp-43 displayed a red color reaction after 1 h in the microaerophilc environment at 37°C. A wider area of color change was observed in all the plates with *H. pylori* samples after 6 h of incubation. The medium became completely red after 24 h and remained stable after the 3rd and 4th days. *Klebsiella pneumoniae* and *Proteus vulgaris* ATCC 13315 showed a color change of the media at 24 h. The slow color change eliminates possibilities of false positive results. Since urease production is one of the major characteristics essential in identifying the bacterium, immediate reaction of the inoculum to urea would shorten identification time. This is beneficial as it leads to rapid diagnosis and at the same time reduces the possibility of false positives.

Growth of *H. pylori* in the different media was observed qualitatively. Good growth of *H. pylori* was observed in the HpNM1 medium. Visible colonies were seen after 3 days of incubation. HpNM2, HpNM3 and HpNM4 medium showed no growth at all. Growth of *H. pylori* in HpNM1 medium is the same as compared to its growth in CBA containing 7% laked horse blood.

Antimicrobial susceptibility testing using HpNM1 and CBA showed the same results in both media. Local isolate, Hp-40, which is metronidazole- resistant gave the same growth rate and reaction in these two media. HpNM1 had urease activity of the bacteria immediately, identification time was shortened. Bacterial growth in this study was confirmed by modified Gram staining, catalase and oxidase test.

In conclusion, the HpNM1 medium is a good substitute for known selective media for growth of *H. pylori*. It was able to sustain good growth as compared to CBA with blood and its immediate reaction to urease facilitated identification of the bacteria. Since other urease producers like *Proteus vulgaris* and *Klebsiella pnuemoniae* did not have an immediate reaction, then false- positive results were eliminated.

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ABSTRACTS OF POSTER PAPERS PRESENTED DURING THE NAST 21st ANNUAL MEETING, 7-8 JULY 1999

MATHEMATICAL, PHYSICAL, AND ENGINEERING SCIENCES

PSEUDO-LEXICOGRAPHIC AND ANTI-LEXICOGRAPHIC ORDERINGS OF PERMUTATIONS AND SOME ALGORITHMS

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Let S={1,2,...,n} and let μ be a permutation of S. Moreover, let $S\mu = \{x \in S \backslash x\ \mu \neq x\}$ be the set of elements of S moved the permutation μ. Define μ_i, $1 \leq i \leq n-1$ to be cycles of length $l = i + 1$ such that $S\mu_i \subset S\mu_{i+1}$. Then $\left\{\prod_{i=1}^{n-1} \mu_i^{d_i} | 0 \leq d_i \leq i\right\}$ generates S_n, the symmetric group n symbols. Moreover, each permutation generated by $\prod_{i=1}^{n-1} \mu_i^{d_i}$ can be indexed by x and represented by π_x, where $x = \sum_{i=1}^{n-1} i!\, d_i$ and $0 \leq x \leq n! - 1$. Define $<$ to be a linear ordering of the permutations π_x, such that π_α presence π_b precedes whenever $a < b$. Let σ_i and α_i be special cycles defined by

$$\sigma_i(x) = \begin{cases} x, & x < n\text{-i} \\ x+1, & n-i \leq x \leq n-1 \\ n-i, & x = n \end{cases}$$

and

$$\alpha_i(x) = \begin{cases} x+1, & 1 \le x < i+1 \\ 1 & , \quad x = i+1 \\ x & , \quad x > i+1 \end{cases}$$

respectively. Then the linear ordering < shall be called *pseudo-lexicographic* ordering if $\pi_x = \prod_{i=1}^{n-1} \alpha_i^d i$, *i.e.*, if $\pi_x = \prod_{i=1}^{n-1} \sigma_i^d i$, i.e., if $\mu_i = \sigma_i$ and *anti-lexicographic* ordering if $\pi_x = \prod_{i=1}^{n-1} \sigma_i^d i$, i.e., if $\mu_i = \alpha_i$.

Algorithms based on these permutation ordering schemes can be easily developed.

FINITE INVERTIBLE LOOPS OF THE COSET PRODUCT TYPE

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A finite *invertible loop* is one in which every element has a unique inverse. The class of all finite invertible loops includes Moufang loops, IP loops, pseudogroups, and groups. This paper shows that every Lagrangian invertible loop $(L; \diamond)$with a non-trivial normal subsystem Z has a unique coset decomposition with respect to Z and is a coset *product* of the form $(L; \diamond) = (E;*)\ X\ (C;\Phi)$, where $(E;*)$ is a loop and $(C;\Phi)$ is a multi- $\varnothing$ system such that $(C;\varnothing_{ij})$ is a quasigroup-type system for every operation $\varnothing_{ij} \subset \Phi$. The analysis of invertible loops as coset products is a useful tool in the study of their structures. This has been applied in the construction and analysis of finite pseudogroups like the Octonion loop (which is associated with the non-associative division algebra of Cayley numbers) and related structures.

Keywords: invertible loop, Moufang loop, pseudogroup, coset product

LATIN SQUARE COMPOSITION OF FACTORABLE GROUPS AND LOOPS

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All factorable finite groups have Cayley tables that are built up of two classes of Latin square blocks: the **cylic** and **Klein** blocks. Using these basic blocks, we can easily construct all known factorable groups like the *symmetric, alternating, dihedral,* and *dicylic* groups. Similarly, we can also construct factorable *loops* like *pseudogroups* with desired properties.

Keywords: finite loops, latin square, cyclic blocks, Klein blocks

CONSTRUCTION OF A FAMILY OF POWER ASSOCIATIVE PSEUDOGROUPS OF ORDER $n=2m$ WITH NORMAL SUBSYSTEMS OF ORDER m

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A pseudogroup *(P;*)* is not an associative invertible loop. Furthermore, it is called *power associative (PA)* if for element a in P, and any integers m and n, then $a^m * a^n = a^{m+n}$. Although this identity is always true for any associative system, this is not always so for non-associative systems like pseudogroups. In this paper, a family of PA pseudogroups was constructed using the *block product method.* The generating systems used was the cycle group C_2 of order 2 and the multi-Φ of order $(m;4)$, where $C = \{1,2,\ldots,m\}$ and $\Phi = \{\varnothing_{ij} \mid i,j = 1,2\}$. The elements of Φ were defined as: *(i)* $\varnothing_{11}$ is an operation for a cyclic group of order m; *(ii)* $\varnothing_{12}$ is a quasigroup operation of order m with left identity element, *(iii)* $\varnothing_{21}$ is a quasigroup operation of order m with right identity element; and *(iv)* $\varnothing_{22}$ is a quasigroup operation such that for all x in C, $x\varnothing_{22}\,x = 1$ and $x\varnothing_{22}y = x\varnothing_{11}y^{-1}$. All members of this family had a normal subsystem of order m.

Keywords: pseudogroups, quasigroup, loop power associative

ASSESSMENT OF INFLUENTIAL OBSERVATIONS IN MAXIMUM LIKEHOOD FACTOR ANALYSIS

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The present paper deals with the analysis of influential observations in maximum likelihood factor analysis. This study is the continuation of the paper of Tanaka and Odaka (1989a, b) who proposed methods of sensitivity analysis in principal component analysis (PFA). The main objective here is to investigate the influence or a small change of data on the result of the analysis. To do this, the theoretical influence functions $I(x; LL^T)$ and $I(x; \Delta)$ for the common variance matrix $T=LL^T$ and the unique variance matrix Δ respectively were derived. To assess the influential observations, some influence measures like the Euclidean norm of $\Delta^{(I)}$ and two scaled norms such as D_{us} and D_{ms}, a quantity similar to the so-called Cook's distance were used in the analysis. Some numerical examples are shown to illustrate the present procedure and a comparison is illustrated with case of principal factor analysis.

Keywords: influencial observations; influence function; maximum likelihood; factor analysis; Euclidean norm; scale; scale-invariant, unique variance matrix; common variance matrix.

PREDICTION OF SEDIMENT MOVEMENT IN LINGAYEN GULF: AN EVALUATION OF APPROACHES AND IMPLICATIONS TO COASTAL STABILITY

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Understanding of the movement of sediments along coastal areas can help in predicting the possible consequences of human activities. It is essential in forecasting shoreline stability, as well as the movement and fate of pollutants entering the coast. However, the importance of sediment transport within the nearshore, hereby referred to as the region between the surf zone and the shoreline, is oftentimes overlooked. Furthermore, numerical circulation models, intended for the offshore regions.

This study established the nearshore and offshore sediment dispersal in Lingayen Gulf using geomorphic and sedimentologic-based parameters. We then compared the results with modeled circulation and wave refraction patterns and dispersal trends defined by remotely-sensed data. The predominant nearshore sediment transport was deduced from geomorphic-based parameters such as splits and deltas, and changes in shoreline position derived from the time series analysis of maps and remotely-sensed images. The net offshore sediment dispersal trends were derived using the granulometry of bottom sediments in the upper 20 cm of the bay floor combined with changes in water depths.

Results indicate a southward transport of sediments along the eastern coast as indicated by the assymetry of Bauang and Aringay River Deltas and growth of the Damortis spit. Utilizing similar features, this continued to a dominantly westward transport direction along the bayhead coast with possible shifts of littoral cells as reflected by changes in the assymetry and direction of mouth bar spits during certain periods. The predominant direction of longshore current suggested by the wave refraction models were consistent with the above results. Trends of deposition in the offshore, defined by changes in water depths and sediment distribution indicate a net northward transport of sediments from Agno and Bued-Patalan Rivers. In a Landsat image, the sediment plumes of the rivers were noted on top of the depositional trends.

In general, there is poor correlation between the sediment dispersal derived from geomorphologic data and remotely-sensed images with water circulation predicted by numerical models. The use of regional winds, depth-integration and absence of tidal influence in the simulations could be the cause of the above disparity.

Keywords: sediment movement, Lingayen gulf, coastal stability, Agno river, shoreline changes, water depth changes, longshore current, geomorphologic indicators, nearshore, offshore

CHANGES IN WATER AND SHORELINE POSITIONS AND THEIR IMPLICATIONS TO SEDIMENT DISPERSAL AND SEDIMENTATION RATES IN MANILA BAY

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Despite the heavy use of Manila Bay and its coasts for fisheries, domestic, industrial purposes, studies addressing the consequences of human modifications along the coastal zone and the potential fate of pollutants entering Manila Bay are few, if not lacking. This study addresses this need.

The predominant nearshore and offshore sediment dispersal pathways in Manila Bay, specifically off Cavite, Manila, and Pampanga, were determined by examining the depositional coastal geomorphic features, wave refraction models, changes in water depths and shoreline positions, and offshore sediment distribution patterns. Water depth changes were used to estimate the rates of sedimentation and establish lateral variations in the rates of sedimentation. High-resolution reflection seismic profiles and piston cores provided information on longer-term sedimentation. The predominant nearshore sediment drift along the eastern coast is to the northeast, along Manila to Pampanga is to the northwest, and along Bataan is to the north. Southwest sediment drift predominates along the Zapote-Kawit coast which is due to wave refraction around the Cavite Split. The flared and relatively stable river mouths fronting the northern fluvial-tidal delta plain indicate the greater importance of onshore-offshore sediment transport along this segment of the coast. Off the eastern portions of Manila Bay, waters move in a general northerly direction. Major sediment sinks occur north of the Cavite Spit and west northwest of the Pasig River mouth. Here, sedimentation rates can be as high as 9 cm/yr.

Variation in the magnitude of shallowing occurs across the three study areas; Pampanga Bay shows the least amount of shallowing while the Pasig River area shows the greatest. The apparent low rates of sedimentation in Pampanga Bay could be due to high subsidence rates in this part of Manila Bay. A general increase of sedimentation rate in the offshore direction is also indicated by the bathymetric changes. This trend implies low retention of sediments near the coast, which might be due primarily to a relative sea level rise in the bay. The seismic lines indicate that the relatively high sedimentation rates along the deeper central portions are not a recent trend. However, this long-term trend is probably controlled by the bay's morphology rather than sea level fluctuations.

Keywords: sediment dispersal, fate of pollutants, changes in shoreline position, changes in bathymetry, Manila Bay, Pampanga Bay, rate of sedimentation, sediment distribtion, Pasig Delta, Cavite Split

PATTERNS OF SEDIMENTATION, SOURCES OF SEDIMENT AND THEIR IMPLICATIONS TO LAGUNA DE BAY'S LIFE SPAN

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Laguna de Bay is used mainly for aqua-culture and is eyed as a source of domestic water for Metro Manila. This study addresses the need to understand

sedimentation processes in the lake because of its implications on the lake's overall water quality and life span.

Comparisons of an updated bathymetric map, constructed from water depth readings acquired in 1997, with 1963 and 1983 bathymetric maps which contain survey results in 1939 and 1968, respectively, reveal that the lake is diminishing. The lake's volume decreased from 2.35 x 10^9 m^3 to 2.26 x $10^9 m^3$, between 1939 and 1968, to 2.16 x 10^9 m^3 by 1997. The rate of volume decreased between 1939 and 1997, is 3.3 x $10^6 m^3$/yr. The average water depth was 2.5 m in 1939, 2.3 m in 1968, and 2.2 m in 1997, the average water depth, between 1939 and 1997, decreased at a rate of 5mm/yr.

Changes in shoreline positions, based on the comparison of the 1963 and 1983 maps, were dominated by shoreline retreat, increasing the lake's surface areas from 959 km^2 to 998 km^2, ^{210}Pb and ^{137}Cs profiles of two vibro-cores, from the central and west lobes, yielded sedimentation rates of approximately 4 cm/yr. Discrepancies with calculated sedimentation rates from bathymetric changes are attributed to subsidence, estimated to be approximately 3 cm yr. Given the shoaling rate of 5 mm/yr, a bulk density of 1.1 g/cm^3 and an 80% water content in the upper 1 m of sediment column, the sediment input is equivalent to 1.1 x 10^6 tons/yr. However, with subsidence accounted, a total sediment input of 7.7 x 10^6 tons/yr. is required. Relative to a previous estimate of watershed sediment yield, the above value is an order of magnitude higher. The discrepancy could be due to an underestimation of the watershed yield and/or unaccounted inputs from domestic, livestock, and industrial sources.

Spatial trends of net bathymetric changes and sediment distribution indicate that: shoaling, area-wise is most prominent in west and central bays; while reworking and erosion predominates the southern portions of south and west bays. The time series of bathymetric changes indicates lateral shifting of depositional and erosional zones. Silt-sized particles predominate the lake's surface sediments. However, clay is abundant in the west bay probably due to a high fine-grained sediment yield of Pasig River and to flocculation induce by seawater intrusion.

Keywords: sediment, bathymetry, subsidence, sediment distribution, Laguna de Bay, shoreline change, sediment input, rate of sedimentation, bathymetric change, lake surface area

SUBSIDENCE AND THE WORSENING FLOOD PROBLEM IN PAMPANGA AND BULACAN

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Towns within the Pampanga delta, from the provinces of Pampanga and Bulacan, have experienced floods that appear to have worsened through time. Deforestation and constriction of channel ways due to siltation and construction of fish pens have been suspected as the culprits. In this paper, we will present the initial results of our work that examines the contribution of subsidence to the flooding problem.

Anecdotal accounts of long-time residents indicate that the floods have become more frequent, higher, and that the waters now recede at a much slower rate. The accounts also indicate that flooding within the coastal towns and adjacent river systems are caused not only by rains but also by tides, and indication that subsidence plays a major role in the worsening problem of floods. In the southwestern parts of Pampanga, some sites now frequently inundated by tides were never reached by tides 30 to 40 years ago. In addition, emergence of water wells, an indicator of subsidence, was commonly observed. The rates of subsidence, based on the emerged well pipes and level of flooding, are estimated to be 3 cm/yr. Prior to the eruption of Mt. Pinatubo, the rates were slightly lower.

Besides flooding, sea water incursion affecting the quality of agricultural lands as well as ground water is another consequence of subsidence. Perhaps, this is the reason for the massive conversion of agricultural lands into fishponds. Ongoing work is aimed at mapping the trend of rates of subsidence, and identifying the causative agents. The results of this study are essential for long-term development plans within the Pampanga delta plain.

Keywords: subsidence, Pampanga delta, Pampanga, Bulacan, groundwater withdrawal, floods, saltwater intrusion, sea level rise, Pinatubo eruption, siltation

UTILIZATION OF LOCAL RAW MATERIALS FOR WHITE EARTHEN WARES AND PORCELAIN BODIES MANUFACTURING

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Whiteware refers to a glazed or unglazed ceramic body which is commonly white and of fine textures. Most of the raw materials used in the manufacture of whiteware bodies in the Philippines are imported despite the presence of raw materials in the country.

White clay from Solsona Feldspar from Pagudpud in Ilocos Norte, and lahar or rice hull ash were used as the main components in the formulation of the following ceramic bodies: sanitary wares, floor tiles, normal porcelain bodies, porcelain bodies and bone china. The materials were beneficiated to remove the magnetic impurities. Test bars were made based on 25 formulations in accordance with the general empirical formula for whiteware bodies and the tri-axial diagram. The test bars were fired at 950°C, 1000°C and 1050°C and the following properties were determined: color, fired shrinkage, total shrinkage, water absorption, porosity, and modulus of rupture.

The results of the test indicate that for sanitary ware body, the 48:30:22 clay:feldspar:rice hull ash formulation was the best, while the formulation 48:27:25 clay:feldstar:lahat body was the best for normal porcelain bodies. The results of the test for porcelain white wares and bone china did not pass the standards. The bodies were tested for glaze compatibility and the resulting glazed bodies were found to be acceptable.

Keywords: clay, earthen wares, feldspar, porcelain, whitewares

PROTEIN HYDROPEROXIDES: PROTEIN DAMAGE INDUCED BY PEROXYNITRITE

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Exposure of proteins to agents such as activated neutrophils, hypochlorous acid, hydroxyl and peroxyl-free radicals can result in the formation of protein hydroperoxides. Protein hydroperoxides can oxidize physiological reductants such as ascorbate and their decomposition can lead to the formation of other free radicals. These findings suggest that protein hydroperoxides, when generated *in vivo*, could cause biological damage, hence the need to find biologicval oxidants capable of inducing the formation of protein hydroperoxides.

Results of this study show that peroxynitrite, a biological oxidant formed by the reaction between superoxide and nitric oxide-free radicals, can induce the formation of protein hydroperoxides. Using the tri-iodide assay, BSA and other protein exposed to peroxynitrite at neutral pH tested positive for the presence of hydroperoxide groups. The newly-formed reactive moieties were attached to the protein and their identity as the hydroperoxide group was confirmed by their reaction with GSH-NaBH4, 2-mercaptoethanol and ascorbate, compounds known to react with hydroperoxides. The yield of BSA hydroperoxides was higher at slightly acidic to neutral pH than at alkaline pH. Presence of metal chelators such as DTPA, EDTA, and NTA did not reduce the yield of BSA hydroperoxide but desferrioxamine caused a 44% reduction. Hydroxyl radical scavenger failed to inhibit the peroxidation of BSA by peroxynitrite at neutral pH. The yield of protein hydroperoxide may be low compared to the concentration of peroxynitrite used. However, the results presented here suggested that peroxynitriti could be a potential biological agent for protein hydroperoxide formation and that protein peroxidation could be another pathway by which peroxynitrite can cause biological damage.

Keywords: protein, hydroperoxides, peroxynitrite, hydroperoxides, oxidants, protein peroxidation, protein damage

HYPOGLYCEMIC ACTIVITY OF SOME FOLKLORE ANTIDIABETIC PLANTS

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Plants have long been used to cure and/or prevent many diseases. The observed efficacy of these plants has made them very popular and in fact, some are considered as "cure-all" medicines. As a first step towards establishing, in part, scientific basis for the folklore use of some of these plants as antidiabetics, 8 folklore antidiabetic plants and 2 edible seaweeds were tested for their hypoglycemic or blood glucose lowering activity.

High blood glucose level was induced in fasted mice by intraperitoneal injection of glucose solution of a dosage of 7.5 g per kg body weight. The blood glucose level returned to the initial level or remained constant using Haemoglucotest strips. The hypoglycemic activity of the plant extracts was determined by comparing the blood glucose levels of the extract-treated mice with those of the control group. Results indicate that *Mimosa pudica* Linn. (makahiya, leaves, and roots), *Solanum nigrum* Linn. (lubi-lubi, leaves), *Luffa acutangula* Roxb. (patola, fruit), *Chrysophyllum cainito* L. (kaymito, leaves), *Basella rubra* L. (alugbati, leaves and stems), and *Blumea balsamifera* (sambong, leaves) have hypoglycemic activity. From these results, these plants can now be considered to contain potential antidiabetic compounds and warrant further studies to isolate and identify the antidiabetic principles.

Keywords: hypoglycemic, antidiabetic, folklore medicine

DESIGN CONSTRUCTION AND EVALUATION OF AN ACID TREATMENT BATH

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An electrically-operated acid treatment bath was designed constructed, and evaluated at DMMMSU-SRDI, Bacnotan, La Union from January 1994 to December 1996 to speed up acid treatment activity in silkworm egg production areas in order to cater the needs of sericulture farmers nationwide.

After a series of tests and improvements, an acid treatment device equipped with electrical and electronic parts was developed. The main assembly is made of stainless angle bar and steel sheets. It has a replaceable acid holder to fit with the desired number of layings of silkworm eggs to be treated.

The maximum input capacity of the designed device is 40 boxes/hr (800,000 eggs/hr) with a power consumption of 1.5 kw-hr. The performance evaluation result showed that the hatching efficiency (laboratory rate) was found to be 93.56% for silkworm hybrids. Economic analysis showed that the cost of treating silkworm eggs is only P4.13 per box with a return of investment of 24.84%. With its promising performance and economic feasibility, the designed machine is best suited for the country's growing sericulture industry.

Keywords: sericulture acid bath machine

PROSPHORUS-31 NMR STUDIES ON *Escherichia coli* AND *Bacillus subtilis*

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The phosphate metabolism in *Escherichia coli* and *Bacillus subtilis* was studied using Phosporus-31 NMR at various temperatures under both aerobic and anaerobic conditions. The pH was determined using the observed chemical shift of orthophosphate and the concentrations of the various phosphates were estimated by integration vs. methylene disphosphonic (MDPA) standard.

Increasing the temperature of the bacterial culture resulted in a decrease in intracellular pH. Addition of glucose also resulted in a decrease in intracellular and pH and was accompanied by the formation of sugar monophosphates. The total soluble intracellular phosphates concentration was estimated to be 2×10^{-17} mole/cell. Intracellular and extracellular orthophosphate was observed, although these appeared to move rapidly between the intracellular and extracellular volumes on the NMR time scale. The rate of utilization of dissolved oxygen in the BOD experiment increased as the concentration of orthophosphate in the test solution increased. This result reinforces the importance of orthophosphate in bacterial metabolism.

Keywords: phosphate metabolism, *Escherichia coli, Bacilus subtilis,* NMR, BOD

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BIOLOGICAL SCIENCES

WILDLIFE INVENTORY OF THE UNIVERSITY OF THE PHILIPPINES DILIMAN AND THE ATENEO DE MANILA UNIVERSITY CAMPUS, DILIMAN, QUEZON CITY, LUZON, PHILIPPINES

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An inventory of the terrestrial vertebrate species of wildlife in campuses of the University of the Philippines Diliman and the Ateneo de Manila University in Quezon City, Metro Manila, was conducted from October 1997 and August 1998. The UP Diliman Campus encompasses 493 hectares while the Ateneo de Manila University campus encompasses 83 hectares.

A total of 76 vertebrate species was recorded in the campuses of UP Diliman and Ateneo de Manila University. This diverse assemblage of wildlife in the study sites is comprised of 6 species of amphibians (1 endemic), 13 species of reptiles (2 endemic), 47 species of birds (7 endemic) and 10 species of mammals (1 endemic).

More than 61% of wildlife species found in the study areas were birds. Historical records of the assemblage of bird species in the UP Diliman area and its environs indicate that six species of birds used to be found in the study sites, are now no longer present

Keywords: biodiversity, UP Diliman and Ateneo de Manila, local extinction, endemic, wildlife

ZOOPLANKTON FROM LAKE TAAL

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Zooplankton were collected from January through August 1997 from three sites in Lake Taal. Water samples (12 L) were collected just below the surface and also near the bottom using a Van Dorn water sampler. Samples were filtered through a 53 μm plankton net and 5 mL formalin was added to each 10-mL collecting bottle.

Plankton collection was analyzed quantitatively using a Sedgewick Rafter counting chamber examined under a Carl Zeiss compound microscope. Triplicate counts were done per sample and the number of individuals per m^3 was calculated for each species. The species identified were one dinoflagellate and 22 rotifers. Also collected were copepods (larvae and adults) and cladocerans. The crustaceans e.g., *Lecane bulla* and *Hexarthra intermedia,* were found in almost all sites. Counts in thousands per m^3 were nauplii, 0-338.9; copepods, 0-138.9; cladocerans. 0-94.5; *B. forficula,* 0-61.1; *B. havanaensis,* 0-56-6; *L. bulla,* 0-208.3; and *H. intermedia,* 0-11.1. Peaks of abundance for the common species occurred around February and June.

Keywords: zooplankton, dinoflagellate, rorifers, copepods, cladocerans, crustaceans, nauplii, Lake Taal.

DISTRIBUTION AND ABUNDANCE OF ATYID SHRIMPS IN VOLCANO ISLAND SHORES, LAKE TAAL, BATANGAS

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The distribution and abundance of atyid shrimps (Family Atydae) were investigated in four sites along the shores of Taal Volcano Island, Batangas, Stations 1, 2 and 3 were on the northern part of the island, while Station 4 was on the midwestern part. Sampling was done for four months: July, October, and December 1995, and January 1996. Collection was done by towing a "pangkalap."

The predominant species collected was *Caridina gracilostris* de Man. Mean densities were highest in Station 4 with values ranging from 203/m^3 to 613/m^3

Mean densities in the other stations were usually <200/m^3. The abundant of atyids in station 4 may be due to its being sheltered from strong winds by the adjacent land mass in the west. This station also had the most abundance littoral submerged vegetation (*Vallisneria gigantea*). Vegetation was present but sparsely distributed in the other stations.

Keywords: atyid shrimps, *Caridina grascilostris, Vallisneria gigantea,* Lake Taal, Taal Volcano Island

LAKE TAAL'S ENDEMIC FRESHWATER SARDINE, *Sardinella tawilis:* SPECIATION IN PROGRESS OR GHOST OF ANCIENT POLYMORPHISM (INSIGHT FROM MOLECULAR DATA)

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Sardines belong to the genus Sardenella of the Family Clupeidae. In the Philippines, seven species under this genus have so far been described to share great morpho-anatomical similarities with one other. Of these seven, importance is being accorded to *S. tawilis* due to its endemicity only to Taal Lake and reports of its fast depletion. In order to delineate this freshwater sardine from marine sardines through identification of molecular markers that could also become basis for future management of *S. tawilis,* analysis of the sardines' mitochondrial DNA was conducted.

Three marine sardines, namely *S. albella, S. longiceps,* and *S. fimbriata,* that are geographically related to *S. tawilis,* were used. Two populations of *S. tawilis* (northern and southern) were studied, Dissected gonald and muscle tissues of the sardines were utilized in the extraction of mitochondrial DNA used in the amplification, cloning, and sequencing of the *cytb* and control region segments. Informative sequences were subjected to phylogenetic analysis using Neighbor-joining and Maximum-parsimony methods.

Results showed that the *cytb* of the sardines was 358 bp long which differed only in one nonsynonymous substitution that led to a change from isoleucine to valine. On the other hand, the presence of an 81-bp insert, a 35-bp tandem repeat present in up to eight copies and conserved sequence blocks (CBSs) in the control region sequences of *S. tawilis* and *S. albella* made them different from the rest of the

sardines. Phylogenetic analysis of the homologous sequences of the control region revealed that from the marine sardines investigated, *S. albella* was closest to *S. tawilis*. However, the possibility of finding closer one should not be dismissed since not all sardines were studied. Also, the two populations of *S. tawilis* were beginning to differentiate from each other as shown by the presence of population-restricted substitutions and by the single mutation in the *cytb*. The northern population was more homogeneous than the other probably due to physical barriers that may hinder gene flow or due to constant use of selective fishing gear in that area.

Overall, results of this study could serve as baseline information for future management of *S. tawilis*. Also, it opens interesting lines of study that would determine whether *S. albella* and *S. tawilis* are just similar to each other or are one and the same species that only became separated during the formation of Lake Taal.

Keywords: sardines, *S. tawilis, S. fimbriata, S. longiceps*, mitochondrial DNA, cytb, control region, conserved sequence blocks (CBS), phylogenetic analysis

A BIOMETRIC STUDY OF *Sardinella tawilis, S. fimbriata,* and *S. albella (Pisces:* Clupeidae)

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Five hundred specimens of *Sardinella tawilis, Sardinella fimbriata* and *Sardinella albella* were collected from Batangas Farmer's market in Quezon City. Thirty-seven biometric characteristics were subjected to factor analysis, discriminant analysis, and cluster analysis.

Results showed that among the 37 morphometric traits, the most consistent character was the fork length, while the most variable one was the length of the ventral fin. Among the meristic traits, the number of rays of the pectoral fin was the most consistent character, while the umber of rays of the anal fin was the most variable character.

Results of the factor analysis showed the presence of seven factors accounting for 77.65% of the variation among the species. Results of the discriminant analysis further revealed that only seven biometric characters sufficed to discriminate efficiently and accurately among the three species of Sardinella considered, namely; *S. tawilis, S. fimbriata* and *S. albella*.

Cluster analysis, which was presented in a dendrogram, revealed that the three Philippine *Sardines* can be distinguished from each other biometrically.

Keywords: *Sardinella, biometric, discriminant analysis, factor analysis, dendrogram.*

FISH COMPOSITION OF THE PANSIPIT RIVER: A COMPARISON WITH THE FIRST REPORT MADE 70 YEARS EARLIER

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Pansipit River is a 10-km single stream channel that serves primarily as an outlet of Lake Taal into Balayan Bay of the South China Sea and flows through the municipalities of Agoncillo, San Nicolas, Lemery and Taal. Commercially-important fish migrate into Lake Taal from Balayan Bay through this river. This study was conducted to determine: (1) the ichthyofaunal species composition of Pansipit River including Palanas River; (2) the abundance and seasonality of the important species under study; and (3) if the fish populations exhibit specific site/habitat preference or longitudinal distribution pattern.

Five sampling sites were established: four were along the Pansipit River (upstream, midstream 1, midstream 2 and downstream), and one in Palanas River, a diverging branch of Pansipit that also empties into Balayan Bay approximately 3 km from the mouth of Pansipit River. Monthly beach seine from February through December 1998 within the Pansipit and Palanas Rivers produced a total 59 species belonging to 37 families. Of the 59 species, 21 were caught exclusively in Pansipit, 26 in Palanas, while 12 were present in both rivers. Pansipit River supports a total of 33 species, 65% of which are migratory. Palanas River, on the other hand, supports a total of 38 species, of these 68% are migratory. Present species composition were evaluated with reference to earlier studies from the past seven years.

Pansipit River serves not only as a transport channel for fish, the villagers use the river for fishing, bathing, and laundering. Fish cages and fish corrals at the Pansipit River have also been erected in some parts of the river. The possible implications of human activities on the ichthyofauna of the river is discussed.

Keywords: fish, taxonomy, migratory, seasonality, distribution, Pansipit River, water quality, environmental pressures, biodiversity, conservation management.

SUBIC BAY FOREST RIVERS: WHAT MOLLUSKS DO THEY SUPPORT?

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Subic Bay is the former base of the U.S. Naval forces in the Philippines. The forest area of the base is approximately 18,000 ha. In 1992, the U.S. Naval forces left and this area was converted into an industrial and eco-tourism zone. The return of the base to the Philippine government paved the way for study of its biota – an activity not possible before due to security restrictions.

Quantitative analysis was conducted at the six major rivers within the Subic Bay Forest from November 1997 to July 1998. Each river system was divided into three sub-sites. Sampling per sub-site was conducted within the 100x100 meter quadrant. Specimens collected were identified based on Jutting (1956), Pace (1973) and Reeve (1860).

The diversity of the freshwater mollusks of the Subic Bay Forest Reserve was limited to two groups only: the Family Neritidae and Thiaridae which includes twenty gastropod species. this might have been possible consequences of the previous eruption of Mount Pinatubo. The species belonging to Family Neritidae are: *Clithon corona, Neritina coromandeliana, N. waigiensis, N. pulligera, Navicella barbonica, Septaria tesellata (S. lineata), S. porcellana,* and *Septaria sp.* The species belonging to Family Thiaridae are: *Thiara (Tarebia) granifera, T. scabra, T. winteri, Thiara* sp. 1, *Thiara* sp. 2, Melanoides asperata, *M. canilis, M. conchilidum, M. costata, M. plicaria, M. uniformis,* and *Sermyla riqueti.* Of the six river systems, Triboa River harbors the most number of species (16/20) followed by Ilanin River (9/20). Only two species were collected from Bayani River. Of the 20 gastropod species, M. asperata was found to be present in all the six rivers. This is followed by *Thiara granifera* (4/6). Nine out of the 20 species (9/20) were present each only in one river.

Melanids and *Neritina pulligera* serve as food to the native people who live within the Subic Bay Forest Reserve. These are commonly cooked with coconut. They also sell these Melanids outside Subic for 10 to 15 pesos per can.

Keywords: Subic Bay Forest Reserve, U.S. Naval Forces, biota, quantitative analysis, biodiversity conservation, Mount Pinatubo eruption, Neritidae, Thiaridae, Melanid

DIVERSITY OF MANGROVE VASCULAR FLORA AND ASSOCIATED MACROFAUNA IN MANGAL COMMUNITIES OF CATANDUANES

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The mangal communities of Catanduanes have not been the subject of various studies on community structure and function. Only fragmental studies have been made in the past. This paper fills in the gaps on the paucity of relevant information about diversity of mangrove flora and fauna in the island province. A taxonomic listing of vascular flora, icthyofauna and molluscan fauna, as well as preliminary data on their occurrence and distribution; species richness; and abundance of the major categories are included in this report.

Keywords: mangrove, macrofauna, mangal community, Catanduanes

OVARIAN CHANGES IN *Apongon themalis Cuvier* IN RELATION TO SPAWNING

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The ovarian changes in *Apogon thermalis* Cuvier from Lake Taal, Batangas were observed from May to January 1997, and from January to June 1998. Mean oocyte diameter and mean ganadosomatic index peaked twice – first in July and another in March. Histological studies of strained parafin section showed various stages of oocyte development: perinucleolar, yolk-vesicle, yolk granule and mature stages. Oocytes at the mature stage were observed in months when the mean ganadosomatic index and oocyte diameters were greatest. Above results indicate the *Apogon thermalis* spawn twice a year with the spawning season occuring between July and August, and between March and April.

Keywords: ovary, *Apogon thermalis,* oocyte, gonadosomatic index, spawning, histology, Lake Taal

PRELIMINARY ANALYSIS OF THE GENETIC STRUCTURE OF GIANT CLAM *(Tridacna crocea)* POPULATIONS FROM NORTHERN PALAWAN AND THE KALAYAAN ISLANDS GROUP (KIG)

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The South China Sea houses one of the most diverse assemblage of marine organisms in the world. Investigations of the genetic variability of selected reef organisms from different shoal and shelf reef systems in the South China Sea provides a basis for establishing affinities of reef-associated organisms within the South China Sea and among the bordering continental reefs. However, information on the genetic structuring of tropical marine organisms, particularly those from local reefs, is currently inadequate. Allozyme variation at five polymorphic loci was examined in three populations of *T. crocea* (KIG, Pangaldauan island and El Nido) to investigate the genetic affinities of the populations in the shoal reefs of the KIG and reefs on the North Western Palawan Shelf. Genetic distance (Nei's D) among populations ranged from 0.005-0.028, and as expected, increased with geographical distance F_{ST} values (mean=0.046) suggest that there is no genetic structring between the populations surveyed. In addition, high N_{am} values (4-6) suggest a high level of mixing of giant clam populations for the neighboring reefs.

Keywords: *Tridacna crocea,* allozyme markers; population genetics; giant clams, Kalayaan

MOLECULAR EVOLUTION OF β-GLUCANASES IN CEREALS

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The diversity, relationship, and pattern of molecular evolution of 23 β-glucanases in cereals were analyzed. The cereal β-glucanase is a structurally diverse class of enzymes with an overall protein sequence similarity of 74%. The size of the mature peptide is typically around 308 amino acids long with an estimated molecular weight of 32 to 35 kDa existing in either acidic and basic forms. Phylogenetic analysis using distance, maximum likelihood, and parsimony methods showed four distinct branches in the gene trees, designated subfamilies A to D. The sequence similarity within subfamilies was 76% for subfamily A, 93% for subfamily B, 87% for subfamily C, and 62% for subfamily D. While subfamily A had β1,3-glucanase activity and subfamily B had β1,3; 1,4-glucanase activity, the catalytic activities of subfamilies C and D are unknown nor can be predicted from their low overall similarities (68% and 60%, respectively) to both subfamilies A and B. Based on sequence homology, an orhologous relationship may exist between six pairs of the β-glucanase genes. The cereal β-glucanase genes exhibited an extreme G+C bias (94.6%) at the codon wobble position. The gene family registered a fast but fairly uniform evolutionary rate, with an overall rate of nonsynonymous substitution $Ka=3.6\pm0.11 \times 10^{-9}$/site/year in which subfamily B evolved slower than subfamily A. Different pairs of genes either observed or violated the molecular clock hypothesis.

Keywords: gene orthology, molecular clock, gene family, β-glucanases, cereals, evolution.

DNA SEQUENCES OF THE MITOCHONDRIAL 16s rRNA and CYTOCHROME B GENE LOCI OF THE PHILIPPINE HANGING PARROT, *Loriculus philippensis*

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The focus of this research is the DNA-based characterization of Colasisi or Philippine Hanging Parrot (*Loriculus philippinenses*) which is endemic to the Philippines. Optimized conditions for DNA extraction and polymerase chain reaction amplification of the 16s rRNA and cytochrome to gene loci were obtained for the colasisi blood samples collected from Laguna and Negros. The amplification of only the 16s rRNA gene fragment was optimized for the Davao colasisi sample. The PCR products were purified and successfully cloned into puc 18 cloning vectors as confirmed by restriction anzyme analysis and DNA hybridization. Cloned genes were subjected to automated DNA sequencing producing DNA sequence data for one of our very own endemic avian species in the country. The sequence data was analyzed to determine relatedness and to study the correlation of the genetic data with the geographic distribution of these parrots in the Philippines. Moreover, this research is one of the very limited efforts in our country to use molecular biology techniques in generating data towards the establishment of a genetic conservation laboratory for endemic wildlife species in the land.

Keywords: endemic wildlife species, sequence homology, Philippine hanging parrot, PCR, cloning, hybridization, 16s rRNA, cytochrome b

MAPPING HOMOLOGS OF DEVELOPMENTAL GENES *ENGRAILED* AND *HUNCHBACK* IN THE RED FLOUR BEETLE, *Tribolium castaneum*

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We have identified the location of the homologs of two developmental genes in *Drosophila melanogaster, engrailed* and *hunchback,* in the genome of the red flour beetle *Tribolium castaneum.* Two goegraphically-separated strains of *T. castaneum.* MMS (with visible morphological mutations) males were mated with T/W (wild type) females and then F_1 female daughters were mated back to their male parent to generate the F_2 mapping population. Portions of *engrailed* and *hunchback* genes in the genomic DNA of the parents, F_1 daughter and F_2 individuals were amplified by PCR (polymerase chain reaction) using opposing primers that correspond to intronic regions of both genes.

Well-amplified PCR products were subjected to SSCP (single strand configuration polymorphism) analysis. Each F_2 individual in the mapping population was scored for the presence or absence of a specific band derived from the T/W female parent. Data obtained were added to an EXCEL file containing the raw data for all RAPD (randomly amplified polymorphic DNA) markers and were analyzed using JOINMAP. The method assigned the location of *engrailed* and *hunchback* in chromosomes 7 and 5, respectively.

Keywords: mapping *Tribolium castaneum,* hunchback, engrailed, PCR, beetle

TRANSIENT EXPRESSION OF SALT-TOLERANCE GENES IN CYANOBACTERIA

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Cyanobacteria or the blue green algae are photosynthetic prokaryotes, many of which are capable of nitrogen fixation. They are deemed important in agriculture because of their role in the maintenance of soil fertility in paddy fields. However, secondary salinization from irrigation has become an increasingly serious and costly problem. Because of the economic importance of cyanobacteria, much interest is currently devoted to studies on the mechanism of salt tolerance in this group of organisms.

The present work elucidated the current hypothesis that stress adaptation is due to the expression of salt-tolerance genes. In this study, proteins from the unicellular forms designated as Lin-20 and Bat-09 were extracted at 0 h (control), 30 min, 1 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, and 10 h after exposure to different concentrations of 240 mM and 360 mM NaCl. The protein profile after electrophoresis using SDS-PAGE of Anabaena Btg 01 at 8 h and 10 h revealed new protein bands. In the case of isolate Bat 8, new bands were found after 6 h and 8 h of treatments. The Lin 20 and Bat 09 isolates did not show any change in their protein profile. Results the earlier data of the group that the filamentous forms are more salt-tolerant than the unicellular ones. These results are also consistent with the reported mechanisms involved in cyanbacterial salt tolerance which imply modification(s) in the synthesis and/or activity of cell proteins to facilitate osmotic adaptation.

Keywords: cyanobacteria, blue green algae, salinization, salt-tolerance, electrophoresis, SDS-PAGE, protein profile, filamentous, unicellular forms, osmotic adaptation

SCREENING FOR ENZYME PRODUCTING BACTERIA USING POLYMERASE CHAIN REACTION AND HYBRIDIZATION

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Enzymes have almost an infinite number of applications and the search for commercially-viable enzyme product often begin with screening for promising bacterial isolates. Locally-isolated protease-producing bacteria which were identified to belong to *Bacillus* species in an earlier study were used in order to develop a DNA-based screening procedure to obtain protease-producing bacteria using polymerase chain reaction (PCR). Primers for PCR that could amplify different regions of a gene for a neutral protease and a region of the subtilisin gene in Bacillus were designed. These primers were used to amplify the genes from the three identified *Bacillus* isolates. PCR profiles were analyzed and the PCR products were purified, labelled, and used as probes for detecting the presence of homologous protease genes using DNA hybridization. The use of PCR and hybridization was useful in detecting bacterial isolates that possibly possess protease genes. Results also indicate that DNA hybridization could be a useful tool not only for detecting protease genes but also for localizing these genes within the bacterial genome. Moreover, a similar screening procedure could be developed for the detection and localization of other commercially-important enzymes.

Keywords: protease gene, PCR, DNA hybridization, Bacillus, localization, primers, extracellular enzymes, neutral protease, subtilisin

OPTIMIZATION OF CONDITIONS FOR *Agrobacterium tumefaciens*-MEDIATED TRANSFORMATION OF RICE

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Plant growth regulators (PGRs), genotype explant type, and tissue culture conditions are some parameters that affect *in vitro* response of rice. We evaluated the response of 6 inbreds, 13 new plant types (ntp), and 6 cytoplasmic male sterile lines

(CMS) for their ability to produce embryogenic calli. Phenylacetic acid (10 mg/L) and 20 mg/L 2,4-Dichlrophenoxyacetic acid (2,4-D) were added to the culture medium as PGR. Spikelets of young inflorescence and mature seed embryos were used as explants to *Agrobacterium tumefaciens*-mediated transformation and incubated in the dark under light conditions.

PAA induced more embryogenic calli formation and plant regeneration to young infloresence (84.15%) than mature seed embryos (45.75%) even during light incubation.

Hygromycin-resistant calli conducted in light was more effective than in the dark conditions. Also, late selection with hygromycin gave a much higher percentage of Hyg^n calli (54.90%) and plants (14.22%) either in the light and dark conditions as compared to selection after Agro infection which ws 48.45% and 0%, respectively. Regenerated LX278 and LX286 plants were placed in the greenhouse. Molecular analysis will be conducted on these regenerants.

Keywords: genetic engineering, rice, crop improvement, binary vectors, *Agrobacterium tumefaciens*

EFFECTS OF TIME AND ENVIRONMENTAL CONDITIONS ON STR PRIMER AMPLIFICATION OF DNA EXTRACTED FROM HUMAN BLOODSTAINS

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The field of forensic science is continually evolving and adapting the latest techniques to be able to analyze the samples obtained from a crime scene. In contrast to whole blood use for paternity testing, biological stains at crime scenes are often exposed to UV (sun) light, humidity, and decay resulting to variable quality and quantity of extractable DNA. In the continuing effort of the UP-PACC DNA Analysis Laboratory to develop DNA Analysis as a tool forensic investigation in the Philippines, the laboratory progressively validates various protocols and procedures for their application in the Philippine setting.

The objective of this study is to determine the length of time and temperatures at which human blood stains can be exposed to yield DNA amplifiable with STR primers. Several 50-microliter blood stains were exposed to varying conditions of temperature (25°C, 27°C, 32°C), and time (3, 7, 14, and 28 days). The DNA from these samples was extracted using a previously valid method of phenol extraction and alcohol precipitation (Kirby, 1992). Sufficient amounts of DNA (70-400 μg/

mL) were recovered after 28 days of exposure. All DNA extracts were amplified at the STR locus HUMFOLP23 (HUMDHFRP2). Apparently, DNA in the blood exposed to temperatures of 25°C-32°C up to 28 days was not significantly degraded at the HUMFOLP23 locus. DNA extracted from bloodstains after 28 days of exposure to different conditions was amplified.

Keywords: human blood, dried bloodstains, validation procedures, DNA analysis. DNA extraction, environmental condition, PCR, HUMFOLP23, DHFRP2, STR

GENERATION AND CHARACTERIZATION OF A RECOMBINANT ANTI-TUMOR HUMANIZED TETRAVALENT ANTIBODY

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Monoclonal antibody (Mab) CC49, a murine IgG1, reacts with the antigen (TAG)-72 expressed in a variety of carcinomas including cancer of the breast, lung, colon, ovary, and others. To reduce the immunogenicity of CC49 Mab in human patients, a humanized CC49 (HuCC49) was generated by CDR grafting. Its relative affinity was 2- to 3-fold lower compared to the murine Mab. To improve the tumor targeting of the HuCC49, we constructed a single gene encoding a single chain consisting of a humanized CC49 diabody attached to human γ1 Fc via the hinge region. The diabody, a bivalent antigen binding structure, was made up of VH/VL and VL/VH domains. In each of the variable domain pairs, the heavy and light variable domains were linked through a short linker peptide, while the two pairs were linked via a 30 residue gly-ser linker peptide to make two antigen binding sites by lateral and noncovalent association of VL of one pair with the VH of the other. Transfectomas expressing the single gene secreted a homodimar of about 160 kDa which reacted to TAG-72, showed cytotoxicity activity, and had a higher functional affinity than HuCC49. This humanized tetravalent antibody molecule is a promising reagent for diagnosis and the therapy of a wide range of human carcinomas.

Keywords: antigen, carcinomas, CDR grafting, diabody, humanized antibody, immunogenicity, monoclonal antibody, recombinant, tetravelent antibody, transfectomas

MULTIPLE ROLES FOR THE N-MYC GNA IN MAMMALIAN CNS DEVELOPMENT

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The N-myc gene belongs to the myc family of cellular oncogenes. It is amplified in many types of human tumors of neural origin such as neuroblastoma and retinoblastoma. The gene product of N-myc is a transcription factor which upon heterodimerization with the Max protein binds to target genes in a sequence-specific manner. To identify the *in vivo* role of N-myc in mice the N-myc gene was specifically ablated in ES cells to produce N-myc targeted mice. Phenotypic analysis of c57Bl/6 embryos at various stages revealed that N-myc homozygous null mutant mice showed defects such as kinky spinal cords with holes or lateral outpocketings in the turnk region and formation of an extracephalic flexure in the dorsal dlencephalon. Neural precursor cell culture studies, immunosaining, as well as RNA *in situ* hybridization analyses using a variety of neural markers all point to multiple roles for the N-myc gene in the formation of the neural tube, organization of the metameric pattern of spinal neurons, development of the cranial ganglia, and in the migration of cephalic neural crast cells.

Keywords: N-myc, knockout mouse, CNS development, cranial ganglia, neural pathfinding, metameric pattern C57Bl/6

PHYSIOLOGICAL RESPONSES OF FOUR MICROALGAL ISOLATES TO CADMIUM

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Four isolates vix., Bat-09 *(Chroococcus)*, Cav-25 *(Desmococcus)*, and CdO-15 *(Chroococcus)* were used to evaluate physiological responses to cadmium treatments of varying levels (i.e., 0.05, 0.5, 5.0, ppm $CdCl_2$) The growth responses of these microalgal isolates were determined through tubidometric analysis and chlorophyll a levels. The uptake of heavy metals by the isolates was determined by Atomic Absorption Spectrophotometry (AAS). All isolates effectively absorbed the heavy metals and uptake increased with concentration within three days. High Performance Liquid Chromatography (HPLC) analyses of protein fractions detected the presence of heavy metal binding polypeptides. This was variable and was not dependent on the concentration of the heavy metals. These isolates are currently being evaluated for bioremediation studies.

Keywords: cadium Chl a, microalgae, AAS, HPLC, polypeptides, bioremediation

HISPATHOLOGICAL ALTERATIONS IN THE INTESTINE OF *Chanos chanos* IN RESPONSE TO MOLLUSCIDE METALDEHYDE

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Metaldehyde is one of the molluscides recommended by the Department of Agriculture-Pesticide and Fertilizer for commercial use to control mollusk, infestation. The effect of metaldehyde on non-target organisms, such as milkfish (Chanos chanos), was studied.

Fingerlings with a mean total length of 5.1 cm were exposed to 3, 30, 60, 150, and 300 mg l^{-1} metaldehyde Porsnail® concentrations for 96 hours. The upper 1/3 of the intestine was fixed and processed for paraffin sectioning. Under light microscopy, sublethal concentrations of metaldehyde induced widening of the lamina propia. Destruction of the intestinal epithelium and detachment of the muscosla layer be-

came visible starting with 30 mg/L, with increasing severity as the toxicant concetration was increased. Hypertrophy of epithelial cells was noted in fish treated with 300 mg l^{-1} metaldehyde.

Keywords: molluscicide, metaldehyde, histopathology, intestine, *Chanos chanos*

GENETIC ANALYSIS OF CHEMICALLY-INDUCED LOSS OF ALBINISM IN MICE

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Albinism is a condition caused by a tyrosinase deficiency and is marked by an inability to form melanin, a dark brown to black pigment responsible for the coloration of the eyes, skin, and hair. In mice, coat color is encoded by genes located in four loci namely; *a* (agouti), *b* (brown), *c* (albino), and *d* (dilute). The wild type C allele encodes the tyrosinase enzyme responsible for melanin production and is dominant over all other mutations at the *c* locus. Albinism thus results from a homozygous (c/c) genotype. We serendipitously generated non-albino mice from both albino parents previously exposed to sodium nitrate when we were originally searching for chemical mutagens affecting the nervous system. All 14 pups of one litter were hyperactive and progressively showed chinchila mottled coat color and black eyes in sharp contrast with their albino (white) father and mother. Subsequent matings of F1 females to albino males revealed that the mutation is a germ-line mutation. The non-albino mice are now being bred to produce inbred and outbred lines for analyzing the mechanisms of nitrate-induce activation of melanogenesis and to identify at which critical point in the melanogenesis pathway did the chemical mutagen interfere in the normal course of melanin suppression in the original albino stock.

Keywords: albino, ICR, mouse, melanin, nitrate, tyrosinase, coat color.

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AGRICULTURAL SCIENCES

DATA MANAGEMENT SYSTEM FOR GERMPLASM

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A new database management system has been developed for the PhilRice Genebank called GEMS-PhilRice Germplasm Data Management System. GEMS is a simple, user-friendly database that allows easy access to the wealth of information about the rice germplasm collection at PhilRice. GEMS holds information on the passport, characterization, evaluation, viability, and inventory of the existing materials. Access to these information simply involve dragging the mouse, clicking buttons and typing search words. Search options are unlimited with regard to combination of target items. Search reports may be generated in various formats such as individual accession profiles or as a group list with selected data sets. Custom searches have been designed for many frequently-requested search items, and for vital data concerning with bright color combinations enlivening every turn. the system can allow worldwide access through the Internet pending the installation of the necessary hardware at PhilRice. Data exchange is straightforward with most of the widely used data formats. Expansion will include storage of plant photographs and enhanced integration with the preparation of planting plans.

Keywords: *germplasm, documentation, database*

CHARACTERIZATION OF HETEROSIS IN PHILIPPINE HYBRID RICE GERMPLASM

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The exploitation of heterosis or hybrid vigor is the goal of hybrid rice breeding. Since genetics is acknowledged to be the basis of heterosis, PhilRice has diversified its hybrid rice germplasm through collaborations with Chinese R & D institutions. These have led to the introduction of new cytoplasmic male-sterili (CMS) and restorer lines into PhilRice's breeding program. Genetic diversity analysis of CMS lines, for example, has revealed nine groups within the PhilRice's CMS materials and a wide array of testers would provide baseline information that would be useful in charting the future of Philippine hybrid rice breeding. Using 850 F_1 derived from eight CMS lines, we examined the maintaining and restoring ability of 450 testers/inbreds. In the average, 19% of the testers were effective restorers while 28% were partial of effective maintainers of eight CMS lines. Crosses made with CMS Lian A were mostly heterotic (34%), and crosses to CMS 28A were mostly sterile (65%). Of the 8 CMS lines, Lian A was the most easily restored while 28A was the most easily maintained. Heterosis levels for ten vegetative and reproductive traits were also analyzed. Trait-wise, more than 20% of the F1 hybrids showed at least 20% superiority over the male parent (MP) for number of productive tillers, spikelet number, grain weight, and grain yield. Distribution of MP heterosis was normal for maturity, plant height, panicle length, grain length, and grain width, and was bimodal or skewed for number of productive tillers, spikelet number, spikelet fertility, grain weight, and grain yield. These results suggest the greater likelihood for selecting heterotic combinations for the latter group of characters. For grain yield, 21% of the hybrids showed at least 20% heterosis indicating that breeding progress is attainable using the gene pool studied.

Keywords: heterosis, genetic diversity, germplasm, CMSD, hybrid rice

MOLECULAR GENETIC DIVERSITY AND HETEROSIS IN RICE

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Molecular markers are powerful tools for assessing genetic diversity at the level of DNA. In hybrid rice, genetic diversity has been acknowledged to be the basis of heterosis or hybrid vigor. Simple sequence repeats (SSRs) or microsatellites, due to their codominant inheritance, are among the DNA marker types most suited for assessing genomic heterozygosity. We examined the relationship of SSR heterozygosity and heterotic potential for eight vegetative and reproductive traits in 48 rice hybrids developed from five cytoplasmic-genetic male sterile (CMS) and ten male parents. These parental lines represented the breadth of genetic diversity in current Philippine hybrid rice germplasm. F_1 heterozygosity was deduced from parental genotypes at 44 microsatellite loci spanning the 12 rice chromosomes. Based on at least 75% genetic similarity, the CMS and male parents clustered into two and eight groups, respectively. SSR heterozygosity and heterotic performance (superiority over the male parent) in the 48 hybrids were significantly correlated for the number of productive tillers per plant (r=0.37*) and leaf area index (r=0.34*). When only hybrids with positive heterosis were analyzed to remove maintainer effects, marker heterozygosity and heterosis were significantly correlated for maturity (r=0.60*), leaf area index (r=0.45*), and number of productive tillers per plant (r=0.43*). However, insignificant correlations were observed for plant height, root length, root weight, harvest index, and grain yield. Correlations were also insignificant when heterosis was based on the check varieties, PSBRc28 and PSBRc72H *(Mestizo)*. These results suggest that while SSRs are useful for genetic diversity assessment, markers unlinked to specific traits may not be very effective in predicting heterotic performance for these traits in the diverse hybrid rice germplasm pool studied.

Keywords: Hybrid rice, heterosis, simple sequence repeat (SSR), genetic diversity, molecular marker

IDENTIFICATION OF GENETIC DONORS FOR RICE SEEDLING VIGOR

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The development of cultivars that perform well under direct-seeded culture requires specific traits such as seedling vigor (SV). SV is essential for optimum stand establishment and competitiveness against weeds. We analyzed the genetic variation for SV in 49 promising parentals under controlled laboratory (slantboard test) and greenhouse (wet and anaerobic) conditions. Highly significant (P<01) differences were observed for shoot, root, mesocotyl, coleoptile lengths using the slantboard test procedure. Under wet and anaerobic conditions, significant differences were observed for shoot length, phenotypic vigor, and emergence vindex. Some varieties, namely, Black Gora, UG-20, and IR64683, performed well under both laboratory and greenhouse environments. Rank correlation analysis showed shoot length under weseeded condition was highly associated with mesocotyl (r=0.54**) and cleoptile lengths (r=0.63**) in the slantboard test. Likewise, shoot length was highly correlated with mesocotyl (r=0.68**) and coleoptile (r=0.82**) lengths in the slantbord test. Highly significant correlations of shoot length with root length (r=0.98**) and emergence index (r=075**) under anaerobic conditions were likewise observed. Indica cultivars were more vigorous under both laboratory and greenhouse environments, compared with japonica types. Nine high-SV genotypes were identified in addition to known SV genetic donors. These include CG-14, CG-17, CG-20, UG-20, Brown Gora, Dular WB, Vandana IR, 64683, and AUS 257.

Keywords: seedling vigor, genetic, emergence, direct-seeding, rice

BREEDING SALT-TOLERANT RICE VARIETIES IN THE PHILIPPINES

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There are two major regions in the country where salinity problem has been reported. One is the Bicol River Basin area in the province of Camarines Sur (37,184 ha) and the other is in the delta plains of Cagayan Valley River Basin (35,000 to

45,000 ha). These two regions and some areas found in Iloilo, Samar, Bulacan, and Pampanga cover more than 100,000 ha of salt-affected areas in the Philippines.

Rice production in the salt-affected areas ranges from 1 to 2 tons/ha only, while yields in favorable areas can reach 5 tons/ha or more. In extreme cases, a total crop failure results. Rice cultivation is one of the alternatives to utilize the idle lands affected by salinity because saline soils are hydrologically, physiologically, and climatically suited to rice.

The location-specific strategy of shuttling breeding materials to the problem areas is a fast and effective approach in identifying lines adapted to coastal areas. Through this strategy, seven promising lines adapted to specific saline areas have been identified and entered in the National Cooperative Test (NCT). One of the lines, PR25989-2-4B, can yield 3.3 tons/ha under salt stress and was recently recommended by the Rice Technical Working Group (RTWG) for pre-release. If acceptable to farmers, PR25989-2-4B will be the first PhilRice-developed line to be nominated to the NSIC as a variety for saline ecosystems.

The prospect for better productivity of salt-affected soils can be attained with the availability of rice varieties tolerant to moderate salinity. However, there is still a need to develop rice varieties that can grow in soils with an electrical conductivity (EC) of more than 6.0 dSm^{-1} during the seedling stage. Apparently, advances in biotechnology could hasten breeding for salt-tolerant varieties

Keywords: breeding, rice, salt tolerant, salinity

SCREENHOUSE EVALUATION OF TRANSGENIC IR72 CONTAINING XA21 GENE FOR BACTERIAL BLIGHT RESISTANCE

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T4 generation of transgenic IR72 lines containing Xa21 genes for bacterial blight resistance were evaluated in the screenhouse for their response to nine races of *Xanthomonas oryzae* and one local Maligaya isolate as compared to untransformed IR72; IRBB21, a conventionally-bred line with *Xa*21 gene; and IR24, a susceptible control in dry and wet season evaluations. Inoculation was done at maximum tillering stage following the clipping method and the % diseased leaf area (% DLA) was measured 14 and 21 days after inoculation. Experiments during the dry and wet season trials revealed that IR24, obtained the highest mean %DLA of 64.50%. The untransformed IR72, which contained some genes for resistance to Xoo showed an intermediate response with an average %DLA of 39.72%. On the other hand, IRBB21,

showed a moderately resistant response, 14.80% DLA. Most of the transgenic lines were resistant to moderately resistant, with an average ranging form 4.68 to 7.12% across the lines and 1.06 to 15.5% across races. The differences among transgenic lines, however, were not significant, which may imply that transgene integration events might have been the same. Race 6 is the most virulent with mean while the Maligaya isolate is the weakest. Molecular analysis is being conducted to determine the genetic constitution of the transgene.

Keywords: transgenic lines, *Xanthomonas oryzae,* Xa21 gene, virulence, susceptible, resistance, diseased leaf area

GENETIC ENGINEERING APPROACH TO DEVELOP PEST RESISTANCE IN RICE

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Genetic engineering is being conducted at PhilRice to improve the pest resistance of high-yielding inbreds, new plant type lines and cytoplasmic male sterile (CMS) B Lines. From collaborators outside the country, we have obtained different pest resistance genes; pZ100 (chitinase and glucanase genes for sheath blight resistance), pTW-a (*pin*II gene for insect resistance), and pB822 (Xa21 gene for resistance to *Xanthomonas oryzae);* and plasmids containing the two reporter genes – green fluorescent protein (gfp) and the β-glucuronidase genes. Initial steps in developing *Agrobacterium tumefaciens* strain EHA 105 to contain the reporter genes and some pest resistance genes have been successfully conducted using the freeze-thaw and electroporation methods. Preliminary results on the use of new strategies in *A. tumefaciens*-mediated transformation of rice cells; the co-transformation system, and the presence of two binary vectors in one strain will be presented.

Keywords: genetic engineering, *Agrobacterium tumefaciens,* transformation, rice.

MANAGEMENT OF SOIL-BORNE DISEASES IN RICE-VEGETABLE SYSTEMS

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During the four years of continuous monitoring of soil-borne pathogens infesting rice-vegetable systems in San Jose, Nueva Ecija, five were found to seriously affect rice and vegetable crops, namely, seedling damping-off of onions (*Fusarium* sp.), bulb rot of onions (*Fusarium sp.* and *Rhizoctonia solani*), pink root of onions (*Phoma terresttris*), bacterial wilt of eggplant, tomato and pepper (*Pseudomonas solanacearum*), and sheath blight of rice (*Rhizoctonia solani*). In Bongabon, Nueva Ecija soil-borne diseases affecting onions were pink root (*Phoma terrestris*) and bulb rot of onions (*Fusarium* spp. and *Rhizoctonia solani*). Twenty out of 47 sampling sites surveyed in Nueva Ecija were positive to pink root, while 4 out of 10 sites in Pangasinan and 2 out of 8 sites in Nueva Vizcaya had pink root. The incidence of bacterial wilt in San Jose City was high in eggplant-growing areas.

In the straw mulch experiment, *R. solani, Fusarium* spp. and *Trichoderma* spp. were detected to be present in the rice straw used as mulch prior to transplanting and carried over to onion. *R. solani* was no longer detected in straw mulch just before harvest.

In vitro evaluation of the five species of *Trichoderma* as potential biocontrol agents showed antagonistic effects to all of the seven soil-borne pathogens. On the potential of *Bacillus* isolates as biocon agents, *Bacillus* sp. 1 showed inhibitory effects to Fusarium 1,3, *P. terrestris* and *Sclerotium rolfsii*, while, *Bacillus sp.* 2 and *Bacillus pumilus* were both effective against *Fusarium 1,3,P.*

The experiment on rice hull burning in San Jose showed non-occurrence of soil-borne diseases in both burned and unburned plots. Moreover, there was a reduction in population of colony-forming units of soil-borne fungi in the burned plots as compared to unburned plots. Compost application did not have any significant effects on the incidence of pink root. Preliminary results in the crop rotation experiment showed that the use of pepper, peanut, mungbean, and cucumber as rotation crops reduced the incidence of pink root in onion.

Keywords: biocontrol agents, bacterial wilt, sheath blight, pink root, mulch, seedling damping off, bulb rot, rotation crops, soil-borne diseases, management.

EARTHWORM COMPLEX IN THE IFUGAO RICE TERRACES

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Over the last 10 years, the earthworm complex has threatened rice production in the Ifugao rice terraces (IRT). They are arrayed with other factors that have been contributing to the gradual disintegration of the vaunted "eighth wonder of the ancient world."

We observed that the damage is not directly on the rice plant in most cases, but more on the soil. Their intense activities in the soil, such as burrowing and making tunnels have caused water seepage and soil erosion. Severe infestation enhances weed growth and collapse of terrace walls. Intense burrowing also damages the roots of rice seedlings and such activities cover up seeds, causing abnormal growth of rice seedlings.

In collaboration with the department of Life Science, Maharishi University of Management. USA, we have discovered several new species of earthworms in the IRT. A brief description and the difficulty to identify them with all known taxonomic keys are discussed. We are currently pursuing two approaches: genetic analysis (DNA sequence), and the extensive search for sexually-reproducing populations in natural habitats, to facilitate the naming of the earthworm species.

Keywords: earthworm, rice, earthworm complex, Ifugao Rice Terraces, genetic analysis, DNA sequence

Meloidogyne graminicola: A NEMATODE PEST FROM RICE TO ONION

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Meloidogyne graminicola, a parasitic nematode of rice, was found to have attacked onions in many areas in Nueva Ecija grown after rice. This is the first report

of *M. graminicola* attacking onions. The nematode is soil-borne and resistance to the nematode has not been observed in several rice and onion cultivars, making this pest a potential threat to rice-onion production systems. If not arrested, it might affect the livelihood of many farmers in Central Luzon where 60% of the fields are planted to onions for export to Japan, Hongkong, and other countries.

Several rice and onion fields were surveyed for incidence of *M. graminicola.* Different vegetable crops were evaluated for resistance to *M. graminicola.* Some management strategies to control this pest to a tolerable level were conducted in farmer's field naturally infested by the nematode.

Mungbean, cucumber, peanut, and hot pepper are potential rotation crops after rice. Nematode population can be controlled by rice hull burning, a technology practiced by many farmers in San Jose City. Mycorrhizae can improve onion seedling height and emergence from the soil.

Keywords: Root-knot, *Meloidogyne graminicola,* onion, incidence, control

Monolepta sp. (Chrysomeliidae: Coleoptera), A NEW PEST OF CORN SEEDLINGS

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A Chrysomelid beetle, identified as *Monolepta sp.*, a new pest of corn seedlings is hereby reported for the first time in the Philippines. This pest was observed in corn experimental plots of the Plant Quarantine Support Laboratory at the Central Experimentation Station, UP Los Baños. This beetle closely resembles the morphological features of corn silk beetle, *Monolepta bifasciata* Homstedt except the black mark which is found on the elytra of the species *fibasciata.* This new pest species has an ochre-brown color. It measures 4.0-4.5 mm. The beetle mimics the damage caused by corn semi-looper. The beetle can cause seedling death with severe infestation. This new pest was also observed feeding on graminacious weeds particularly kabit-kabit (Tagalog), sabung-sabong (Ilocano), *Eleusine indica* (L) Gaerntn, and agingay (Tagalog), marapagay (Ilocano) *Rotboellia exaltata* L.f.

Keywords: Chrysomelid beetle, corn seedlings, new pest, corn, Monolepta sp.

Macrosiphum (Sitobion) miscanthi Takashi: A NEW APHID ATTACKING SUGARCANE (HOMOPTERA: APHIDIDAE)

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Of the 345 varieties of sugarcane, seven harbored the aphid, *Macrosiphum (Sitobion) miscanthi* Takahashi, namely: B50112, BM 357, CP 65357, H 60-11902, Phil 58260, Q 44 and Q 114. As a virus vector, the aphid was found to infest the sugarcane arrows only, unlike the wooly aphid, *Ceratovacuna lanigeraa,* which infested both the leaves and the stalks. The pollen from arrows was collected early in the morning by tapping the inflorescence on a funnel-like cardboard. Specimens were collected and mounted for identification. The aphid was aliencolae and completed its life cycle within the arrows. Descriptions and other ecological notes are provided.

Keywords: Aphidedae. *Macrosiphum (Sitobion) miscanthi* Takahashi, sugarcane

COMPETING ABILITY AND CONTROL OF *Echinochloa colona* (L.) LINK IN COTTON

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Echinochloa colona (L.) Link is one of the most prevalent weeds in cotton. This weed causes profound reduction in yield because of its severe infestation, rapid growth, fast and numerous tiller production, and great competitive ability. It is more competitive than *Cyperus rotundus* L. and *Trianthema portulacastrum* L. causing a reduction in seedcotton of 74%.

Information on the competing ability and growth stage of *E. colona* which is most susceptible to herbicide will aid in the determination of a precise timing of application in order to obtain maximum control of the weed. Hence, two pot experiments were conducted in Batac, Ilocos Norte to determine the competing ability of this weed in cotton supplied with varying nitrogen levels and identify the stage of growth that can be effectively controlled by fluazifo-butyl.

Increase in height, lead production, nitrogen content, and seed output of *E. colona* was observed as nitrogen level was increase from 35 to 105 kg/ha. *E. colona* plants that grew with cotton were shorter; produced shorter panicles, fewer tillers, panicles, spikes, and seeds, and exhibited earlier tillering, flowering and panicle production than in monoculture.

Height of cotton, number and weight of bolls also increased at high nitrogen, Boll production was reduced by 54.5% where *E. colona* competed with the crop for the whole season. Crop yield reduction was due to decreased nitrogen content, shorter cotton plants with fewer and smaller bolls.

Effective control of *E. colona*, plants before they completed with cotton was attained when application was done at the 4th and 8th leaf stages. As a result, taller plants, increased number and weight of bolls were obtained.

The overall implication of the study is that for maximum utilization of nitrogen fertilizer applied, weed control is a necessity.

Keywords: *Echinochloa colona* (L.) Link, fluazifop-butyl, bolls, seedcotton

Hispodonta sp. (Alticinidae: Chrysomeliidae) A NEW PEST OF BANANA

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The presence of *Hispodonta sp.* an obscure pest of banana in the Philippines, has caused alarm for the first time. The flat, ovoid larva of this beetle scarifies midrib and sterm tissues. It also feeds on unrolled leaves creating parallel perforations when opened. It is usually found in the moist portion of the bracts. The adult beetle is moderately sized with the elytra slightly broadened posteriorly. It makes a horizontal straight line feeding marks on the opened leaves. It has been observed also on unrolled central leaf. This pest is now rampant in Benguet Province and Baguio City. It has also reached the provinces of Kalinga and Apayao. The bioecology of this pest and extent of damage in the Cordillera are being studied further.

Keywords: Cordillera, Hispodonta sp., banana, Benguet, bio-ecology, new pest, Chrysomellid beetle, Hispinae, Philippines

THE ENDANGERED GIANT WILD HONEYBEES *(Apis dorsata Fab.)* HABITAT AND BEHAVIOR

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The habitat and behavior of giant wild honeybees *(Apis dorsata* Fab.) or "pukyutan" were studied in six types of ecosystems namely: dipterocarp, mangrove, mossy, pine, and agroforest in Northern Philippines from 1995 to 1998. Field observations together with wild honey harvesters were made in order to gather benchmark information needed in developing appropriate conservation strategies for this endangered species and how they can be managed to pollinate other crops.

"Pukyutan" thrives in all the three regions in Northern Philippines but prefer to build nests in the Cordillera, particularly Benguet Province, where it is cooler and with distinct wet and dry seasons. The order or preference for building nests is dipterocarp forest followed by dipterocarp-pine forest, agroforest or fruit trees nearby houses, and mangroves, where there are food sources and suitable nests sites. They do not build nests in pine, mossy and molave forests, and on open agricultural areas, ranches or grasslands but may forage in these areas when there are flowers in bloom.

"Pukyutan" builds one nest with one comb on partially shaded and either alive or dead trees. They prefer tree trunks with smooth, fine-textured and clean barks; 15 to 30 cm in diameter and inclined between 10 to 50°; at least 2 m long; and 1 to 12 m high above the ground surface.

The mature adult bees transfer to other places when environmental conditions become unfavorable and when food is scarce. They are less gentle but they sting people only when they are disturbed. A colony consists of thousands of bees (eggs to adult stages) and all of them are killed during honey harvest due to improper harvesting technique, forest fires, and environmental pollution.

Keywords: giant wild honeybee, *Apis dorsata*, "pukyutan", honey, honeybees

PHILIPPINE SPECIES OF *Illeis* Mulsant (COLEOPTERA: COCCCINELLIDAE: COCCINELLINAE: PSYLLOBORINI)

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The Psylloborini group plays an importance role as a biological control agent feeding on aphids, white flies, mites, and fungi. Among the ladybird beetles, this group has been considered mycetophagous. There are about two genera, namely: *Psyllobora* Timberlake and *Illeis*. Timberlake existing in the Philippines. Fortunately, the genus Illeis is presented locally by two species. This includes the endemic *I. luzonica* Timberlake and the introduced *I. koebelei* Timberlake. On the other hand, *I. koebelei* is represented by two subspecies, namely, *I. k. koebelei* Timberlake and *I. k. amamiana Miyatake*. Descriptions and key to the species and subspecies are provided.

Keywords: coccinellidaee, Psylloborini, Illeis spp., I. luzonica timberlake, I. koebelei koebelei timberlake, I. koebelei amamiana Miyatake, mycetophagous

EL NIÑO AND ITS EFFECT ON FIELD POPULATION INCREASE OF MIGRATORY LOCUST

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Noticeable increases in the field population of migratory locust, *Locusta migratoria manilensis* Meyen were observed during the El Niño in 1998 in the Visayas, Mindanao, Luzon, and Palawan. there was also parallel population biodiversity build-up on other species of grasshoppers such as *Valanga nigricornis* and *Gastrimargus marmoratus*.

The drying up of surroundings during El Niño caused the congregation of migratory locusts in remaining green patches. Subsequent population build-up followed. This phenomenon of migratory locust population build-up was observed in Bulacan, Tarlac, Pampanga, Nueva Ecija, Zambales and Pangasinan in Luzon. Aggregation of migratory locust in the Visayas, particularly Negros Oriental and Occidental, was observed to have originated in sugar cane fields adjacent to creeks or river tributaries where green grasses abound. In Mindanao, population groupings were observed in green patches just after corn harvest at General Santos City. Population increases in Narra, Palawan was also observed in remaining green covers and migratory forms attacked corn and rice.

Population build-up in Luzon was not successful due to continuous rain. Thus, mating, feeding and related activities were interrupted by strong rains. Population build-up in Mindanao and Visayas were successful due to short duration rains from May onwards. These rains favored the growth and reproduction of migratory locust in the green grasses in the case of Mindanao and the sugar cane fields in the Visayas. Hence, the migratory locust population reached migratory forms which are still attacking crops in Negros Oriental in the Visayas and in General Santos City in Mindanao.

Other grasshopper species also showed elevated population level but not as high as those of the Locust species.

Keywords: migratory locust, Visayas, Mindanao, Luzon, Palawan, bio-physical, El Niño, population, *Locusta migratoria manilensis*

INSECTICIDAL PROPERTIES OF SEVEN INDIGENOUS PLANTS AGAINST THE ORIENTAL MIGRATORY LOCUST ON SUGARCANE

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The oriental migratory locust, *Locusta migratoria* manilensis Meyen, poses a threat to the sugar industry with the recent severe infestation of vast areas of sugarcane in the country. It defoliates sugarcane completely, leaving it with only the midribs. To suppress locust population, the use of chemicals is still recommended. Recent findings, however, report the potential of insecticidal compounds occurring naturally in plants and their great promise as alternative to synthetic chemicals. Hence, crude extracts from leaves of seven indigenous plants were tested on the locust.

At 10% concentration, neem (*Azadirachta indica* A. Juss.), kakawate (*Gliricidia sepium* Jacq.), oregano (*Coleus amboinicus* Lour.), and serpentina (*Andrographis*

paniculata (Burm. F.) Rees) extracts sprayed on sugarcane showed growth inhibitory, repellent, antifeedant, eradicative, and protective properties against the locust. None of the hoppers on *A. indica* developed into adults and had 100% mortality seven days after treatment. Locusts on *A. paniculata, C. amboinicus,* and *G. sepium* treatment were shorter and lighter with prolonged nymphal development and has lower growth index than those on marigold *(Tagetes erecta),* sambong *(Blumea balsamifera* (L.) D.C. Prodr.), and chichirica *(Cantharanthus roseus* (L.) G. Don extracts. In free and no choice tests, these four plants had repellent and antifeedant actions on the hoppers and flyers. *A. indica* consistently protected sugarcane from the locust, but the efficacy of the other plants decreased with the length of time. The insecticidal properties of these indigenous plants can be considered in the formulation and implementation of integrated locust management strategies in future infestation.

Keywords: locust, *Locusta migratoria* manilensis, sugarcane, *Azadirachta indica, Gliricidia sepium, Coleus amboinicus, Andrographis paniculata,* growth, inhibitory, repellent, antifeedant

DIVERSITY AND FORAGING BEHAVIOR OF INSECT POLLINATORS OF COMMON FRUIT TREES IN CENTRAL LUZON STATE UNIVERSITY

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Assessment of the diversity of insect pollinators of common fruit trees in Central Luzon State University was conducted to identify the pollinators of the common fruit trees and to observe the foraging behaviors of these pollinators.

Results show that there were 16 insect pollinators identified and observed visiting the flowers of the common fruit trees in CLSU. They were classified under 3 orders, 13 families, and 16 genera.

Among these pollinators *Phormia regina* (blow fly) got the highest value in density, dominance, frequency, and importance value followed by *Eristalis sp.* (syrphid fly).

The foraging activities in the insect pollinators on the flowers of common fruit trees were also studied. Results revealed that mostly pollinators randomly visited the flowers and some stayed on the flower for a few seconds while others stayed for only a few minutes. Some visited and stayed for a long time on flowers which were not yet pollinated or contained rewards such as nectar and pollen which are sources of food for pollinators.

Over all, the species diversity value of these pollinators was 78, which indicates that they are diverse due to the multiple number of flowers of fruit trees which they can pollinate and secure as their food.

Keywords: diversity, foraging, behavior, insect, pollinator, reward, nectar, pollen, density, dominance, frequency

DEVELOPMENT OF SILKWORM, F1 HYBRIDS FOR SEMI-ARID CONDITION

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This research aimed to develop silkworm F_1 hybrids that could be used for year-round cocoon production and perform better than locally-available varieties in terms of cocoon yield and quality. Six bivoltime and three multivoltine purelines were developed and evaluated for their combining ability using Dialle! Analysis of Griffing's Method 1 (Parents, F_1s, and Reciprocals) at the Sericulture Research and Development Institute, Bacnotan, La Union from 1990-1997. The performance of the promising silkworm hybrids were also evaluated in farmer's fields.

The results of the combining ability tests revealed that the promising hybrids are: for single cocoon weight, DMSU 101 X DMSU 115, DMSU 100 X DMSU 103, and DMSU 102 x DMSU 103, for cocoon shell percentage, DMSU 100 x DMSU 101 and DMSU 103 x DMSU 115, for cocoon yield per box, DMSU 102 x DMSU 103, DMSU 100 x DMSU 107, DMSU 101 x DMSU 103, DMSU 101 x DMSU 107, and DMSU 101 x DMSU 115, for cocoon filament length, DMSU 101 x DMSU 102, DMSU 100 x DMSU 115, and DMSU 103 x DMSU 107.

The cocoon yield performance of the developed F_1 hybrid DMSU 101 x DMSU 115 and its reciprocal was better than the highland silkworm hybrid when reared at the Sericulture Extension Site in Sta. Maria, Ilocos Sur. The same hybrid combination and its reciprocal, as well as the F_1 hybrid of DMSU 115 and DMSU 103, proved promising when reared in the other extension site in Ilocos Norte.

Keywords: Silkworm F_1, hybrids, Grifting's Diallel Analysis, promising hybrids; single cocoon weight; cocoon shell percentage; cocoon yield box

EXTRACTION AND CHARACTERIZATION OF TOBACCO SEED OIL

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Oil from seeds of three tobacco types (Flue-cured: reams 266, Burley: 21 and Native: Sammaha) were extracted and analyzed for physical and chemical properties. Oil content yield by solvent extraction method using petroleum ether and hexane as solvents ranged from 29 to 44%. Extraction using haxane yielded more oil than when petroleum was used as solvent for all samples.

Physical and chemical characteristics namely: refractive index, color, specific gravity, saponification value, unsaponifiable matter, iodine value, peroxide value, lenoleic acid, calorific value, and hydroxyl value were determined.

Fatty acid analysis and acid value were likewise determined.

Keywords: Tobacco seed oil, physical characteristics, chemical characteristics, solvent extraction, calorific value

USE OF ORGANIC SUBSTANCES TO PROMOTE ANTHER CULTURE RESPONSE IN RICE

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Coconut water, cocogro (coconut water growth hormone extract, BIOTECH, UPLB) and banana powder (Sigma Chemical Company, St. Louis, MO, USA) are some organic substances that can be utilized to promote anther culture response in rice. In this study, we evaluated the effect of coconut water (150 mL/L) and varying levels of cocogro (5, 10 20 mL/L) and banana powder (1, 5 10 g/L) on the anther culture response on rice. Desiccated and undesiccated anther culture-derived calli were used as experimental materials.

Incorporation of coconut water as an additional source of plant growth hormone in regeneration medium, enhanced green plant regeneration in responsive japonica rice variety, Taipei 309, especially when coupled with tissue desiccation. Similarly, enhanced green plant regeneration was obtained from 3 of the 5 F_1 crosses

evaluated in the medium supplemented with cocogro as sole source of plant growth hormones, replacing NAA and kinetin. Across genotype, cocogro, at 5 mL/L level, yielded the most number of calli which regenerated green plants. Reduced number of albino plants were obtained from desiccated calli cultured in media with 5 and 20 mL/L cocogro. Moreover, combining cocogro with tissue desiccation reduced the incidence of tissue browning (necrosis). Banana powder treatment did not enhance greenplant regeneration for the two F_1 crosses evaluated. However, callus necrosis was markedly reduced (47-100%) with increasing level of banana powder in the medium.

Keywords: anther culture, rice, coconut water, cocogro, banana powder, tissue desiccation, callus, green plant regeneration, albino plants necrosis

THE ACCEPTABILITY OF TILAPIA *Oreochromis mossambicus* SPREAD

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In the present situation where the catch is nearing the maximum sustainable yield and aquaculture is constrained both in space and environmental concerns, the most direct and immediate contribution to increase food supplies is by reducing post-harvest losses (Santos, 1995). Tilapia *Oreochromis mossambicus*, being prolific, thus rampant in every region is considered unutilized due to its off flavor taste. This study sought to formulate tilapia spread with different flavors, e.g. lemon, coconut wine (tuba), and ethanol.

Tilapia *Oreochromis mossambicus* were purchased fresh from the CFT fishpond and transported to the research laboratory. They were washed with potable water, dressed and precooked at 95°C for 30 minutes. It was cooled, flaked, and treated with different flavors, e.g. lemon, coconut wine (tuba) and ethanol. They, including the control sample, were then individually heated for one hour until dry. The flavored samples were mixed with mayonnaise, pickle relish, pimiento, sugar, and salt. The mixtures were packed and processed for 45 minutes at 10 lbs pressure. The finished products were subjected to descriptive and consumer testing and analyzed using ANOVA at %% level of significance, proximate composition, and cost analyses. About 50 consumers and 10 trained people compressed the test panel.

Results of the preference test showed no significant differences between the newly processed tilapia spread with different flavors and the control sample. After three months of storage, tilapia spread treated with coconut wine (tuba), lemon and

the control sample were not significantly different from one other but significantly different from samples treated with ethanol, that ranked last based on its mean. After 10 months of storage, tilapia spread enriched with lemon ranked first based on its mean but was significantly different from the other samples due to its rancid flavor. Thus, the accepted product was tilapia spread with lemon flavor, which contained 10.44% protein, 20.20% moisture, 27.80% fat, 1.62% ash and 39.95% carbohydrates. The production cost per bottle with a net weight of 225 grams was only P16.45, whereas samples treated with coconut wine (tuba), ethanol, and the control cost P16.35, P16.90, and P16.30, respectively.

Keywords: tilapia, *Oreochromis mossambicus*, processing

Bacillus cereus var. IMPROVED SENSORY AND PROTEIN QUALITIES OF FERMENTED "TINABAL MOLMO *"(Scarus spp.)"*

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Bacterial starter culture has been added in some fermented products to hasten fermentation. *Bacillus cereus var.* and *Pediococcus pentosaceus* isolated from "tinabal molmol" were used in the fermentation of this product. The fish samples, parrot fish or locally called fish were inoculated with the young culture of *B. cereus* and *P. pentosaceus* after 24 hours of salting. The inoculum rate was about 10% by volume of the starter culture based on the weight of the fish. The inoculated slated fish were allowed to ferment for 15 days at room temperature (28 $\mp$ 2°C). The fermented fish were subjected to sensory evaluation and amino acid analyses through High Pressure Liquid Chromatography. Inoculation of pure culture of *Bacillus cereus var.* improved the flavor and essential amino acid content of the fermented fish.

Keywords: tinabal molmol, starter culture, inoculum, fermentation, sensory evaluation, laboratory panelist, Bacillus cereus var., Pediococcus pentosaceus

LEVELS AND SOURCES OF SIGNIFICANT WASTEWATER PARAMETERS IN SELECTED SUGAR MILLS IN THE PHILIPPINES

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Wastewater samples in two sugar mills were analyzed for physico-chemical analysis in 1996 to February 1998. The significant wastewater parameters (BOD, COD, pH, TSS, oil, and grease) were analyzed using standard and alternative analytical procedures.

The study focuses on the levels and sources of significant wastewater parameters for possible baseline information on the physico-chemical characteristics for sugar mill waste. Correlation study between BODs and COD was also conducted and statistical results revealed that there is no direct relationship between the two parameters established using sugarcane wastewater.

The examination of trends in these parameters, major sources as well as their point of entry into, and possible pathways through, the waste treatment plant and hydrological cycle is discussed. It also reports the first attempt to determine the efficiency of the individual wastewater treatments plants, (WTP) of selected sugar mills visited, processes involved, and some management practices in mitigating the impacts of water pollution.

Keywords: BOD, COD, sugarcane wastewater, WTP, pH, TSS, baseline information

CUMULATIVE EFFECTS OF THE EXISTING LAND USES ON THE WATER RESOURCE OF THE SAND DUNES OF ILOCOS NORTE

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Cumulative effects of the existing land uses (LUs) on the quantity and quality of the water resources in the different portions of the sand dunes in Ilocos Norte was assessed from December 1997 to Febuary 1998. The LUs that depend on groundwater resources are residential, tourism-based, and agricultural.

The static water table observed in the study area was confined to shallow well areas (SWA). Estimated water supply based on pumping test and estimated renewable safe groundwater yield based on recharge rate, were comparably substantial. Current LUs used only 4% of estimated supply of 67.15 million liters per day (M lpd) or estimated renewable safe yield of 74.80 m lpd (based on recharge rate). Future expansions of different LUs based on different scenarios indicate a big increase in water demand. However, estimated safe groundwater yield far exceeds this demand. In fact, a full-range development of the sand dunes (based on current uses) shows a water demand of 50.93 M lpd, which is 68% of the available safe yield.

Analysis of the sand dunes' current groundwater also showed a level of quality demanding slight restrictions for domestic use. Nitrate concentration, for instance, exceeded the permissible level in most parts of the residential area. Evidence of contamination with coliform *(E. coli)* was also found in most of the areas but still passed the permissible limits for most uses.

The study predicts that the continuous expansion of various uses in the area will affect the available supply not only due to massive extraction but also due to contamination. This dilemma should serve as a challenge for the sand dune's users to manage every activity in a manner that would not adversely affect the water supply and quality, so that the full range of benefits that this resource can offer is maintained and sustained.

GROWTH, FEED CONVERSION RATIO, AND SURVIVAL OF THE PHILIPPINE ABALONE *Haliotis asinina* CULTURED IN NET CAGES AT DIFFERENT STOCKING DENSITIES

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The effects of different stocking densities on the growth, feed conversion ration, and survival of two size goups of the Philippine abalone *Haliotis asinina* were determined. Three culture trials were conducted in net cages installed in a sheltered cove in Guimaras Province. Trials 1 and 2 were conducted using 15-20 mm abalone juveniles for 150 days, whereas trial 3 was conducted using 35-50 mm abalone for 180 days. The animals were fed sufficient amounts of the red alga, *Gracilariopsis bailinae* (=*G. heterochada*) throughout the experiment.

The results showed an inverse relationship between growth (length and weight) and stocking density. At high densities, stacking restricted movement and feeding of animals. Hence, food limitation was one of the factors that affected growth of abalone at high densities. Another factor that contributed to slower growth of aba-

lone at high densities was rate of water flow. Water movement stimulates feeding and, therefore, growth of abalone. Feed conversion ratio was not influenced by density but was observed to be higher for larger animals. Survival was not significantly affected by density.

Net cages are appropriate for culture of *H. asinina*. This study showed *H. asinina* can reach commercial size of about 60 mm in one yar. The results also showed that growth of *H. asinina* can be sustained using a single-species diet as food source. An economic analysis is important in choosing the best stocking density for commercial production.

Keywords: Philippine abalone, *Haliotis asinina;* stocking density, feed conversion ratio; survival; cage culture

CULTURE OF "LATO" *Caulerpa lentillifera* IN PONDS USING AN ARTIFICIAL SUBSTRATE

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Caulerpa lentillifera, locally known as "lato" is one of the few seaweed species of high demand in local and foreign markets. In recent years, the demand for fresh and salted Caulerpa and other seaweeds has increased considerably. In some areas of the country, the supply of Caulerpa is harvested from the wild in the coastal or marine waters. Extensive cultivation of Caulerpa in enclosed ponds has already been carried out commercially in Cebu, particularly in Kalawisan, Mactan Island and a few other places in the country. However, the development and or improvement of culture techniques to further increase Caulerpa is needed as the prospects of mass production of this seaweed is promising. The study aims to culture Caulerpa in ponds using an artificial substrate, and to determine the efficiency of the artificial substrate.

The study was conducted at the CSCST College of Fisheries Technology fishpond project in Carmen, Cebu. Knotless nylon nettings or V-net (0.5 x 20 m) was used as artificial substance. Ten (10) strips were prepared and installed in rows about 1 m perpendicular to the pond bottom. Caulerpa seedldings were planted by sticking 500 grams each in between the substrate into the mud at about 50 cm intervals. Water parameters such as salinity, temperature, depth, and pH of the water was monitored every week. After 62 days, the length of shoots was measured, weighed, and analyzed using T-test at 0.5 levels of significance.

After 62 days of culture, the mean measurement of shoots using substrate was significantly different from the control. However, the net weight production was not

significantly different from each other at 0.05 level of significance. The results revealed that an artificial subtrate improved the quality of the Caulerpa in terms of the physical appearance and the number of shoots developed at the stem of the plant. The shoots developed were dark green in color and were longer compared the control.

Keywords: *Caulerpa lentillifera*, lato, seaweeds, fishpond, substrate

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HEALTH SCIENCES

VALIDATION OF TWO STEP DIFFERENT EXTRACTION FROM SEMINAL FLUID MIXED WITH EPITHELIAL CELLS

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DNA typing of biological material has become one of the most powerful tools for personal identification and has gained worldwide acceptance since 1985. With recent advances in DNA typing, the UP-PACC DNA Analysis Laboratory was established to develop the national capability to respond to this growing need for accurate identification. One of the most common sources of DNA samples obtained from sexual assault cases is a mixture of seminal fluid and epithelial cells. To initiate advancement in the investigation of such cases, this study was performed to assess the feasibility of current protocols in extracting DNA from such biological sources. These protocols on differential lysis were tested. A single mixture of fresh semen and epithelial cells was utilized for conformity in all the three protocols. After the DNA was extracted from the female and male fractions, it was amplified at STR locus HUMFOLP3 (HUMDHFRP2). Analysis of the amplified DNA showed complete separation of the two fractions. All three protocols were suitable for DNA extraction, however, use of the modified FBI protocols yielded higher amount of the needed substance (100μg/mL). Alleles were detected and the sources of the mixed stain were cnfirmed by matching the results with corresponding female and male blood donors.

Keywords: seminal fluid w/ epithelial cells, human identification, two-step differential extraction procedures DNA typing, male/female fractions, differential lysis. PCR, HUMDHFRP2, HUMFOLP23, STR

BIOASSAY OF PARALYTIC SHELLFISH POISONING (PSP) TOXINS FROM TOXIC BACTERIA *(Micrococcus sp.)* USING BRINE SHRIMP *Artemia salina* (Linnaeus)

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Paralytic Shellfish Poisoning (PSP) toxins is a group of related neurotoxins derived from toxic single-cell planktonic organisms known as dinoflagellates. Mollusks infected with any of these organisms may cause food poisoning when ingested. Different bioassay techniques have been developed to determine toxicity levels of mollusks infected wth these dinoflagellates. Studies have also shown a relationship between toxic dinoflagellates and bacteria associated with them. In this study, toxic bacteria (*Micrococcus sp*). isolated from the toxic dinoflagellate *Pyrodinium bahamense var. compressum* was cultivated for production of PSP toxins. The toxins were extracted and identified using spot test and thin layer chromatographic (TLC)- Fluorometric method. Two different bioassay techniques were used to detemine toxicity levels of the extracted toxins. Using the mouse bioassay technique, BALB/c mice were injected intraperitoneally with 1 mL of crude extract while in *Artemia salina* bioassay, 0.5 mL of the crude extract or its dilutions were added to four plates of saline medium containing 10 brine shrimps *(A. salina* nauplii) each. Toxicity levels for mouse and *A. salina* were measured in terms of mouse units and mortality rate, respectively. Spot test of the extract showed a retardation factor (Rf) of 0.79. Death did not ensue in mice and consequently, toxicity in terms of mouse units could be determined. In *A. salina*, addition of the crude toxin extracted to the medium resulted to mean mortalities of 87.14%. Further dilution of the toxin extract to 100 folds resulted to mean mortalities of 43.91%. Further studies are being done to determine concentration and biological activity of the crude extract, as well as to develop a Lethal Dose 50 (LD_{50}) for the *A. salina* bioassay.

Keywords: paralytic shellfish poisoning toxins, spot test, thin layer chromatography-flourometry, retardation factor, intraperitoneal injection, bioassay, mouse unit, mean mortality, lethal dose 50, *Artemia salina nauplii*

DETECTION OF *Mycobacterium tuberculosis* BY POLYMERASE CHAIN REACTION

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A polymerase chain reaction assay was used to amplifyh a 123-bp region of the IS 6110 insertion element, in which multiple copies occur n the mycobacterial genome, for the diagnosis of pulmonary tuberculosis. Sputum samples from patients with clinical diagnosis of pulmonary tuberculosis, were evaluated by PCR. Results were compared with those obtained by microscopic examination of concentrated smear stained with acid-fast-kinyoun stain, and radiometer (BACTEC) culture. All smear and culture positive samples were PCR positive, indicating that PCR could be a good tool for the rapid diagnosis of mycobacterial infectious diseases.

Keywords: polymerase chain reaction, *Mycobacterium tuberculosis*, IS 6110, insertion element, acid-fast, kinyoun stain, radiometric, BACTEC culture, mycobacterial genome, infectious diseases

MICROSATELLITE DNA POLYMORPHISMS IN FILIPINOS

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Microsatellite DNA are soft tandemly repeating polymorphic sequences distributed throughout the human genome. Genetic variation of microsatellite loci have been studied extensively in many populations worldwide for phylogenetic analysis, genome mapping and linkage analysis, and identity testing in the medical and forensic scienes. This study reports genetic variation in three Philippine enthnolinguistic populations based reports on allele frequencies at the HUMF13AO1, HUMFES/FPS and HUMvWA STR loci.

Genomic DNA samples of consenting individuals from the Cebuano, Ilocano, and Pampango language groups were analyzed using polymerase chain reaction and automated fluorescence-based product detection. While all three Philippine populations were found to share the most frequent alleles at these loci, interpopulational variation was observed on the frequency distribution of the other alleles. Comparison with other Asia Pacific allele frequency data showed significant differences, indicating that these microsatellite loci applicability of the allele frequency data for forensic identity testing, calculations for ramdon-match probability, likelihood ratio, exclusion power of the loci, and power of paternity exclusion were performed. Values suggest that the data generated can be contributed to the national genetic database to be used as basis for forensic casework in the country.

Keywords: human genetic variation, microsatellite DNA, Philippine populations

IDENTIFICATION OF HCV GENOTYPES IN FILIPINO POPULATION BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM

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Hepatitis C virus (HCV) is recognized as the major etiologic agent of most cases of acute and chronic non-A, non-B liver diseases and infects around 1% of the general population worldwide. At least nine major genotypes have been documented and a significant number of data indicate that correlation exists between HCV genotypes with clinical course, virulence, and response to interferon therapy. This study aims to identify existing genotypes in the Filipino population by restriction endonuclease cleavage of the RT-PCR amplified 5' non-coding region of the HCV genome. Renal transplant patients, blood donors, and patients undergoing hemodialysis and blood transfusion were included in the study if their serum was found reactive with anti-HCV by second generation EIA. RNA from serum was extracted and a reverse transcription RT-PCR was performed in a single tube assay using nested primers from the highly conserved 5' non-coding region of the HCV genome. Restriction digestion using (a) *Rsa*I and *Hae*III, (b) *Hin*fI and *Mva*I, and (c) *Scr*FI was carried out on secondary PCR products. After electrophoresis through a 3% agarose gel, restriction patterns were determined by comparing digestion products with established genotypes.

Preliminary results show that out of 29 serum samples reactive with anti-HCV by ELISA, 14 were confirmed positive by RT-PCR. Subsequent RFLP analysis of 14 samples identified 12 as genotype 1, 1 genotype 2 and 1 genotype 4. Our ongoing work is designed to correlate the identified HCV genotypes with the degree of liver derangement, biochemically and histologically.

Keywords: Hepatitis C. Virus, HCV genotypes, interferon therapy, restriction endonuclease cleavage, RT-PCR, restriction digestion, restriction fragment length polymorphism, second generation EIA, single tube assay, nested primers

BINDING PROFILE TO NEOGLYCOPROTEIN PROBES IN CERVICAL CARCINOMAS

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When affected by oncogenic cells, layers of the cervix exhibit changes in structure and composition. Histochemical changes manifesting qualitative and quantitative alterations of cellular and extracellular glycoproteins may be detected using biotinylated probes. Histochemistry through probe-receptor-color reaction using lactose, mannose, N-acetylgalactosamine, N-acetylglucosamine, as well as hyaluronic acid and fucoidan allowed detection of receptor sites in the tissues obtained from Metro Manila hospitals. Cancer tissues showed greater affinity to lactose while normal tissues showed positive reaction to mannose. Binding profile to the outer probes was highly variable. Lactose and mannose are feasible biomarkers for carcinoma and normal cervical tissues, respectively.

Keywords: binding profile, cervical carcinoma, lactose, mannose, fucoidan, hyaluronic acid, N-acetylglucosamine, N-acetylgalactosamine

BINDING PROFILE OF A LECTIN AND NEOGLYCOPROTEIN PROBES ON NORMAL AND THYROID CARCINOMAS FROM FILIPINO PATIENTS

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Although mortality due to thyroid cancer is not really high, very little is known about it except for its prevalence among Filipino women. Moreover, epidemiological studies of thyroid cancer in the Philippines reveal that incidence of the disease has been on the rise in the past decade. In its respect, thyroid tissues representing 20 normal and 34 papilliary carcinomas were processed for histochemical staining using biotinylated concanavalin A, a mannose-specific lectin and six neoglycoproteins also biotinylated and conjugated to BSA-made histochemically inert. Localization of receptors to the probes was made possible by using peroxidase conjugated avidin-biotin complex and the chromogenic substrate DAB. Results revealed that the probes, concanavalin A is a very promising histochemical marker for transformed thyroid. All of the normal specimens did not stain with the probe but nearly 60% of the thyroid papillary carcinoma were positive for con A receptors. Thus, mannose containing receptors, which are undetectable in normal thyroid gland epithelia, are expressed by cancerous epithelium. The signal becomes stronger in more advanced carcinomas. This information can be used for diagnostic purposes as well as in the development of therapeutic tools for thyroid cancer.

Keywords: lectin, concanavalin A, avidin-biotin complex, papillary carcinoma

BREAST CANCER WITH P53 GENE MUTATION

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This study is the first report on the histopathology of breast tissues in Filipinos who have mutation in p53 gene. Patients from the Philippine General Hospital

(PGH), East Avenue Medical center, Veterans Memorial Medical Center were screened by the team from the Philippine Nuclear Research Institute for p53 mutations. Both qualitative and quantitative histopathologic parameters were identified and recorded.

Analyses show that 86% of the Filipino patients have invasive ductal carcinoma, 6% have invasive globular carcinoma, 6% have phallodes tumor, and 2% have mucinous carcinoma. Ductal carcinoma exhibits nuclear features which are markedly different from those of Caucasians. These may be the ethnic-specific profile of Filipino patients. These histopathologic qualitative and quantitative parameters are our original contribution to supplement the morphological routine in malignancy grading.

Keywords: breast cancer, p53 gene, histopathology, carcinoma, malignancy, grading

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SOCIAL SCIENCES

EDUCATING FOR CRITICAL THINKING: REVIEW OF LITERATURE

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This paper summarizes ideas on critical thinking from literature reviewed at Harvard University library while the author was a visiting scholar during Spring 1998. Researches on critical thinking submitted by the author's students at PLM were content analyzed supplemented by group interview.

Ruggiero proposes three strategies for thinking instruction in the 21st century. Sternberg and Lubart think that developing creativity in children and in adults involves teaching them to use six resources: intelligence, knowledge, intellectual style, personality, motivation, and environmental context. Sternberg also discusses eight easy ways to fail in teaching critical thinking. These eight fallacies obstruct the teaching of critical thinking before we even begin and make it easy to fail. The effects of these fallacies on the teaching of critical thinking are both insidious and pernicious. The best way to promote critical thinking in an English course, in Social Studies, and in teacher education is to involve students in class discussions in which they have the opportunity to raise issues, clarify their thoughts, and test their ideas against their classmates.

In studying teaching techniques at various US schools and school districts, Brown and his colleagues at the Education Commission of the State evaluated classroom "thoughtfulness" on eight criteria. Strother points out in his paper that researches know that for students to cultivate thought they need to ask questions often and freely, become actively involved for long periods with problems that make sense to them, and engage in activities in which the teacher plays the role of coach.

In Defense of Vague Assignments, Wolfe drives that purposeful vague assignments often can force students to make their own decisions, define, and solve emerging problems, practice inquiry, discover their own purposes for writing, develop their own organizational patterns, and become secure, confident, and able

observers, readers, writers, and thinkers. In short, vague assignments can help students learn how to learn, how to appreciate a work esthetically, and how to respond personally to their reading and writing. Garrison presents his conceptual model for developing critical thining in adult learners which include problem identification, problem definition, exploration, applicability, and integration while Schulam points out that great minds (for 7-10 year old children) start with great questions. Rath offers 14 "thinking operations"-dimensions of higher-order mental functioning that may serve as guides for developing classroom activities-for exercising thinking. Teaching for thinking is more effectively carried out when thinking tasks are used in concert with teacher's reflective, analytical, and challenging responses.

Keywords: critical thinking, creativity, education

BENGUET FARMERS ADOPT AGRICULTURAL TECHNOLOGIES

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Benguet farmers have grown and matured in that they want to try new farming technologies. They say that traditional and modern ways of farming can complement each other. Gone are the days when farmers clung to traditional practices.

The study surveyed farmer-respondents in Benguet who adopted mature technologies recommened by various government agencies in the province. Results of the study showed that the respondents were convinced that environmental factors, natural and man-made calamities, and beliefs affected their decision to adopt technologies.

Majority of the farms were rainfed. The problems encountered by the farmers which affected their decisions to adopt technology were: cold weather, soil erosion, and lack of water or irrigation. Majority of the respondents had low incomes.

The respondents said they wanted to be assured of market outlets for their produce since they invested capital and labor. the recommendations of the study include the following: the government should generate or develop technologies aimed at industrializing the agriculture industry. There is a need to come up with techologies for food processing, packaging, and marketing. The government should conduct research and extension activities in the farmers' fields to enable them to see for themselves the technologies introduced and establish hands-on demonstration farms so that farmers can aquire the necessary skills.

Keywords: Benguet farmers, agriculture, technologies, agricultural technologies

ATTITUDES TOWARD BIODIVERSITY CONSERVATION OF MANGROVES AMONG COASTAL DWELLERS IN CATANDUANES

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The island province of Catanduanes in the Bicol Region has only about 1,735 ha of classified mangrove area (approximately 1.6% of the total mangrove area of the Philippines or 16% of the mangrove areal extent in Luzon.) Although the mangrove area is relatively smaller compared to other island provinces in the country, it has enormous significance on the econology and economy of this typhoon-prone province.

Current studies indicate that there are at least 20 major and minor mangal elements found in the mangrove areas which are characterized by both riverine and wave-dominating *allochthonous* materials. It appears that diversity of the mangrove vascular flora and macrofauna associated with this coastal marine ecosystem have long been subjected to stresses, i.e., typhoons, tidal surges and anthropogenic factors. Recently, there is now an increasing interest to involve the local people in various mangrove reforestation projects.

The purpose of this paper is to provide a small contribution to mangrove research by examining the attitudes of coastal dwellers, specially teachers toward biodiversity conservation of mangroves. An understanding of their attitudes about the values of conserving mangroves will aid in formulating appropriate educational initiatives to encourage sustainable use, rational management, and biodiversity conservation. We hope to provide a case example which will stimulate further research on the socio-cultural factors related to success in mangrove reforestation or regeneration projects.

Keywords: Catanduanes, mangrove, reforestation

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