TRANSACTIONS of the NATIONAL ACADEMY OF SCIENCE and TECHNOLOGY

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Volume XV

Republic of the Philippines
National Academy of Science and Technology
TAPI Building, DOST Complex, Bicutan,
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WELCOME ADDRESS

Senator Shahani, our honored guest, Dr. Gloria, honorable Secretary of Science and Technology, national scientists of the Philippines, fellow academicians, esteemed co-workers in science, ladies and gentlemen:

First, allow me to congratulate our new Academicians on their election to the National Academy of Science and Technology.

The focus of our annual meeting this year is the Filipino. Two years ago, we had an extensive presentation and discussion on how to manage our environment. We analyzed how badly our environment has degenerated -- our forests, mountains, lakes, rivers, cities and seas. There were many suggestions on what to do.

Last year our topic was how to mitigate natural disasters. The Mt. Pinatubo calamity and, before that, the Ormoc catastrophe, focused our attention on the need to mobilize our brains and efforts toward coping with and alleviating if not preventing the sufferings from such disasters. Again, a good number of suggestions were put forward.

The task force assembled to study and organize these recommendations for better implementation made a striking fundamental observation: not one of these suggestions can be implemented unless the people are willing. The solutions suggested are all, as we say, in the books. They have been known for a long time; they have been repeated over and over. But they have not been carried out.

Take the diseases that are water-borne, food-borne and insect-borne; they continue to rampage through our population. There is no reason why we continue to suffer from them when personal hygiene and sanitation of our surroundings and environment could practically eradicate them. In China, Mao succeeded in almost eradicating pests and insects purely by the individual efforts of each person in a community. Why cannot we do the same? Why is it that we are content to just talk and talk, argue, maybe even quarrel most of the time and afterwards do nothing?

Today, we will try to study this person, the Filipino, and see how best to handle him. After we have analyzed his strong points and his weak points, his habits and what makes him tick, perhaps we may discover how best to manage him.

I wish us all a fruitful and successful conference.

CONRADO S. DAYRIT
President

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THE POWER OF HUMAN VALUES

(Address of SENATOR LETICIA RAMOS SHAHANI before the National Academy of Science and Technology at its 15th Annual Scientific Meeting, 7 July 1993, Manila)

It is my honor to speak before the National Academy of Science and Technology, an organization representing the nation's outstanding scientists and thinkers. This occasion is particularly significant not only for me but for the entire nation as well because of the theme adopted by the Academy this year: "Filipino: The Key to the Solution of His Problems." Being the proponent and principal author of the Moral Recovery Program which has been adopted as a government program by the Ramos administration, I take a measure of pride in noting that the Moral Recovery Program has served as an inspiration in the 15th Annual Scientific Meeting of NAST. The recognition and realization of the importance of values formation in nation-building by such respected and learned group of people like you strengthen my conviction that the need to re-evaluate and re-cast the Filipino's moral value system is as pressing as any other issue our country faces today. The challenge now is on our part, to use all resources available so that others may benefit from the full potential and challenge offered by the program.

The Moral Recovery Program

At this point, allow me to give a brief history of the modest beginnings of the Moral Recovery Program which has now gained ground to become a major facet of the Ramos administration.

In September 1987, I filed a resolution urging the Senate to undertake a study of the strengths and weaknesses of the Filipino character. My premise was to identify the positive qualities for nation building. That initiative produced a report, prepared by a distinguished group of Filipino scholars from the Ateneo and the University of the Philippines. The report recommended that in order to fully utilize the good qualities of our people, the weaknesses or negative qualities have to be removed or rectified. The good qualities can then be used for the good of our daily lives over a sustained period of time. The rest is history. The reception that the report got can very well be described by the status of the MRP as a government program today. Indeed, it is obvious that the Senate Moral Recovery Program has served to fill a hunger among our people who are anxious and worried about where we are heading and what we should do next. "Ano ba talaga ang nangyayari?" is a

common question. In other words, the individual Filipino unlike his or her counterpart during more prosperous times some 40 or 30 years ago can no longer lead a separate and protected life. The problems of society as a whole have become shared personal problems of individual members, problems such as short-sighted decisions on the debt management strategy, flash floods caused by environmental degradation, the great number of street children nationwide, brutally violent initiation rites in schools – all these affect the Filipino's daily existence and outlook in life. We, poor, middle-class, rich, all seem to be in the same sinking boat. If our economic problems are massive, so are our ethical and psychological dilemmas.

In essence, the Moral Recovery Program is a movement which aims to mobilize Filipinos for nation-building through practical exercise of human values in our daily lives as citizens, and to awaken us to the power of these values in achieving our individual and national goals. Those values are free of charge; we do not have to borrow, nor to beg regularly and constantly from the outside world to obtain them; we only have to look inward, internalize these values for our own self-transformation, then externalize them for our individual lives and for building our nation. To use current terminology, the Moral Recovery Program seeks to empower people – the poor, the middle-class and the rich – through the sustained application of human values and virtues to overcome our problems and build our country in accordance with our collective vision. We can also see the Program as an attempt to complete the complex picture of nationalism. If nation-building has its political, economic and cultural dimensions, it also has its moral and ethical imperative. This imperative is a most compelling dimension of nation-building. It goes beyond mere legislation of anti-graft measures or Congressional investigations of wrongdoing in the Government. We need to go back to the basics and ask the fundamental questions: what is our vision of ourselves and of Filipino society? how do we achieve that vision despite overwhelming odds? what key values are needed to attain our goals? I submit that this vision and the strategies and political will needed to realize it should constitute the main framework to build this nation. Nothing less will do. This combination of vision and action is the key to our national survival, rebirth and renewal. In this context, the Moral Recovery Program becomes a major ingredient of an alternative strategy for national development.

Ethics and Politics

The close interrelationship between ethics and politics is obvious in our many problems – our large foreign debt; the state of permanent disrepair of our roads and public toilets; graft and corruption in Government; the perennial squabbling and intramurals between Government bodies; and bureaucratic inefficiency. Chronic problems in such vital areas as agriculture and industry, rural development and land reform could be overcome if some of the values such as love of country, discipline, honesty, accountability and teamwork were practiced on a daily basis in Government offices and political circles, as well as by the people themselves.

Vision

The over-all vision I have for our country has the following essential elements: reverence for all forms of life and the primacy of human values; a priority given to cultivation of the spiritual and cultural life of the nation; the democratization of power, resources and wealth; the right combination of a free market economy and Government intervention in appropriate areas at appropriate stages to provide for the basic needs of its citizens; a Government which works for the good of the people, the development of our agricultural resources and an environmentally conscious industrialization plan; a well-implemented agrarian reform program; respect for human rights, including the rights of women; and an independent foreign policy within the framework of global cooperation. In other words, we should have a vision which represents strong combination of human dignity, sustainable development and appropriate economic growth; national interest; and global orientation. A tall order indeed, but a vision must inspire over the long-term, shed light in the midst of darkness and make possible the seemingly impossible.

Individual and National Transformation

At this point, we come to the question: what is to be transformed or changed – the structures of society or the individual? In my view, both should be transformed, each dynamically affecting the other, but the starting point is always the individual, or a group of individuals within institutions. The empowerment of the poor must come from the poor themselves; the poor must help themselves; others can only help them to help themselves. There is a welcome opportunity in this country to help empower the poor, and such empowerment is vital to the creation of more just social and economic structures.

Human Values: Powerful Building Blocks

It is obvious from what I have said that human values are powerful building blocks in the development of a nation. Yet the non-economic and non-budgetary dimensions of progress and growth, i.e., the moral and cultural elements, have been conveniently overlooked or disregarded by the learned technocrats and theoreticians of development perhaps to make way for smooth, non-controversial discussions of the development process. The technocratic and neutral language of development, which has evolved from the agenda of international institutions, has eclipsed the moral choices which have to be made in the development process. Terms like equity, social justice, distributive justice when repeated over and over again without any explanation of the painful ethical choices which have to be made by individuals and governments in order to achieve them cannot touch the hearts and minds of the populace – the rich, the middle-class and the poor, on whom the burden of transformation rests. Development is, after all, a grassroots-oriented process and a challenge in mass mobilization, for the people and not for political expediency.

A similar observation can be made of our country where the study of the law is the favorite road to success of the best and the brightest. By using legalisms and citing provisions of the law as solutions to issues of right and wrong, we conveniently close the door to the need for a deep reflection on the dialectic of thesis, antithesis and synthesis in human history. Reality becomes external, verbal and theoretical. No wonder many of the values to which even the most righteous among us pay lip service have no power to transform individuals, because such values are not internalized and made part of their heart and soul, nor externalized into lifestyles and professional careers.

The Missing Links: Values as Part of Vision and Strategy

Where are the missing links in our value and cultural system which are partly responsible for our currently retarded position? The goal of development so far has eluded us; at present we remain the basket case among ASEAN countries. A common but embarrassing observation raised by our Asian neighbors, which has almost become a chorus in our region is: "What has gone wrong with the Philippines? Thirty years ago, your country was one of the most advanced among the Third World countries in Asia; now you have become the maids, the waiters and the illegal migrants of the world, and classified by the IMF as 'prolonged user' of its funds."

One of these missing links is love of country or nation-building. Filipinos, as pointed out in the Moral Recovery Program, take care of themselves and their families first, but allegiance to the nation and the flag is not yet characteristic of them. Compare this to the attitude of the Japanese or Koreans or Thais who have utilized their pride in their history as a people to shape their priorities and pace of development. How do we make the Filipino care for and love this country? This is an important question for this forum as it is we Filipinos, who must and should be responsible for our own destiny. As it is, so ingrained over the years is the habit of automatically asking assistance from the outside world that we have forgotten our own potential for self-empowerment and lost our national pride.

Another missing link which is so important in nation-building is the capacity to implement efficiently and decisively policies and plans. Internationally, Filipinos are recognized for their talents in conceptualizing plans and programs; for this reason, our kababayans are much in demand in international organizations. Those talents, unfortunately, are not matched by a capacity to translate visions and ideas into concrete realities. Dreams can become real only through a well implemented strategy. Is this incapacity on our part a product of our long national history, where we saw our colonial masters giving orders from on high to their brown subordinates? Whatever might be the origin of this typical characteristic, it could also be a result of our protective attitude toward our children, many of whom are brought up by yayas or relatives. It is also a stinging commentary on our colonial educational system which apes the progress of the West without being able to

match the vigor of its intellectual life and the rigor of its scientific traditions. Another missing link so important to the advancement of our collective existence is the need for coordination, consensus and integration of policies and programs. We seem to be forever beginning anew, hardly finishing anything which we can evolve into enduring traditions and institutions. Like Penelope of Greek mythology, we seem condemned to be deliberately undoing what we have accomplished in the past to start all over again. This has been variously identified as the "crab mentality," the *kanya-kanya* syndrome, the culture of *palakasan at pataasan,* and the adversarial approach which stems from an attitude of extreme personalism. No wonder our foreign friends are puzzled and ask: "What do you Filipinos really want?"

GAP Between Public Image and Private Conduct

Closely related to the inability to implement policies and plans is the gap between public image and private conduct or personal lifestyle. In other words, Filipino culture inordinately tolerates contradictions between public image and private morality. In my view, individuals, particularly the leaders of the community and nation, must demonstrate in their daily lifestyle, their commitment to simplicity and accountability, in terms of the food they eat, the clothes they wear, the kind of motor vehicles they use and the family life they live.

These missing links which I have described should be restored and put in proper perspective as ethical values have a way of complementing, reinforcing and balancing one another. It seems development demands a certain amount of perfection and completeness; the piecemeal and fragmented approach cannot and does not work.

As to structures needing transformation, two sets of classification have to be examined. First, is the individual, family, organizations, community and nation; second is the elite, the middle-class and the poor. Specifically, I suggest that we examine more closely the current status of the Filipino family in the hopes of strengthening it in view of its disintegration due to poverty.

Our vision for the future cannot be complete without reference to the notion of sustainable development. As a concept, sustainable development originated in the West, but it has now become an accepted topic among developing countries. In the Philippines, non-governmental organizations have already pioneered in making us aware of sustainable development.

Why the current emphasis on sustainable development? In 1991, the gospel of economic growth for the sake of growth was no longer tenable. Conventional development in terms of constantly increasing production to satisfy unending demands of a consumer society can no longer be sustained nor maintained. The era of sustainable development in its essential meanings that I have articulated is upon us. It is now our task to relate and reconcile sustainable development with our vision of individual dignity, human values and economic growth. Lester Brown, the

well-known environmentalist, defined sustainable development as one that "satisfies its needs without jeopardizing the prospects of future generations. Inherent in this definition is the responsibility of each generation to ensure that the next one inherits an undiminished natural and economic endowment." According to Brown, a sustainable world must be achieved within the next 40 years, by the year 2030; otherwise, environmental deterioration and economic decline will pull the world into a downward spiral of social disintegration. By the year 2030 many countries will opt for solar-based systems, not one powered by coal or natural gas. The world in 2030 can achieve a more equitable and secure economy if the Third World debt is reduced to the point where the net flow of capital from industrial to developing countries is restored. Sustainability can be achieved by the year 2030 if the use of recycled materials is promoted, a shift of resources from military programs into life sustaining activities such as reforestation and soil conservation is made, and a balance between human and natural resources is reached. This movement toward a sustainable society will require a new set of individual priorities and values. Materialism, status-seeking and the amassing of personal riches and national wealth will have to be drastically eschewed. In other words, in speaking of values within the framework of sustainable development, we are not just dealing with legalistic anti-graft and anti-corruption measures; we are going more deeply, more profoundly into the relationships of values and development, where human survival, indeed, the future of the human race will depend on the kind of values people will internalize in their daily lives.

We Must Transform Ourselves First

Friends, I have touched on many themes in this address but the main message of the Moral Recovery Program is that we must transform ourselves first before we can transform our nation. Let us not wait for others to begin this transformation; the margin of error is getting narrower. The time frame given us is getting shorter. For us Filipinos, the place and time is here and now. We shall not fail in this undertaking of individual and national transformation if we have faith in the power of human values to help us achieve our vision of human dignity, nation-building and sustainable development.

Finally, I would like to share with you a verse which actually served as seminal seed for the Moral Recovery Program:

"Watch your thoughts, they become words;
Watch your words, they become actions;
Watch you actions, they become habits;
Watch your habits, they become character;
Watch your character, for it becomes your destiny!"

Thank you and good day!

Symposium Papers

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VALUES AND DEVELOPMENT

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INTRODUCTION

The theme of this year's 15th Annual Scientific Meeting of the National Academy of Science and Technology is very timely. At no point in our history has there been a need to re-examine the issues and processes in the social transformation of the Filipino.

The purpose of this paper is to discuss (or better still, to ventilate) certain issues which, to my mind, have contributed much to the transformation of Filipinos away from their native grounds. This transformation (some call it colonization) has been the subject of long debates – its impact on our society has been scrutinized in detail more than a hundred times over.

I do not intend to repeat the subject of colonization here, although it is very tempting to do so. It is the most visible aspect of our historical experiences which transformed our institutions and, to borrow James Fallow's phrase, "damaged our culture."

But I will not do it here – the subject matter has already been wrung dry of its meaning and significance. Rather, I shall examine certain elements of our society and culture which have remained intact underneath this historic veneer and see whether we can make these elements surface and use them to "prime up" our national development.

I am referring here to our traditional values which we have constantly blamed for our inability to develop. They are barriers to our efforts to nation building. That is why, critics of our society and culture are quick in saying that these values are sources of "ills plaguing our society" and of the "weakness in Filipino character."

I have a different view from these critics. I do not see these values as causes of the "ills plaguing our society"; neither do I accept that these traditional values account for the "weakness of Filipino character."

On the other hand, I believe that our negative views about our values cause the "ills now plaguing our society"; they have held back the momentum of

development everytime we make a good headstart. It is our disdain of these values which dulls our senses and blurs our vision of the future. It is our refusal to harness these values in nation building which accounts for much of our difficulties in making this nation move forward.

As one bureaucrat said in an interview:

> *"Sa totoo lang, suyang-suya na ako d'yan sa pakikisamang iyan, sa utang na loob na iyan, sa awa na iyan. Magagandang katangiang pambahay ang mga iyan. Ngunit sa opisina – ang mga katangiang iyan ang sanhi ng kalokohan, pagkawalang disiplina, graft and corruption. Sobra na, dapat palitan na ang mga ugaling iyan."*
>
> Rough translation: "Truthfully, I am fed-up with those traits – *pakikisama* (getting along with others), *utang na loob* (debt of gratitude), awa (pity). These are beautiful traits to use at home. But in the office – these are the sources of mischief, lack of discipline, graft and corruption. It is too much. Those values have to be changed."

Let us change these values. For what? Western ones? But our colonizers tried that before – for 400 years under Spain and 50 years under the United States and apparently they did not succeed. After they left, we continued to use their value-system and look at the results – we have not gotten off in spite of our bold schemes at development. Let us review some of these. For example:

1. A long time ago, we said that political independence could not only set us free but could also stimulate economic growth and development. So we revolted against the Spaniards in 1896-98; fought the Americans in 1898-1905; resisted the Japanese in 1942-45; and in 1991 removed the last bastion of American dominance in the country – the US military base in Subic.
2. We also argued that education was it – and the Americans helped us acquire Western education. Literacy was said to be the key to development. Our educational system, patterned after the American system, became the envy of other Asians. In fact, we became the training center of other Southeast Asians. The College of Agriculture in Los Baños and the International Rice Research Institute are among the finest institutions in the world. That is why, Thailand, Malaysia and Indonesia, among others, sent their students here to study agriculture.

   Ironically, however, our economy did not grow as expected and we have to import rice from Thailand.

3. When we received political independence from the United States immediately after world War II, we stressed community development as the best stimulator of economic growth. Feeder roads were built to link the villages to towns, artisian wells were constructed to give the rural people potable water, the villagers were taught the skills to become better agents of change and so forth.

   After the euphoria, economic growth and development did not take place. We were back to square one: poverty and underdevelopment continued to prevail.

4. Then, we introduced land reform as the harbinger of growth. It was the centerpiece of the administrations of Presidents Macapagal, Marcos and Aquino. However, it did not bring about the growth it promised; it compounded our economic problems.

5. Ferdinand Marcos declared Martial Law and worked for the creation of a New Society. The battlecry was" "This Country Can Be Great Again" because we have recast the structure of society which causes our economic sufferings – the elite.

   But the outcome of the 20-year experiment of 20-year centralized power did not push the frontier of development to any greatness at all. The outcome is now history.

6. Today, we look at foreign investments as the key to economic growth and modernization. To insure its success this time, we are now engaged in a national moral recovery program – hoping to create a better environment to sustain whatever growth takes place.

   But our headstarts seem to be fraught with frustrations: political in-fighting, brownouts, congested streets, lack of water supply, etc.

ISSUES

What I have just presented is the darker side of our past record in national development. The optimistic side is that we are still here, despite all the natural and socio-political disasters which hit us during the last 30 years. What is the secret? Why can't we make this nation move forward a bit faster than how it is doing today? What holds us back everytime we gain momentum in development?

There is no singular answer. My personal view, based on my long years of active participation in research and academic teaching, as well as consulting in both public and private organizations, is that there is one fundamental flaw in our approaches to social transformation: we have not founded our approaches deep into our native grounds. We have not harnessed our traditional values to serve as inner reinforcements of the edifice of progress we have been attempting to build, such that everytime we add one more story of development, the structures fall apart.

We cannot build castles on sands nor strong walls without reinforcements. Ask the architects and the engineers and they will tell you that the height of the building is proportionate to or is dependent upon the depth of its foundation. The foundations of our past development schemes were not deeply rooted on our native grounds. They were merely laid out on the surface. That is why everytime we add another story of progress, the structures collapse and we are back to square one.

But it is a different phenomenon when it comes to our traditional institutions and values. They have been subjected to all kinds of acculturative pressures and yet they are still with us. They are firmly ingrained in our minds and deeply embedded in our hearts such that even the long years of colonization have not seriously altered them. The visible changes we see are merely at the veneer, propped by fad and fashions of the time. But underneath this veneer, our core values and institutions are intact – waiting for us to surface in our consciousness and to harness for nation building.

THE CHALLENGE

It is the harnessing of these core values and institutions for nation-building which is the real challenge of our times. We find it difficult to accept these values because we have been looking at them from negative points of views. These views do not give us better appreciation of Filipino behavior in its proper framework, context and meaning.

For example, many critics see Filipino conformity to traditional values as passivity, subservience and lack of initiative (i.e, "we are even patient with long hours of brownouts and government inaction"). These critics also consider the high premium we place on reciprocity as "scheming", concern for consensus as "lack of leadership", silence borne out of deference or sensitivity to feelings of others as "concealed dishonesty", firmness and discipline as "authoritarianism", kinship loyalty as "nepotism" and so on.

These critics also hold the view that Filipino norms – like *hiya* (politeness, shame), *pakikisama* (cooperation), *utang na loob* (debt of gratitude), *bahala na* (responsibility) and others – are primary sources of "ills in our society and weakness in our national character."

There are many other examples wherein critics do not seem to see anything positive in Filipino traditional values and institutions, especially those values which highlight the basic and unique Filipino ways of thinking, believing, feeling and acting.

But suffice it to say, at the outset, that these "critical views" about and definitions of Filipino traditional values, have not been helpful in broadening our knowledge of our society and culture. Neither have these views been helpful in nation-building.

Although intended to enlighten us about ourselves, as Filipinos, these views have succeeded only in confusing us, in producing basic incongruences within our social system, in transforming our otherwise positive values into negative psychology that denies us the moral will to realize our full potentials as individuals and as members of the national community.

These incongruences must be resolved. This negative psychology must be corrected. They have confused us for so long now. They have also held us back from harnessing efficiently our value-driven potentials for excellence.

To rectify these errors in social transformation, the following initial steps are suggested:

First, let us demystify the superiority of Western models and restore our lost confidence in our indigenous paradigms. Unless we do this we will never realize our potentials as a people, individually or collectively.

Second, let us look at our traditional values more positively than we do now. There is nothing wrong with them, in spite of what critics say. These values are what hold us together as a people. They are sources of our inner strengths, ethical principles, moral judgments and cultural ideals. It is only our way of looking at these values which is wrong; and, this view shaped by past colonial biases, has influenced us to use them wrongly.

Third, let us examine the functions of our values more in terms of the logic and moral authority of our tradition and less in terms of the logic and legal authority of our borrowed models. Our traditional values still form the bases of our collective sentiments and world view as a people.

Fourth, let us focus our academic and civic endeavors at discovering the inner strength of our culture instead of continuously looking for its weakness. This is not saying that the traditional system has no weakness. Admittedly, it has. But if we persistently load our consciousness with ideas of weakness, we will never realize our real strength. Moreover, self-criticisms in the past have never been helpful.

And **fifth,** let us incorporate the positive features of our values in our textbooks and teaching strategies in schools, in managing our public and private organizations, in advertising our products, in writing stories and in producing programs for radio, television and the cinema.

It is only in proceeding on these steps that we can harness our traditional values and institutions to support our efforts at social transformation. Let us take some examples.

Let us focus our attention on familism. Critics say it is one of the sources of "corruption in society" and of "the weakness in Filipino character." These critics have their reasons for associating familism with these "ills in society."

But if we look at familism in terms of its positive function, such association is wrongly placed. Familism is deeply ingrained in Filipino minds and hearts. It is central to Filipino world view. Thus, if familism has to prevail as a principle of transformation, it has to be used as a tool of teamwork in modern organizations; it must not be used to favor family members. Doing so is a violation of the original meaning of familism which is to protect the honor of the family. Doing so is not the right way to use the concept of familism; it is not only unethical, it is also immoral. It violates the principle of "delicadeza."

Rather, familism must be used, at the personal level, in working hard to bring honor to the family by not engaging in activities (like taking advantage of one's position in the company or bureaucracy) that will embarass or shame the family. At the organization, it must be used as a principle of mutual protection -- i.e., attending to the welfare of the workers and working for the interest of the company.

In this way, familism serves as a means of social control against nepotism rather than its "originator", "facilitator" and "protector". It is in this way, too, that we can restore to the concept of familism the respectability it deserves.

Other norms may be redefined and harnessed as tools for restoring our confidence in ourselves, thereby strengthening our sense of identity in, pride of and commitment to our national ideals.

Pakikisama can be used as a tool for public relations, teamwork and cooperation. There is no teamwork without *pakikisama.*

Hiya can be harnessed as an instrument of motivation, discipline and quality performance. It is *nakakahiya kapag hindi natin nagampanang mahusay ang ating tungkulin* (It is embarassing if we do not do quality work.)

Bahala na can be used as moral force of calculated risk and our *amor propio* as the inner strength to win, as demonstrated by our athletes in the 1991 and 1992 Southeast Asian Games. They were disadvantaged in all fronts but they brought home gold and honor to the country. As the Philippine Daily Inquirer wrote in its Dec. 5, 1992 editorial:

> *We will never know what fires lit in the heart of the Filipino that drove him to run as though the wind lashed at his back. Maybe Rizal was right. Maybe* ***amor propio*** *did wonders to the Filipino. Maybe what drove him to rebel against the most iniquitous rule was the same thing that drove him to rebel against the most inequitable odds. For suddenly, in our glorious moment, the Filipinos were one again, pouring into the game sites and cheering their countrymen on.*

Suddenly, in our glorious moment, the Filipinos were proud again, flying their flags, hurdling their hurdles, making their wild dash to victory.

Indeed, we will never know in detail what fires were lit in the hearts of our athletes. But we agree with the editorial writer that *amor propio,* often brazenly condemned as negative norm, was what "did wonders to the Filipino." The *hiya,* as pointed out, can also be turned into strong motive force which enabled them (i.e., the athletes) to achieve peak performance. *Dapat hindi tayo mapahiya* (We ought not to be embarassed or shamed.) was among the challenges the coaches gave the athletes before the games began. The athletes understood the exhortation, accepted the challenge, felt the need to excel and performed well.

Even in government offices where our traditional values are notoriously misused and condemned, there are bureaucrats who can use the same so-called "negative values" to their advantage. As one bureaucrat in an interview said:

By invoking the other person's ***pakikisama*** *and by establishing a feeling of* ***utang ng loob*** *to me and what I stand for. I was able to get my subordinates to cooperate efficiently. These norms can be used to counter the very negative practices which we say they cause. It is a matter of using them to your advantage than condemning and throwing them away.*

BACK TO THE BASICS

In other words, if we are to provide proper direction and hasten the positive transformation of the Filipino, we have to go back to the basics. We have to relearn the positive functions of our values and rediscover their inner strength.

Let us be reminded that the lack of appreciation of the positive strength of our cultural values is what holds us back each time we gain momentum in national development. We are afraid we might not sustain our momentum. We do not trust our sentiments and abilities. Thus, each time we make progress, we tend to check ourselves whether or not we are in the right path we set. We indulge in "too much analysis until we suffer from academic and bureaucratic paralysis" – we cannot move. If ever we succeed to move, it is too late – events have already overtaken us.

In the process, we become frustrated. This frustration, in turn, leads us to become cynical about our abilities to perform well. We have become a nation of cynics whose highest delight is to bash our cultural dignity by downgrading our traditional values and by extolling the virtues of the West. We beat our breast with joy each time we spot errors in our values and doubt whether or not there is merit in looking for positive strengths in our values. Many of us believe that there is none. We have concluded that we are not progressing because our culture is "damaged", our "character is flawed and weak" and we "lack the moral will" to do things right.

Let us be reminded further that it is also this cynicism which prevents us from harnessing our traditional values for development. Thus, we cannot marshal societal support for our programs because there are no values -- sentiments and attitudes -- in them which could evoke in many of us a deeper sense of identity, pride and commitment to national development. Without these basic motive forces, no progress can ever be attained.

CONCLUDING REMARKS

Let us go back to the basics. Let us remember that, as someone has said before, "The people who do not honor their native tradition can never hope to build a nation." Indeed, nation-building begins from the heart -- in sentiments, attitudes and values of the people desiring to build that nation -- before it is rooted as intellect in the mind or expressed as skills in the hand.

SYMPOSIUM I

Symposium Title	:	**Values and Development**
Moderator	:	**Academician Apolinario D. Nazarea**
Rapporteur	:	**Academician Salcedo L. Eduardo**
Speaker	:	**Dr. Felipe Landa Jocano**

SUMMARY

Dr. Jocano's paper dealt with values and development, concentrating on traditional values. These values have been blamed by others as the cause of our inability to develop but he believed otherwise. For Dr. Jocano, there are no negative Filipino values. It is rather our negative views about our values, our unwillingness to view these values positively as well as our continued refusal to harness these values for development which cause the "ills now plaguing" the Filipino society.

There was no singular answer as to why we have not moved forward as we ought to. However, one fundamental flaw in our approaches to social transformation was identified: "We have not founded our approaches deep into our native grounds"; "we have not harnessed our traditional values to serve as inner reinforcements of the edifice of progress we have been attempting to build."

RECOMMENDATIONS

1. Demystify the superiority of Western models and restore lost confidence in indigenous paradigms.
2. Look at traditional values more positively.
3. Examine the functions of these values more in terms of the logic and moral authority of Filipino tradition and less in terms of the logic and legal authority of borrowed models.
4. Focus academic and civic endeavors at discovering the inner strength of our culture instead of continuously looking for its weakness.
5. Incorporate the positive features of Filipino values in all aspects of our activities.

Trans. Nat. Acad. Sci. Technol. 15: 19-28. 1993

TRANSFORMING THE HEALTH SECTOR: ISSUES IN HEALTH CARE FINANCING IN THE PHILIPPINES

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INTRODUCTION

A review of recently available data reveals several disturbing trends regarding several indicators of health status in the Philippines (Herrin et al., 1993). Little progress has been made in reducing infant mortality in the 1980s, and regional differences have persisted. Chronic diseases, such as diseases of the heart, diseases of the vascular system and malignant neoplasm have emerged as major causes of death even while infectious diseases, such as pneumonia, tuberculosis and diarrhea are still the major causes of death. The nutritional status of specific subgroups of the population, notably preschool children and pregnant and lactating mothers, remains poor.

The slow improvement in health status during the past decade can be understood in terms of the interplay of several proximate and underlying socioeconomic determinants. The major proximate determinants (those that directly affect health status) include health care utilization, fertility, nutrient intake and environmental sanitation. The socioeconomic determinants, on the other hand, include household income and level of education. A review of the indicators of these determinants reveals the following. First, health care utilization has remained low despite the expansion of public and private health care services. This is partly evidenced by the very large proportion of reported deaths (60% in 1989) without medical (physician) attendance. Secondly, fertility levels have declined at a slower rate than those of neighboring countries, such as South Korea and Thailand. It is well known that high fertility is associated with high infant and child mortality. The slow decline in fertility is due primarily to low levels of contraceptive use, particularly of the more effective methods of contraception. There was no increase in contraceptive use between 1986 and 1988 when the national family planning program was non-operational. Thirdly, in addition to chronic deficiencies in dietary and micronutrient intake, the prevalence and duration of breastfeeding (an important factor in the health and nutrition of infants) have declined between 1978 and 1983,

especially among younger women, among women with low level of education and among women in the rural areas. There is no information to indicate whether this decline has been arrested in the most recent period. Finally, environmental sanitation conditions are still poor among a large proportion of households as indicated by the lack of a safe water supply and sanitary toilets.

Underlying the slow progress in the proximate determinants are slow economic growth, the persistently high poverty rates in the 1980s and the slow improvements in education. In the 1980s, per capita GNP declined by an average of 1.7 per cent annually, while the poverty rate was still close to 50 per cent in 1988. While the literacy rate of the country's population 10 years old and over was 90 per cent in 1989, the "functional" literacy rate (defined as those who can read and write and compute) was only 73 per cent. It is likely that the functional literacy rate for older people, say those 25 years old and over who currently represent the parents, is even lower. The level of education, particularly of mothers, has been found to be an important factor in the efficient use of health services for infants and children in particular, and in the effective promotion of health for the entire family in general

As suggested in the foregoing discussion, health care utilization is only one of many important factors affecting the health status of the population. With health care utilization as a factor affecting health, there is a popular belief that low levels of health care utilization stem largely from the inadequacy of health services to meet the needs of the growing population. Likewise, expanding health services, especially to the underserved population, would require additional financial resources. Since the government is faced with increasing budgetary constraints, it is argued that alternative ways of financing this expansion of health services must be found. Common in discussions on health care financing is a tendency to over-emphasize the need to generate additional financial resources by designing new schemes to the neglect of an equally important and related concern -- how to make the health sector more efficient and equitable. It can be argued that additional resources can be generated within the health care system itself without introducing new financing schemes by simply making the system perform more efficiently.

In another vein, it is increasingly being recognized that the economic performance of the health sector in terms of efficiency and equity is intimately linked to the way the system is financed. The existing financing schemes may be in fact the source of some of the observed inefficiencies and inequities of the system. Thus, it is possible to introduce new financing schemes and design them in ways that will promote greater efficiency and equity of the health system in addition to generating resources for health. Viewed in this light, discussions on new financing schemes cannot be separated from discussions of resource allocation in general.

The purpose of this paper is to outline basic issues in health care financing from the larger perspective of transforming the health sector toward greater efficiency and equity in resource allocation. This is done in the next section. The paper then describes what we know about health care financing in the Philippines, suggesting that we know so little with respect to both sources and uses of health care financing,

as well as the impact of specific health financing schemes on the efficiency and equity of health sector performance. The paper ends with a brief discussion on possible reforms that could be undertaken both within and beyond the health sector, and on where such reforms will likely lead us in future. The general areas of scientific research needed to support policy reforms with respect to health care financing from a broad resource allocation perspective are noted as they arise during the discussion of issues.

II. BASIC ISSUES IN HEALTH CARE FINANCING

Health care financing is not just a question of raising funds to finance the expansion of health services or of recovering the cost of existing services; nor is it simply containing or reducing costs. Since health care financing affects the efficiency and equity of health sector performance, it is necessary to view health care financing issues within a broader resource allocation perspective. Viewed from this perspective, health care financing involves the following basic issues or questions:

1. What health services are to be financed? This question, in turn, includes the following questions: what services are to be produced (output-mix); how should such services be produced (input-mix); and who should use such services and how are such services to be used (utilization)?
2. Who should finance what health care services? This question involves issues regarding the proper role of the public and private sectors in financing.
3. How should health care services be financed? This question involves consideration of alternative private and public modes of financing health care: first, narrowly, with respect to the criteria of financial efficiency and financial equity; and second, broadly, with respect to their impact on the economic performance of the health care sector.
4. How much financing should the health sector receive relative to the other sectors of the economy (intersectoral resource allocation)?

Below we describe some basic principles that could guide our attempt to answer these questions.

What Health Services are to be Financed?

It is obvious that one would want to finance only those health services that are most effective in generating health impact from a given level of resources; those that are produced at least cost; and finally, only those that will actually be used by the population with health needs to produce the expected health impact.

These considerations lead us to issues regarding service structure (output mix), the production of specific services (input mix) and the utilization patterns of health care facilities and services.

Health Service Structure. The health sector can produce various kinds of services that will improve health status. Broadly, these services would include community health services and personal health services. Community health services include such services as public information and education, health surveillance, environmental health services and research and training. Personal health services, on the other hand, include both outpatient and inpatient care. Given the resources available for the health sector, how much should be allocated to each of these different services?

For a given level of resources available, greater efficiency in resource allocation can be achieved by producing that combination of services that is the most effective in improving health status. We shall call this type of efficiency the ***service structure efficiency.*** This implies, in part, choosing the combination of health services of given quantity and quality that adequately addresses the most important health problems/diseases. Choices once made are difficult to reverse once the health service infrastructure is set in place. It is, therefore, important to inform such choices with the findings of scientific research on the persisting and evolving patterns of disease as well as on the demonstrated relative cost-effectiveness of alternative health service.

Health Service Production. A particular health service, whether community or personal service, can be produced using different kinds of inputs, e.g., different types of human resources, medical equipment and facilities and drugs. What resource inputs are needed? In what combination should these be employed in the production of health services of given quality? Greater efficiency can be achieved by choosing that combination of health inputs that is the least costly among alternative combinations given the relative prices of various inputs and given the prevailing medical tehcnology. We shall call this type of efficiency ***service production efficiency.***

The effective application of this principle requires not only the consideration of substitution possibilities among different types of inputs that already exist, but also the expansion of the range of substitution possibilities through research and careful experimentation. Moreover, there is a need to review certain established practices in health care provision that, while designed with lofty objectives, such as ensuring high quality of service, unintentionally tend to restrict input substitution, and hence, the achievement of greater efficiency.

Health Service Utilization. A common approach adopted by many governments in response to the perceived unmet health needs of their population is the provision of more facilities and services closer to where people live. The approach, however, has not been found to be entirely successful. Instead, one finds the seemingly incongruous coexistence of unmet needs and inappropriate or excessive use of services, on the one hand, and of underutilized service capacity in

certain health facilities and overcrowding in others, especially at the higher levels of the delivery structure, on the other.

Greater ***service utilization efficiency*** can be achieved if: 1) those with real health needs as medically defined, do seek and get care; 2) those who do get care do not demand or are not provided excessive or unnecessary services as medically defined; and 3) those who do seek care for real health needs seek and are provided with necessary services at the most appropriate health facility in the service delivery structure. In addition, greater ***access equity*** can be achieved by ensuring that health care is obtained by those who need them irrespective of income or geographic location.

It is necessary to understand, through careful research and analysis, both the demand and supply factors influencing health service utilization. This in turn is important to achieve greater service utilization efficiency through: 1) the design of mechanisms or incentive structures which will modify the behavior of consumers and providers with respect to service utilization; and 2) the design of a delivery structure which will optimize the utilization of services and facilities.

Who Should Finance What Health Services?

In some countries, health care expenditures are financed largely by the government mainly through tax revenues, while in others, they are financed largely by the private sector through user fees or health insurance. What should be the respective roles of the private sector and the public sector in health care financing? In particular, which services should best be financed by the public sector and which services should be left to the private sector to finance?

Public sector financing to improve the efficiency of health sector performance is appropriate in such cases where information is imperfect, or when there are externalities in consumption or production or where services are simultaneously enjoyed by all. The presence of externalities in the consumption of certain health care services, e.g., immunization, could lead to underutilization of such services. Efficiency in utilization could improve with public subsidies to consumers of such services. Moreover, there are certain health services or activities, such as health education/information through mass media, epidemic control and vector control where the benefits can be enjoyed by all including those who do not pay for such services. In this case, the private sector is unlikely to provide such services due to the difficulty of collecting payments from the users. To ensure that such services are provided, government financing is necessary either to directly provide such services or to subsidize private producers. Public sector financing can also be justified on equity grounds. Government financing may be needed to improve equity of access to services either by subsidizing people who could not otherwise finance the high cost of services due to low incomes, or by subsidizing private producers to provide services at lower costs that are within the reach of lower income groups.

How Should Health Services be Financed?

Health care services can be financed publicly through various means: direct and indirect taxes or a payroll tax in social insurance schemes. Private financing can also take several alternative forms: user chargers, employer-based financing, private insurance, community financing and capitation schemes. Which of these alternative financing modes should be adopted?

In deciding which of these alternative financing modes (or combinations thereof) are to be adopted, it is necessary to examine each in relation to the various criteria of efficiency and equity, first with respect to the financing scheme itself, and second with respect to its impact on health sector performance. Among health care financing schemes that can generate the same level of financial resources, the more efficient scheme is the one that requires lower administrative cost of generating such financial resources. We shall call this type of efficiency ***financial efficiency.*** Moreover, among alternative schemes, the scheme wherein beneficiaries with higher incomes make progressively higher contributions relative to those with lower incomes would be a more financially equitable scheme than other schemes where the beneficiaries are required either to contribute amounts proportional to their incomes, or worse to contribute equal absolute amounts toward the financing of helath services. We shall call this type of equity ***financial equity.***

In addition to the criteria of financial efficiency and financial equity, the choice of which scheme or combination of schemes to adopt must be based on a careful assessment of its ability to promote efficiency in service structure, service production and service utilization as well as promote equity in access. Among health care financing schemes, the scheme or combination of schemes that promotes greater efficiency and equity in these aspects of health sector performance is to be preferred to those that do not.

How Much Financing is Needed?

The question policymakers frequently ask and in which they show great impatience when a definite figure cannot be readily provided is: how much should the country spend for health services?

A basic principle based on economic theory can be given: greater efficiency in resource allocation can be achieved by allocating relatively more of the additional resources available to those sectors that offer larger contribution to social welfare and relatively less of the additional resources to those sectors that offer smaller contributions to social welfare. We shall call this type of efficiency ***intersectoral resource allocation efficiency.*** This is perhaps the most abstract of all performance indicators. Unfortunately, there is no practical procedure that can be routinely applied to measure this performance indicator (de Ferranti, 1983). The problem lies in the difficulty of measuring social welfare of which health is but one of the components. This problem persists even when the concept of social benefit is

narrowly defined in terms of health status improvements. This is due largely to the difficulty of adequately measuring health status on the one hand, and the relative contributions to health status improvements of various health-promoting sectoral activities, on the other.

Several approaches have been used in the past to deal with this question. These include inter-country comparisons of total health care spending as a percentage of GNP, and intra-country comparisons of historical trends in health care expenditures vis-a-vis other expenditures, either in terms of percentage of GNP or rates of growth, in conjunction with other information, such as the trends in mortality and morbidity, changes in disease patterns, access to health care delivery services, changes in population composition and others. Although none of these approaches is entirely satisfactory, each may provide some basis for making qualitative judgments as to whether or not, at the margin, more spending on health services is likely to promote greater social welfare than if the same resources were spent elsewhere.

III. HEALTH CARE FINANCING SYSTEM IN THE PHILIPPINES

The Current System

The Philippine health care financing system consists of a number of financing sources. These include: 1) central government-financed services; 2) local government-financed services; 3) social insurance (Medicare); 4) private health insurance including Health Maintenance Organizations (HMOs); 5) employer-financed health services; 6) community-based health financing schemes including cooperatives; and 7) direct household spending. At present, our information regarding both the amounts financed from each of these sources and the uses of financing is quite limited. We also know little about the impact of each financing scheme on consumer and provider behavior which eventually influences efficiency and equity of health sector performance according to the criteria described earlier.

Recent attempts have been made at estimating total health care expenditures and sources of financing (Intercare, 1987; Solon, et al. 1992; Herrin et al., 1993). Preliminary estimates show that total health care expenditures for 1985 and 1991 were less than 2 per cent of GNP (Table 1). The estimates are obviously biased downwards because they do not include expenditures from other sources for which no information is available, i.e., local government, employer-financed services and community financing. The expenditures from these other sources are probably small compared to those for which we had some information, hence a rough estimation of around 2 per cent of GNP might be a reasonable first approximation of total health expenditures in the Philippines. Real GNP per capita increased between 1985 and 1991, hence total spending per capita for health was also increasing in real terms during this period, But real GNP per capita in 1985 and 1991 was lower than it was in 1981. Hence, if health expenditures in 1981 were also around 2 per cent of GNP, then real per capita spending has declined during the 1985-1991 period.

About half of total health sector expenditures are financed by households in terms of direct payments to providers. While this implies, on the one hand, that households can and do pay for health services, it also means that those without money incomes to pay for services can not easily get access to such services (inequity in access). Moreover, among the very poor, paying for health services would likely involve shifting present or future resources away from alternative uses which could have both short-run and long-run adverse consequences, e.g., borrowing money to pay for health care now could mean less consumption in the future or less investment in other forms of human capital, such as the education of children.

About 40 per cent of total health sector expenditures were from the government, financed largely from taxes. In view of the regressiveness of the current tax structure, the burden of financing public sector expenditures falls heavier upon lower income groups than the highest income groups (financially inequitable).

Social insurance thorugh Medicare is still a minor source of health sector financing, constituting 8 per cent of total health sector financing in spite of its having been in operation for 20 years. A major factor in the slow growth of social insurance is the slow structural transformation of the economy which makes it extremely costly (financially inefficient) to expand Medicare coverage to the population outside of the formal employment sector.

Possibilities for Expanding Resources for Health Care

There are several possibilities for expanding resources for health care while achieving greater efficiency and equity in the health sector. The first set of possibilities lies beyond the health care sector. The first is tax reform and administration: getting more taxes from higher-income groups who are currently undertaxed to make the burden of taxation more equitable and at the same time raise more tax revenues to finance government activities, particularly health. This improves financial equity while generating additional resources. The second is economic reforms to speed up growth and development that takes into account the large labor force and agricultural potentials of the country. With faster structural transformation, the expansion of (the reformed) Medicare Program will be facilitated. With increased incomes and equitable distribution, demand for health services provided by the private sector will increase, thus facilitating the growth of the private sector particularly in the hospital industry. The growth of the private sector, in turn, will relieve government from providing hospital services in urban areas, thus facilitating the reallocation of government resources to rural health services.

The second set of possibilities lies within the health sector. The most important of this is the reforms in the Medicare Program: restructuring contributions and benefits, better fund management (to manage it like a health fund rather than a social security fund), strict enforcement of enrolment of employees to the program, expansion of memberships outside of the formal employment sector through the

use of cooperatives (e.g., rural electric cooperatives) or other large organizations and the reduction of fraudulent claims. These reforms would make the Medicare Program more financially efficient and equitable and better able to provide additional benefits and to expand coverage in tandem with structural transformation. Also, it would be prudent to proceed with caution with respect to user charges until we have more information on their impact on utilization, and on community-based financing schemes. That is, unless we are sure that government intervention can do a better job than current community initiatives.

With these reforms, we might see in the future a national financing system with the following structure: 1) government funds (about 20 per cent of total financing) will be used mainly for public good, for preventive care and for the hospital care of the poor in rural and urban communities; 2) social insurance will constitute the main source of financing (about 60 per cent of total financing), mainly for hospital services (Cost containment measures, however, will have to be developed to reduce the excessive use of health care services which could arise from the scheme.); 3) user fees in the form of co-payments and direct payments for inexpensive outpatient care and drugs will constitute about 15 per cent of total financing; and 4) other sources of funds (about 5 per cent of total financing) will serve to supplement the three main sources above especially in emergency cases during calamities.

IV. SUMMARY AND CONCLUSION

The basic issues in health care financing viewed from the larger perspective of resource allocation invariably include such questions as: what health services are to be financed, who should finance what health care services, how should health care services be financed and how much financing is needed? The ability to address these questions in the Philippine context is greatly hampered by the lack of basic information on the health care sector, ranging from the basic information on health outcomes to the structure of the delivery system particularly the private sector component. There is also a dearth of information on the production and cost structures of major service providers, the utilization of health care services and sources and uses of health care financing.

The generation of such basic information is urgently needed to address the above questions, and more urgently so to address specific policy questions that are now being deliberated upon by health policymakers, health administrators and legislators. These specific policy questions are: a) how well did the current social insurance program for government and private employees perform in providing health benefits to its members and what reforms are needed to improve performance; b) with an increasingly overburdened government budget, can the cost of publicly provided health services be recovered and by what means; and finally c) given that a large segment of the population is still not covered by any type or risk coverage or health insurance, how can the benefits of the existing social insurance scheme be expanded to cover the rest of the population or what is the most appropriate way to develop a national health insurance program?

Table 1. Estimated total health care expenditures by source of financing: 1985 and 1991 (in current prices)

Source	1985		1991	
	Billion Pesos	*Per cent of Total*	*Billion Pesos*	*Per cent of Total*
Government	3.78	38.4	7.42	36.3
Households	5.36	54.5	10.94	53.6
Medicare	0.55	5.6	1.73	8.5
Private insurance	0.15	1.5	0.33	1.6
Total	9.83	100.0	20.09	100.0
Percent of GNP	1.64		1.63	

Source: Department of Budget and Management, Family Income and Expenditure Surveys of 1985 and 1991, Philippine Medicare Commission and Gamboa, R.M., 1991, *Background Paper on Health Insurance in the Philippines*, Report prepared for the United States Agency for International Development as reported in Herrin, A.N., A.D. Kraft, O.F. Picazo, O.C. Solon, M.M. Taguiwalo and M.S. Zingapan, *Health Sector Review: Philippines*, Health Finance Development Project Monograph No. 3, Department of Health, Republic of the Philippines and United States Agency for International Development.

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SYMPOSIUM II

Symposium	:	**Issues in Health Care Financing in the Philippines**
Moderator	:	**National Scientist Jose Encarnacion Jr.**
Rapporteur	:	**Dr. Anna Miren Gonzales-Intal vice Fr. Bienvenido F. Nebres**
Speaker	:	**Dr. Alejandro N. Herrin**

SUMMARY/HIGHLIGHTS OF DISCUSSION

Health, being a produced commodity, involves the use of resources. Hence, the issue is resource allocation. In this regard, health care financing is not just a matter of raising funds but that the economic performance of the health sector in terms of efficiency and equity is intimately linked to the way the system is financed.

There are four basic issues in health care financing from the larger perspective of transforming the health sector toward greater efficiency and equity in resource allocation. The research challenge is that we know so little about the sources and uses of health care financing. These basic issues are:

1. What health services are to be financed? What appropriate mix of primary and tertiary health care services should be provided by the government or the private sector to various clientele groups?
2. Who should finance such sources? What is the efficient and equitable mix of public and private sector financing of the appropriate mix of health care services?
3. How should health care services be financed either by the government or by the private sector in terms of financial efficiency and equity?
4. How much financing should the health sector receive relative to the rest of the economy?

Dr. Herrin described some basic principles in addressing the above questions. With respect to what health services are to be financed, the objective is to finance only those health services that are most effective in generating health impact from a given level of resources; those that are produced at least cost; and those that will actually be used by the population with health needs to produce the expected health impact.

On the issue of how health services should be financed, the choice of which mode of government and private sector financing depends on: a) which provides the most financial resources at the least administrative cost; and b) which forces encourage the rich health care beneficiaries to make progressively higher contributions than the poor health care beneficiaries.

On the question of how much financing is needed, Dr. Herrin points out that this is the most difficult to answer because there are no clear-cut methodologies to use. Also, it is difficult to measure the impact of improvements in health status relative to other social investments.

With respect to the health care financing system in the Philippines, preliminary estimates showed that health care expenditure by the government and households was less than 2 per cent of national income in 1991. About one-half of total health expenditures is paid for by households directly to health care providers; about 40 per cent is provided by government through taxes which is regressive; and of the rest, about 8 per cent is provided by social insurance through MEDICARE.

There are several possibilities for expanding resources for health care. The first set of options involves reforms outside the health care sector, specifically, improved taxation and tax administration and economic reforms to speed up economic growth and more rapid structural change. The second set of options involves reforms within the health sector. These are reforms in the MEDICARE program and the need to study user charges and community-based financing schemes.

Several issues, were raised during the open forum: 1) the tentativeness of Dr. Herrin's data due to lack of systematic data on health care financing in the Philippines; 2) the efficiency of Philippine health expenditures for every peso spent and how this compared with those of other countries; 3) the emphasis on preventive health services thereby reducing the cost of health care; 4) how the primary health care program is to be financed; and 5) the wisdom of the devolution of health, education and social services to local governments whose personnel may not be very knowledgeable in these areas. Preventive and health promoting activities affecting the whole nation need to be centralized while unique health issues and problems might be better localized or decentralized.

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FILIPINO VALUES IN PHILIPPINE DEMOGRAPHIC TRANSFORMATION

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INTRODUCTION

Most people think demography is just mathematics in disguise – a form of dry social accounting that tallies numbers of people in a given place and time from national censuses and surveys. However, once exposed to the subject, many change their minds and come to appreciate the profound impact demographic forces have on societies. Many do so only after recognition that it is likewise fascinating because it deals with many personal elements of a woman's and a man's life. For the same reason, it is a discipline both controversial and misunderstood.

It is controversial because in essence, it deals with the most intimate elements in one's life: sexuality, reproduction, geographic mobility, life span, mortality among others. All these are highly emotional events imbued with joy, pleasure, pain and anxiety.

It is often misunderstood because it is at one and the same time a discipline, an interdiscipline and a subdiscipline. It is clearly a discipline because it has its own body of concepts, techniques, journals and professionals associations. On the other hand, it is also very much an interdisciplinary field because it draws its subject matter and methods from many disciplines including sociology, economics, biology, geography, history and the health sciences. And still, it is considered a subdiscipline within some of the major disciplines. In most universities, demography programs are housed within the sociology department, perhaps because population phenomena have been linked to social causes and consequences for so long now.

Wherever the place of demography in the social sciences the fact is there are demographic events that raise significant questions requiring careful examination. Indeed, if people are not interested in these demographic questions, they are not interested in themselves. The urgency of dealing with the country's demographic phenomena has never been more true than during the past half-century, a period of unprecedented social and demographic change.

In line with the theme of this annual meeting, allow me to comb through some major issues and processes in social transformation as they relate to what I prefer to call social demography. At this instance, I am convinced there are at least two pertinent issues in the field of population studies which have significant implications for social transformation. The first is the Filipino woman's status as it relates to reproduction and woman's reproductive health. The second is adolescent sexuality as it pertains to the youth's overall development.

Of all the things women are supposed to be, being a mother comes first. Here, the connection with nature is indubitable: only women can become mothers. As such, women's reproductivity is as powerful a cultural myth as their perceived non-productivity. Becoming and being a mother is held out as the primary feminine goal in the 1990s, as it was in the 1920s or in the 1850s. This brings us to concerns about women's status, reproduction and maternal health.

Maternal mortality has recently become another indicator of disadvantage within and between the developing and developed countries. Over 99 per cent of the estimated 500,000 maternal deaths that occur annually take place in developing countries, with ratios ranging from 760 per 100,000 live births in West Africa to 120 in East Asia (WHO, 1988). By definition, a maternal death is defined as the death of a woman while pregnant or within 42 days of a termination of pregnancy, from any cause related to or aggravated by the pregnancy or its management but not from accidental or incidental causes (WHO, 1977). What is unfortunate is that most maternal deaths could have been prevented if only these women had access to reproductive health information and services.

Vigorous efforts to advance women's social and economic position could probably guarantee for them the right to reproductive health. Even when the commitment to women's overall equality seems firmly entrenched, at least in the language of women and development plans, the question of control over reproduction remains a highly explosive issue.

Questions are many. So are the arguments. The more important questions are embodied in the central problem of reconciling individual human rights with government actions to influence population trends. More crucial is the question of who, between husband and wife, is to determine, freely and responsibly, the number and spacing of children. Here, the Filipina is caught in a tension between local customs and norms and individual preferences. One has to recognize the fact that once reproductive health is understood to involve more than just the physiological workings of a woman's womb, men have the power to shape the world in which women live.

When all the rhetoric is done with, the true key to improving women's reproductive health is AUTONOMY. This means enabling women to assume control over their reproductive lives by entrusting to them the authority to decide when to have a child and how many to have. Women should not merely subscribe to men's desires and dictates. Perhaps, the commitment to women's autonomy – giving them ability to decide about reproduction based on access to adequate information and

appropriate services and on emancipation from a husband-oriented decision-making process – is the value that can bridge cultural divides and ideological borders. In a society where the norm is that women are subject to men who are perceived as the AUTHORITY FIGURE, most Filipino women in reality have no opportunity for real individual choice.

Given the normative structures surrounding decisions about reproduction that promote over-dependence, if not a false sense of resignation, to authority figures such as the PADRE DE FAMILIA, a commitment to woman's autonomy can greatly increase control over their lives and can inspire self-discovery. This is an essential ingredient if women are to contribute to socially desirable development directions and goals such as improved maternal and child health. Ultimately, the problem of too rapid a population growth can be modified by an awakening of Filipino women, sustained by a favorable response and support to a basic value modification by Filipino men.

One cannot talk about women and development in the Philippines if Filipino women are still obsessed with "good" and "attractive" physical appearance to please men as a major health concern (Baltazar, 1992). Neither would it be to the credit of women and development advocates if women are exposed to the risk of HIV infection just because of high levels of non-monogamous sexual contact and resistance by Filipino males to use protective devices for safe sex. This situation is even worse among commercial sex workers who are "unable to bargain for safe sex with their partners and allow the customers to determine how the services will be rendered" (Dominguez et al., 1992). For as long as women view the locus of control over their lives as residing outside of themselves, low self-esteem could impair the much needed awakening of women necessary for improved maternal and child health. Consequently, reduced probabilities of infant and child deaths may not be forthcoming. Then it becomes rational to have a large family size to ensure replacement of deceased children. How then can our demographic transformation from a regime of high fertility to a regime of low fertility proceed?

Adolescent Sexuality and Development of the Youth

That lifelong values and habits are developed during the formative years of adolescence is incontestable. As such, the adolescent period in one's life is distinct from other life cycle stages and leaves much of its character in later years.

Adolescence is said to be the process through which an individual makes the transition from childhood to adulthood. Some demographic changes in the country -- like increased rural to urban migration coupled with sweeping social changes brought about by modernization such as longer stay in school – have resulted in increased incidence of early sexual activity among the adolescents aged 10 – 24 years. While the transition into sexual activity is a natural transition made by nearly all humans, it is the timing and the circumstances surrounding the transition which have significant implications for the welfare of the young adolescents.

Childbearing by women below 20, i.e., adolescent fertility, is not a new phenomenon in world history. What is new in the past decade or so is the growing recognition of, and concern over, the adverse health, social, economic and demographic effects of adolescent fertility. Given the current stock of adolescent population estimated to be at least a third of the total population in 1990 and a relatively high fertility rate among women in the reproductive ages, adolescent fertility needs to be addressed to cushion its impact on population growth. Even if fertility rates drop, the sheer numbers of adolescent women mean a continued impact on population growth. Cushioning the demographic as well as social and economic impacts of adolescent fertility entails that adolescents, boys and girls, are ensured adequate education, including family life and sex education, to instill in them positive and healthy attitudes toward sexuality.

Today's youth encounter social, technological and demographic changes that have in recent years weakened family and social structures required for the development of positive attitudes toward sexuality. Such attitudes are necessary where the objective is to reduce early pregnancies. There seems to be a dual character in the average Filipino male who demands a virgin for a wife while wanting to "deflower" a girl when given the chance. In a survey of adolescents aged 10-24 in 1982, data showed early ages at first premarital sex, ranging from 13 to 17 years (Raymundo, 1982).

It used to be that boys were warned by parents against taking advantage of girls because they might be drawn into an unwanted marriage. Girls on the other hand, were often likened to plates or mirrors, which once "broken", cannot be put together again. But in a fast changing society, the Filipino parent-child dyad has been loosened by many existing alternative non-kin social allies in an open community. Of significance to the adolescent is the peer group or the "barkada" which could effectively erode the "close family ties." Where the peer group acts as confidant on personal experiences such as first sexual encounter, one can expect inadequate and inaccurate information on sexuality and reproductive behavior and its consequences on the individual and the general society.

Pregnancy and childbirth are likely to interrupt if not totally disrupt educational and career opportunities. Whatever the case, adolescent fertility spells some form of socioeconomic cutbacks in the future. Early pregnancies likewise have been implicated in a host of other social problems ranging from increasing school drop-out rates, rising numbers of households headed by single women, persistent poverty and one that is transmitted across generations.

The prominence of unwanted pregnancies as leading to abortion certainly merits attention. The struggle between conformity to cultural traditions of refraining from sexual activity outside marriage and escaping the strong social stigma of being an unwed mother and exposure to health risks is indeed reflected by Abad and Sandoval (1990). Perhaps unknown to the Filipino public, abortion ranks as the third highest among the 10 leading causes of hospital admissions in the Philippines: about 8.2 per cent of abortion cases are admitted in government hospitals and that

one out of every four (24 per cent) maternal deaths in the Philippines is attributable to induced abortion.

With an eye to the future, let us assess what values need to be strengthened among adolescents, in battling the ill consequences of early and unplanned adolescent pregnancies. This could forestall what could be a generation of a "reproductive underclass" comprised of disadvantaged teen mothers.

We have a deeply ingrained attitude reflecting basically a risk-taking behavior which at its negative aspect is a kind of blind faith in fate. To my mind, this *bahala na* attitude is an escape from decision-making and planning for the future. Some sociologists have alluded to it as the principal factor for the absence or lack of social responsibility and a paralysis of collective action for the common good. This, in fact, could be the reason for the apparent inability of the Filipino to transcend a certain brand of preoccupation with the self. Thus we almost always feel the powerful presence of a *kanya-kanya* mentality.

Nothing is more appropriate than the *bahala na* attitude which holds two population subgroups – i.e., the women of reproductive ages and the young adolescents – together at a demographic crossroad: planned or fate-driven pregnancies? Who could be more predisposed to this risk-taking behavior than the young adolescent – bombarded by media's effective tools for promoting acceptability of modern values such as sex before marriage? Who could be more resigned to fate about having as many children as God allows, not recognizing that the marital sexual act is not necessarily there for procreation alone than the typical submissive Filipino housewife?

CONCLUSION

No social transformation is without a parallel demographic revolution in fertility norms. Once again we are confronted with the difficulty of slowing population growth quickly under the present trying circumstances. Attempting to decelerate population growth when living standards are deteriorating is one of the most politically complex undertakings our government must face. What we should strive for is a value reformulation. At the heart of this value reformulation is the development of a sense of collectivity above and beyond the self. Amidst such a demographic emergency, one can shift the focus of childbearing decisions. Couples should be made to understand the need to go beyond the narrow confines of self-interest. This means shifting concerns from how many children they would have who could support them in their old age to how the size of their family would affect the world in which their children would live. This should enable us to grapple with a social reality that there are indeed external costs to every individual action. Too much of such costs can endanger the common good and hamper the much needed demographic transformation for the country's overall development.

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SYMPOSIUM III

Symposium Title	:	**Filipino Values in Philippine Demographic Transformation**
Moderator	:	**National Scientist Carmen C. Velasquez**
Rapporteur	:	**Academician Carmen Ll. Intengan**
Speaker	:	**Dr. Aurora E. Perez**

SUMMARY/HIGHLIGHTS OF DISCUSSION

The discussion highlighted the need for more rights of women in their role as mother.

Parenthood is a dual responsibility of both father and mother.

RECOMMENDATIONS

Men should be included in the formulation of population programs.

There is a need to reformulate demographic values especially as they affect the health of mothers.

Trans. Nat. Acad. Sci. Technol. 15: 39-40.1993

CULTURE, ARTS AND NATION

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This paper is divided into three parts: 1) the concept of the Philippines as nation; 2) the importance of the Filipino culture; and 3) the physiognomy of a national culture.

There are three terms that we are talking about here: culture, arts and nation. Perhaps we have to begin with the basic working definition of each of these.

Culture is the totality of a people's way of life produced by their struggle with nature and their interaction with one another and with outsiders. I believe this anthropological definition is the best definition we can get. Culture includes the performing arts (music, dance, theater), the literary arts (poetry, prose), visual arts (metal craft, pottery, painting, sculpture, graphic arts), architecture, the broadcast arts (radio, TV, print media) and film.

Culture is shaped by man's surroundings, specifically by the struggle with nature. An example is the very interesting art form that has developed because of the kind of geography that we have: the church structure. One foreign scholar called this form of church architecture earthquake baroque. Where European churches would be long and high, most Philippine churches would be long and squat. Why? Because we have earthquakes. And churches were built long before we had reinforced concrete and steel. These churches were built using coral rocks, sometimes adobe, built one on top of another.

Churches had to be built in such a way that their staying power was insured during earthquakes. They had walls that were thicker than usual -- about one-and-a-half to two meters, sometimes three to four meters thick. They were also not very high and they had buttresses also made of stone. Lastly, these churches had very small and very few windows. Why is this so? Because a window is a breach in the wall. The more windows you have, the more chances of the walls collapsing. Filipinos have, over the centuries, devised a system of decoration that fools the eye. Through paintings all around the walls, for example, a low and squat church could look deep and wide.

So our churches, although some people call them Spanish churches, are not Spanish churches. They are Philippine churches. The concept of the church has been adapted to Filipino conditions first, because of the earthquake phenomenon and second, because of our motiff and our own decoration.

What about interaction with outsiders? When you have groups or tribes who need to protect themselves, they develop cultural forms that strengthen the tribes. One good example would be the epic. The epic, in times past, functioned as a way of rallying the people around one epic hero, like Lam-ang who represented a particular tribe. The epic's social function therefore was to set up a hero for a tribe who would be the epitome of all the important values that those tribe people should have. And it is a rallying point for them, their identity. What does this culture therefore include? It includes all the systems -- economic, political, religious, social and artistic – which propagate the beliefs and values which may reaffirm, modify or change this very system.

The second concept is the arts. Arts include the works which express the best thoughts and emotions of a people and which are marked by integrity and intelligence. Art could be music, dance, theater, beautiful textiles, carvings, architecture, film, radio, television, comics like Kenkoy. Art form should include all forms, whether in the ethnic, in the Hispanic or in the American tradition that the Filipinos now use to express their own ideas and emotions. Good art is characterized by excellence and integrity.

Nation is composed of the people of a territory, united under a single government. It is a state, a stable, historically developed community of people, with a territory, economic life, distinctive culture and language in common. The Philippines is a nation because of the Filipinos' commonality of experience in history and in contemporary society. We all know Ferdinand and Imelda, Ninoy and Cory; we all know EDSA, the AFP, the NPA, *coup d'etat,* the vigilantes.

SYMPOSIUM IV

Symposium Title : **Culture, Arts and Nation**
Moderator : **Academician Jose R. Velasco**
Rapporteur : **Academician Magdalena C. Cantoria**
Speaker : **Dr. Nicanor G. Tiongson**

SUMMARY/HIGHLIGHTS OF DISCUSSION

1. The terms culture, arts and nation were defined.
2. The Philippines is a state, but because it has no distinctive culture, it has not yet attained the status of a nation as defined. The country has to develop a national identity in politics, economy, education and media.
3. There is a need for a Filipino national culture which is the soul and strength of a nation. This has to be consciously developed. The identified problems are: 1) cultural fragmentation; 2) impairment of racial memory; 3) culture still inchoate; and 4) the perception that "the Filipino is negotiable."

RECOMMENDATIONS

1. Cultural engineering can be done by: 1) identifying and emphasizing those aspects of the Filipino way of life which should be saved and strengthened; and 2) supporting and encouraging patronage of the arts. Filipino artists should shape and express the national soul by their art.
2. Some suggestions to develop a national culture are: 1) create a national awareness of this vision; 2) disseminate information about the various ethnic groups to promote knowledge and understanding of these communities; 3) recognize the important artists and the character of the works of each; and 4) offer incentives to artists in the form of awards and grants.
3. Efforts to develop a national culture should be divided among persons in different fields in government and in the private sector. All these should start at the same time.

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Technol. 15: 43-52.1993

DISCIPLINE, CULTURE AND INFORMATION SYSTEMS COMPUTERIZATION AND BEHAVIORAL TECHNOLOGY IN GOVERNMENT AND SOCIETY

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ABSTRACT

The broad-spectrum complexity of our national crisis is regarded as characteristic of the rise and growth of population and urban centers. The existing structures and tools of government management do not constitute an adequate means for handling the intricacies of administration and the volume of information quickly and analytically across distance and time, belonging as they do to an era of small organizations and traditional communities. The new information systems technology is proposed for consideration.

A culture of information technology needs a concerted effort in development and therefore will imply an official statement of policy of entry into the Information Age at the highest levels of authority. The reasons for this are conceptualized and examples in the discipline of government and society are alluded to as possibilities that might yield a more adequate information systems technology.

Recommendations are made for establishing the mechanisms for the following 1) a continuing study of the impact of information technology on Philippine culture, all in the service of policy; and 2) supporting and designing the implementation and execution of policies in (1) by the executive and legislative branches of government.

INTRODUCTION

Our Problem

The national crisis seems to have grown to unmanageable proportions: an entire culture is at bay.

We feel the need for some larger understanding of our situation. Is there another way of looking at the awesome diversity and depth of our problems? At graft and corruption in government? At control of crime and law enforcement, malfeasance in the judiciary, at tax collection and smuggling? At poverty? At

environmental degradation and the breakdown in moral standards? Is there a broader conceptualization of the cultural processes in these problems that is both definition of our situation and its solution?

There is a point made by Gunnar Myrdal about transitional, urbanizing societies, "soft states" he calls them, that have not as yet achieved firm institutional control over the softer, intimate relations of an older tradition. Some behavioral scientists call them *loose societies* as distinguished from *tight societies,* where rules, regulations and the law are very much more developed as established norms of conduct. In tight societies, highly-evolved urban complexes and the sanctions on social behavior are more visible, clearer and probably well internalized such that discipline is easier to enforce and maintain.

Why is the Filipino so much more disciplined and productive when working abroad? Is it because the signals, cues and perceptual framework of membership in foreign communities are much clearer in showing him what will happen for what he does or plans to undertake? Maybe the environmental information system, in some broad meaning of the term, is more definitive and relatively compelling in its operation.

There was a time when our societal information system, as well as that of the government, may have been good enough, perhaps about half a century ago. But the world burgeons and moves faster, the mobility and growth of populations accelerate at furious rates, human motivations ramify in all directions, destroying old values and personal boundaries. The resulting loss in social control reveals, in its enormity, problems that challenge our intelligence and our courage.

The Printed Page and the Telephone

One of the most explosive events in the evolution of information systems was the invention of the printing press – the multiplication and distribution of information in quantity. Civilizations were built and developed from books, pamphlets, leaflets, magazines, newspapers, a phenomenon which is described in MacLuhan's **The Gutenberg Galaxy.** Alongside radio and television and the telephone, libraries, mass media, business and industry, bureaucracies and educational and legal systems were spawned and shaped into the institutions that they are today.

The Micro-porcessor and Modern Telecommunications

The advent of the computer and modern telecommunications is creating a new revolution in information systems management that is changing the way people think and do things all over the world. It has become a race to the future. And our country will have become engulfed into the vortex of the inescapable.

Micro-processors have become not only incredibly faster and much more capacious in the systematic collection, storage and retrieval of information in large

quantity but also in the analytical processing of data. The possibilities of improving management and administration of complex situations are almost limitless and are now before us as a new option.

Indeed, there is no problem of choice here because, as noted previously, this is but a question of now or later. Like the printed page, the telephone, radio and television, the computer will soon become a part of our daily lives.

Business and industry here today, by force of necessity, have begun entry into the Information Age and are at the forefront of developments in this field. The computer is not everything there is in management, but it is now more than ever essential for staying competitive. The automatic teller machine of the banking system is a remarkable development in national networking of the computer.

Many government agencies and departments have chosen to computerize and are in various stages of development, all the way from announcements in the newspapers of their intentions, purchases of stand-alone computers, plans and proposals for local or national networking of mini computers and mainframes, or some combination of the foregoing. To name just a few being drawn into the tide of the new revolution in information systems: the Bureau of Internal Revenue, the Bureau of Customs, the Philippine National Bank, the Professional Regulations Commission, the Social Security System, the Government Service Insurance System and the National Census and Statistics Office. The Armed Forces of the Philippines is going to build a national networking information system for quick-response operations and administration. But still there are many government units, some of them revenue collection agencies, that are just limping into the circle, perhaps without any comprehension of their own possibilities with an effective information system.

Schools for computer training are sprouting all over, mostly in centers of population, the major towns and cities. Colleges and universities are now offering computer science degree courses, but the top-rate computer scientists of the country, most of them trained in the best institutions of higher learning abroad, are leaving the country or serving with the multinationals. This seems particularly true of our good programmers or software experts who are now engaged by corporations based in Japan, Switzerland or the United States. Our country seems to have some fair level of readiness to enter into computerization. Still, we are just at the threshold.

The Larger Arena of Information Systems

It is easy to forget that the information system of computer-telecommunication language is but a part of the larger information system that has been developing throughout all of history: from the alphabet and the spoken word, and all sorts of signal systems invented by man in order to communicate and establish forms of social control and governments, to its present stage exemplified by the printed page, radio, television, the telephone and satellite transmission. One scientist considers information as equivalent to energy, energy in the environment that

causes humans to respond. Consider those special arrangements in external world that effectively evoke desirable behavior. Social scientists give special attention to the study of environmental contingencies of human response. These environmental factors are a special area for the psychologist, sociologist, anthropologist, the social engineers, and, oftentimes, without our recognizing it, also the management experts, since indeed the problems we have are in the end problems of management. The poets, philosophers and the humanities at large, as an important part of the knowledge pool, are engaged in the same task in some subtle and deeper sense of indirect management of information; but we will not consider this point for now. Instead we shall try our hand at some of the more recent developments in behavioral science that might have a bearing on our problem.

The Cybernetic System: Quick and Relevant Feedback

The idea of monitoring is a special case of feedback to the observer of information existing in the field of observation. We monitor in order to determine what course of action to take. It is obvious that if the information feedback is fragmentary or too much delayed in time, the information may have become irrelevant for useful action.

But monitoring also has other special effects. Awareness that human behavior is being monitored functions as a corrective or guide: its presence makes for an element of social control, an important feature of the traditional, small communities which have all but disappeared in the maze of urbanizing societies.

Furthermore, feedback is probably the very essence of supervision and management.

More importantly, however, feedback for actions taken has been demonstrated to affect such actions quite dramatically. In our work, we call this effect **immediate reinforcement of behavior.** It does not matter whether the feedback is aversive or gratifying; its impact on behavior is lasting and immediately understandable.

The Unpredictable Schedule

One of the more recent developments in experimental work on behavior, which in principle is capable of generating broad possibilities of application in social control, is that of the variable or unpredictable schedule of sanctions for undesirable or desirable action, whichever is the object of control. Some units of our government have already started utilizing unpredictable schedules, spot checks they are called, during the anti-smoke belching campaign in the streets. The Bureau of Internal Revenue uses the random check for its tax reviews. Done systematically, the unpredictable or variable schedule of contact between the individual and any agency applying the schedule has been demonstrated to exert a very powerful effect on the individual's behavior.

Quite apart from the foregoing observations, the unpredictable schedule allows for an unusual economy in personnel, effort, time and equipment and particularly in the spatial and temporal deployment of encounters between the schedules which can be programmed in diverse ways at will. Since no one knows who will be next in the encounter, the effect on the group would be quite pronounced. The situation now develops a form of discipline where paying attention to the requirements of the law and deciding carefully as to possible consequences becomes important in maintaining clarity of perception and action because the choices are clearer and better defined.

Instructions, Signs and Appropriately Designed Environmental Markers

When instructions are clear and easily understood (in the streets, in buildings, in offices, on paper and posters, in the classroom and wherever), people generally pay more careful attention to and follow what is indicated by the instructions. This is true only, however, if the consequences of following or not following are significant or important to the individual. These consequences are called sanctions, and should always be provided for even if only intermittently. The intermittent sanctions maintain disciplinary control and may be completely benign in character (such as a reminder here and there) or very severe (as in a court indictment that leads to a corresponding penalty). There are levels of sanction in between these two extremes naturally before disciplinary internalization matures into a sense of social order (It should be noted here that the technology of intermittency of sanctions is an area of research that invites serious consideration, since its findings are probably directly applicable to many of our problems.).

To repeat the same points indicated above, there should be a systematic study of our public markers and posters, or more generally, of all instructional signals in the environment, such as those in offices, streets, public places, buildings and so on, the enforcement of which could be monitored at the local level. The environment would be less confusing then because reminders and directives in effect would be all over the place and the public would have some clearer idea of orderly compliance with whatever rules and regulations are expected to be observed. It is necessary in principle to begin with external supports to disciplined behavior in order to approximate and establish gradually the conditions of public shame and individual conscience.

The Externalization of Discipline

Practically all values are formed in the earlier years of the individual through the external social environment and only very gradually are these external elements of control eliminated. By then, we say that the values have been internalized and have acquired a functional autonomy of their own. Whether or not the values of the small community, the family and the kinship system will transfer to and survive

under the new conditions of urban life may be debated and discussed. However, the evidence which is all around us, allows us to conclude that these values have been eroded and have even become a difficulty under the pressure of conditions different from those traditional, soft cultures. This is especially true of discipline as a value.

The Quick-response System; Sharper Contingencies of Management

Many courses of action require information based on what is currently happening in the field operation. All monitoring and supervisory functions consider this kind of information as desirable: not only must the information be adequate and accurate but also contemporaneous. Any delay in its transmission may render the information useless or lessened in value. Contemporaneity of information is one big argument in favor of computerization and telecommunications, but that is not all at this point. Continuity of feedback in time on all actions taken allows a larger panorama of the field of operation and structure of solutions to become more obvious. The way we think out our problems would have undergone a radical alteration because the information technology actually has the potential to alter possibilities of humans responding to situations. In other words, contingencies of management become sharper, clearer as to results, and they indicate where to put human intelligence to optimal use. The current revolution in corporate management, wherein they are re-engineering their institutional structures and information systems to respond directly and quickly to consumer requirements, is one of the most important examples that show the power of a quick-response system due to the new information system technology.

Redundancy in Distributed Information

A noted professor once observed that the ultimate protection against any dictatorial control is the free and widest possible distribution of knowledge and information of every kind to the people. This says a good deal about transparency in social and management processes, for it makes a statement on the preservation of the general social contract which binds society together as a polity, about the fundamental rationale of all governments and organized life. Independent sources of information, or redundancy in the information pool, is one of the most remarkable possibilities that the new information technology may create for us, because in some sense it restores an important feature of social control in traditional societies.

Who Checks the Checkers? Supervising the Supervisors

An important problem in current day Philippine public administration is the question of monitoring the monitors. Another problem with similar characteristics is: how do we enforce the law on the law enforcers themselves? These problems

may partly reduce themselves fundamentally to: 1) the creation of informational structures that automatically make all transactions and processes more visible and accessible to more people on a continuous basis,; and 2) cross-linking the organization or institution with an independent, outside element on a research basis. The possibility of the first suggestion (1) perhaps has a matching tool in the new information system technology, while (2) deserves special attention because of the highly compartmentalized nature of the bureaucracy.

They have confining boundaries that are jealously guarded from being tampered with for reasons of self-interest. Setting up a research arm to study the bureaucracy will be met with vigorous resistance because the subculture they have set up for themselves will be directly threatened. However, more liberal attitudes now are in the air: higher administration is very much interested in coordination and cooperation between departments, with non-government organizations (NGOs) and colleges and universities. While a general statement of government policy about putting an independent research arm within a department or unit may not be necessary, this provision for an effective countercheck or audit should be considered. This problem will certainly be encountered in the course of decentralization of government. The devolution of authority in favor of local governments will meet with similar problems and one way out would be to develop an adequate networked system of information technology before more resistance to innovation sets in. The wide spread in territorial distance will force networking for the Department of Interior and Local Goverrunent (DILG) in the end anyway.

The "Fear of Being Caught" vs. Getting Away With It

In one of the more serious articles published during the recent debates on the proposed bill providing for the death penalty for heinous crimes, evidence was adduced to the effect that the death penalty most likely would be ineffective as a deterrent because of a flawed system of law enforcement and administration of justice. The criminal in effect gets away with it. It was argued that it is the "fear of being caught" that will deter the criminal. It is difficult to disagree with this point of view. But what is this "fear of being caught" essentially?

For all practical purposes, it would be quite difficult to keep track of every violator or potential violator of the law. But sampling out a small subset of these violators and meting out immediately the just penalties make for a public statement that should instill a greater respect for the law. The two necessary and sufficient conditions for striking "fear of being caught" are: 1) the identification and apprehension of actual violators; and 2) the definitive imposition of just penalties on the guilty, preferably made known to the public, even if those caught are few in number. People will learn that an inevitable consequence for the violator would be too costly, or, at least, very highly inconvenient. The conceptualization of "fear of being caught" utilizes generation effects of the actual catch.

Furthermore, conditions of greater transparency created by an adequately programmed information system would considerably reduce the temptation to violations of conduct.

New Perspectives, Change and Resistance

Old habits have an inertia all their own; a change in perspective may be a real danger to lives that have been enculturated into an older information system but which have been outdated by the complexities of modern life. Problems multiply and ramify in never-ending complications, and we solve them the same way people before our own time solved theirs. Computerization and networking certainly will change the way we think and act, even our roles and perhaps our image of ourselves. This is a very difficult subject matter, but it is only fitting to suggest the magnitude of the task before us. As noted previously, resistance may take explicit forms in such excuses as that the new technology would be a very expensive affair and that there are other priorities for our scarce resources. The point is that the development of a new information system for the government is a priority. The world is moving inexorably into the global information system in the first place. But more realistically, it could be a fundamental and basic solution to many of our problems of managing the government seen as an organic part of a cultural phenomenon, problems which will yield answers probably only if the new tools of technology are allowed to express the intelligence and creativity of our people. It is a key solution that has the widest possible effects, is versatile and has the power to telescope space, time and distance. It will definitely change our cultural ways and we should prepare for the prospects of this new reality very carefully.

The theory is that any form of development can be accelerated by entering into the Information Age right away and pulling the industrialization and agricultural phases along with it. The expensive outlay for establishing an information system on a national scale should more than pay for itself eventually in terms of the new discipline in government and productivity and efficiency in administration.

Official Statement of Policy

Within the global framework, the nation bids fair to become part of the new revolution in information technology, of the Information Age. Business and industry in the country have moved into computerization and networking ahead of the others, because they know it is the wave of the future, the only way to survive the global competition. The government is very much slower and has been fragmentary in its response. There are a few bright spots, but they seem to be just reacting to situational problems of the moment without the conceptual equipment of a larger developmental program that should enable them to intellectually prepare for the compelling dialectics of historical forces that are already at the threshold knocking insistently at the door for recognition.

Clearly, the state itself must lead in framing an effective program of development for entry into the Information Age. A vigorous statement of policy declaring that the entire government has been formally launched into Information Technology will place every department, bureau, unit and agency of the government

within a single framework, moving in harmony and coordination with every part of the administration machinery. State universities and colleges, the entire educational system will be tasked with the intellectual preparation of our most precious capital asset – our human resources. It will be necessary to study on a continuing basis the total human impact of our entry into the Information Age, as I am sure there will be unique cultural problems of encounter with this innovation.

Commission on Information Systems Policy

It would be necessary to have an independent Commission on Information Systems Policy under the Office of the President of the Republic. The objectives of this Commission would be to study, research on and formulate policies relating to a balanced scientific and humanistic development of our culture as it enters the Information Age. The Commission shall have administrative working personnel and its members shall receive appropriate emoluments and expense privileges for work rendered. A broad spread of the disciplines shall compose the membership of the Commission, as follows:

anthropology
sociology
psychology
economics
political science
education
linguistics
law
health sciences
2 generalists as members-at-large
mass communication
literary arts
history
performing arts
physics
biology
chemistry
mathematics
philosophy

Joint Executive-Legislative Commission on Information Systems and Technology

For the implementation and execution of policy, this Commission will create enabling legislation for entry and sustained development in information systems technology for the country, particularly in the bureaucracy itself. There will be programs in human resource development, national problems of ethical and security regulation that will require legislation, and above all, budget problems and the search for financial resources. The membership will be on an ex officio basis, suggested as follows:

The President or his/her representative;
Senate Chairman on Science and Technology (S & T);
House of Reprsentatives Chairman on S & T;
Director, National Computer Center;
Director, University of the Philippines Computer Center;
Secretary, National Economic and Development Authority;
Director National Census and Statistics Office;
Commissioner, Bureau of Internal Revenue;
Commissioner, Bureau of Customs;
Secretary, Department of Budget and Management;
Secretary, Department of Interior and Local Government;
Secretary, Department of Transportation and Communication;
Secretary, Department of Science and Technology;
Secretary, Department of Education, Culture and Sports;
Secretary, Department of Health;
Secretary, Department of National Defense;
Secretary, Department of Justice;
Director, Philippine National Police; and
President, University of the Philippines System.

This virtually is the cabinet of the President together with key legislators and some experts in computers and telecommunications.

Planning and design for the entire government could be initiated without too much delay. Since projects of this kind take time even just to begin in some small way, it is essential to start early. Government must have the plan and the political will to develop its financial resources for the purpose: loans, transfers in allocation or priorities, grants, donations, taxation, for short term, medium term and long term parts of the project. There shall be an overall plan that sets standards, provides for development and growth, step by step, but leaves important choices to be made at lower levels, with every unit in the same general direction, integral to each other, coordinated and related but independent and free.

SYMPOSIUM V

Symposium Title :		**"Discipline, Culture and Information Systems Computerization and Behavioral Technology in Government and Society**
Moderator	:	**Academician Bienvenido O. Juliano**
Rapporteur	:	**Academician Tito A. Mijares**
Speaker	:	**National Scientist Alfredo V. Lagmay**

SUMMARY

The paper is an attempt at broadening the cultural processes of problems in "loose societies" where rules, regulations and the law are not as well developed as established norms of conduct as in tight societies." Graft and corruption in government, crime and law enforcement, malfeasance in the judiciary are manifestations of loss of social control and lack of social discipline.

Solutions to these problems might be viewed from the perspective of improving information systems in society and developing an information culture in all aspects of social behavior to instill discipline and respect for the law and established norms of conduct.

The Philippines, in the author's view, has not yet caught up with the Information age in which the world lives today. Like printing, the telephone, radio, television, technological advances in computer and telecommunication systems are becoming a part of our daily lives. In the Philippines, however, we are still in the threshold of these developments. The relatively few who are well trained in information technology are either leaving the country or are serving multinationals.

Information systems and their management touch every aspect of human activity. The psychologists, sociologists, anthropologists, social engineers and all those at the core of technological and scientific investigations – even the poets, philosophers and artists -- are to a certain extent engaged in information management. An application of information in social control is in the "quick and relevant feedback" mechanism it can provide as basis for useful action. In monitoring human behavior as feedback for actions taken, the behavioral scientist calls it immediate reinforcement of behavior. Examples of these include "spot checking" (unpredictable scheduling) in the anti-smoke belching campaign and "random checks" in tax reviews. These exert powerful effects on individual behavior while permitting economy in the use of resources such as personnel, time and effort.

When possible consequences become clear in violations of rules and regulations, discipline is easily engendered. The sampling out of a few criminals and meting out immediate just punishment for their acts will do more to deter would-be criminals and instil more respect for the law than the "fear of being caught" argument such as the death penalty propounded by some legislators.

Disciplined behavior needs external support under the environment of constant and clear reminders (i.e., information) for an orderly compliance of rules and regulations in society. As a means toward formation of good values, discipline needs to be internalized for it to acquire a functional autonomy of its own.

It is also said that the widest possible distribution of information and knowledge (transparency) ultimately protects society from dictatorial rule. Also, unimpeded access to information in government bureaucracies could provide counterchecks on undesirable performance.

Computerization and networking can change the way we think and act, in fact, change our cultural ways. While it may be expensive, the development of a new information system for government should be given priority.

RECOMMENDATION

The author suggests the creation of a: 1) Commission on Information Systems Policy to study, research on and formulate policies relating to a balanced scientific and humanistic development of our culture as it enters the Information Age; b) Joint Executive-Legislative Commission on Information Systems Technology to create enabling legislation for entry and sustained development in information systems technology for the country.

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REGIONAL CULTURE AS PART OF PHILIPPINE NATIONAL CULTURE

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INTRODUCTION

In my way of thinking, regional culture refers to the language, the folk traditions, the literature, the arts and the lifestyle of people living in the various regions of the country.

These regions are mainly defined by geographical boundaries, although, as in the case of Ilocano culture, the boundaries are defined not merely by geographical limits but also equally important by linguistic considerations.

Ilocano regional culture, for instance, was originally limited to the culture of the Ilocos region in the *original* Ilocano provinces of Ilocos Norte, Ilocos Sur and La Union. Lately, however, Ilocano regional culture has since been expanded to include the culture of Ilocano-speaking inhabitants living in the province of Abra, which was originally a Tinguian territory, or to that of the formerly non-Ilocano speaking provinces of Pangasinan, Tarlac, Nueva Ecija, Cagayan, Isabela, Nueva Vizcaya, Benguet, Bontok, Ifugao, Kalinga-Apayao and Zambales – what some Ilocanos proudly call the "Ilocanized" provinces. In fact, Ilocano culture has even been extended beyond the Philippines, in some parts of California, Hawaii and Guam.

In this paper, therefore, I would like to use the Ilocano regional cultural experience as basic frame of reference in my discussion of a regional culture which has since become part of Philippine national culture. Not that it is the best regional model possible (although partial Ilocanos might be inclined to think so), but that it is the regional cultural experience with which I am best familiar.

I would also like to pinpoint certain areas of a regional culture which are now a part of national culture, and point out certain directions which a regional culture might take in its efforts to preserve and disseminate itself at the same time that it enriches Philippine national culture.

I would like to begin by saying that regional culture, far from being divisive, complements, even enriches the broader fabric of Philippine national culture. Indeed, national culture is nothing but the sum total of all the local or regional cultures taken together.

I also hasten to add that he who loves his regional culture is no less nationalistic, nor less patriotic for doing so.

As I have had occasion to say elsewhere, love "of a country or nation does not obviate nor does it obliterate love of a town, province or region. On the contrary, one who does not love his own town, province, or region – or the traditions intimately intertwined with the historical developments of that town, province or region will find it extremely difficult, if not impossible, to love a larger – and even more mythical – body politic known as a nation" [Marcelino A. Foronda Jr. *Some Notes on Philippine Historiography* (Manila: United Publishing Co., 1972), p. 12].

Certainly, if we are to have a more perceptive, a more complete and, therefore, a much more genuine love of Philippine national culture, it is inescapable that we should first of all love the culture of our town, province or region. One complements the other. Far from fostering regionalism, we may say that if one is to understand, and consequently appreciate the complex entity that is Philippine culture, it is inevitable that people should first know the culture of their own town, not to say their province, or region, that forms part, as it were, of the much larger mosaic that we now call the Philippine national cultural experience (An adaptation of *loc. cit.*).

In truth, we may never achieve a fuller understanding of the Philippines and the culture of its people if we fail to study the culture of our towns, our provinces and our regions. And it is this culture of our towns, provinces and regions that we call regional culture, to distinguish it from general, national or universal culture.

To be sure, the Ilocanos share many cultural traits with their countrymen of whatever regional grouping: close familial ties, hospitality, respect for elders and a sense of fatalism.

But the Ilocanos also have some typical characteristics, which may indeed contribute to the stereotype of the Ilocanos, as they appear in their literary works or as they live in actual fact, the virtue of thrift and of hardwork, a sense of adventure and a pioneering spirit, ingenuity and an extraordinary persistence in eking out a living from their barren soil.

To be sure, such traits are expressed in their own culture. Their language, for instance, is characterized by a certain hardness and bluntness thus reflecting the harsh life of the Ilocanos; a definite contrast to the declamatory tone of the Tagalogs, or the wistful, somewhat effete intonation of the Ilongos, which, for their part, somewhat manifest the easy life of both Tagalogs and Ilongos. The Ilocanos' song, the **Pammulinawen,** which has almost become the national anthem of the Ilocano nation, has a sprightly, martial spirit, a direct contrast to the soulful and sentimental kundiman of the Tagalogs, or the equally sentimental balitaw of the Visayans.

The same vigorous Ilocano spirit is reflected in their pre-Hispanic epic, the **Biag ni Lam-ang,** although the version as handed down to us was only written down in the latter part of the nineteenth century and thus inevitably carries some Spanish influences and, therefore, certain refinements so typical of the Spanish colonial period.

But possibly an effective medium in Ilocano literature to show these influences – an intense love of the Catholic faith, the refinements of the effete upper class, Chinese mestizo Vigan society, thus somewhat lacking the sturdy Ilocano regional spirit – was Leona Florentino. She was, according to Gregorio F. Zaide, "the first poetess of the Philippines" [For a study of her poetry, see my work, Dallang: *An Introduction to Philippine Literature in Ilokano and Other Essays.* Belinda A. Aquino, Ed. (Honolulu, Philippine Studies, Asian Studies Program, University of Hawaii, 1978), pp. 36-42].

If one were to look for the earthy, down-to-earth spirit of the Ilocanos, one has to read the stories of Manuel Arguilla written, strangely enough, not in his native Ilocano but in English [See his *How My Brother Leon Brought Home a Wife and other Stories.* with an *Introduction* by A.V.H. Hartendorp. (Manila: Philippine Book Guild, 1940), 245 p.]. And if one were to seek the middle class values of Ilocano families living beyond the confines of the original Ilocos, one has to read the stories of Gregorio Brillantes, also written in English and set against a Tarlac background [See his *The Distance of Andromeda and Other Stories.* (Manila: Benipayo, 1960, 265p.].

And while the Ilocano experience in communities beyond the seas has been depicted lately in short stories written by Ilocano authors writing in Ilocano in Hawaii [See Pacita Cabulera Saludes and Mario A. Albalos, Eds. *Bullalayaw: Antologia dagiti Nangabak iti Salip iti Sarita iti 1976-1977.* (Honolulu: Gumil-Hawaii, 1978), XIV, 187p.], it is, I think, the Ilocano writer Carlos Bulosan who has best depicted the tribulations of the early Ilocano immigrants to the United States in his book in English, **America is in the Heart** [Earlier published in New York by Harcourt (c1946); later reissued in Manila by Alberto S. Florentino (1973) and in Seattle and London by the University of Washington Press (1973 and 1975)].

No less significant writing has been done in the Ilocano language to depict the Ilocano experience; in fact, a considerable body of significant literature has been produced and still continues to be produced in that language for the past many years.

The Ilocano experience during the revolution against Spain has been described by Marcelino Peña Crisologo in his novel **Mining, wenno Ayat ti Cararua** [*Mining wenno Ayat ti Cararua.* (Vigan 1932), 450p.)], while Ilocano immigration to Cagayan and to the United States has been depicted in novels written by Arsenio T. Ramel [See his *Ti Maingel ti Kabambantayan,* a novel serialized in *Bannawag,* XVI, 27 (February 7, 1955) to XVII, 31 (May 5, 1956)] and Marcelino A. Foronda Jr. [See my novel *Ramut iti Gangannaet* serialized in Bannawag, XXV, 1 (August 19, 1963) to XXV, 40 (May 18, 1964), respectively].

The most ambitious literary work on Ilocano immigration, however, has been undertaken by the well-known fictionist Francisco Sionil Jose, whose tetralogy of generations of Ilocano immigration to Pangasinan has been published [See his *The Pretenders and Eight Short Stories* (Manila: Benipayo, c1960), 328.; *My Brother, My Executioner* (Quezon City: New Day Publishers, 1973), xi, 187p.; *Tree* (Manila:

Solidaridad Publishing House, c1978), v, 133p.; *Po-on, A Filipino Novel* (Manila: Solidaridad Publishing house, 1984), 204p.; and *Mass, A Filipino Novel* (Manila: Solidaridad Publishing House, c1979), 232p.] While written in english, Jose's novels will, for many years to come, remain as practically a definitive study in Philippine fiction of what happens to an Ilocano immigrant – whether to Pangasinan or to Pobres (Forbes? Park) – and to his descendants.

But Ilocano regional culture is also expressed in painting and sculpture, in folk and practical arts like embroidery and the Ilocano cuisine, as well as in religious institutions.

Filipino art connoisseurs always think of the Ilocano painter Juan Luna when they refer to Philippine painting. But the ordinary viewers may find it difficult to call his art "typically" Ilocano, or typically Filipino, whatever that means. For Luna followed the prevailing international style of his times, in much the same way that Ilocano painter Lamarrosa and the late Ilocano printmaker Ray Albano follow the international style of today.

What would pass for typically Ilocano art could well be the intricately embroidered satin robes of religious statues – the **santos** – done in gold or in silver thread, which were the handiwork of anonymous Ilocano embroiderers during the Spanish colonial period.

Embroidery was cultivated as a utilitarian art form even in our very own times, although in more recent years it has become patently secular, as evidenced in the richly colorful table cloths, bed spreads, napkins and window curtains many of which found their way into foreign markets, mainly in the United States where adventuresome Ilocano immigrants had settled down.

This art form is all but wiped out now, with the advent of cheap machine embroidered materials.

Sculpture has up to more recent times been cultivated in the Ilocos (sculptors from San Vicente, Ilocos have a regional reputation). Today, many of those so-called tourist "Igorot" art are actually done by Ilocano artisans in the Ilocos. During the Spanish colonial period, however, anonymous Ilocano sculptors decorated the altars of their churches with their own works, a good example of which is the flat statue of San Vicente Ferrer done in silver by an Ilocos Sur artisan. But most Ilocano sculptors used softwood and hardwood as medium for their work, a good example of which is an angel's statue, which is polychromed, done in La Union

All the sculptors are not known by name, but a rare exception was Juan Nepomuceno Tolentino from Magsingal, Ilocos Sur who did an interesting retable for the main altar of his hometown church, where it was installed.

Painting was cultivated in Ilocos during the Spanish colonial times, although, as in the case of the nameless Ilocano sculptors, Ilocano painters of the times also remained anonymous. Most of their work have not survived the ravages of time, or if they did, they still have to be brought to the attention of art and cultural historians. Lately, however, the 14 paintings commemorating the Basi revolt by Esteban Villanueva of Vigan have been publicized. These primitive paintings, done in the

second half of the nineteenth century, are characterized by vivid style and a charm typically their own, and are among the very few surviving examples of paintings during the period. Other paintings by Esteban Villanueva, who was a businessman and a self-taught painter, have still to be discovered.

Ilocano dishes, on the other hand, do not generally have the refined embellishments of the Pampango kitchen, for instance, again possibly reflecting the harsh life in the Ilocos. The ordinary Ilocano peasant, for instance, relishes practically raw or near raw dishes. The kilawen, half-cooked goat's or cow's meat sliced thin, raw fish in vinegar, raw tomatoes eaten with bagoong, are favorites in his kitchen. The folkloric pinakbet (eggplants, ampalaya and othe vegetables cooked with bagoong and few slices of pork) is a runaway favorite of Ilocanos and, has in effect, become to the Ilocano table what Pammulinawen is to Ilocano music.

Another Ilocano favorite is the papait or pinapaitan, made up of chopped innards of either cow, carabao or goat, with small slices of meat thrown in for good measure, and cooked with vinegar soup and with papait, which is the predigested grass in goat's, carabao's or cow's bile.

Ilocano culinary ingenuity might be evidenced in the fact, as a Tagalog writer once put it, that the Ilocano can actually turn one medium-sized goat, slaughtered, into a complete feast; the hump as roast, the shoulders as stew the innards as pickled aperitif.

But the Ilocano, like his other brothers of the other regions of the country, does not live by rice and fish, or for that matter, pinakbet or papait alone. He is, like the Bicolano, deeply religious after his own fashion. By a process of adaptation, the Ilocano has significantly welded together Hispanic Christianity and some of his pre-Spanish folk beliefs. He has, for instance held on to what is believed to be pre-colonial rituals meant to propitiate angered spirits and has given these rituals a Christian coloring, as in the *kuskusip* (a corruption of St. Joseph) and the *atang*, during which ritual food is offered as gifts to propitiate angered spirits.

These religious practices indicate the Ilocano reaction to the imposition of institutionalized Christianity. This reaction would be institutionalized in the many revolts in the Ilocos during the Spanish era and colorum movement among the Ilocanos of Pangasinan, if not in some Rizalist cults of today, many of whose members are actually Ilocanos.

But a more formal reaction to the Catholic faith was the establishment of the Philippine Independent Church by Aglipay and his fellow Ilocanos, notably the writer and nationalist Isabelo de los Reyes in 1902.

Other Ilocanos, however, like the University of the Philippines president and Supreme Court Justice Jorge Bocobo, and the senator, statesman and educator Camilo Osias joined the Methodist and other Protestant churches earlier established by the American ministers in the Ilocos, and to this day many of the leaders and members of these non-Roman Catholic churches including President Fidel V. Ramos are Ilocanos.

Most Ilocanos, however, opted to remain in the church of their forefathers, and today many Catholic archbishops, bishops, priests and religious sisters work not only in the Ilocos but also in other parts of the country and even abroad.

One can, indeed, speak on and on about the multifaceted aspects of Ilocano regional culture, which have richly contributed to the rich mosaic that is Philippine national culture in general. But Filipinos living in the other regions of the country can also do the same thing with respect to their equally rich and variegated cultures, and which have, needless to state, also enriched and continue to enrich the multicolored tapestry that is the culture of our country.

To be sure, there are many other manifestations of Ilocano culture, but which could not be mentioned here for lack of time. But the above-mentioned manifestations of Ilocano culture have transcended the limited confines of the region, and have since become part of the rich granary of what we call Philippine national cultures. That these manifestations of Ilocano regional culture have enriched Philippine national culture goes without saying, and in their own way will help define the limits and boundaries of Philippine national culture.

Still, it might be necessary to mention here that the Ilocano is fiercely in love with his own language, and takes pride in speaking it and in writing his own literature in it. It is, thus, that Ilocano is spoken not only in Ilocos but in many parts of the country and beyond, and the language has become the lingua franca of certain non-Ilocano areas. As noted above, Ilocano literature is being written by Ilocanos living in the Ilocos, in the Ilocanized provinces and even in Hawaii, Guam and California. Far from fragmenting the Filipino linguistic experience, the love of a regional language like Ilocano enriches what is hoped to become the Filipino national language, and can significantly contribute to the creation of an eventual Filipino national literature, both of which are necessary concomitants to the emergence of a truly Filipino national culture.

The creation of a Filipino national language, and, hence, a Filipino national literature is inevitable, although this will take some time. After all, the evolution of the English or for that matter the Spanish (as distinguished from Castillian) languages and the creation of their literatures were not done in a day, so to speak, but in hundreds of years. And I venture to say that something similar to this, although of a much shorter time, is going to happen to Filipino. Already, much more inter-regional travel and more widespread mass media like comics, movies, radio and TV are helping bring this about. While loving his own regional language, and considering it as his own priceless heritage, the Ilocano, and for that matter, the Cebuano, the Ilongo or Pampango, will eventually pick up Filipino, and contribute his own share in its emergence evolution and development.

Nevertheless, the culture – and this includes the language and literature – of the various regions will have to be preserved. Ilocano culture and those of the other regions were developed at some given place and at some given point in our history, as, indeed, they are still being developed, and as such they are not expected to conform to a certain, rigid pattern favoring one linguistic group at the expense of

others, which would be, as Leopoldo Y. Yabes once said, some kind of cultural imperialism.

To be sure, the development of these reigonal cultures should not be dismissed unfairly as being regionalistic; on the contrary, they should be encouraged to develop, for in their own development will consist their very contribution to the emergence and development of a truly Filipino national culture.

Understandably, the non-verbal expressions of the spirit of these regions like music, the folk or ethnic dances and the works of regional painters and sculptors, by their very nature and through the use of modern mass media techniques shall be easy to disseminate, and shall, thus, be more readily available to other Filipinos not belonging to their linguistic groups, than say, for instance, the regional literatures. The problem is intelligibility, i.e., a novel in Ilocano is not intelligible to a Visayan or Pampangueno, and vice versa. There is thus the need to have the outstanding literary works in the various regions translated into English or Tagalog (Tagalog, however, inspite of the mass media, is still a foreign language to many non-Tagalogs, like the Cebuanos, for instance.) if they are to be made available to a much wider audience. While at first blush, this might seem to be such a gigantic task, it is not so if scholars and students of literature are ready to dedicate their efforts to this task, as it is already being done at De La Salle University and other universities.

Indeed, the task of translation and dissemination of vernacular literatures from the different regions shall demand the dedication of scholars [See my work *Kutibeng: Philippine Poetry in Iloko, 1621-1971* (Manila: De La Salle Unviersity, 1976), viii, 153p. and Elena G. Maquiso, *Ulahingan: An Epic of the Southern Philippines*. (Dumaguete City: Silliman University, c1977), x. 315p.] as it shall be the task of graduate students and of institutions of higher learning. Fortunately such work has been undertaken by graduate students of the country's universities [Much work has been done along these lines, some of which might be mentioned here. The late Jose Resurreccion Calip's Ph.D. dissertation, The Ilocano epic *Lam'ang: A Critico-Anthropological Analysis* (Manila: University of Santo Tomas, 1957), Angel A. Cacio's M.A. thesis, *Pagsasalin sa Pilipino ng 'Biag ni Lam-ang' at Pag-aaral ng Matandang Kalinangang Iloko na Inilarawan ng Epiko* (Manila: Dalubhasaang Normal ng Pilipinas, 1969) and Pilar G. Encarnacion's M.A. thesis *The Novels in Iloko of Marcelino Peña Crisologo: A Critical Study with Special Emphasis on Mining wenno Ayat ti Cararua* (Quezon City: University of the Philippines, 1957) are among the efforts of doctoral and masteral students to study, and thus make better known to others, aspects of Ilocano literature.

Other studies of other vernacular and regional literatures like Juan S. Aguas' M.A. thesis *A Study of the Life of Juan Crisostomo Soto with Special Reference to Alang Dios* (Quezon City: University of the Philippines, 1995), which is a study of the outstanding Pampango dramatist, and Gaudiosa M. Ochotorena's Ph.D. dissertation *Ag Tubig nog Keboklagan (The Kingdom of Keboklagan): A Subanon Epic* (Manila: University of Santo Tomas, 1972), to name just two, are among the earlier and later attempts to study Philippine vernacular literatures.

All the above-mentioned doctoral and masteral theses, except Aguas' and Ochotorena's works, remain unpublished. But by and large the results of these efforts have remained in manuscript form gathering dust in dark unfrequented corners of some graduate school library. What is equally important is to publish them, in popular versions, if necessary, as what has been started by the Cebuano Studies Center at the University of San Carlos.

This leads us to the regional centers for the study of the various regional cultures such as the Cebuano Studies Center of the University of San Carlos, the Samar-Leyte Studies Center of the Divine Word University in Tacloban, Bicol Studies Center of the University of Nueva Caceres in Naga and the Van Vactor Research Center specializing in Filipino-Muslim studies attached to Dansalan College in Marawi, among others. It is interesting to note that these centers are attached to private institutions, although state-run institutions like the Mindanao State University, also in Marawi, and the U.P. Iloilo Ilongo Studies Center and the Mariano Marcos State University Ilocano Study Center in Batac are also engaged in activities analogous to those of these centers.

It will be the task of these centers and others which hopefully will be established in other regions, to help preserve our regional cultures, not only to preserve, but also to develop and disseminate them. In so doing, these centers, rather than fragment the Philippine cultural experience, shall contribute significantly to the development of the multi-colored mosaic that is Philippine national culture.

SYMPOSIUM VI

Symposium Title : **Regional Culture as Part of Philippine National Culture**
Moderator : **Academician Rafael D. Guerrero III**
Rapporteur : **Academician Leopoldo S. Castillo**
Speaker : **Dr. Marcelino A. Foronda Jr.**

SUMMARY

Regional culture considering geographical limits and linguistic considerations includes: language, folk traditions, literature, arts and life style. The Ilocano region is an example of regional culture being a part of the national culture. He avers that the Ilocanos share many cultural traits such as close familial ties, hospitality, respect for elders and a sense of fatalism with those from the other Philippine regions.

One question raised was "What is it in the Ilocano that makes him successful in his migratory activities?" The large population in the area limited by Caraballo/ Cordillera mountains made the Ilocanos migrate to other areas (provinces) where they worked very hard to support themselves. Moreover, Ilocanos have an operational sense of thrift, "matipid or kuripot", which makes them rich.

Dr. Foronda suggested that regional centers for the study of the various regional cultures such as those in Cebu, Tacloban, Naga, Marawi and others be created and those already operational be expanded to preserve, develop and disseminate the regional cultures as part of the Philippine national culture.

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CHOICES

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What makes life today complicated and difficult as well as challenging and interesting is the opportunity to make choices. In ancient or tribal cultures, there was hardly any choice except with the very basic sets of right versus wrong; good versus evil; life or death. Life was much simpler then; decisions and choices were dictated by rigid guidelines. Civilization and the renaissance opened the minds of men to heretofore unexplored aspects of his existence. *Cogito ergo sum* pointed out the primacy of the use of one's mind. Since then the use of the mind has created the never-ending process of change. Choices bring about change usually in the name of progress. Individuals have chosen progress in their personal and environmental lives. The twentieth century, from the year it began to its closing in seven years, is a virtual turn-about from ancient times. Life is now a continuous series of events requiring changing previous choices and making new ones, reconciling modernity and tradition or facilitating degrees of breakaway from the latter.

Human beings living in contemporary times, to keep in step with life around them, need to respond to change around them, as well as the issues raised by change, whether these affect them directly or not. Chances are they will be. They are told also that if not them, then the generation coming after them will be. If they retreat to noninvolvement, they risk becoming irrelevant, deviants, eccentrics or psychologically impaired beings. By refusing to accommodate change, they risk unhealthy psychological consequences.

What does making choice entail? Some choices are easy, simple and effortlessly made. Others may require some reflection but can stand postponement for some time, involve little risk for a possible costly mistake or cause only minimal psychological pain. In short, they are not important and are not likely to cause any insomnia or mood aberration.

Choices which are the most difficult to make are those which put ourselves on the line, which endanger our self-esteem and appear to violate our sense of identity. They raise doubts as to who we are and what we believe in. Such choices resoundingly hit home, namely our inner selves, and assume the form of psychological conflict and personal pain.

Psychic economy demands that a choice be made, otherwise a toll is paid by the individuals' psychological well-being which they may ill afford. Conflict resulting in indecision or paralysis of action needs resolution; otherwise prolonging the state brings people nearer to impaired health. There is little to surpass the pain of a self divided, of inability to make a choice because one is pulled with equal force in opposite directions. Behavioral aberrations soon follow (For example, one of the cardinal sysmptoms of schizophrenia, a malignant disorder, is precisely such a state; individuals allow such opposite forces to co-exist by removing their emotional components).

Forced to make a choice, individuals with their own unique repertoire of coping behavior, summon the proper rationalization to accompany their choice and their subsequent behavior. Healthy rationalization guards one's sanity, premised on the assumption that one is well aware of the conflict, has considered alternative resolutions and has made the choice. The reason may not be **the** reason, but nonetheless, a good enough reason (e.g., a man resigns from his job for reasons of personal health, etc.). Painful though the choice might be, it is one the person can live with; his self-respect and self-esteem are intact. Any psychological damage is reparable. He remains whole.

What portends psychological disaster is when individuals, to minimize the pain and expedite relief, deceive themselves and go on as if no conflict exists. Not only do they employ denial and self-deception, but they also unwittingly sacrifice, by avoiding self-confrontation, their psychological integrity, or their wholeness.

By pushing the conflict out of awareness, they hide, as it were, a loaded gun in the bottom drawer. They flee from painful consideration of alternatives. They choose to bury their heads in the sand. Denial does not get rid of the pain; they still pay the price of going around it. They curtail their freedom of choice, become inflexible and stiffen up, as those who have perceived a grave threat, and posture to shield themselves from battle. They control any damage to their ego by becoming even more rigid than they already are. The more they bristle, the more brittle they become. Aspects of their lives, which may have been pleasurable or humanizing, are sacrificed lest these make them more vulnerable. They move about tense and guarded, narrow down their relationships, are extra-careful not to arouse any hostility within themselves or without. They are overly sensitive and they increase their guard about any possible doubt that they are reacting in the right way. They have in a sense reduced themselves to robots.

Such persons who deny conflict, who try to get away from dealing with painful choices, become dysfunctional as persons. Within their relationships, they continue to deny feelings and guard against confrontation.

How about compromise? It is a key word these days whenever choices are complicated or difficult. "Life is nothing but a series of trade-offs," as one woman in her 50s opined, to explain her being spouseless and childless. The problem with compromise is that it is seldom fully acceptable; it is a cover-up or worse, a sell-out. Some regret lingers or some lowering of one's self-respect occurs, with

self-recriminations. It is comparable to a draw, shaking hands with your adversary after a bloody fight. You keep remembering how you could have fought more effectively, how victory could have been more decisive. And the pain recurs, even more so when your adversary is yourself.

How do private and personal choices participate in social and national change? Asia, it is now conceded, is critically different from the West (U.S., Britain) because it places importance of the society over the individual, with the one recognized exception of the Philippines. Asian leaders have at one time or another pointed to the lack of progress in the Philippines, its cellar position among Asian nations as traceable to its adoption of western democracy with its emphasis on individuals grafted upon an Asian nature which is group-oriented.

The result is one of indecision, confusion and resorting to improvisation as a solution to crisis situations. The individuals compete with society for their own ends. The choices are made at the individual level and do not find their way upwards toward the policymakers. Conversely, ideals and values at the leadership level hardly have relevance at the level of the individual. Media are on the side of the individual; they go by ratings or number of satisfied customers.

The critical choice to be made by individuals is whether or not they will whittle down or shelve aside individual illusions and concentrate on the common good. The forces which are anti-social are well organized: organized crime, organized dissent against the establishment, organized corruption. The forces battling them need to be even better organized to gain any success. The battlefield continues to be the individual psyche.

The choices of the individuals do not emerge as such in resultant group or social activity. As we know, a mob, a group, a community, a nation, each attains a dynamic of its own. Individuals may start out with a noble motive; the mob may turn this into savage revenge. People may feel guilt initially, and in contrition seek to help redress wrongs, only to end up punishing and controlling others. Invested with patriotism, people may end up with less lofty motives. Such is the complex and contradictory nature of human emotion and behavior.

It is no surprise that the person taking a stand on any issue can no longer be guided by true-blue ideals. Reality has to be plugged into the equation. And the standard reply to the question of "Which side are you on?" is the catch-all "It all depends." From this point on, the criteria get blurred. Depends on what? To assess potential leaders, what do we look for? If they smoked marijuana in high school? That they garnered more votes in the elections than their accusers? That the people being judged are speaking against revered tradition? The logic becomes tortuous, truth becomes elusive and may even be the first casualty.

Individuals, in self-examination, return to their conscience and ultimately to their "gut" feelings. "To thine own self be true" is still a valid axiom for mental health. Otherwise, they may find themselves in a corner, burnt-out or alienated, feeling betrayed or at the very least, confused.

If the signals sent by society are confusing, what must this mean to the very young adults waiting in the wings? They will soon be making the choices of their lives. On many issues pressing on their minds, the jury is still out and cannot help them make a decision. They do not have the luxury of time.

At this point, people look to their leaders for help in clarifying the choices. How the leadership is perceived may leave people still confused, indifferent or at least may set them thinking. No matter how convincing or credible leadership is, the individuals may still deviate from the side leadership is on. For example, is the condom campaign successful? Violence still reigns in media.

Which brings us full circle back to the individuals and their own psyche. To make a rational choice with full understanding of what it means, to exercise autonomy over themselves in making such a choice, to be unwavering in their conviction that no matter how lonely their voices are in the crowd, they can still make a difference as human beings. Such is the mandate for each of us.

SYMPOSIUM VII

Symposium Title	:	**Choices**
Moderator	:	**Academician Solita F. Camara-Besa**
Rapporteur	:	**Academician Lourdes J. Cruz**
Speaker	:	**Dr. Lourdes Vera-Lapuz**

SUMMARY

The Filipino is unique among Asians in not placing the importance of society over the individual. The lack of progress in the country may be traceable to its adoption of western democracy with its emphasis on individuals, grafted upon the group-oriented Asian nature. The conflict between the individual good and the social common good leads to compromise, which oftentimes is not satisfactory. The individual in making a choice must return to his conscience and be true to himself.

RECOMMENDATIONS

The individual must: 1) make a rational choice with full understanding of the alternatives; 2) exercise his autonomy over his own self in making such a choice; 3) be unwavering in his conviction that no matter how lonely his voice is in the crowd, he can still make a difference as a human being.

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SCIENCE AND TECHNOLOGY NICHES FOR THE PHILIPPINES

ANTONIO C. ABAYA
The Manila Chronicle

In the last six years, from 1986 to the present, the Philippines has had two, maybe three, master plans for science and technology. One was prepared by Dr. Antonio Arizabal, President Aquino's first Department of Science and Technology (DOST) Secretary.

But barely a few months after he submitted his Science and Technology master plan, he was replaced by Ceferino Follosco as Secretary for Science and Technology, who proceeded to draft and submit his master plan.

I do not know what the differences are between the Arizabal master plan and the Follosco master plan. But I think I can safely hazard the opinion that it does not make any diffcrene what those differences are because we now have a new Secretary of Science and Technology in Dr. Ricardo Gloria who, we can safely predict, will soon present his master plan for science and technology to President Ramos, if he has not done so already.

The point I am driving at is that under our present political system, any master plan for anything is doomed to failure, irrelevance or total amnesia, even before it starts.

Our constitution decrees a change in leadership every six years – formerly four – and that means changing not only the president and vice-president but also the top echelons of the bureaucracy. This is done in the absence of a top-caliber civil service, as in Britain and its former colonies; such is capable of, and constitutionally mandated to, carry out long-range planning and the execution of those plans, even though the Prime Minister and his Cabinet are subject to change at almost any day.

In our political system, every new president and his Cabinet throw away the master plans, projects and priorities of the previous government, in favor of their own master plans, projects and priorities, which in turn are also thrown away by the next government.

Consequently, we are forever starting with step one and step two, unable to get to step three and step four. And this applies to science and technology as it does to other fields.

Let me cite an example from my own family's experience. My father was appointed by President Quirino in 1951 to the board of directors of NASSCO, the National Steel and Shipyards Corporation, the state corporation that was tasked to build the country's first steel mill and first shipyard.

To this day, 42 years and 7 presidents later, the steel mill and shipyards of NASSCO have remained unintegrated, long since overtaken by the more recently established but more determined steel mills and/or shipyards of South Korea, Taiwan, Singapore, Malaysia, Thailand and Indonesia.

Compare this with the experience of Indonesia, whose science and technology master plan, conceived by the Secretary of State for Research and Technology, Dr. Baharuddin J. Habibie, has been in continuous implementation for 17 years now. It is geared toward making Indonesia a fully industrialized country by the year 2026.

I was in Indonesia in 1989 as a guest of the Indonesian government. I was shown some of their science and technology infrastructure, most of which had been envisioned and built, and still managed, by Dr. Habibie, whom I had the pleasure of talking with for one hour.

In 1989, Indonesia was already fabricating helicopters and fixed wing aircraft, including a 35-passenger, two-engine passenger airliner. This airliner, co-designed and co-manufactured by Indonesians and Spaniards, was fabricated from raw aluminum slabs, not merely assembled from imported components.

The aircraft factory in Bandung also fabricates components for Boeing 727 and 767 airliners, as well as for the F-16 Flying Falcon, the top-of-the-line fighter-bomber of the US Air Force and Navy, in service with many other air forces around the world. The Indonesians also make components for Rolls Royce aircraft engines.

The Indonesians also showed me their shipyards in Surabaya, which produce oil tankers and container ships for their maritime industry, as well as warships for the Indonesian Navy. Also in Surabaya is a diesel engine plant that manufactures, not just assembles, diesel engines of up to 6000 hp and electric generator sets of up to 4500 kva.

I was also shown their telecom industry in Bandung, where telecommunications equipment are manufactured. Such equipment include earth stations for their four space satellites, as well as telephone switching equipment for up to 40,000 lines each.

Backstopping all this is a science and technology research center – PUSPIPTEK – in Serpong, a 1,000-hectare facility with complete facilities for 700 scientists and engineers. PUSPIPTEK has modern research facilities in chemistry, physics, metallurgy, nuclear science, propulsion, electronics, computers, etc. It even has state-of-the-art wind tunnel to support its aerospace industry.

The genius behind this science and technology quantum leap by Indonesia -- Dr. Habibie -- graduated summa cum laude in aircraft design from the Technische Hochschule in Aachen, Germany. After graduation, he worked for the famous Messerschmitt aircraft company, where he rose to become vice-president for research, with 80 German engineers under him.

While working for Messerschmitt, Dr. Habibie borrowed money from the company and he used this loan to finance the advanced education abroad of a number of Indonesian scientists and engineers who became his cadre for building Indonesia's science and technology infrastructure.

By 1989, Dr. Habibie had sent 4,000 young Indonesians to study science and engineering in North America, Western Europe and Japan, financed up to 80 per cent by the World Bank.

Dr. Habibie told me how in the early 1970s he was sent to Manila by his company to help start an aircraft industry here, on request of President Marcos. He did what he could for a while, he said, but there was no master plan for local fabrication, only the assembly of imported components. Naturally, he did not give away his master plan, which he started to implement for his beloved Indonesia when President Suharto summoned him home in 1976.

The Philippines needs a genius like Dr. Habibie to envision and implement our industrialization according to a master plan.

But it is my contention that even if we had a Dr. Habibie and a master plan for science and technology, they would be wasted here because our political system does not allow any long-range planning and implementation, however brilliant, for longer than six years.

Our political systems condemns us to mediocrity forever, unless and until it is drastically changed.

If I were running the science and technology establishment in the Philippines, I would forget about ambitious master plans because they would be thrown away by my successor anyway. It would be futile to plan anything beyond six years in this country. Our political system militates against it.

I would instead concentrate on finding three or four niches under the following considerations and pour all resources into these three or four niches:

a. There must be a real and desperate need for the object of the research;
b. The object of the research must use indigenous resources that are available in great abundance;
c. The object of the research must result – or at least have the potential of resulting – in a qualitative change in society in the shortest possible time; and
d. Close collaboration with a technologically advanced country is essential to hasten the research process.

Under the above parameters, and given that only at most six years are available under our political system, I would concentrate on just the following:

1. **Low-cost. pre-fabricated mass housing.** I realize that Secretary Gloria recently inaugurated some low-cost housing units, 35 sq. m. in area, and costing only P80,000 per unit.

But this is not what I have in mind. The low-cost mass housing that I envision needs research in the matter of materials and design, the ideal being a design whereby components are mass-produced by specialist production units and then assembled *in situ* like giant Erector sets, each one in a matter of hours, not days or weeks.

This means that a production unit will fabricate only roof trusses, another only roof components, another only windows, or only doors, or only wall components, or only stairs, etc. All will be according to strict specifications so that they can be assembled into single detached houses, duplexes, quadruplexes, or row houses, as the case may be.

A massive program of low-cost, pre-fab mass housing, backed by constant research in materials and design technology, would change for the better the landscape of our towns and cities, and provide a better quality of life for the poorest of our poor. It can even turn out to be a lucrative export industry.

2. **Alternative sources of energy.** Blessed with abundance of sunshine the whole year round, this country should be at the forefront of research in solar energy, which, based as it is on a limitless resource, is the energy of the future. But research must be conducted now.

Except for a modest effort with the German government, there seems to be no serious effort at research on solar energy, whether thermal or photovoltaic. I propose a joint effort with the South Koreans or the Japanese on thermal solar energy, rather than on photovoltaic solar energy.

Research on thermal solar energy is within our means because it deals with techniques for more efficient focusing of the sun's rays on a black box to boil water into steam that will turn the turbines of a generator. Research on photovoltaic solar energy, on the other hand, involves potentially expensive search for materials more efficient than, say, selenium or gallium arsenide in converting photons of light into streams of electrons.

There is also room for research on wind energy, perhaps in collaboration with existing wind energy research efforts in California and Hawaii. I have seen the wind energy research facility near Livermore in Northern California. Dozens of wind generators dot the barren hills. But these are all horizontal-axis windmills that need much open space.

Perhaps Philippine research on wind energy can concentrate on wind generators with vertical axes, using fabric or metal foil sails instead of propeller blades. Assuming equal outputs, a battery of three vertical-axis wind generators, one on top of the other in a structure, can theoretically generate three times as much as one horizontal-axis generator occupying an area of equal size.

Finally, on the matter of alternative sources of energy, there should be serious efforts to support the pioneering work of Filipino inventor Rudy Lantano in developing a fuel mix that is 85% diesel fuel and 15% alcohol.

There is nothing revolutionary about this. During the Japanese occupation, my father, who was a mechanical engineer, used diesel fuel mixed with coconut oil for the engines and vehicles of his company.

Lantano's breakthrough is achieving an apparently stable mixture without using a chemical additive, catalyst or wetting agent. . . only a vigorous agitation of the mixture. Physical chemists can theorize that the induced turbidity caused the different hydrocarbon molecules to link together temporarily in a stable and evenly distributed mixture.

Whatever the scientific explanation for Lantano's breakthrough, it is made even more beneficial by the apparently more complete combustion that the mixture undergoes. Lantano's empirical data, based on the performance of cargo trucks in a Batangas sugar refinery and of buses on the EDSA route in Metro Manila, claim a virtual elimination of pollution in the form of black fumes so common among jeepneys and buses in Metro Manila.

Lantano's breakthrough can not only reduce our dependence on imported oil and decrease pollution in our cities. It can also generate employment in the countryside by creating a market in the cities for anhydrous alcohol produced from sugar cane. It can save our moribund sugar industry and create hundreds of thousands of jobs in the rural areas. And it can also become a lucrative export industry.

3. **Electric rail transport.** I propose that the Philippines strive to attain world class expertise in electric rail transport, in the same way that Indonesia attained world class expertise in below-50-passenger, propeller-driven air transport.

Indonesia did it through a four-step process devised by Dr. Habibie:

a. Enter into an agreement with a foreign partner to assemble imported components of the partner's product for sale and use in the domestic market and part of the global market;
b. Gradually fabricate locally made and more of the imported components;
c. Enter into an agreement with the foreign partner to co-design and co-manufacture a completely new product, to be marketed in the shared global market; and
d. Using the experience gained in steps a, b and c, design and manufacture a completely new product without any foreign help, for sale in the global market.

Thus has Indonesia scored a breakthrough in a technology formerly reserved for much more advanced countries. If Indonesia can do it in the aerospace industry, there is no reason, aside from our limiting political system, why the Philippines cannot do it in another industry, like electric rail transport.

Why electric rail transport? From a combination of several factors, Metro Manila is becoming asphyxiated by both pollution and an antiquated public transport system. Our jeepneys and buses are totally inadequate for our needs and are injurious to our health.

The only solution is to switch to electric rail transport in a few years, after we have solved the power crisis. Furthermore, dwindling oil reserves as well as growing concerns over pollution and global warming make this a universal solution to a growing universal problem.

In other words, in the next few years, there will be a growing market worldwide for electric rail transport systems, and the Philippines could choose this early to establish world class expertise in this technology. That is, if we can get our minds away from politics for a while.

In closing, I repeat my proposal that we forget about grandiose master plans for science and technology, which our political system will not allow us to implement anyway. Instead I suggest that we channel our energies and resources into only three niches and concentrate on these niches. These are: a) low cost, pre-fab mass housing; (b) alternative sources of energy, such as solar, wind and alco-diesel; c) and electric rail transport systems.

Thank you for your attention, and good day.

SYMPOSIUM VIII

Symposium Title : **Science and Technology Niches in the Philippines**
Moderator : **Academician Ernesto O. Domingo**
Rapporteur : **Academician Clara Y. Lim-Sylianco**
Speaker : **Mr. Antonio C. Abaya**

SUMMARY

Master Plans in Science cannot work out in the Philippines because of our political system. The best approach will be to harness the idea of a working genius in a particular field like what they do in Indonesia.

Develop deeply some niches such as:

1. Alternative energy sources like solar and wind energy and a combination of alcohol and diesel fuel (Lantano's);
2. Low cost housing that can be set up in hours; and
3. Electric rail transit development.

RECOMMENDATION

Adopt the proposal of Mr. Antonio Abaya.

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INTELLECTUAL DISCIPLINE FOR THE FILIPINOS

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Flying high over the Carribbean on Delta Airlines, I was reading the Safety Instructions which said, "In case of emergency put the oxygen mask on first and then put the oxygen mask on your child." The reason was "If you black out first, you would not be able to put the oxygen mask on your child." Much intrigued by this way the Americans think, I asked myself, "How will the Filipino mother think? Will she not first put the oxygen mask on her child?" The question brings up the theme of this present paper which has to do with two modes of mind or two ways of thinking: the objective-rational way and the subjective-intuitive way.

Another experience. In cooperation with the DAP I was giving a human development seminar to the top officials of the Development Bank of the Philippines to demonstrate the process of consensus-formation. To start off the process, I would give the participants a simple arithmetic problem to solve individually and then to see if they could then reach consensus. The problem went like this: "I bought a carabao for P6,000. I sold it for P7,000. I bought it back for P8,000. I sold it for P9,000. How much money did I gain?."

Less than 25 per cent of the group came up with the correct answer, which was P2,000. The shocking thing was that the chairman of the board gave P1000 as his answer and insisted on being right because as he said, "You buy at P6000 and sell for P7000, you make P1000. You buy back at P8000, you lose the P1000. You sell at P9000, you gain P1000. So the answer is P1000 gain." His assistant, a woman, gave the answer as "No gain, no loss. You buy P6000, you lose money, you sell P7000, you gain money. You buy P8000, you lose money. You sell P9000, you gain money. *Patas*. No gain, no loss." This was said in all seriousness, with some anxiety at the possibility of being wrong and at having gone against her boss. Yet they and the majority were wrong.

I gave the same problem to sixth, seventh and eight-graders at the International School. Ninety-five percent of the students gave the right answer on the first try. I gave it to a Chinese group with similar results. One Chinese boy explained how he

got it: "I have a money box. I buy P6000, *labas.* I sell P7000, pasok. I buy again, P8000, *labas.* I sell P9000, *pasok.* P14000 *lumabas,* P16000 *pumasok.* So, P2000, gain." It was a beautiful combination of rational and intuitive thinking, probably the result of receiving early math education on the abacus.

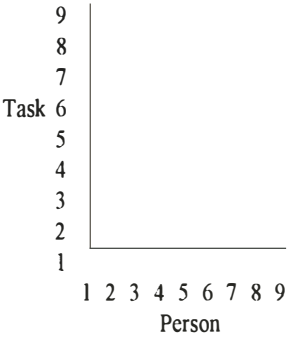
THEORY

At this point it is not my intention to arouse the ire of the nationalists by asserting that Americans and Chinese are more intelligent than Filipinos. The question is not intelligence but the quality of intelligence. In the 1950s, psychologists still thought of intelligence as a unitary ability, an IQ. With the development of factor analysis, we have come to realize that intelligence is made up of several factors which may be considered as distinct abilities. Different cultures differ in the composition of these abilities according to how that culture evolved in response to the challenges of its times. Filipinos have developed the subjective-intuitive factor of inteligence and have not fully developed the objective-rational.

In the 1970s, as a result of Nobel Prize winner Sperry's left-brain, right-brain experiments, emphasis was given to dividing mind functions into two: left-brain functioning which has to do with the verbal, rational, problem-solving ways of thinking, and the right-brain functioning which has to do with the intuitive, emotional, artistic, etc. ways of thinking. What Sperry seems to have proven is that the brain locations are the seat of these functions when they occur, but not necessarily that they determine a man's intelligence or even the quality of his intelligence. Left-brain and right-brain are but shortcut ways of describing functions of the mind which have long been known to psychologists. The syndromes are the same even when different words or concepts are used to describe them. This two-syndrome view of the mind was already forseen as early as the early 20th century by Carl Jung who divided personalities into intuitive and rational. Since then, personalities, mind functions, attitudes, abilities, etc. have been classified into similar dimensions, for instance:

task-orientation	–	person-orientation
scientific	–	artistic
obsessive-compulsive	–	hysterical
objective	–	subjective
head	–	heart

Different disciplines may have given different names to their mind-sets according to their own experiences but in general they agree on these two syndromes. Furthermore, they agree on seeing these two dimensions as fusing into a whole. For example, task-orientation and person-orientation can be plotted on a two-dimensional graph with abscissa and ordinate each numbered from one to nine like this:



Thus a subject scoring 9-1 can be considered a TOB (task-oriented bastard) and a subject scoring 1-9 would be a BH (bleeding heart). Of course, the holistic person might score a high 8-7 or a more retarded 2-3, balanced but low.

All this is the theoretical model relevant to our theme, the Transformation of the Filipino, trying to locate him on a point between the ordinate which we can call objective-rational and the abscissa which we can call subjective-intuitive. It is not our intention to determine in actual number the Filipino mind processes (though this can be done easily by social scientists), but rather to give a clinical picture of how the Filipino mind works, with a view to transforming what can be transformed in order to adjust to the demands of the 21st century. Following Lee Kuan Yew's call for social discipline, this psychologist is calling for intellectual discipline. To survive in the modern world the Filipino has to learn to think rationally, objectively, scientifically and to develop left-brain functioning.

The Underdeveloped Left-Brain Function

For over 10 years, I had the pleasant task of selecting candidates for college scholarships of the Insular Life from all the regions of the Philippines. Every year we tested over 2,000 high school valedictorians, salutatorians and first honorable mentions, and interviewed the finalists. To test the quality of thinking, I might ask a question such as, "Suppose while riding in an airplane, I simultaneously dropped one kilo of solid steel and one kilo of loose, fluffy cotton. Which package would hit the ground first?" More than half of these "bright" high school students would answer, "The steel and the cotton would hit the ground at the same time."

These are the Filipinos who have been over-educated by the system to think by rote, in slogans and cliches. In the course of their education, they have been taught "scientific' answers but they have not developed the rationality (and common sense) to look at objective reality and know how objective reality works, which is the true science. The educational system has taught them to memorize a lot of words but did not develop basic scientific thinking. Certain regions of the

Philippines, in particular Eastern Visayas and Southern Mindanao, exhibited this thinking among their high school graduates.

The individual has not been taught to trust his own objective observation. For instance, in the comprehensive examinations for a Master of Arts degree in Psychology, I asked the following question, "If one researcher can jump one meter high, how high can 10 researchers jump?" Three of the ten answered, "Ten meters high."

If one were to analyze the answer of these three, one may see that in their eagerness to please the examiner, they tried to use their weakest part which was the verbal-analytic. They failed to use their own intuition which was really the strongest part of themselves. They came out with an answer which was against their own common sense and which they would, if confronted, say was wrong: the researchers jumped 10 meters high. Wrong analysis and/or poor expression.

In a similar way, to analyze the thinking process of college students, I have asked the question, "Which is bigger, a circle or a square? About a fourth will answer, "The square is bigger because it has four corners." Another fourth will answer, "The circle is bigger because it goes beyond the sides of the square. Another fourth will answer, "I don't know. I was poor in geometry." Only a fourth can verbalize the answer, "It depends on the size. A big square is bigger than a small circle and a big circle is bigger than a small circle." One can see how rational analysis and/or verbal expressiveness, both left-brain functions, seem to be lacking.

The cultural predominance of right-brain thinking over the left brain manifests itself on the one hand in the Filipino's cultural artistry, his spontaneity in music and dance (Most of the musical bands in Southeast Asia are composed of Filipinos.). On the other hand, this is also manifested in his poor handling of modern technology and in his inability to plan for the future. The present crisis in electrical power gives testimony to this. In the first place, it was the lawyers, not the scientists who made the decision on the nuclear power plant. There was inability to plan and there was inability to handle the technological problems when they arose.

This low-level rational-objective, problem-solving quality of thinking shows itself in the way Filipino culture has responded to other 20th century social needs. Take for instance the transportation system. There are no railways or other forms of mass transportation needed to link the nation together, leading further to a way of thinking which may be called the barrio or small town mentality. Jeepneys crawl at snail's pace in the towns, stopping anywhere for passengers. Routes and terminals have not been nationalized, with the exception of a few towns like Subic. The jeepney itself stands as an incarnated symbol of cultural thinking.

The jeepney was originally a step in modernization when it replaced the *calesa.* But it froze in its early form and remained the way it was in the post World War II Liberation period. It has grown slightly from *limahan* to *waluhan* on each side. But it has kept (except in the U.P.) the huge sign in front: "Lyn-Lyn," "Katas ng Saudi," "2 Sisters," etc., while the jeepney's destination is relegated to a tiny sign behind the windshield. (It is interesting that U.P., the bastion of rationality,

has made the jeepneys that pass through the campus place their destination in big letters on the jeepney's forehead, a big step toward rationalization). But the windshield remains crowded with "borloloy," with signs and slogans narrowing the driver's view. And the two tin horses over the motor, symbolizing speed, complete this symbolic monument to the subjective-intuitive Filipino mind. It is a lovely symbol – it is not much but it is ours.

We come to the theme of this paper: that we must raise a new generation that is capable of thinking scientifically and rationally, away from slogans and rote thinking. The emphasis here is on scientific thinking, objectively, rationally, not on having more classes in science where students are made to memorize "The earth is round like a ball." Scientific thinking can be taught even in non-science classes where the emphasis is put on producing the specific word, the exact image, and of course, in correct grammar. Students can learn to do critical thinking, observation, can know when data are insufficient, can withhold judgment when necessary, can push the truth when it deserves to be pushed. This is left-brain thinking.

A sign of our own lack of analysis is that our human development trainers emphasize right-brain thinking. They do so in rote imitation of American trainers, never thinking that Americans need right-brain improvement in order to be more "human." But the Filipino needs more left-brain thinking to be "humanized" or more explicitly, a holistic merging of the two dimensions at a more balanced level, a 9-9 rather than a 9-1 or a 1-9.

The thinking of the Filipino is right-brain thinking; the subjective-intuitive aspect of the mind occupies a dominant position over the objective-rational. This condition does not necessarily mean that the Filipino is inferior to, let us say, the German or American. It merely means that the Filipino has a way of seeing reality and mentally manipulating it differently from the German or American. But it is good to be aware of this quality of thinking of our people because we can understand why we are ranked among the happiest people in the world (cf. Newsweek), why we do not worry about the future (e.g., that Laguna de Bay is the last possible source of fresh water for Manila yet it is fast becoming polluted), why the culture has given over to the Chinese and Americans the task of running manufacturing plants and megamalls or why we always call on foreign experts to solve our problems in science, medicine traffic and technology.

Why does a population prefer left-brain thinking over right-brain thinking or right-brain thinking over left-brain thinking? Is the difference genetic, cultural or both? There is not enough evidence to say. But certainly, culture has at least a large influence in shaping one's thinking preferences as may be seen from the notable differences between the Filipinos brought up in the United States and Filipinos brought up in the Philippines. Likewise, individual introspection brings out the correlation between early experiences and life careers. For instance, taking my present interest in Psychology, I can trace this interest to early experiences in my father's Physiology laboratory at the U.P. College of Medicine where I practically grew up amid the calculators and the experimental animals. My father taught me

how to hypnotize a chicken when I was only eight years old. It seems most natural to drift into empirical psychology. Early experiences have much to do with ways of thinking.

This is the culture that we have developed in the Philippines and which we also find in Indonesia and Malaysia. It is interesting to note that the most economically advanced nation in the ASEAN is Singapore, which is basically Chinese and which by the highly discliplined social behavior of its populace shows them to be highly objective-rational and left-brained.

Consequently, the question can be asked: Should we Filipinos be happy with the way we are, bring up our children in the traditional values, teach them *pakikisama, utang na loob,* etc. the way the nationalists teach in the schools? Should we remain happy in our subjective-intuitive ways of thinking? The answer that this paper suggestes is: *Yes,* but at the same time we must develop our objective-rational side, raising our scientific and technological skills to meet the challenges of modern times. Actually, whether we like it or not, the culture will change in this direction.

SHIFTING TO THE LEFT BRAIN

How does one develop objective-rational thinking in the Filipinos? The total answer is still hidden from us. Maybe exposure to the modern world by itself will force the nation to evolve its way of thinking to meet its challenges. For instance, the power shortage and all the failed attempts to solve this problem politically will force the nation to enter into the nuclear age and to think science and technology rather than politics and law.

However, this psychologist may make the following suggestions: teach the Filipinos the three Rs: reading, 'Riting, 'Rithmetic on a higher level.

Reading

The Filipino simply does not read. Our neighbors in China, Japan and Korea have crowded bookstores. Xiamen, for instance, has a bookstore four stories high, stocked with books from the equivalent of Pepe and Pilar all the way to the highest levels of literature, science and philosophy. And the store is crowded with eager readers. And each apartment has its own little collection of books. In the Philippines, even today's college students cannot or do not read. When assigned scientific articles for them to read and evaluate, there are feelings of helplessness and cries of dismay because they simply do not know how to teach themselves from the printed page. In the absence of reading, self-learning becomes impossible. As one participant in a human development seminar said in defense, "All I needed to know in life I learned in kindergarten." And the only answer one can give is "Yes." And thus the nation remains backward, the basket case of East Asia.

The Filipinos must read a lot if they have to survive as a nation in the modern world.

'Riting

Verbal ability is closely related to rational thinking because words give a person control over his world. The ability to manipulate mental symbols, i.e. language, has a double effect. It allows the speaker to organize his own thoughts, to improve rationality, to make his unconsious, conscious. Secondly, it allows social communication and makes possible thinking and acting in groups. As the saying goes, "One learns from what one says rather than from what one hears."

Thus, besides reading, proper verbal expression develops rationality: the apt word in the proper context, to write and speak with clarity, force and interest. Honeybees can work together as a hive because they have their own ways of communicating with one another. Filipino human beings must use words and language to work together with one another and with the modern world.

There is the particular problem: which language to use to develop the objective-rational ability of the Filipino. The emerging national language, Taglish, is on its way to becoming language. But it has a long way to go. For now it is not understandable to farmers on the one hand, or to English-speaking foreigners, on the other. It has no stable rules of syntax nor an accepted dictionary of clear-cut meanings. And so, for the language of instruction, I suggest a bilingual policy that even the Tausugs and Maranaws can accept. Let there be two languages, Tagalog and English. Set as goal of education equal facility in both and if possible let there be no mixture of both. The upper class in Manila schools have succeeded in producing such bilingual speakers and thinkers. It may be possible to broaden this base. Otherwise, we will end up in the 21st century with a nation whose higher socio-economic class speaks English and Tagalog or Cebuano, and a lower socio-economic class that understands only the local dialect and a kind of chabacano like that of Papua New Guinea. (And whether we like it or not, English is the language of modern science as well as of airports, air controllers and computers. The world has changed a lot from the time of Napoleon, Bismarck and Jose Rizal.)

'Rithmetic

Quantified thinking is the basis of modern science. Up to now, Philippine culture has not adequately evolved this form of objective-rational thinking but has relegated this function to the Chinese and Americans in its midst. This is why manufacturing and megamalls are the creation of minds brought up in a different culture, which demands hard quantitative thinking from them.

It is safe to assume that the Filipino mind is not anatomically nor physiologically inferior to that of the Chinese or Americans. The deficiency in

mathematical functioning can be seen as cultural in origin: 1) it is taken for granted that mathematics is hard; 2) there are no real demands for Filipinos to study math or do problem-solving; and 3) the teachers, themselves, being part of this culture, do not practice quantitative thinking and accordingly do not demand this kind of accurate and exact thinking. (to do so would be "kulit," unpopular and undemocratic.)

And yet accurate, objective observation should be a major objective of all scientific thinking. Respect for data is something learned. It is not learned by being allowed to copy a classmate's experiment just to fulfill an academic requirement. The mind never learns to tell fact from fiction. There is no felt need to take pains to verify facts. All this because they were taught to write reaction papers, not to discover facts. As a result, we end up with a whole slew of newspaper columnists but no real reporters. What is needed then is culture change. The setting up of science high schools is a step in the right direction. Then it is a matter of political will by the government to bring about change in the teaching of math and science in the nation. Whoever sets up and enforces such a policy will have to be a cultural non-conformist. But there is always the possibility that such a person may get through the Commission on Appointments.

The question remains then: how can we make this change in the teaching of science and mathematics when the whole culture is against it? To change the whole culture seems an impossible task, but it may be possible (following the Chinese example, cf. Time) to set up islands in the culture where a select school made up of select teachers and students can develop scientific thinking in the population. An honors high school or a regional science high school in every region can do much to introduce scientific thinking in the public school system. But national change will be very slow.

By way of digression: The Philippine Science High School was the result of the vision and persistence of one man, Dr. Torabaya, a simple math professor in New York University in the 1950s. With help from Dr. Frank Co Tui and later from Dr. Paulino Garcia, almost single-handedly he set up this school which may be studied now as a model on how to develop the mathematical and scientific abilities of the Filipino. It has become the model for various science high schools all over the Philippines, and even if the financial constraints of these other schools limit the extent to which they can emulate Philippine Science (which is a very expensive school), the quality of change is there and we begin to reserve capable young Filipinos from mediocrity in science and technology.

Lee Kuan Yew of Singapore, when asked what the Philippines needed to become a Newly Industrialized Country (NIC), gave Social Discipline as his answer. This paper is saying the same thing. Social discipline is the other side of the coin with intellectual discipline on the other side. Is it possible to develop this intellectual discipline in the Filipino? We certainly hope so!

SYMPOSIUM IX

Symposium	:	**Intellectual Discipline for the Filipinos**
Moderator	:	**Academician Ruben L. Villareal**
Rapporteur	:	**Academician Faustino T. Orillo**
Speaker	:	**Fr. Jaime Bulatao, S.J.**

SUMMARY

The paper mentions two modes of mind or two ways of thinking: the objective-rational way and the subjective-intuitive way.

On the subject of intelligence, the question is not intelligence per se but the quality of intelligence. Intelligence is made up of several factors which may be considered as distinct abilities. Different cultures differ in the composition of these abilities according to how that culture evolved in response to the challenges of its times. Filipinos have developed the subjective – intuitive factor of intelligence; they have not fully developed the objective – rational.

The paper cites Sperry's left-brain, right-brain experiments which gave rise to the division of mind functions into two: left brain functioning which has to do with the verbal, rational, problem-solving ways of thinking, and the right-brain functioning which has to do with the intuitive, emotional, artistic, etc. ways of thinking.

Following Lee Kuan Yew's call for social discipline, Fr. Bulatao is calling for intellectual discipline. To survive in the modern world, the Filipino has to learn to think rationally, objectively, scientifically and to develop left-brain functioning.

The cultural predominance of right-brained thinking over the left-brained kind manifests itself on the one hand in the Filipino's cultural artistry, his spontaneity in music and dance. On the other hand, this is also evident in his poor handling of modern technology and in his inability to plan for the future. The present crisis in electrical power gives testimony to this.

The low-level rational-objective, problem-solving quality of thinking shows itself in the way Filipino culture has responded to other 20th century social needs as exemplified by the country's transportation problems.

The paper suggests – teach the Filipinos the three R's: Reading, 'Riting, 'Rithmetic on a higher level.

READING

Unlike the Chinese, Japanese and the Korean, the Filipino simply does not read or does not read enough. In the Philippines, even today's college students cannot or do not read. When assigned scientific articles for them to read and evaluate, there are feelings of helplessness because they simply do not know how to teach themselves from the printed page. In the absence of reading, self-learning

becomes impossible. The Filipino must read a lot if he is to survive as a nation in the modern world.

'RITING

Verbal ability is closely related to rational thinking. Words give a person control over his world. The ability to manipulate mental symbols, i.e. language, 1) allows the speaker to organize his own thoughts, to improve rationality, to make his unconscious, conscious; and 2) allows social communication and makes possible thinking and acting in groups. As the saying goes; "One learns from what one says rather than from what one hears." With regard to language, Fr. Bulatao suggests a bilingual policy that even the Tausugs and Maranaws can accept. Let there be two languages, Tagalog and English. Set as goal of education equal facility in both and if possible let there be no mixture of the two.

'RITHMETIC

Quantified thinking is the basis of modern science. Up to now, Philippine culture has not adequately evolved this form of objective-rational thinking but has relegated this function to the Chinese and Americans in its midst.

It is safe to assume that the Filipino mind is not anatomically or physiologically inferior to that of the Chinese or the American. The deficiency in mathematical functioning can be seen as cultural in origin: 1) it is taken for granted that mathematics is hard; 2) there are no real demands that Filipinos study math or do problem-solving; 3) the teachers themselves, being part of this culture, do not practice quantitative thinking and accordingly do not demand this kind of accurate and exact thinking.

A culture change is needed. The setting up of science high schools is a step in the right direction toward solving the aforementioned shortcoming in the scientific and rational thinking of the Filipino.

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A MIE LIDAR SYSTEM FOR ATMOSPHERIC MONITORING: DESIGN CONSIDERATIONS

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ABSTRACT

With the growing urbanization of Metro Manila and other cities, there is an increasing need to monitor the atmosphere and environment. The laser radar or lidar technique has been shown to be highly flexible and capable of providing accurate multidimensional measurements of atmospheric and meteorological parameters over a wide area. LIDAR, the acronym for "light detection and ranging", is an active laser remote sensing system which employs the radar principle to give range-resolved measurements of the atmosphere from a single location. In particular, the Mie lidar system utilizes the phenomenon of elastic Mie scattering to monitor the atmosphere of aerosols, detect and measure particulate emissions from vehicles, observe clouds, and measure stratospheric aerosol layers consisting primarily of sulfate particles.

This paper discusses and analyzes the different parameters required to set up a highly sensitive two-wavelength Mie lidar system. Using the scattering lidar equation and known system specifications, the received power and signal-to-noise ratio are calculated and plotted with range, in order to predict the range capability and sensitivity of the system. The system is divided into the laser transmitter, the optical receiver, and the signal processing system, and the requirements of each are analyzed and discussed. Results of sensitivity measurements are presented. Because of their sensitivity and power, laser-based environmental sensors are able to provide high resolution and versatility.

INTRODUCTION

With the growing urbanization of Metro Manila and other cities, there is an increasing need to monitor the atmosphere and environment. Monitoring of pollutants near the ground level is necessary in order to determine the quality of air that man breathes. Both ground-level and higher-level monitoring must be performed in order to develop mathematical models for predicting air quality for varying circumstances. Stratospheric gases and particles affect man in a less direct, but equally important way.

In general, laser remote sensing is one of the active areas of laser applications and it benefits a major sector of the general populace. It is one area where lasers can make a significant impact in the Philippines.

A result of careful and thorough study, this paper gives an overview of the LIDAR technique and Mie lidar systems, and discusses and analyzes the different parameters required to set up a highly sensitive two-wavelength Mie lidar system.

THE LIDAR TECHNIQUE FOR ENVIRONMENTAL SENSING

Advances in the field of lasers gave rise to the active remote sensing technique called the Laser Radar or LIDAR, the acronym for "light detection and ranging". The LIDAR technique is characterized by the introduction into the atmosphere of well-collimated laser beams of high irradiance and spectral purity.

In a manner analogous to the microwave radar technique, the time between the transmission of the laser pulse and the arrival of the scattered return signal can be directly related to the range at which the scattering occurred. Thus, the laser radar is capable of range-resolved measurements.

The atmosphere, in addition to the major gases N_2, O_2, Ar, water vapor and CO_2, contains trace constituents, solid particles, and aerosols. Most of these constituents modify the transmission of electromagnetic radiation through the atmosphere. There are various ways by which the transmitted light interacts with the atmospheric constituents. These processes include Rayleigh scattering, Mie scattering, Raman scattering, resonance scattering, fluorescence, absorption, and differential absorption and scattering. The decision to use a particular technique is based on the information required from environmental species. This is obtained from measurements of physical parameters of the scattered radiation.

Lasers afford great flexibility of operation and can monitor with high resolution a wide variety of pollutants. The scope of lasers in environmental sensing is extensive. They can be used to undertake concentration measurements of both major and minor constituents in the atmospshere, detection and mapping of specific constituents as required in pollution monitoring, and airborne mapping of fluorescent substances such as chlorophyll or oil slicks in or on lakes and oceans. Furthermore, these observations can be made remotely with both spatial and temporal resolution from the ground or from mobile platforms such as boats, helicopters, aircraft, or satellites.

Figure 1 is a diagram of the basic laser radar configuration. In most systems, a pulsed laser is employed as transmitter and the laser radiation is collimated and transmitted into the atmosphere. As the laser energy passes through the atmosphere, it interacts with various scatters such as aerosols, gas molecules and solids. A fraction of this energy is backscattered and collected by a receiving telescope, transmitted through an optical filter or monochromator, and transferred to a photodetector. The electrical signal is recorded by a transient memory recorder in a

range-gated fashion and then transferred to a computer. The spatially-resolved data of the atmospheric scatters are calculated by a signal processing computer and recorded on a display terminal. The range of the scattering volume can be uniquely determined from the recorded signal as a function of time, and the backscattering properties, such as particle concentrations, are derived from the magnitude of the signal.

THE MIE SCATTERING LIDAR

The Mie LIDAR system uses the developed technique of Mie scattering. This is the efficient elastic scattering process whose cross section can be so large that even low concentrations of dust or aerosols can be detected. The Mie scattering scheme provides direct observation of the transmitted frequency of atmospheric elastic backscattering from which the presence, location and distribution of particles, such as dust, smoke and cloud, can be determined. This lidar system, either mobile or stationary, is capable of observing the tropospheric environment and collecting information over a wide area.

Since the first laser radar observations were reported in 1963, the Mie scattering lidar has been studied and developed as a new tool for understanding atmospheric processes in connection with the transport and convection of pollutants and aerosols from natural and man-made sources. Early laboratory experiments with the lidar, such as those of Fiocco and de Wolf (1968), and Fernald et al. (1972), attest to the system's capability to measure aerosols in the atmosphere with high spatial and temporal resolution. On the other hand, Davis (1969) used the lidar to observe clouds and obtain direct information on cloud heights and thicknesses.

Single-wavelength Mie lidar observations lead to measurements of the volume backscattering coefficient which provide direct information on the presence and location of smoke plumes, dust, among others. Collis and Russell (1976) point out that this measurement has little practical significance in itself for air pollution monitoring. Information on mass concentration, or number concentration of particle size distribution, is of far greater importance. Such information, however, can not be directly obtained by remote single-wavelength lidar observations. Multiple wavelength or bistatic lidar observations can provide information on these quantities. Two-wavelength lidar systems consist of long and short wavelengths, and explore the wavelength dependence of the backscattering and extinction coefficients to provide such direct information as mean particle size. A convenient choice is a single laser system such as the Nd: YAG which has emissions at 1.06 μm and its second harmonic at 532 nm. For example, Uthe (1982) has shown that the Nd:YAG laser system would be useful in evaluating particles of sizes less than 1 μm from the ratio of long wavelength to short wavelength extinction coefficients.

Any preliminary analysis of the system begins with the lidar equation which is classified according to the target and optical interaction process. For Mie scattering, the equation is:

$$P(R) = P_0 \, l \, K \, A_r \, \beta(R) \, Y(R) \, T_0(R)^2 / R^2,$$

where P(R) is the received power, l is the effective length of the scattering volume corresponding to one-half of the laser pulse length (l = ct/2, t is the laser pulse duration), K is the optical efficiency of the transmitting and receiving optics, Y(R) is the geometrical overlap factor between the laser and telescope receiver field of view, A_r is the receiving telescope area, $\beta(R)$ is the volume backscattering coefficient of the target scatterers, $T_0(R)$ is the single path transmittance of the laser beam through the atmosphere which depends on the total extinction coefficient of the atmospheric scatterer at the laser wavelength and R is the range.

The detection sensitivity of the system can be determined by the voltage signal-to-noise ratio (S/N), which is simply the ratio of the signal to noise current. The signal current is the number of photoelectrons generated by the detector per unit time and is directly proportional to the power received P(R). In this context, noise represents the false signals received by the system and can reduce the accuracy of the measurements. In laser sensing, noise may come from statistical fluctuations of signal and background radiation, thermal generation of photoelectrons in the absence of light and the thermal agitation of photoelectrons. Depending on various conditions, all these contribute to the noise current and can affect the detection sensitivity of the lidar system. In the case of the photomultiplier tube (PMT) as detector, considering shot-noise limited conditions, the governing equation is

$$\left(\frac{S}{N}\right)_v = \sqrt{\tau_g \eta \lambda m / hc} \, \mu P_r / \sqrt{P_r + 2P_b}$$

where τ_g is the gate time of observation for a single laser pulse, η is the quantum efficiency of the photodetector, m is the total number of laser shots within some predetermined observation time, μ is the photomultiplier noise factor. P_b is the background power, and is

$$P_b = B(\lambda) \, \Delta\Omega_r A_r K \, \Delta\lambda$$

where $B(\lambda)$ is the spectral radiance of background light, ΔQ_r is the solid angle of the receiver field of view, and $\Delta\lambda$ is a the spectral width of the receiver. Here the boxcar integration technique is to be preferred to accommodate a wider range of optical signals for lidar operation. The range capability of the system is determined from a plot of $(S/N)_v$ vs. R, as in Figure 2. Note from the graph that a system using

a flashlamp-pumped Nd:YAG laser emitting at 532 nm as specified may reach a maximum altitude of 40 km at nighttime under clear air conditions.

Figure 3 presents a schematic diagram of the highly sensitive two-wavelength Mie scattering lidar system that is to be developed locally. The system consists of three main parts: the laser transmitter, the optical receiver and the signal processing system. For the purposes of system design, it is essential to consider the requirements of each.

The Laser System

In general, the choice of the laser transmitter must consider the following for highly sensitive laser remote sensing: atmospheric optical transmittance; optical interaction involved; availability of high power and efficient laser sources and highly sensitive detectors and detection techniques; and eye-safety conditions. For the two-wavelength Mie lidar, a flashlamp-pumped Nd:YAG laser is a suitable choice because it satisfies the requirements of power and wavelength.

The beam emitted by the laser is directed through appropriate output optics toward the target of interest. The function of the output optics is threefold: to improve the beam collimation; provide spatial filtering; and block the transmission of any unwanted broadband radiation, including the emission that arises from some lasers.

The design of the beam collimator and expander system is mainly determined by the laser beam divergence, the beam spot size and the opening angle of the telescope. The components of the system are so chosen to satisfy the condition on the geometrical overlap factor. The geometrical overlap factor is unity where the field of view of the receiver optics overlaps the laser beam. In practical terms, this is so, provided the divergence angle of the laser beam is less than the opening angle of the telescope. The other required optics are reflectors with high reflectivities at the laser wavelength. Often a small fraction of the laser pulse is sampled to provide a zero-time marker (a reference signal with which the return signal can be normalized should the laser's output reproducibility be inadequate) and a check on the laser wavelength where this is important.

The Optical Receiver

The telescope mirror collects the backscattered radiation and reflects it onto the detector via an aperture or iris, and a dielectric interference filter. The size of the receiver's aperture is directly proportional to the power received and it depends to a large extent on the technique and range involved. Majority of lidar systems make use of reflecting (Newtonian or Cassegrainian) telescopes and require some kind of secondary mirror to direct the backscattered signal onto the detector. Such secondary mirror is not employed in the receiving optical system shown in Figure 4. As mentioned earlier, the opening angle of the telescope is a significant parameter to consider and can be readily measured. The same parameter determines the size of

the iris which is placed at the focal spot. The radiation gathered by the receiver optics is passed through some form of spectrum analyzer on its way to the photodetection system. The spectrum analyzer serves to select the wavelength interval of observation and to provide adequate rejection of all off-frequency radiation. It can take the form of a monochromator, polychromator or a set of narrowband spectral filters.

The choice of the photodetector is determined by basic characteristics which include spectral response, quantum efficiency, frequency response, current gain and dark current. The wavelength of the signal to be detected constitutes the primary factor in selecting the photodetector to be employed. Usually, a photomultiplier tube (PMT) is used as detector, particularly at visible laser wavelengths where the detector quantum efficiency is 10%, or better. In the near-infrared, the quantum efficiency of PMTs is about 0.1% or less. Thus, in this wavelength region, it is practical to consider another type of detector such as the photodiode. A few systems (Hansen and Spinhirne, 1982; Salemink et al., 1985) have considered this idea. For the near-infrared component of the backscattered signal, this system uses a high responsivity silicon avalanche photodiode-preamplifier module with noise equivalent power of 8×10^{-14} $W/\sqrt{Hz}$ at 1060 nm. The module consists of a silicon avalance photodiode, a high frequency amplifier, a temperature sensing element and associated circuity for temperature compensation of the photodiode responsivity – all of which are in hybrid form and in a hermetically-sealed modified 25-mm diameter package.

If the lidar is to be efficient in long-range observations, the detector's sensitive surface should be located in the focal plane of the telescope. The detector must be centered on the optical axis of the collecting mirror such that each point on its sensitive surface receives radiation from the mirror. Focusing optics may be required to compress the lidar signal down to the detector size.

In a two-wavelength lidar measurement using an Nd:YAG laser, the emitted beam consists of the fundamental at 1.06 μm and its second harmonic at 532 nm. In such a set-up, the collected backscatter passes through a harmonic separator so that the near-infrared portion is focused on the photodiode while the visible received signal is imaged on the photomultiplier tube. In front of each photodetector is a bandpass interference filter centered at the corresponding wavelengths.

Signal Processing System

The signal from the photodetector may be processed through analogue or digital techniques. In the analogue approach, raw data is displayed as the backscattered signal intensity as a function of elapsed time on a wide-bandwidth oscilloscope. The data can be digitized by means of fast dual-waveform digitizers which together with a computer make real-time data processing possible.

OPERATION AND PERFORMANCE OF THE SYSTEM

After the Mie lidar system is put together, it is then ready for alignment and measurements. The lidar system alignment proceeds by adjusting the optical components for optimum overlap of the laser beam and the receiving telescope at the farthest range possible with the emitted laser power. The position of the detector assembly is also adjusted in search of the optimum position of the telescope's focal point. The corresponding changes are monitored with the oscilloscope. The main object of the system alignment is to receive the maximum signal from the farthest range possible with the emitted laser power. Figure 5 presents typical oscilloscope data resulting from sensitivity measurements. One was obtained under clear air conditions from 500 meters, while the other was obtained with clouds from an altitude of 2.4 km.

ACKNOWLEDGMENT

This study was made possible by an Asahi Shimbun Research Fellowship (1990) under the direction of Professor Takao Kobayashi of the Department of Electrical and Electronics Engineering of Fukui University in Japan.

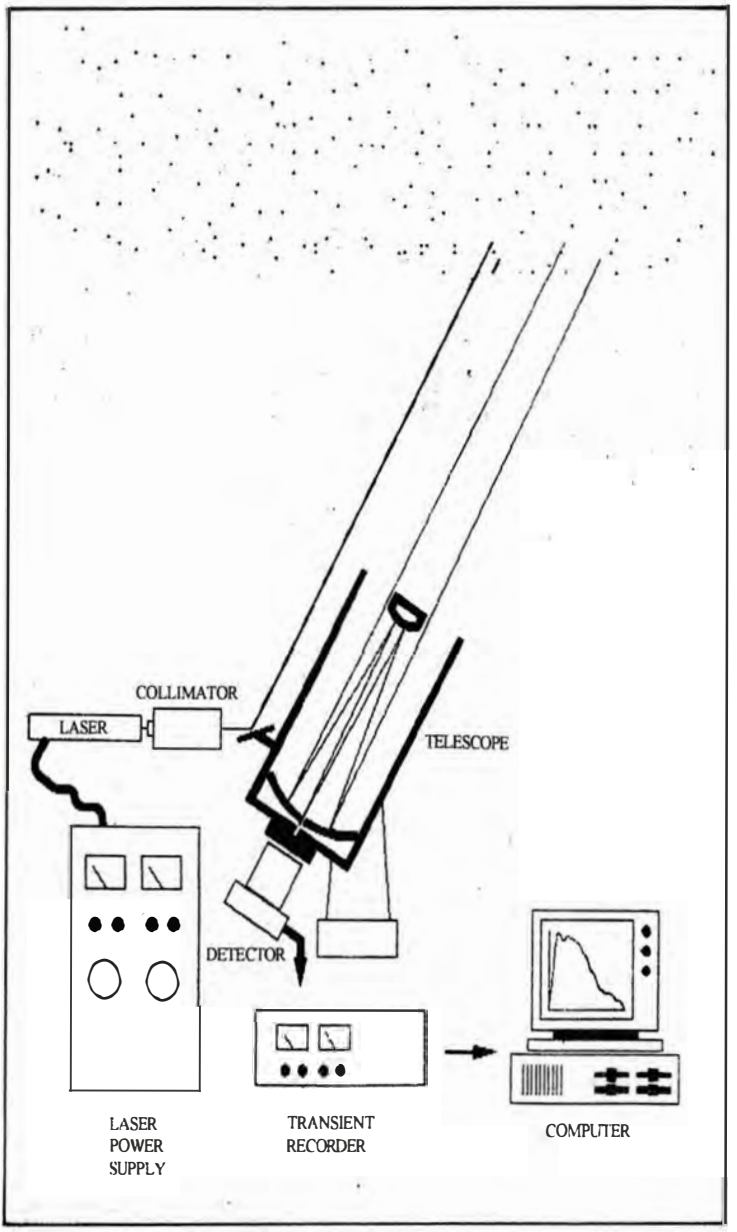

Figure 1. **The basic laser radar configuration consists of the laser transmitter, the optical receiver and the signal processing system.**

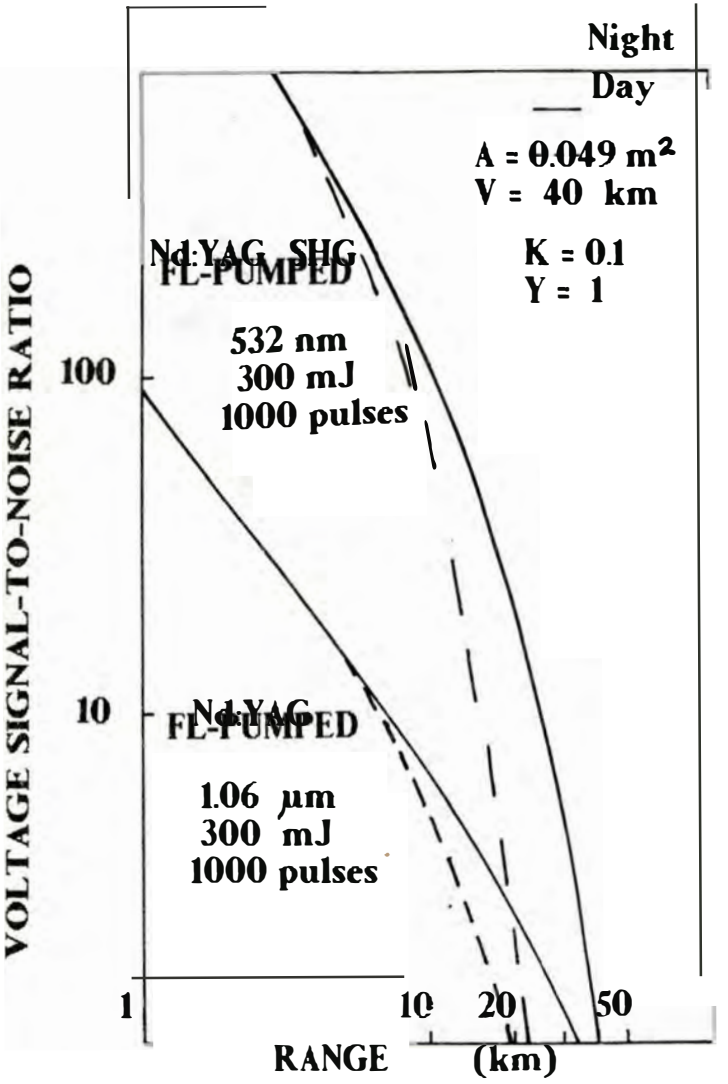

Figure 2. Range dependence of voltage signal-to-noise ratio for detecting molecular Rayleigh scattering with a two-wavelength Mie lidar using the fundamental and second harmonic beams of a flashlamp-pumped Nd: YAG laser

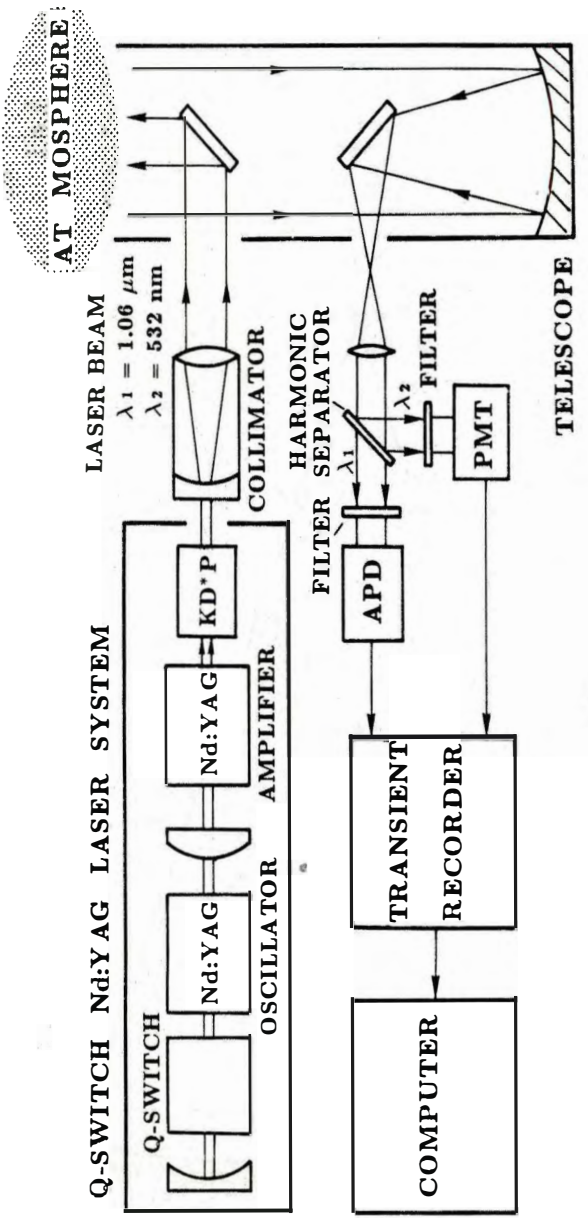

Figure 3. The highly sensitive two-wavelength Mie scattering lidar system

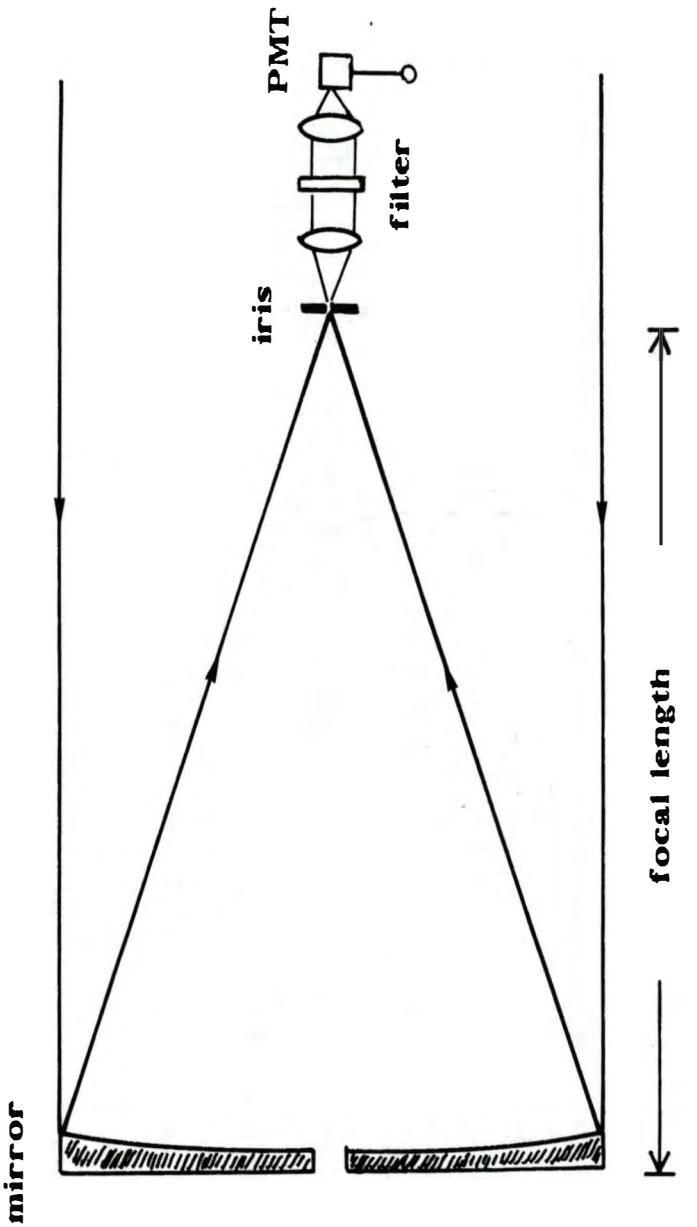

Figure 4. The telescope mirror collects the backscattered signal and reflects it onto the detector through an aperture and an optical filter.

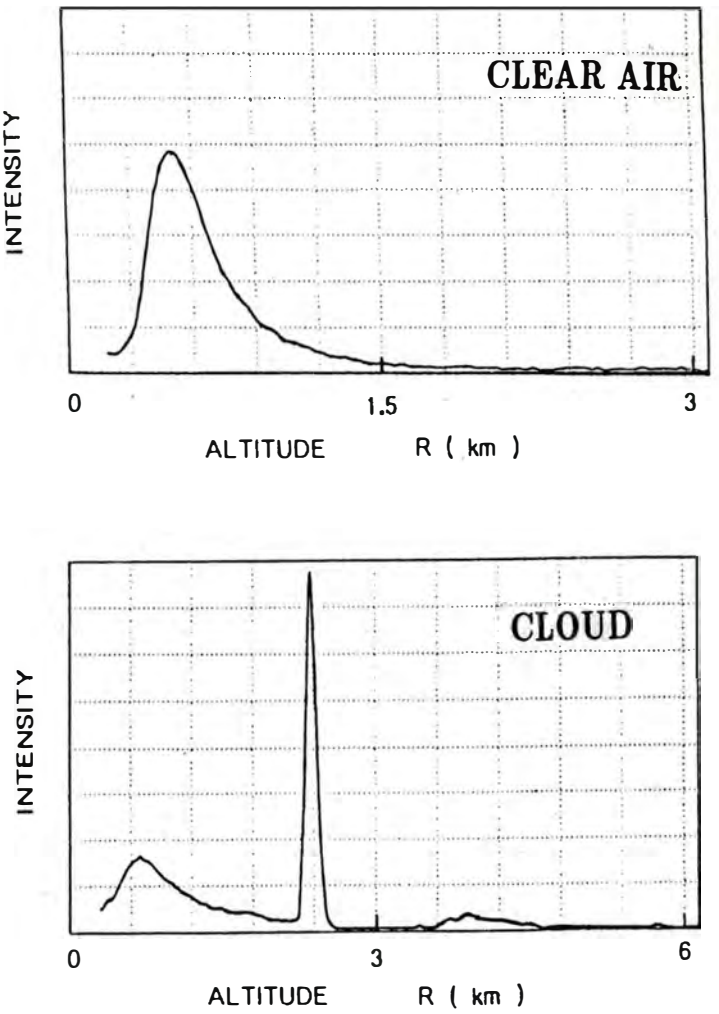

Figure 5. **Typical oscilloscope data resulting from sensitivity measurements of aerosols and clouds**

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SINGULAR GRAPHS: THE SUM OF TWO GRAPHS[1]

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ABSTRACT

The *sum* of two graphs G and H, denoted by $G + H$, is the graph obtained by taking disjoint copies of G and H and then adding every edge xy, where x is a vertex in G and y is a vertex in H. The sum $G + H$ of two graphs may be singular or non-singular, independently of the singularity or non-singularity of G and H.

Here, G and H are limited to complete graphs, paths and cycles. Formulas for det $A(G+H)$ are derived and consequently, necessary and sufficient conditions for the sum to be singular are obtained.

INTRODUCTION

By *graph* G is meant a pair $G = \{V(G), E(G)\}$, where $V(G)$ is a nonempty set of elements called *vertices*, and $E(G)$, is a set of 2-subsets of $V(G)$ called *edges*. The *adjacency matrix* of a graph G with vertices $v_1, v_2, \ldots, v_n$ is the $n \times n$ matrix $A(G) = (a_{ij})$, where $a_{ij} = 1$ if v_i and v_j are adjacent, and $a_{ij} = 0$ otherwise. The graph G is said to be *singular* if $A(G)$ is singular, i.e., det $A(G) = 0$; otherwise, G is said to be *non-singular*.

The *sum* of two graphs G and H, denoted by $G + H$, is the graph obtained by taking disjoint copies of G and H and then adding every edge xy, where $x \in V(G)$ and $y \in H$.

The graph with n vertices where each vertex is adjacent to the remaining $n - 1$ vertices is called the complete graph of order n, denoted by K_n. The *path of order n*, denoted by P_n, is the graph with n distinct vertices $x_1, x_2, \ldots, x_n$ and whose edges are $x_i x_{i+1}$ for $i = 1, 2, \ldots, n-1$. The *cycle of length n*, denoted by C_n is the graph obtained from the path P_n by adding the edge $x_1 x_n$.

This paper studies the sum $G + H$ of two graphs G and H where G and H are any of the graphs K_p, P_q and C_r. Formulas for det $A(G + H)$ are derived and singular graphs $G + H$ are characterized.

[1]This paper is part of a research project supported by the National Research Council of the Philippines, 1992-1993.

SUM OF TWO GRAPHS

Rara (1991) has computed for the determinants of $G + H$ where G and H are any of the graphs P_n, C_n, K_n with only three exceptions, namely, $C_m + C_n$, $C_m + K_n$ and $K_m + K_n$. The last sum is trivial since $K_m + K_n = K_{m+n}$. The problem on the first two sums is settled here.

The adjacency matrix of the sum of two cycles C_m and C_n has the following form:

$$A(C_m + C_n) = \begin{bmatrix} A(C_m) & M \\ N & A(C_n) \end{bmatrix}$$

where M and N are matrices of 1s having sizes m X n and n X m, respectively. For convenience, any matrix all of whose entries are equal to c will be called a *c-matrix*.

If A is any m X n matrix, denote by R_i its *ith* row and by *Cj* its *jth* column. The operation of interchanging rows (columns) u and v will be denoted by $R_u \leftrightarrow R_v$ ($C_u \leftrightarrow C_v$). The operation of adding to R_i the linear combination of rows $c_1R_1 + c_2R_2 + \ldots + c_mR_m$ will be denoted by $[c_1R_1 + c_2R_2 + \ldots + c_mR_m] + R_i \rightarrow R_i$.

Theorem 1. If $m \equiv 0 \pmod 4$ *or* $n \equiv 0 \pmod 4$, *then* $C_m + C_n$ is singular.

Proof: Without loss of generality, assume that $m \equiv 0 \pmod 4$. Let $A = A(C_m + C_n)$ be the adjacency matrix of $(C_m + C_n)$. If the row operation $[-R_2 + R_4 - \ldots - R_{n-2}] + R_n \rightarrow R_n$ to A is applied, the *nth* row is clearly reduced to a zero row. Clearly, this row operation preserves the determinant of A. Thus, $C_m + C_n$ is singular. ■

Lemma 1. *Let n be odd and* $A = A(C_n)$. *Then A is transformed to the diagonal matrix diag*(1, 1,..., 1, 2) *by the following sequence of 5 determinant-preserving operations:*

(a) $R_1 \leftrightarrow R_2, R_2 \leftrightarrow R_3 \ldots, R_{n-1} \leftrightarrow R_n$

(b) $[-R_1 + R_3 + \ldots + (-1)^{\frac{n-1}{2}} R_{n-2}] + R_{n-1} \rightarrow R_{n-1}$

(c) $[-R_2 + R_4 + \ldots + (-1)^{\frac{n-1}{2}} R_{n-1}] + R_n \rightarrow R_n$

(d) $[-\frac{1}{2} R_n] + R_{n-1} \rightarrow R_{n-1}, [-\frac{1}{2} R_n] + R_{n-2} \rightarrow R_{n-2}$

(e) $[-R_{n-1}] + R_{n-3} \rightarrow R_{n-3}, [-R_{n-2}] + R_{n-4} \rightarrow R_{n-4}, \ldots, [-R_3] + R_1 \rightarrow R_1$

Proof. Verification is straightforward. ■

Lemma 2. *Let J be an $n \times r$ 1-matrix, where n is odd. Then the sequence of operations (a)-(e) transforms J to a matrix whose first n-1 rows form a $\frac{1}{2}$- – matrix and whose last row forms a 1-matrix.*

Proof. Verification is straightforward. ■

Lemma 3. *Let $A = A(C_n)$, where $n \equiv 2 \pmod 4$. Then A is transformed to the diagonal matrix diag$(1, 1, \ldots, 1, 2, 2)$ by the following sequence of 5 operations:*

(a) $R_1 \leftrightarrow R_2, R_2 \leftrightarrow R_3 \ldots, R_{n-1} \leftrightarrow R_n$

(b) $[-R_1 + R_3 + \ldots - R_{n-2}] + R_{n-1} \rightarrow R_{n-1}$

(c) $[-R_2 + R_4 + \ldots - R_{n-1}] + R_n \rightarrow R_n$

(d) $[-\frac{1}{2} R_n] + R_{n-1} \rightarrow R_{n-1}, [-\frac{1}{2} R_n] + R_{n-2} \rightarrow R_{n-2}$

(e) $[- R_{n-1}] + R_{n-3} \rightarrow R_{n-3}, [- R_{n-2}] + R_{n-4} \rightarrow R_{n-4}, \ldots, [- R_3] + R_1 \rightarrow R_1$

Moreover, the above sequence of operations reverses the sign of det A.

Proof. Verification is straightforward. ■

Lemma 4. *Let J be an $n \times r$ 1-matrix, where n is odd. Then the sequence of operations (a)-(e) transforms J to a matrix whose first n-2 rows form a $\frac{1}{2}$ – matrix and whose last two rows form a 1-matrix.*

Proof. *Verification is straightforward.* ■

Theorem 2. *Let $m \equiv 1$ or $3 \pmod 4$ and $n \equiv 1$ or $3 \pmod 4$. Then* det $A(C_m + C_n) = 4 - mn$.

Proof. By applying (a)-(e) to the first m rows and to the last n rows of $A(C_m + C_n)$, the following matrix is obtained.

$$A' = \begin{bmatrix} I'_m & M \\ N' & I'_n \end{bmatrix} = \begin{bmatrix} 1 & & & & & \frac{1}{2} & \frac{1}{2} & \cdots & \cdots & \frac{1}{2} \\ & 1 & & & & \frac{1}{2} & \frac{1}{2} & \cdots & \cdots & \frac{1}{2} \\ & & \ddots & & & \vdots & \vdots & & & \vdots \\ & & & 1 & & \frac{1}{2} & \frac{1}{2} & \cdots & \cdots & \frac{1}{2} \\ & & & & 2 & 1 & 1 & \cdots & \cdots & 1 \\ \frac{1}{2} & \frac{1}{2} & \cdots & \cdots & \frac{1}{2} & 1 & & & & \\ \frac{1}{2} & \frac{1}{2} & \cdots & \cdots & \frac{1}{2} & & 1 & & & \\ \vdots & \vdots & & & \vdots & & & \ddots & & \\ \frac{1}{2} & \frac{1}{2} & \cdots & \cdots & \frac{1}{2} & & & & 1 & \\ 1 & 1 & \cdots & \cdots & 1 & & & & & 2 \end{bmatrix}$$

where, by Lemmas 1 and 2, $I'_m = \text{diag}(1, 1, \ldots, 1, 2)$, M' has $[\frac{1}{2}\ \frac{1}{2}\ \frac{1}{2}\ \ldots\frac{1}{2}]$ in the first $m-1$ rows and $[11\ \ldots 1]$ in the last row, $I'_n = \text{diag}\ (1, 1, \ldots, 1, 2,)$ N' has $[\frac{1}{2}\ \frac{1}{2}\ \frac{1}{2}\ldots\frac{1}{2}]$ in the first $n-1$ rows and $[11\ \ldots\ 1]$ in the last row. Multiply rows m and $m+n$ by $\frac{1}{2}$ to get the matrix

$$A'' = \begin{bmatrix} I''_m & M'' \\ N'' & I''_n \end{bmatrix}$$

where I_m, I_n are identity matrices and M'', N'' are matrices whose entries are all equal to $\frac{1}{2}$. If the operations $[-\frac{1}{2}R_1 - \frac{1}{2}R_2 - \ldots - \frac{1}{2}R_m] + R_i \rightarrow R_i$ for $i = m+1, m+2, \ldots, m+n$ is applied, the resulting matrix is block upper triangular and has the following form

$$A^* = \begin{bmatrix} I_m & M'' \\ O & I^*_n \end{bmatrix} =$$

$$\begin{array}{ccccccc}
1 & & & \frac{1}{2} & \frac{1}{2} & \cdots & \frac{1}{2} \\
& 1 & & \frac{1}{2} & \frac{1}{2} & \cdots & \frac{1}{2} \\
& & \ddots & \vdots & \vdots & & \vdots \\
& & 1 & \frac{1}{2} & \frac{1}{2} & \cdots & \frac{1}{2} \\
& & & 1 & \frac{1}{2} & \frac{1}{2} & \cdots & \frac{1}{2} \\
& & & p & q & q & \cdots & q \\
& & & q & p & q & \cdots & q \\
& & & \vdots & \vdots & \ddots & \cdots & \vdots \\
& & & q & \cdots & q & p & q \\
& & & q & \cdots & q & q & p
\end{array}$$

where each entry in the diagonal of I^*_n is $p = 1 - \frac{m}{4}$ and each off-diagonal element is $-\frac{m}{4}$. It follows that $\det A^* = 1 - \frac{mn}{4}$ and consequently, $\det A(C_m + C_n) = 4 \det A^* = 4 - mn$. ■

Using a similar argument and Lemmas 3 and 4, the following theorem can be established:

Theorem 3. *Let* $m \equiv n \equiv 2 \pmod 4$. Then $\det A(C_m + C_n) = 16 - 4mn$.

By a similar argument and using Lemmas 1, 2, 3 and 4, the following theorem can be established:

Thereom 4. *Let* $m \equiv 1$ or $3 \pmod 4$ and $n \equiv 2 \pmod 4$. *Then* $\det A(C_m + C_n) = 2mn - 8$.

Theorems 1-4 may be summarized as follows: *The sum* $C_m + C_n$ is singular if and only if C_m or C_n is singular.

Consider now the sum $C_m + K_n$. In [4, 6, 7], it is shown that if x and y are two distinct vertices in a graph G with $N(x) \setminus y = N(y) \setminus x$, then $\det A(G) = -2 \det A(G \setminus x) - \det A(G) \setminus \{x, y\})$. Assume that $n \geq 2$ and apply this result to the graph $K_n + G$. Then by mathematical induction, get

$$\det A(K_n + G) = (-1)^{n+1}[n \det A(K_1 + G) + (n-1) \det A(G)]$$

Applying this to the graph $G = C_m$,

$$\det A(C_m + K_n) = (-1)^{n+1} [n \det A(K_1 + C_m) + (n-1) \det A(C_m)]$$

Now, $K_1 + C_m = W_m$, the wheel of order $m + 1$. Therefore,

$$\det A(C_m + K_n) = (-1)^{n+1} [n \det A(W_m) + (n-1) \det A(C_m)]$$

In [7], the following formulas are given:

$$\det A(W_m) = \begin{cases} 0, & \text{if } n \equiv 0 \pmod 4; \\ n-2, & n \equiv 1 \pmod 4; \\ 2, & n \equiv 2 \pmod 4; \\ -n & n \equiv 3 \pmod 4. \end{cases}$$

$$\det A(C_n) = \begin{cases} 0, & n \equiv 0 \pmod 4; \\ 2, & n \equiv 1 \pmod 4; \\ -4, & n \equiv 2 \pmod 4; \\ 2 & n \equiv 3 \pmod 4. \end{cases}$$

Therefore, the following theorem has been proved:

Theorem 5.

$$\det A(C_m + K_n) = \begin{cases} 0, & \text{if } m \equiv 0 \pmod 4; \\ (-1)^{n+1}(mn + 4n - 2), & \text{if } m \equiv 1 \pmod 4; \\ (-1)^{n+1}(6n - 4), & \text{if } m \equiv 2 \pmod 4; \\ (-1)^{n+1}(-mn + 2n - 2), & \text{if } m \equiv 3 \pmod 4. \end{cases}$$

It is easy to check, based on the above result, that if $m \not\equiv 0 \pmod 4$, then $\det A(C_m + K_n) \neq 0$. Thus, the following corollary.

Corollary. *$C_m + K_n$ is singular if and only if $m \equiv 0 \pmod 4$.*

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IMAGING 2-D OBJECTS HAVING FRACTAL EDGES: CHARACTERISTICS

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ABSTRACT

The image characteristics of a two-dimensional object bounded by fractal-like edges were investigated. The two -dimensional (2-D) object is formed using the star of David as initiator and the von Koch snowflake as generator. The fractal complexity of the object edges is analyzed up to the 10th iteration. The image of the 2-D object is computed by convolving the 2-D object with a sombrero function representing a circular pupil function. Image characteristics, particularly the degradation of details, are analyzed by studying the effects of convolution on the image area and circumference, as well as fractal (Hausdorff-Besicovitch) dimension of the edges of objects formed at various stages of iteration.

PERSPECTIVE

Two general spatial features are of interest when observing a two dimensional object. These are its surface and edges. Surface properties are of particular importance in reflectance, refractive index and contour studies, while an accurate knowledge of edge boundaries is essential in the determination of object sizes, shape and location.

The edge usually lacks the finer features of the true object due to low-pass filtering by the imaging system. These losses of edge details comprise the accuracy with which the object area and location are measured.

The aim of this work was to study how bounded objects with fractal-like edges looked when imaged. Fractals are known to exhibit the property of self-similarity (Feder, 1988) and are thought to be the appropriate geometry to describe objects that occur in nature (Stevens, 1990). More recently, the microstructures with complicated shapes have been studied and experimented on (Langer, 1992). Therefore, an understanding of their imaging properties may lead to new and more effective signal recovery techniques.

according to $Dx = \lambda f/D$ where f is the focal length of the lens and λ is the illumination wavelength.

Figures 4A and 4B show the images of Figure 1 for different diameters of the pupil: D = 32 pixels and D = 256 pixels, respectively. Note that accompanying the decrease in diameter of the circular pupil is the loss of objects details. This smoothing effect is a consequence of diffraction limited imaging. The images in Figure 4 were generated by taking the inverse Fourier transform of the resulting products between the pupil and the Fourier transform of the object.

Figures 5A and 5B illustrate how the image area and circumference are affected by the imaging process at various diameters of the pupil. Note that the image area is multivalued with respect to D, but the circumference rises monotonically with D. Both graphs are normalized with respect to values associated with the object.

Figure 6 presents the effect of pupil diameter on the fractal dimension of the image. The fractal dimension of the image is lower than that of the object. This decrease is expected as the convolving effect of the pupil function with finite diameter leads to the smoothing of edge details. Note further, that the effect of the pupil diameter on the fractal dimension is nonlinear within range of diameter values considered. The fractal dimension rises monotonically with D.

CONCLUSION

This study aimed to find correlations between the fractal dimension of the image and the perceptible effects of area and circumferential changes as the object with known fractal dimension is imaged using a circular pupil of known diameter. Experimental results show

that object smoothing, which is a consequence of diffraction-limited imaging, affects distinctively the object area and circumference. The image circumference increases monotonically as the diameter of the convolving pupil is increased. On the other hand, the image area before finally increasing, exhibits an inflection point around D = 48 pixels. As the D is increased, it is expected that the image area will asymptotically approach the value of the object area.

Results also indicate that fractal dimension is affected nonlinearly by imaging. There is a monotonic dependence of the fractal dimension on pupil diameter.

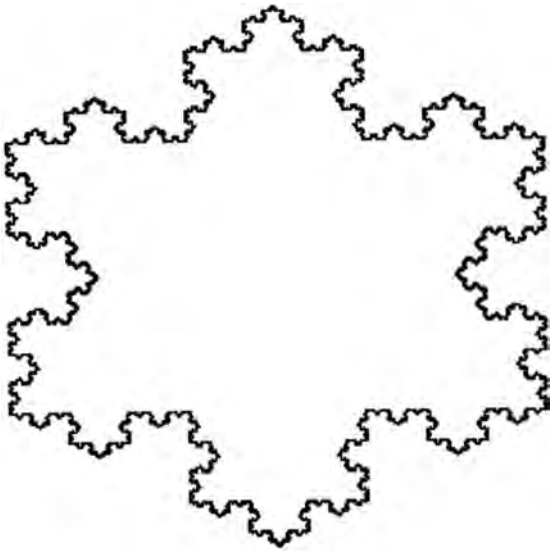

Figure 1. Edge profile of the Star of David initiator after the 6th iteration of the von Koch snowflake generator

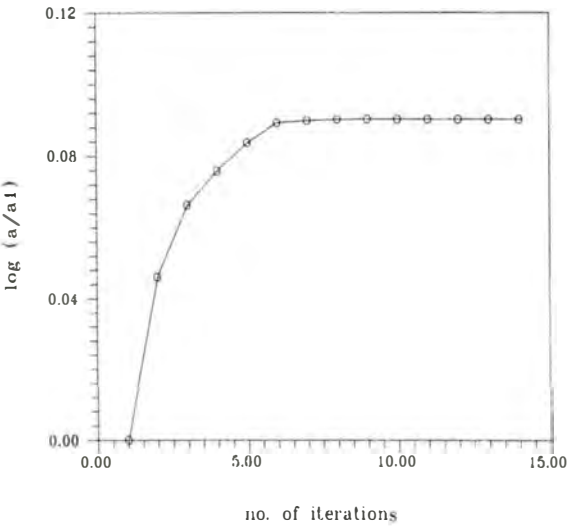

Figure 2(a). Dependence of object area with the order of iteration

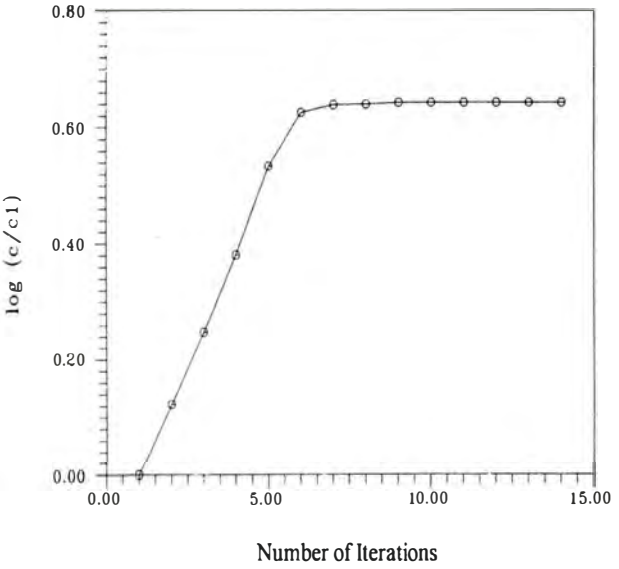

Figure 2(b). Dependence of object circumference with the order of iteration

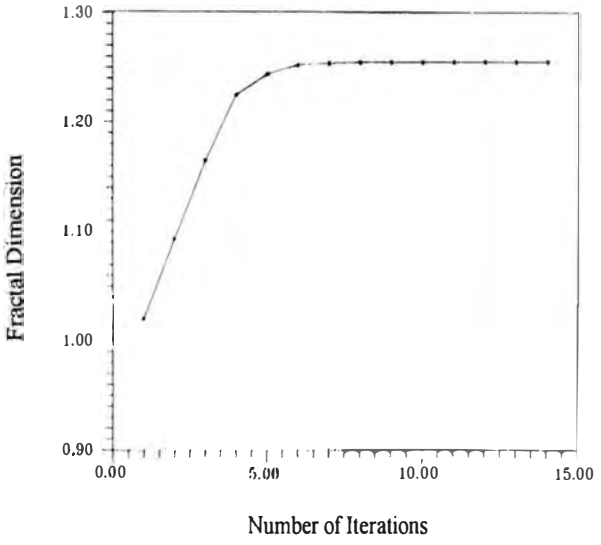

Figure 3. Relationship between fractal dimension and order of iteration

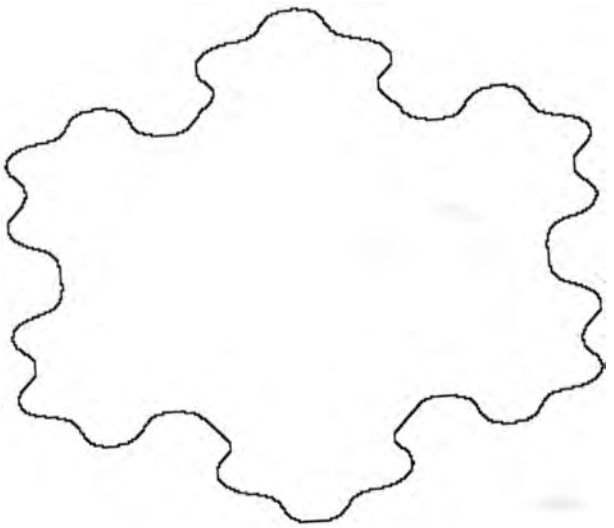

Figure 4(a). Effects of imaging using circular pupil on the object (6th iteration); pupil diameter = 32 pixels. Object used for imaging is that shown in Figure 1.

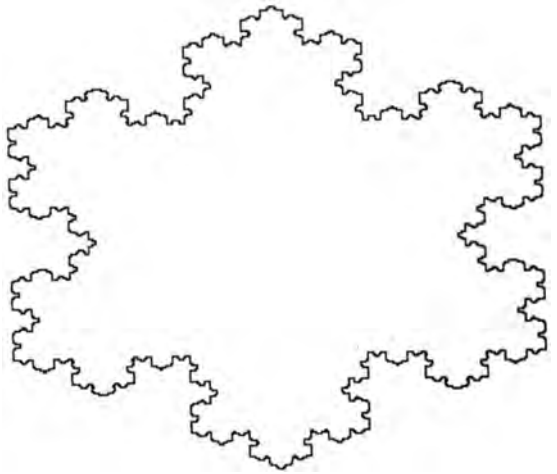

Figure 4(b). Effects of imaging using circular pupil on the object (6th iteration); pupil diameter = 256 pixels. Object used for imaging is that shown in Figure 1.

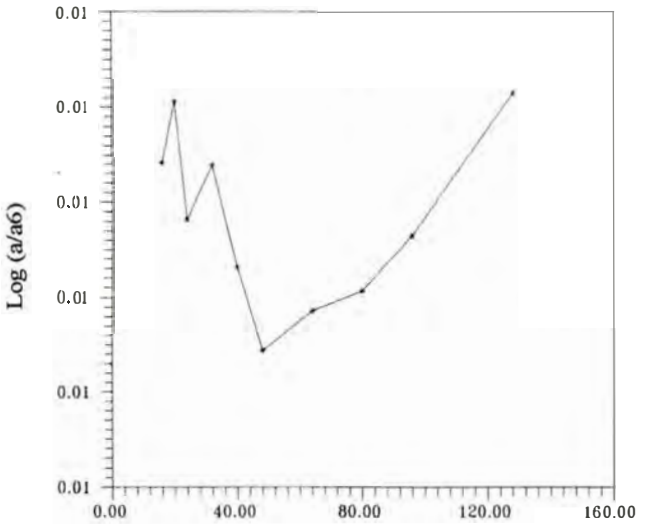

Figure 5(a). Dependence of image area with pupil diameter

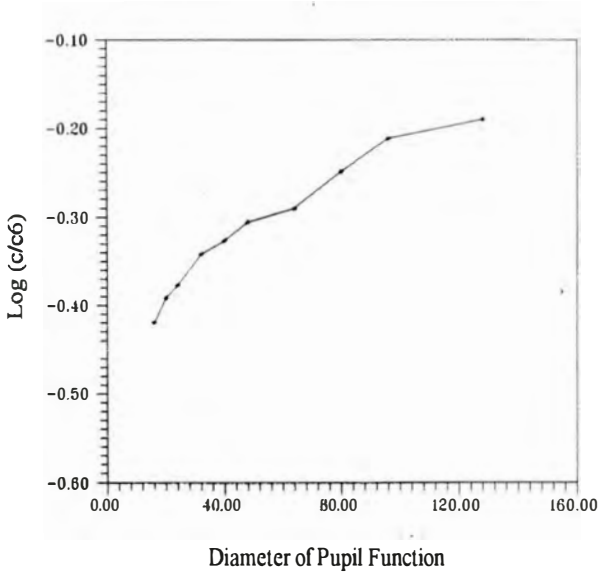

Figure 5(b). Dependence of image circumference with pupil diameter

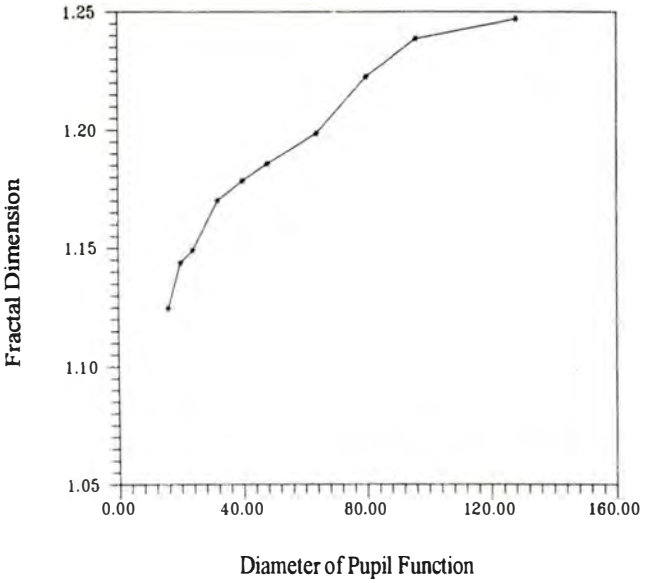

Figure 6. Relationship between image fractal dimension and pupil diameter

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CLONING AND CHARACTERIZATION OF SUCROSE SYNTHASE cDNA FROM RICE SEED

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ABSTRACT

Two partial cDNAs for the rice sucrose synthase 1 (RSs1) were isolated from a cDNA library constructed from developing seeds using a cDNA to the maize Shrunken-1 (Sh-1) gene as a probe. These two cDNAs were fused at an internal Sac I site to produce a complete cDNA for the enzyme which was 2627 nucleotides (nts) long with an open reading frame 2424 nts long and coded for 808 residue peptide. High homology (94%) to the maize Sh-1 gene, in addition to some preliminary expression data, indicates that this cDNA represented an endosperm-specific form of the enzyme in rice. This endosperm specific sucrose synthase cDNA will permit elucidation of the role of sucrose synthase in starch biosynthesis in the developing rice seed. Strategies for doing this are discussed briefly in the text.

INTRODUCTION

Starch is the predominant form of carbon and the major determinant of grain yield in rice and other cereals. Since sucrose is the primary form of translocated carbon in higher plants, the ability of the developing seed to cleave sucrose and use it to synthesize starch can be a measure of "sink" strength, i.e., the ability of the seed to synthesize starch as reserve carbohydrate. Sucrose-starch transformation is an important theme of plant biochemistry. Yet, little is known about the molecular mechanisms underlying the seed-specific expression of key genes in starch biosynthesis and how these are coordinated with those of other pathways, especially that of storage protein biosynthesis in the developing rice grain.

The molecular mechanisms of starch biosynthesis in the developing rice grain are not well understood. Recent evidence indicates that sucrose synthase (EC 2.4.1.13, ADP-glucose:D-fructose-α-D-glucosyltransferase), which catalyzes the reversible conversion of sucrose to UDP/ADP-glucose and fructose, could be the major enzyme that provides UDP/ADP-glucose to the growing starch chain in amyloplasts.

Although it is commonly accepted that ADP-glucose pyrophosphorylase (ADPGP) is an important regulatory enzyme in the biosynthesis of starch in plants (Okita, 1992; Smith and Denyer, 1992; Kleczkowski et al., 1991), recent evidence shows that sucrose synthase could be the key enzyme in starch biosynthesis in the amyloplast (starch synthesizing plastids). ADP-glucose, the product of ADPGP, is the preferred glucose donor to the growing starch chain. Akazawa's lab in Nagoya has shown: (a) the presence of an adenylate transporter in the inner membrane of amyloplasts from Sycamore cells (*Acer pseudoplatanus*) that is capable of transporting ADP-glucose and is tightly linked with starch biosynthesis (Ngernprasirtsiri et al., 1989; Pozueta-Romero, et al., 1991a 1991b); and (b) that ADP-coupled cleavage of sucrose by sucrose synthase could be a major source of ADP-glucose in the cytosol (Pozueta-Romero, 1991c). The allosteric modulation of sucrose synthase by sucrose is an interesting property of the enzyme that could play a key regulatory role in sucrose to starch transition.

Sink strength is acknowledged to be important in determining yield in plant crops. As translocated sucrose arrives at the recipient cell sink, sucrose is fed into the metabolic pathways by either sucrose synthase or invertase (Cronshaw et al., 1986). Studies on certain plant sinks (potato tuber, lima bean seed) show that sucrose synthase is the dominant sucrose cleavage enzyme rather than invertase (Sung et al., 1989; Huber and Akazawa, 1986). Therefore, sucrose synthase could be a biochemical index and determinant of sink strength in the developing rice seed.

The key roles of sucrose synthase in starch biosynthesis and as a biochemical determinant of sink strength emerging from this evidence, point to the need of using molecular techniques to elucidate the role of this enzyme. Thus, it is proposed that these two important roles be elucidated by blocking the expression of the sucrose synthase gene in a constitutive and endosperm-specific manner by transformation of antisense sucrose synthase DNA constructs. Likewise, the expression of the gene could possibly be increased by using the strong cauliflower mosaic virus (CaMV) 35S promoter. The phenotypes induced by these transformations could then be observed. As a step toward this, the cloning and characterization of the sucrose cDNA from rice are reported, as is the construction of sense and antisense DNA to be used for transformation.

MATERIALS AND METHODS

cDNA Library and Screening

A λgt 10 cDNA library made from the milky stage rice seeds (gift from Dr. Susan Wessler) was screened using a cDNA for the maize shrunken -1 (Sh-1) as a probe (Gupta et al., 1988). After screening more than 4×10^4 plaques, 12 positive clones were isolated, 5 were plaque purified and the Eco RI inserts of the largest

two, F1 and G1a, were subcloned into the pBluescript vector (Stratagene) and sequenced. All other common recombinant DNA techniques were done according to Sambrook et al. (1989).

DNA Sequencing and Computing Program

The dideoxy chain termination method of Sanger et al. (1977) was employed, using the Sequenase kit from US Biochemical Corporation. The DNA data were analyzed using the Intelligenetics program at the ENZYME/ARGUS cluster of the Biochemistry Division, Department of Molecular and Cell Biology, University of California at Berkeley.

RNA Preparation and RNAse Protection Assay

Seeds at different stages of development, leaves and roots were collected from IR 36 and BKN rice plants grown in the greenhouse. Total RNA was prepared and Northern analysis was carried out using the procedure of Ausubel et al. (1987). RNAse protection assay was done using the Lysate Ribonuclease Protection Kit from US Biochemical Corporation.

RESULTS AND DISCUSSION

The two largest Eco RI fragments F1 and G1a, obtained from purified plaques, were subcloned and sequenced. They were found to have high sequence homology to the maize Sh-1 gene. F1 contained the 3' 1,842 nts of the coding region and 193 nts of 3' flanking sequences including the TAG and polyadenylation sites. G1a contained the 5' 2379 nts of the coding region including the translation start site and 10 nts of the 5' flanking region. These cDNAs shared a 100% homologous overlap of 1799 nts corresponding to nts 1-1799 of F1 and nts 591-2389 of G1a. Beyond this point F1 continued to code for a sucrose synthase protein while G1a contained the first 63 nts of intron 15 of rice RSs1 gene described by Wang et al. (1992).

A complete cDNA for sucrose synthase was constructed from F1 and G1a by fusing the 5' 700 nts of G1a to the 3' 1927 nts of F1 at a Sac 1 site located at nt 698 in G1a and nt 108 of F1. This complete cDNA was 2627 nts long and it contained an open reading frame of 2424 nts coding for a protein of 808 amino acids. The sequence of this cDNA and the predicted protein sequence it encodes are presented in Figure 1.

A comparison of the predicted protein sequence with that of other sucrose synthase genes showed that it had the highest homology (94%) with the endosperm-specific maize enzyme coded by the Sh-1 gene (MSs1) and was essentially identical to the coding regions of RSs1 (Wang et al., 1992). This information, together with

preliminary Northern blot data, indicates this cDNA represents the endosperm-specific form of sucrose synthase, RSs1, in rice seed. An alignment of the two proteins RSs1 and MSs1 appears in Figure 2. This sucrose synthase is distinct from RSs2 reported by Yu et al. (1992), the tissue-specificity of which has yet to be established.

Expression of Sucrose Synthase in Various Organs of Rice Plant

Figure 3 shows the Northern analysis on the leaf, stem and seeds of variety IR36 and variety BKN, which is submergence tolerant. Expression in BKN seed was higher than that in IR36 (all lanes contained 20 ug total RNA). In IR 36, expression was strongest in the seed (a mixture of different stages) followed by the stem and a very weak expression in the root. It is interesting that although IR 36 leaf showed no expression of sucrose synthase, the leaf in submergence tolerant BKN showed some expression, although it was weaker than that of the seed.

This was confirmed in another Northern analysis done on BKN shown in Figure 4. Increasing expression was evident from early through late maturation seed and the extent of expression in the leaf was about equal to that of the mid-maturation seed. Anaerobic stress induces increased expression of MSs1 RNA in maize (Springer et al., 1986) and sucrose synthase RNA in potato (Salanoubat and Belliard, 1989). The expression of RSs1 in the seed, as well as in the leaf of the submergence tolerant BKN, is interesting, and sucrose synthase could be one of many enzymes induced in the leaf in response to submergence.

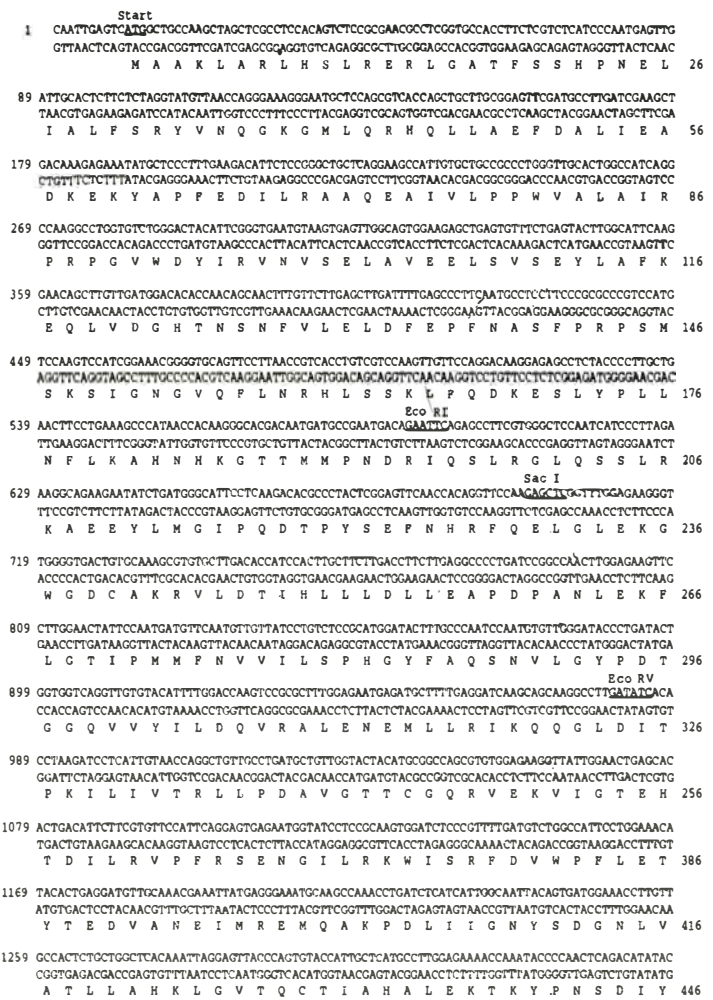
Construction of Constitutive Sense and AntiSense Sucrose Synthase

To elucidate the role of sucrose synthase in starch biosynthesis in rice endosperm, it is proposed that various constructs in the sense and antisense direction driven by the strong plant constitutive promoter CaMV 35S and by the endosperm specific glutelin promoter (Kim and Wu, 1990) be transformed into rice. Polyadenylation signals are provided by the nopaline synthase DNA 3' end (NOS) in all six constructs shown in Figures 5A and B, which have been constructed and sequenced and which are expected to drive the synthesis of sense and antisense sucrose synthase RNA in all organs of the rice plant. Constructs C and D are similar to A and B except that the glutelin promoter had been inserted into the -90 site of CaMV 35S which provides the transcription initiation site. These constructs are designed to drive the strong synthesis of sense and antisense RNA in the endosperm. Insertion of a cotyledon specific soybean β-conglycinin promoter into CaMV35S imparts seed specific enhancement to the constitutive promoter when transformed into tobacco plants (Chen et al., 1988). Constructs E and F are driven by the glutelin promoter with RSs1 coding region in the sense and antisense orientation. These constructs are designed to test if the glutelin promoter, which is derived from one of about 10 genes in a small multigene family coding for the rice main storage protein (Kim and Wu, 1990; Takaiwa et al., 1991), could cause the blocking or enhancement of sucrose synthase expression in the rice endosperm.

ACKNOWLEDGMENT

We acknowledge with appreciation the rice cDNA library from Dr. Susan Wessler, University of Georgia; the maize Sh-1 cDNA from Dr. Prem Chourey, University of Florida; and the rice glutelin promoter from Dr. Ray Wu, Cornell University. We especially thank Dr. Gurdev Khush of IRRI for his support and encouragement, and for providing seeds of IR36 and BKN.

Figure 1.



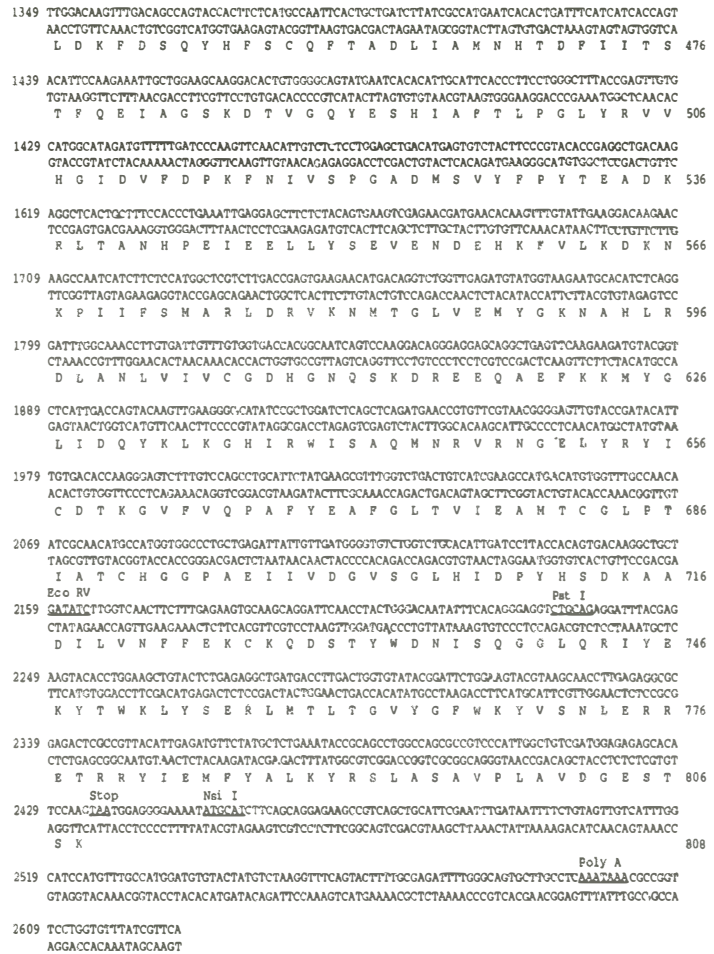


Figure 1. **Sequence of the cDNA for RSs1 and its predicted protein sequence. Important features are underlined. The nucleotide sequence reported here appears in the EMBL Database under the accession number Z15028.**

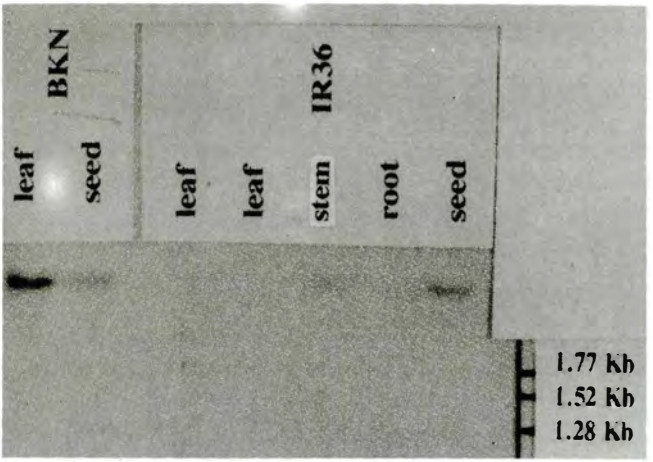


Figure 3. Northern blot analysis of RNA from various organs of rice varieties IR36 and BKN. Each lane contained 20 ug of total RNA and probed with a 2.0 Kb Eco RI fragment of F1.

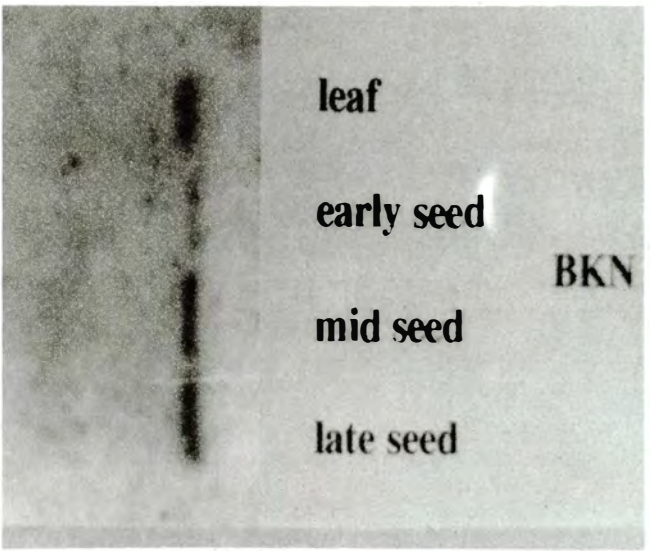


Figure 4. Northern blot analysis of seed and leaf RNA from rice variety BKN. Each lane contained 20 ug of total RNA and was probed with a 2.2 Kb Pst I fragment of the RSs1 cDNA.

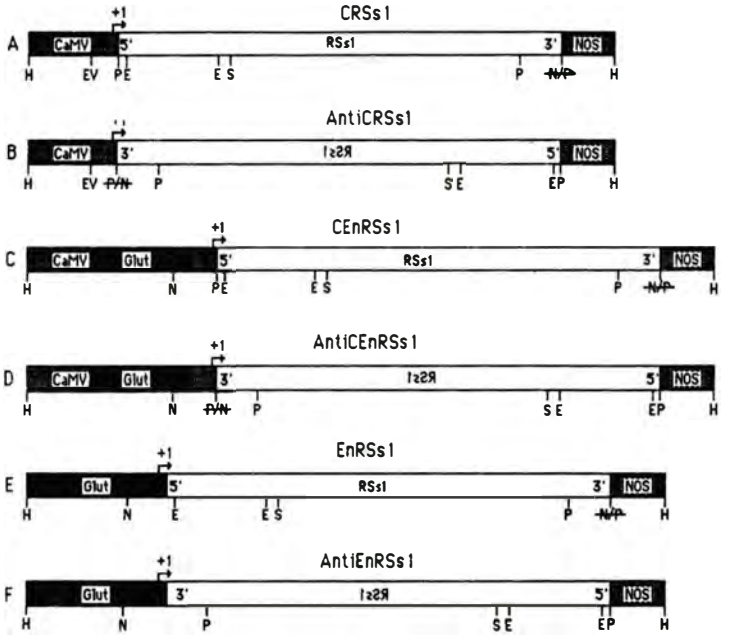


Figure 5. **Proposed DNA constructs for stable transformation into rice to elucidate the role of sucrose synthase in starch biosynthesis in the rice plant and developing endosperm. The coding regions (open) contain the full coding region for sucrose synthase 1 in the sense (RSs1) or antisense (1sSr) orientation. The polyadenylation signal is provided by the nopaline synthase gene (NOS) 3' end in all constructs (hatched). Labeled restriction sites are: Eco RI, E; Eco RV, EV; HinD III, H; Nsi I; N; Pst I, P; Sac I, S.**

- **A. CRSs1. RSs1 sense strand driven by the cauliflower mosaic virus (CaMV) 35S constitutive promoter**
- **B. AntiCRSs1. RSs1 antisense strand driven by CaMV 35S**
- **C. CEnRSs1. RSs1 sense strand driven by a chimeric promoter composed of the endosperm specific elements of the rice glutelin promoter (black) inserted into the -90 site of CaMV 35S which provides the transcription initiation site**
- **D. AntiCEnRSs1. RSs1 antisense strand driven by the same promoter as C above**
- **E EnRSs1. RSs1 sense strand driven by full length glutelin promoter (black)**
- **F. AntiEnRSs1. RSs1 antisense strand driven by same promoter as E above.**

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CONSTRUCTION OF ALL CAYLEY ALGEBRAS OF ORDER 2^r BY THE ZSM PROCESS

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ABSTRACT

The existence of Cayley Algebras of order 2^r is established by construction. These are real division algebras which include the real numbers R (order 2^0), the complex numbers C (order 2^1) and the quaternions H (order 2^2) all of which are associative - and the Cayley numbers O (Order 2^3) which are nonassociative. This paper shows that all of these real division algebras have a common structure exemplified by the Cayley numbers and they all belong to a single family composed of classes of Cayley algebras of order 2^r, where r is any positive integer. This is done by introducing the ZSM Process to construct all of these algebras.

INTRODUCTION

In 1845 A. Cayley constructed a remarkable *real division algebra* of order 8 (now known as the *Cayley numbers* O) which is *nonassociative, noncommutative, normed* and contains as subalgebras the *quaternions* H (order 4), the *complex numbers* C (order 2) and the *real numbers* R (order 1) itself. G. Frobenius proved in 1878 that the only real associative division algebras of finite order are H, C and R, all of which are normed. Attempts to determine other normed real algebras of finite order led A. Hurwitz in 1898 to the theorem that the only algebras of this type are of orders 1, 2, 4 or 8. In 1947, A. Albert showed that these are again R, C, H and O. Then in 1957, R. Bott and J. W. Milnor finally proved that the only finite dimensional real division algebras are of orders 1, 2, 4 and 8. Pursuing more general considerations, L. Dickson introduced in 1923 a general method (called the *Cayley-Dickson Process*) and used it to construct the class of order 8 real division algebras which includes O as its prototype. This paper shows that all real division algebras of order 2^r (like R, C, H and O) belong to a family of classes of *Cayley algebras of order* 2^r. This interesting family is constructed by introducing the ZSM Process thereby proving the theorem that: *There exists a class of Cayley algebras of every oder* 2^r, *where r is any positive integer.*

DIVISION ALGEBRAS OF ORDER 2^r

Consider the algebra $A_r = \{V, F; +, x, \otimes, \otimes,.\}$ over the field $F = R$ of real numbers. Take as the basis of the n-dimensional vector space V the set $E_n = \{e_i/i=1,\ldots,n\}$ of n *basis vectors* over which the binary operation $\otimes$ is defined by the $\otimes$-*matrix* $M_r(E_n) = (m_{ij})$, i,j = i,...,n, where

$$m_{ij} = e_i \otimes e_j = z_{ij} \cdot e_k \qquad \text{Eq. (1)}$$

$e_i, e_j, e_k \in E_n$, and $z_{ij} \in F$. Every vector of this algebra can be expressed uniquely as a linear combination of the n basis vectors in E_n. Thus, if a. $b \in A_r$, then

$$a = \sum_{i=1}^{n} a_i \cdot e_i \quad \text{and} \quad b = \sum_{j=1}^{n} b_j \cdot e_j \qquad \text{Eq. (2)}$$

where $a_i, b_j \in F$. *Vector multiplication* is defined by *bilinearity* and the matrix $M_r(E_n)$ so that the product $a \otimes b$ of any two vectors a, $b \in A_r$ is given by the expression

$$a \otimes b = \sum_{ij=k}^{n} f_{ij} \cdot (e_i \otimes e_j) = \sum_{ij=k}^{n} f_{ij} z_{ij} \cdot e_k \quad (k=1,\ldots,n) \qquad \text{Eq. (3)}$$

where $f_{ij} = a_i b_j$ and the index ij=k means that the sum is to be extended over all pairs of indices i,j for which the relation holds: $e_i \otimes e_j = z_{ij} \cdot e_k$. This can be expanded into

$$a \otimes b = \sum_{ij=1} f_{ij} z_{ij} \cdot e_1 + \sum_{ij=2} f_{ij} z_{ij} \cdot e_2 + \ldots + \sum_{ij=n} f_{ij} z_{ij} \cdot e_n \qquad \text{Eq. (3.1)}$$

By definition, an algebra A_r over a field F is a *division algebra* if it has a *unity* for vector multiplication and every non-zero vector $a \in A_r$ has a unique *inverse* $a^{-1} \in A_r$, that is, $a \otimes a^{-1} = a^{-1} \otimes a = e_1$, where e_1 is the unity of $\otimes$-multiplication. Such a vector a^{-1} exists in A_r if a vector a*, called the *conjugate* of a, can be defined such that $a \otimes a^* = a^* \otimes a = (N(a) \cdot e_1$, where N(a), called the *norm* of a, is a positive element of the field F. If such a vector a* can be defined in A_r, then $a^{-1} = a^*/N(a)$ fulfills all the requirements of an inverse of $a \in A_r$.

To determine the necessary and sufficient conditions for the inverse a^{-1} of a to exist in A_r, first form the products $a^* \otimes a$ and $a \otimes a^*$ by means of Eq.(3.1). For a* to be the conjugate of a,

$$a \otimes a^* = a^* \otimes a = \sum_{i\,j=k} f_{ij} z_{ij} \cdot e_k = \begin{cases} N(a) \cdot e_1 & \text{if } e_i \otimes e_j = z_{ij} \cdot e_1 \\ \text{zero} & \text{if } e_i \otimes e_j = z_{ij} \cdot e_1 \end{cases} \qquad \text{Eq. (4)}$$

where $N(a) = \sum_{i\,j=1} f_{ij} z_{ij}$ (summed over all i,j for which $e_i \otimes e_j = z_{ij} \cdot e_1$), $f_{ij} = a_i a_j^*$, and a_i, a_j^*, are the field coefficients of a, a*. These equations constitute the necessary and sufficient conditions for a^{-1} to exist in A_r. Any vector a* that satisfies Eq. (4) is a conjugate of a. If $a^* \in A_r$, then it follows that $a^{-1} \in A_r$.

Consider once more the three well known real division algebras: the *Cayley numbers* O (order 2^3), the *quaternions* H (order 2^2) and the *complex numbers* C (order 2^1). Since O contains H and C as subalgebras, then they all share a number of basic properties in common:

1. They all have orders (or dimension) of the form $n = 2^r$, where r= 1,2,3.
2. Their basis vectors $e_i \in E_n$ satisfy the following set of fundamental equations:

$$\begin{aligned} e_i \otimes e_i &= e_i^2 = -e_1 && (\text{if } i \geq 2) \\ e_i \otimes e_1 &= e_1 \otimes e_j = e_i && (\text{for all } i) \\ e_i \otimes e_j &= -e_j \otimes e_i && (\text{if } i \neq j,\ i,j \geq 2) \end{aligned} \qquad \ldots\ldots \text{ Eq. (4)}$$

3. Any vector $a = a_1 e_1 + a_2 e_2 + \ldots + a_n e_n$ $(a \neq O)$ has a conjugate a*, a norm N(a), and an inverse a^{-1} given by

$$a^* = a_1 e_1 - (a_2 e_2 + \ldots + a_n e_n) \qquad \text{Eq. (5)}$$

$$N(a) = a_1^2 + \ldots + a_n^2 \qquad \text{Eq. (6)}$$

$$a\text{-}1 = \frac{a^*}{N(a)} \qquad \text{Eq. (7)}$$

These properties are clearly exhibited by the matrix $\mathfrak{M}_3$ shown in Figure 1 which defines an algebra $\mathfrak{U}_3$ isomorphic to O. Here, the submatrices $\mathfrak{M}_1$ and $\mathfrak{M}_2$ define algebras isomorphic to C and H, respectively. Moreover, if the sign coefficients of the entries of $\mathfrak{M}_3$ are separated into another matrix Z_3 (E_8), then the resulting matrix $S_3(E_8)$ can be seen to have the structure of the Klein group $<E_8; o>$ of order $n = 2^3$ shown in Figure 2. Note that $\mathfrak{M}_1$, $\mathfrak{M}_2$ and $\mathfrak{M}_3$ have the form $\mathfrak{M}_r = (m_{ij})$, where $m_{ij} = e_i \otimes e_j = z_{ij} \cdot e_k$, e_i, e_j, e_k represents basis vectors, and $z_{ij} = \pm 1$ are sign coefficients. This means that $\mathfrak{M}_r$ is simply the matrix representation of Eq. (4), where r = 1, 2, 3. These equations, however, do not completely define the operation $\otimes$ over the basis vectors in E_n. Rather, they constitute a set of *necessary conditions* that define a class of algebras of which the Cayley numbers are the protoype

The conditions given by Eq. (4) for any $n = 2^r$, where r is any positive integer can be generated to form a $\otimes$-matrix $M_r(E_n)$ such that Eqs. (5), (6) and (7) hold. To do this, introduce two special matrices $Z_r(E_n)$ and $S_r(E_n)$ of the same dimensions nxn which shall be called the *sign matrix* and *structure matrix,* respectively. The sign matrix is defined as: $Z_r(E_n) = (z_{ij})$, i, j = 1,...,2^r, where $z_{ij} = \pm 1 \in F$ (the real numbers +1 and −1). On the other hand, the structure matrix is defined as: $S_r(E_n) = e_{ij}$), i,j=1,...,2^r, where $e_{ij} = e_i \circ e_j$, which defines the *abelian p-group* $\prec E_n, \circ \succ$ of order 2^r (where $e_i^2 = e_1$ for all $e_i \in E_n$ and e_1 is the identity element) which shall be called the *Klein group* of order 2^r. Next, introduce the *star product* * of any two n X n matrices $A = (a_{ij})$ and $B = (b_{ij})$, as the n X n matrix $A*B = (c_{ij})$, i,j=1,...,n, where $c_{ij} = a_{ij}b_{ij}$. Now, form the star product of $Z_r(E_n)$ and $S_r(E_n)$ obtaining: $Z_r(E_n)*S_r(E_n) = (c_{ij})$, i,j=1,...,$2^r$, where $c_{ij} = z_{ij} \cdot e_{ij} = z_{ij} \cdot (e_i \circ e_j)$. If we let $Z_r(E_n)*S_r(E_n) = M_r(E_n)$ and set $m_{ij} = c_{ij}$. Then write:

$$\left.\begin{aligned} M_r(E_n) &= Z_r(E_n)*S_r(E_n) = (m_{ij}) \\ m_{ij} &= e_i \otimes e_j = z_{ij} \cdot (e_i \circ e_j) \end{aligned}\right\} \quad \text{Eq. (8)}$$

This matrix $M_r(E_n)$ defines the operation '$\otimes$' over the elements of the set E_n.

Consider the matrices $Z_3(E_8)$ and $S_3(E_8)$ shown in Figure 2. If their star products, $Z_9(E_8)*S_3(E_8) = M_3(E_8)$ is formed, one finds that $M_3(E_8)$ has the same structure as $\mathfrak{M}_3(E_8)$. Moreover, if the submatrices $M_2(E_4) = Z_2(E_4)*S_2(E_4)$ and $M_1(E_2) = Z_1(E_2)*S_1(E_2)$ are similarly formed, one observes that they are also structurally similar to $\mathfrak{M}_2(E_4)$ and $\mathfrak{M}_1(E_2)$, respectively. This shows that all of the real division algebras C, H and O can be defined by $\otimes$-matrices of the type $M_r(E_n)$ defined by Eq. (8), where $n = 2^r$. Note that the Z-matrix $Z_3(E_8)$ shown in Figure 2(a) satisfies the following equations: $M_r(E_n)$ such that Eqs. (5), (6) and (7) hold. To do this, introduce two special matrices $Z_r(E_n)$ and $S_r(E_n)$ of the same dimensions n x n which are the *sign matrix* and *structure matrix,* respectively. The sign matrix

$$z_{ii} = -1 \text{ (if } i \geq 2)$$
$$z_{i1} = z_{1i} = +1 \text{ (for all i)} \ldots\ldots\ldots\ldots \quad \text{Eq.(9)}$$
$$z_{ij} = -z_{ji} \text{ (if } i \neq j,\ i, j \geq 2)$$

which simply corresponds to the sign coefficients of Eq. (4). The structure matrix S_3 (E_8), on the other hand, defines the Klein group $<E_8 ;o>$ of order n = 8. This group contains the Klein group $<E_4 ;o>$ and $<E_2 ;o>$ as subgroups which are defined by the submatrices $S_2(E_4)$ and $S_1(E_2)$, respectively. Thus, as noted earlier, the real division algebras O, H and C have a common *substratum:* the Klein group of order $n = 2^r$.

It is clear from the above discussions that the construction of the ⊗-matrix $M_r(E_n) = Z_r(E_n)*S_r(E_n)$ satisfying Eq. (4) can be carried out for any value of $n = 2^r$, where r is any positive integer. Such a matrix, in turn, can be used to construct a real division algebra A_r of order $n = 2^r$ which we shall call a **Cayley algebra of order** 2^r. In such an algebra any vector $a \neq O$ has a conjugate a* of the form given by Eq. (5), a norm N(a) given by Eq. (6) and an inverse a^{-1} given by Eq. (7).

THE ZSM PROCESS

To construct the ⊗-matrix $M_r \equiv M_r(E_n)$, first form a sign matrix $Z_r \equiv Z_r(E_n)$ that satisfies Eq. (9). Note, however, that there are many such sign matrices $Z_{r,k} \equiv Z_r(E_n)_k$ that satisfy these equations. Using Eq. (9), write: $Z_r = Z_{r(+)} + Z_{r(-)}$, where $Z_{r(+)}$ is symmetric while $Z_{r(-)}$ is skew. The skew matrix $Z_{r(-)} = (z_{ij})$ is such that $z_{ij} = -z_{ji}$ if $i \neq j$ and $i, j \geq 2$; otherwise $z_{ij} = 0$. Because of this the set $\mathcal{Z}_r$ of all Z-matrices $Z_{r,k}$ satisfying Eq. (9) has exactly $N(\mathcal{Z}_r) = 2^m$ distinct elements, where $m = \sum_{i=2}^{n-1} (n-1)$ and hence $k=1,\ldots,2^m$ With the aid of Eq. (8), these 2^m matrices $Z_{r,k} \in \mathcal{Z}_r$ can be used to construct 2^m ⊗-matrices of the form

$$M_{r,k} = Z_{r,k} * S_r \qquad (k = 1,\ldots,2^m), \qquad \text{Eq. (8.1)}$$

where $S_r = S_r(E_r)$ defines the Klein group $<E_n ;o>$ of order 2^r. These 2^m matrices $M_{r,k}$ form a set $\mathcal{M}_r$. Call this method of construction the ZSM Process.

Every $M_{r,k} \in \mathcal{M}_r$ defines a real division algebra $A_{r,k}$ of order $n = 2^r$. Hence, there are 2^m algebras of this type forming a set $\mathcal{A}_r$ which defines the class $\mathcal{C}[\mathcal{A}_r]$ of *Cayley algebras of order* 2^r. These 2^m algebras, however, are not all distinct. Since $M_{r,k} = Z_{r,k}*S_{r,k}$ defines ⊗ over E_n, then if P_π is an n X n permutation matrix associated with the permutation π on the n numerals $1,\ldots,n$ representing the n rows/columns of $Z_{r,k}$ it follows that the algebra $A^{(\pi)}_{r,k}$ defined by

$$M^{(\pi)}_{r,k} = (P_\pi Z_{r,k} P_\pi)*S_r = Z^{(\pi)}_{r,k} *S_r \qquad \text{Eq. (10)}$$

is isomorphic to the algebra $A_{r,k}$ defined by $M_{r,k}$, that is $A^{(\pi)}_{r,k} \cong A_{r,k}$. This isomorphism is determined by the one-to-one correspondence to the

$$\begin{array}{lccccccc} A_{r,k}: & 1 & 2 & 3 & \ldots & i & \ldots & n \\ & \updownarrow & \updownarrow & \updownarrow & & \updownarrow & & \updownarrow \\ A^{(\pi)}_{r,k}: & \pi & \pi 2 & \pi 3 & \ldots & \pi i & \ldots & \pi n \end{array}$$

of the elements of their sets of basis vectors, where there are set $i = e_i$ and $\pi i = e_{\pi i}$ for simplicity. Although there are n! possible n×n permutation matrices P_π, only (n-2)! of these preserve the form of Z_r under the transformation: $Z_r \rightarrow Z^\pi_r = P_\pi Z_r P_\pi$. Thus, given any matrix $Z_{r,k} \in \mathcal{Z}_r$, there are also (n-2)! matrices $M^{(\pi)}_{r,k} \in \mathcal{M}_r$ that are *structurally equivalent* to $M_{r,k}$ and which define isomorphic algebras $A_{r,k}$. Hence, the Set $\mathcal{A}_r$ has at most $2^m/(n-2)!$ non-isomorphic (or distinct) Cayley algebras of order 2^r. Some of these algebras can also be obtained by the so-called *Cayley-Dickson Process* and are called *Cayley-Dickson Algebras*. The ZSM Process, on the other hand, enables one to obtain all of the 2^m members of the class $\mathcal{C}[\mathcal{A}_r]$ of Cayley algebras of order 2^r, where r is any positive integer. Thus, the following important

Theorem. There exists a class of Cayley algebras of every order 2^r, where r is any positive integer.

Every algebra $A_{r,k}$ in the class $\mathcal{C}[\mathcal{A}_r]$ contains a series of r-1 sub-algebras of orders $2^1, 2^2, \ldots, 2^{r-1}$ which belong respectively to the classes $\mathcal{C}[\mathcal{A}_1], \mathcal{C}[\mathcal{A}_2], \ldots, \mathcal{C}[\mathcal{A}_{r-1}]$. This means that $\mathcal{C}[\mathcal{A}_r]$ contains all of these smaller r-1 classes as subclasses in which each class $\mathcal{C}[\mathcal{A}_x]$ is contained in the next larger class $\mathcal{C}[\mathcal{A}_{x+1}]$. In general, since r is any positive integer, then there is an infinite number of classes which form an ascending series:

$$\mathcal{C}[\mathcal{A}_1] < \mathcal{C}[\mathcal{A}_2] < \ldots < \mathcal{C}[\mathcal{A}_x] < \mathcal{C}[\mathcal{A}_{x+1}] < \ldots < \mathcal{C}[\mathcal{A}_r] < \ldots$$

This infinite series constitutes the Cayley family of real division algebras in which each class $\mathcal{C}[\mathcal{A}_x]$ determines a subfamily consisting of the finite ascending series: $\mathcal{C}[\mathcal{A}_1] < \mathcal{C}[\mathcal{A}_2] < \ldots < \mathcal{C}[\mathcal{A}_x]$.

The class $\mathcal{C}[\mathcal{A}_1]$ contains only $2^0 = 1$ member A_1 which is isomorphic to the complex numbers C. $\mathcal{C}[\mathcal{A}_2]$ has $2^3 = 8$ members of which only four are nonisomorphic. On the other hand the class $\mathcal{C}[\mathcal{A}_9]$ has 2^{21} members all of which are nonassociative; at least 720 of these are isomorphic to the Cayley numbers O. Any algebra belonging to a class $\mathcal{C}[\mathcal{A}_r]$ in which $r \geq 2$ is noncommutative. And if $r \geq 3$, it is always nonassociative.

To illustrate the construction of Cayley algebras of order 2^r by the ZSM Process, consider the case of the 2^m algebras $A_{2,k}$ where $r = 2$ and $n = 4$. Here, $m = \sum_{i=2}^{3} (4\text{-}i) = 3$ and $N(Z_2) = 2^3 = 8$. Figure 3 shows the eight matrices $Z_{2,k}$ $(k=1,\ldots,8)$ which, together with the matrix S_2 shown in Figure 2(b), are used to form the matrices $M_{2,k}$ $(k=1,\ldots,8)$ shown in Figure 4. These matrices can be used to construct eight Cayley algebras $A_{2,k}$ of order $2^2 = 4$ forming the set $\mathcal{A}_2$ which defines the class $\mathcal{C}[\mathcal{A}_2]$. It can be shown that $A_{2,3} \cong A_{2,7}$, both of which are associative and $A_{2,1} \cong A_{2,5}$, $A_{2,2} \cong A_{2,6}$, $A_{2,4} \cong A_{2,8}$ all of which are nonassociative. The smallest nonassociative real division algebras are therefore of order $2^2 = 4$. Note that if the permutation matrix P_α represents the permutation $\alpha = (23)$ on the numerals 1234 representing the 4 rows/columns of $Z_{2,3}$, then $M^{(\alpha)}_{2,3} = (P_\alpha Z_{2,3} P_\alpha)^* S_2 = M_{2,7}$. Hence, $A_{2,3} \cong A_{2,7}$. In fact it can be shown that both $A_{2,3}$ and $A_{2,7}$ are isomorphic to the algebra Q of quaternions. Also, of the eight algebras in A_2, only $A_{2,3}$ and $A_{2,7}$ are associative and normed.

As a final example, Figure 5 shows the matrix $M_{4,p}$ which defines the Cayley algebra $A_{4,p}$ of order $n = 2^4 = 16$ belonging to the class $\mathcal{C}[\mathcal{A}_4]$. This is a real division algebra containing O, H and C. It is nonassociative and noncommutative, and it is not normed.

The Cayley algebras of order 2^r, where $r \geq 3$, are not just curiosities but they have important applications in both pure and applied mathematics. Thus, Eric Temple Bell remarked: "In passing, it seems rather remarkable that such a truncated algebra as [that of the Cayley numbers] could have any physical significance, but it has been applied to the quantum theory."

SUMMARY

This paper discussed real division algebras and showed that they have a common underlying structure exemplified by the algebra of Cayley numbers. This observation led to the construction of Cayley algebras of order 2^r, where r is any positive integer. In doing this, the ZSM Process was introduced using two special matrices (the sign matrix and structure matrix) to construct another matrix (the x-matrix) that defined the Cayley algebras of order 2^r. These algebras were shown to form a family of classes which established the existence of a class of Cayley algebras of every order 2^r where r is any positive integer.

	1	2	3	4	5	6	7	8
$\mathfrak{M}_1$	2	-1	4	-3	6	-5	-8	7
	3	-4	-1	2	7	8	-5	6
$\mathfrak{M}_2$	4	3	-2	-1	8	-7	6	5
	5	-6	-7	-8	-1	2	3	4
	6	5	-8	7	-2	-1	-4	3
	7	8	5	-6	-3	4	-1	2
	8	-7	6	5	-4	-3	2	1

Figure 1. The ⊗-matrix $\mathfrak{M}_3(E_8) = (m_{ij})$, where $m_{ij} = e_i \otimes e_j = z_{ij}$, which defines the real division algebra $\mathfrak{U}_3$ of order $2^3=8$ isomorphic to the *Cayley numbers*

	+	+	+	+	+	+	+	+
Z_1	+	−	+	−	+	−	+	−
	+	−	−	+	−	+	−	+
Z_2	+	+	−	−	+	−	+	−
	+	−	−	−	−	+	−	+
	+	+	−	+	−	−	−	+
	+	+	+	−	−	+	−	−
	+	−	+	+	−	−	+	−

(a) $Z_3(E_8)$

	1	2	3	4	5	6	7	8
S_1	2	1	4	3	6	5	8	7
	3	4	1	2	7	8	5	6
S_2	4	3	2	1	8	7	6	5
	5	6	7	8	1	2	3	4
	6	5	8	7	2	1	4	3
	7	8	5	6	3	4	1	2
	8	7	6	5	4	3	2	1

(b) $S_3(E_8)$

Figure 2. (a) $Z_3(E_8) = (z_{ij})$, i j = 1,...,8, is a special *sign matrix;* for simplicity, ± = ±1. (b) $S_3(E_8) = (e_{ij}\ i,j=1,\ldots,8$, where $e_{ij} = e_i \circ e_j$ is the *structure matrix* of the Klein group $\langle E_8;\circ\rangle$ or order 8; $v=e_v$.

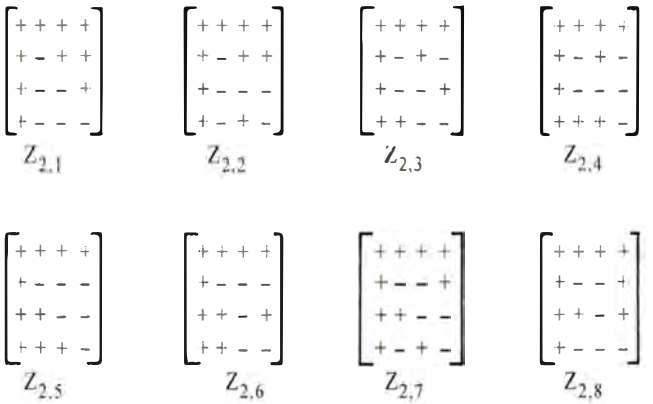

Figure 3. Eight possible Z-matrices $Z_{2,k}$ that can be used to form eight ⊗-matrices $M_{2,k}$ (shown in Figure 4) satisfying Eq. (8)

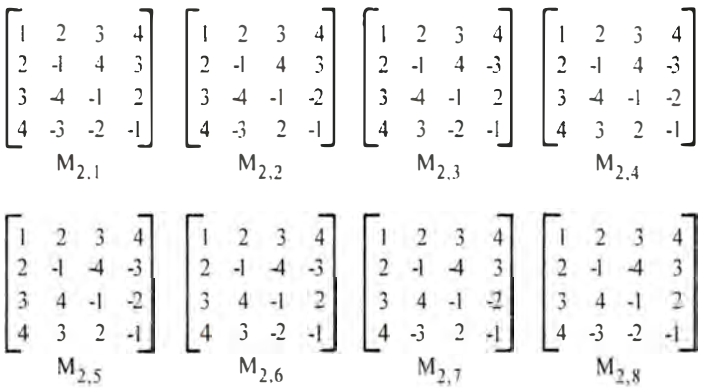

Figure 4. Eight ⊗-matrices $M_{2,k}$ satisfying Eq. (4). Note that $M_{2,3}$ and $M_{2,7}$ are both isomorphic to the algebra Q of quaternions.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
M_1	2	-1	4	-3	6	-5	-8	7	10	-9	12	-11	14	-13	16	-15
	3	-4	-1	2	7	8	-5	-6	-11	12	-9	10	-15	16	-13	14
M_2	4	3	-2	-1	8	-7	6	-5	12	-11	10	-9	16	-15	14	-13
	5	-6	-7	-8	-1	2	3	4	-13	14	-15	16	-9	10	-11	12
	6	5	-8	7	-2	-1	-4	3	14	-13	16	-15	10	-9	12	-11
	7	8	5	-6	-3	4	-1	-2	-15	16	-13	14	-11	12	-9	10
M_3	8	-7	6	5	-4	-3	-2	-1	16	-15	14	-13	12	-11	10	-9
	9	-10	11	-12	13	-14	15	-16	-1	2	-3	4	-5	6	-7	8
	10	9	-12	11	-14	13	-16	15	-2	-1	4	-3	6	-5	8	-7
	11	-12	9	-10	15	-16	13	-14	3	-4	-1	2	-7	8	-5	6
	12	11	-10	9	-16	15	-14	13	-4	3	-2	-1	8	-7	6	-5
	13	-14	15	-16	9	-10	11	-12	5	-6	7	-8	-1	2	-3	4
	14	13	-16	15	-10	9	-12	11	-6	5	-8	7	-2	-1	4	-3
	15	-16	13	-14	11	-12	9	-10	7	-8	5	-6	3	-4	-1	2
	16	15	-14	13	-12	11	-10	9	-8	7	-6	5	-4	3	-2	-1

Figure 5. This ⊗-matrix $M_{4,p}$ defines a Cayley algebra of order n = 16. Note that its submatrices M_3, M_2 and M_1 have the same structures as those of O, H and C.

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LECTINS AND LECTIN RECEPTORS FROM *ACANTHAMOEBA* SP.

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ABSTRACT

Acanthamoeba isolates from soil and water samples have been characterized for the presence of lectins and lectin receptors. Crude soluble protein extracts have been shown to agglutinate neuraminidase-treated and non-treated fixed horse erythrocytes and fixed rabbit erythrocytes. Purification was done using alpha-methyl-mannoside-agarose column, with a protein peak that showed one band in native PAGE and two bands in SDS-PAGE slightly above the 66 Kd region. Con A-FITC stained the plasma membrane, and binding was inhibited by alpha-methyl-mannopyranoside. By Con-A blotting of the soluble and membrane-bound proteins on nitrocellulose, molecular weights of the glycoconjugates were determined.

INTRODUCTION

The increasing frequency of chronic amebic keratitis among contact lens wearers and a number of cases of granulomatous amebic encephalitis infections have led to the cell and molecular research of the bioactive molecules in *Acanthamoeba* (Ma et al., 1990; Matias, et al., 1991). A significant class of recognition molecule called lectins has been the subject of studies in many biological systems including the parasitic protozoa, bacteria, viruses, plants and higher animals (Sharon and Lis, 1989).

Lectins are proteins or glycoproteins of non-immune origin that agglutinate cells and precipitate complex carbohydrates or both (Goldstein, et al., 1980). Lectins have been implicated in many biological activities. For instance, the phagocytosis of mammalian erythrocytes and yeasts by *Acanthamoeba castellanii* has been shown to be mediated by the mannose-sensitive, carbohydrate-binding sites on the amoeba cell surface (Brown et al., 1975; Allen and Dawidowicz, 1990). So far, glycoproteins have been detected in the plasma membrane of *A. castellanii* (Paatero and Gahmberg, 1988). But the precise nature of this lectin-like activity in *A. castellanii* has yet to be understood.

Are these suspected carbohydrate-binding sites on *Acanthamoeba* cell surface which may be present in the cytoplasm indeed lectins? Hence, there is a need to characterize this kind of recognition molecules in *Acanthamoeba*. Moreover, the detection and characterization of the endogenous lectin receptors have been shown to serve as indirect evidence of the occurrence of lectins in *Naegleria* (Matias et al., 1990). Likewise, studies on lectin receptors may provide important clues and advance our present knowledge on the biological functions of lectins in nature (Rademacher et al., 1988).

The present study endeavors to: 1) isolate and characterize lectins from the Philippine *Acanthamoeba* isolate; and 2) detect the presence and distribution patterns of endogenous lectin receptors in trophozoites and cysts.

The significance of this work is focused primarily on the biochemical and cytochemical characterization of these ubiquitous free-living amoebae which are potentially pathogenic to man. Among others, it will provide a basis for further studies on the exact nature of the carbohydrate-binding sites of *Acanthamoeba* which have been suggested to act as lectins. Furthermore, the identification of a local source of lectins, which can be mass produced and which can be developed as direct probes for the detection and localization of the cytoplasmic or cell surface glycoconjugates, will be of commercial importance.

MATERIALS AND METHODS

Acanthamoeba isolation and cultivation

The *Acanthamoebae* were isolated from the different water samples at the University of the Philippines, Diliman, Quezon City; Ateneo de Manila University; and Novaliches according to the standard method for isolating these amoebae from natural sources (Page, 1988). The isolation was done by inoculating water filtrate to non-nutrient agar plates lawned with 24-hour culture plasmid free *Escherichia coli* (DH1). The isolates were cloned using a Skermann micromanipulator. The cloned isolates were cultured axenically in Page's protease-peptone-glucose liquid medium (PPG). The cells were harvested after 72 hours incubation (log phase) at 37°C. The cells were washed thrice with amoeba saline (Page, 1988). A final wash was done using 0.1 M Tris-CH1 and the resulting cell pellet was stored at -20°C until use.

Lectins

Protein Extraction. The cells were disrupted using a sonicator. This was done by dipping the probe 10 times in the chilled test tube with the cells in extraction buffer (1 mM Phenyl methyl sulfonyl fluoride, 1 mM dithiotreitol, 1 mM ethylene diamine tetra-acetic acid and 1 mM a-amino hexanoic acid in .1 M Tris-HC1) for 10 sec, after which the cells were checked for 100% cell disruption. The cell lysate was centrifuged at 32,000 rpm for one hour at 4°C using Beckman T1-100 Ultracentrifuge. The supernatant was collected and labeled as soluble cytoplasmic extract and stored at -20° C. The membrane-bound proteins were isolated according to the method used by Matthews et al. (1986). Total protein concentration was determined according to the method of Bradford (1976).

Hemagglutination Assay. Glutaraldehyde fixed and neuraminidase-treated rabbit and horse RBC were used. Five ml of fresh erythrocytes were washed three times in PBS (150 mM NaCl, 10 mM phosphate buffer, pH 7.2) and centrifuged after each washing at 1000 rpm for 5 min and resuspended in the same buffer. Two percent RBC suspension was used in the assay. The extracts were serially diluted with PBS buffer in micro-titer V-plates in a total volume of 100 ul. Twenty-five microliters of the 2% RBC was added to each well and incubated in 37°C for one hour. (If the extract has a hemagglutination activity, a flat carpet of RBC will be formed at the bottom of the plate. In case of negative reaction, a clear red dot will be formed at the center of the plate. The reciprocal of the maximum effective dilution rate was expressed as the titer. Determination of the sugar specificity was performed by serially diluting the sugar in PBS with the extract (already diluted to at least 8 times) before adding the RBC to the hemagglutination mixture.

Affinity chromatography. Cell extracts that showed positive lectin activity were purified using alpha-methyl- mannoside-agarose (Sigma) column. The lectin extract was applied directly to a 5 ml column and washed with PBS. The lectin was eluted with PBS containing 0.2 M of the corresponding carbohydrate. The fractions containing the lectin were pooled, dialyzed against PBS and tested for hemagglutination activity. The purity of the lectin was determined by native PAGE and SDS-PAGE analysis according to the modified method of Laemlli (Matias, 1991).

Lectin Receptors

Lectin blotting. The proteins separated by SDS-PAGE were transferred onto a nitrocellulose membrane (S&S, 0.45 um) using an LKB (2117) Multiphor II Electrophoresis System (1986). After which the strips of nitrocellulose membrane were washed five times for 5 min with PBS (with 0.1% Tween- 20) and blocked with 3% BSA in PBS-Tween 20 for one hour. Blots were washed five times and incubated with 3 ml of peroxidase-labeled lectin (10ug/ml) overnight at 4°C and were washed as above. For the control, the peroxidase-labeled lectin was pre-incubated with the alpha-methyl-mannose at 0.5 M for one hour. The blots were visualized with 10 mg

diaminobenzidine, 0.5 ml 1% $CoCl_2$, 19.5 ml 0.5 M PBS and 50 ul 30% H_20_2 which was added prior to use. After color development the blots were washed with distilled H_20, air dried and photographed immediately.

Lectin fluorescence assay. (Hill et al., 1981). For lectin receptor localization, the cells were cultured in petri dishes for 72 hours. The cells were harvested and then washed with PBS three times. The cells were incubated with fluorescein isothiocyanate (FITC)-labeled lectins (100 ug/ml) for one hour and washed three times. The cells were suspended in 1 ml PBS and mounted onto glass slides and sealed with nail polish. Observations were done using a Carl Zeiss fluorescence microscope.

The control sugar inhibition of lectin-FITC binding to *Acanthamoebae* was done by incubating both lectin and *Acanthamoeba* trophozoites with 100 mM of monosaccharides for 30 min before performing the fluorescence assay.

RESULTS AND DISCUSSION

Soluble protein extracts from 11 strains of *Acanthamoeba spp.* were tested for hemagglutination activity (HA). Strains W_4 and W_3 gave positive agglutination results against 1.5% rabbit erythrocytes. These *Acanthamoeba* strains that were positive for hemagglutination test were the isolates from an artesian well in Novaliches. Cyst morphology, isoenzyme patterns and mtDNA digestion phenotypes showed that these two strains could be of the same species (Natividad et al., 1993, unpublished data) indicating that lectin activity could be used to verify inter-strain characteristics.

The soluble cytoplasmic protein extracts of the W_4 strain tested for HA against neuraminidase treated horse erythrocytes showed strong hemagglutination activity (Fig. 1). Neuraminidase is a hydrolytic enzyme that cleaves the terminal N-acetyl-neuraminic acid (sialic acid), thus, exposing the neutral sugars of the glycoconjugates of the erythrocyte plasma membrane. Lectins that may be specific to these sugars could freely bind and exhibit strong hemagglutination reaction. The presence of lectins, which showed specificity to mannose fructose, was demonstrated in the phagocytosis of horse erythrocytes and yeasts by *A. castellanii* (Brown et al., 1975). The occurrence of intracellular lectins with mannose-receptors in *A. castellanii* has been suggested to mediate the delivery of soluble mannose-rich molecules to the degradative compartments, particularly the lysosomes (Allen and Dawidowicz, 1990). The present work on the local isolation of lectins in *Acanthamoeba* has been based in this study, especially on the purification step. The alpha-methyl-mannoside agarose matrix was used as affinity column.

Successful elution of an *Acanthamoeba* lectin with a protein concentration of 2.87 ug/ml was obtained (Fig. 2). The protein was eluted with 0.2 M alpha-methyl-mannoside. One band was obtained under 6% non-denaturing polyacrylamide gel electrophoresis (Fig. 3A). These data suggest that a homogenous protein was

obtained. Further analysis of the protein by 12% SDS-PAGE electrophoresis revealed two slightly separated bands above the 66 kd region (Fig. 3B). These were suspected to be the subunits of eluted lectin.

The specificity of binding between the Con A-FITC and the cell surface-mannose moieties could demonstrate the topographical distribution of the lectin receptor sites (Stevens and Kaufman, 1974; Gonatas and Avrameas, 1977). Lectin receptor studies using fluorescein isothiocyante-labeled Con A showed fluorescent patches around the plasma membrane and cytoplasm (Fig. 4). The fluorescence could be quenched completely with 0.5M alpha-methyl-D-mannopyranoside. Brighter fluorescence was observed on the sites of adherence between the *Acanthamoeba* (W_4) cysts and trophozoites.

The clustering of lectin receptors at the site of aggregation of cells has been demonstrated at the ultrastructural level using ferritin-labeled Con A by Stevens and Kaufman (1974). High concentration of the conjugate was observed routinely on the membrane areas immediately adjacent to and at contact points of both agglutinated avirulent (*A. castellanii* Neff) and virulent (*A. culbertsoni* A-3) strains; but the presence of an electron dense, amorphous material with associated ferritin-labeled Con A was occasionally found in agglutinated avirulent amoebae. Areas more distal to the contact regions were relatively free of conjugate (Stevens and Kaufman 1974). It was also observed that these lectin receptors are much concentrated at the uroidal region of the trophozoite and they adhere to the substrate, leaving a fluorescent trailing. They are completely absent in the pseudopodia and hyaline cap of the cell (data not shown).

Surface lectin receptors on a *Naegleria philippinensis* were localized using Con A-FITC to be uniformly distributed throughout the surface of the trophozoite, cyst wall and cyst pores. It was suggested that lectins can aid in understanding some of the taxonomic and morphogenetic problems within this group of free-living amoebae (Matias et al., 1990).

For the Western blot analysis of lectin receptors, the soluble cytoplasmic extracts showed three major bands between 29 to 66 kd molecular weights (Fig. 5). On the other hand, the plasma membrane extract also contains three major bands at the 40 to 80 kd molecular weight range. The appearance of these bands supports the occurrence of lectin receptors as evidenced by the strong fluorescence observed upon whole cell incubation with Con A-FITC. If purified and labeled with peroxidase or FITC, it may be used to detect the presence of intrinsic *Acanthamoeba* lectins and demonstrate the lectin-lectin receptor interaction.

Con A-peroxidase detection method was completely inhibited by the addition of 0.5M alpha-methyl-D-mannopyranoside. These lectin receptors are believed to be glyco-conjugates in the form of glycoproteins or glycolipids. The presence of glycoproteins in *Acanthamoeba* plasma membrane was demonstrated using periodate/NaB^3H_4 and galactose oxidase/NaB^3H_4 radiolabeling techniques (Paatero and Gahmberg, 1988). In addition, a diffusely labeled region with Mr of 55,000 to 75,000 seen on electrophoresis was suggested as glycolipids (Paatero, 1989).

Several bands for lectin receptors have been observed in the soluble cytoplasmic extracts of other *Acanthamoeba* strains used such as *A. Lenticulata,* C_{13} (an enviromental strain) and H-1 (pathogenic strain) (Fig. 6). The qualitative and quantitative similarities and differences exhibited in their Con A receptor patterns obtained through the lectin-peroxidase conjugate could serve as "glycogram" of the isolate to a particular lectin (Gonatas and Avrameas, 1986). Similar studies showed that glycoprotein patterns of highly pathogenic and weakly pathogenic *Naegleria fowleri* were distinctly different upon detection with Con A/WGA-peroxidase (Scott et al., 1989). Indeed, the great diversity of carbohydrate structures associated with the soluble and surface-bound glyco-conjugates poses the same diversity and significance in their biological function. The evidences show that carbodydrates may serve as markers of cell differentiation, development, pathological states and potential modification of the activities of proteins to which they are attached (Rademacher et al., 1988).

ACKNOWLEDGMENT

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Figure 1. **Neuraminidase-treated horse erythrocytes strongly agglutinated by the soluble cytoplasmic protein extract from *Acanthamoeba* sp. (W-4). (Bar=20 um)**

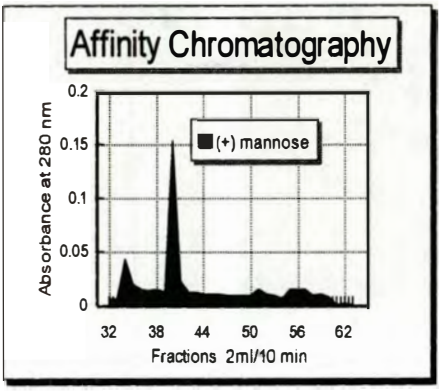

Figure 2. **Protein peak eluted from an affinity column for mannose-binding lectins. Soluble cytoplasmic proteins were loaded and bound proteins were eluted with 0.2 M alpha-methyl-mannopyranoside in PBS (.15 M NaCl, 0.01M phosphate buffer, pll 7.2).**

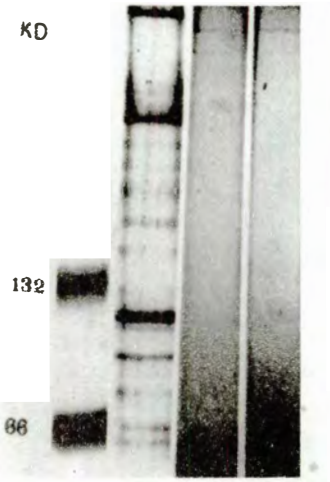

Figure 3A.

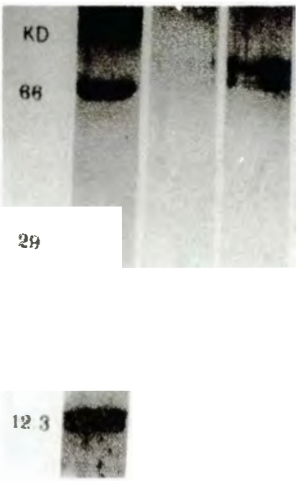

Figure 3B.

Figure 3A. **Native PAGE profile of the *Acanthamoeba* sp. (W-4) protein eluted from mannose agarose affinity column. Lane 1- Molecular weight markers (BSA dimer, 132 kd; BSA monomer, 66 kd); Lane 2-Soluble cytoplasmic protein extract; Lane 3 – Single band of the eluted mannose binding protein of *Acathamoeba* sp. (W-4) (Visualized by silver staining)**

Figure 3B. **SDS-PAGE profile of the protein eluted from mannoside-agarose affinity column. Lane 1- Molecular weight markers (BSA, 66 kd; carbonic anhydrase, 29 kd; lysozyme, 12.3 kd); Lanes 2-3- *Acanthamoeba* sp. (W-4) mannose-binding protein sub-units at the 66-70 kd region (Visualized by silver staining)**

Figure 4A.

Figure 4B.

Figure 4A. *Acanthamoeba* sp. (W-4) trophozoites stained with Concannavalin A-fluorescein isothiocyanate (Con A-FITC) showing the lectin receptors concentrated at the plasma membrane particularly at the point of adhesion between the two cells

Figure 4B. The same cells viewed under phase contrast light microscope (bar = 10 um)

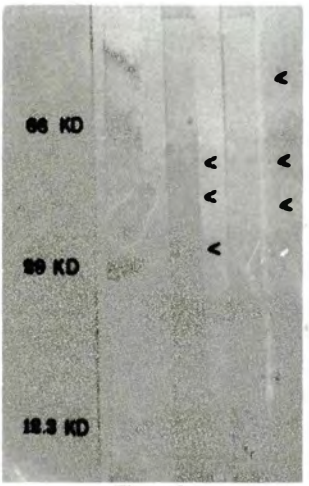

Figure 5.

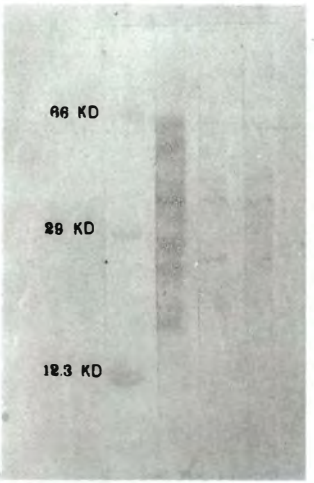

Figure 6.

Figure 5. Lectin (Con A) receptors of the *Acanthamoeba* sp. (W-4) soluble proteins (Lane 2) and plasma membrane proteins (Lane 3) separated by 12% SDS-PAGE visualized by Con A-peroxidase on nitrocellulose membrane; Molecular weight markers stained with India Ink: BSA, 66kd; carbonic anhydrase, 29 kd; lysozyme, 12.3 kd (Lane 1)

Figure 6. Lectin (Con A) receptors of *A. lenticulata*, C-13, and H-1 from the soluble cytoplasmic proteins separated by 12% SDS-PAGE and visualized by Con A-peroxidase on nitrocellulose membrane. Lane 1: Molecular markers; BSA, 66 kd; carbonic anhydrase, 29 kd; lysozyme, 12.3 kd; Lane 2: *A. lenticulata;* Lane 3: C-13 (UP, Diliman); Lane 4:H-1 (Hamburg, Germany)

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MORPHO-HISTOCHEMICAL STUDIES OF COMMONLY USED MEDICINAL PLANTS IN BUKIDNON

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ABSTRACT

This survey on the extent of herbal healing in the province of Bukidnon conducted from May 29, 1986 to January 17, 1987 shows that folk healing with herbal medicine is prevalent. Based on their availability, 20 species were described morphologically and identified, namely: Mangga (*Mangifera indica* L.); Kapayas (*Carica papaya* L.); Gabon [*Blumea balsamifera* (L.) D.C.]; Hilbas (*Artemesia vulgaris* L.); Mansanilya (*Chrysanthemum indicum* L.), Anghelika [*Bryophyllum pinnatum* (Lam.) Kurz]; Busikad (*Cyperus kyllingia Rotto*); Tubatuba (*Jatropha curcas* L.); Kogon (*Imperata cylindrica* L.); Tanglad (*Andropogon citratus* D.C); Mayana (*Coleus blumei* Benth:); Hierba buena (*Mentha cordifolia* Opiz); Abukado (*Persea americana* Mill); Gumamela (*Hibiscus rosasinensis* L.); Bayabas (*Psidium guajava* L.); Kamunggay (*Moringa oleifera* Lam.); Pandan (*Pandanus odoratissimus* L.f.); Buyo (*Piper betle* L.); Sinaw-sinaw (*Peperomia pellucida* L.) and Luy-a (*Zingiber officinale* Rosc.).

Histochemical tests were likewise made from the different organs of the plants to determine the presence and localization of active principles in the plant tissues.

Histochemical findings revealed that the active constituents present in the commonly used medicinal plants were alkaloids, tannin, oxalic acid, formic acid, fats and oils, amygdalin, saponin, arbutin and tartaric acid. These active principles were localized in the epidermis, cortex, vascular bundles and mesophyll of the plant tissues.

The presence of the active constituents was recorded as: 0 = absent; 1 = rare; 2 = abundant; and 3 = very abundant.

INTRODUCTION

Herbal healing in Bukidnon, as learned from the author's personal interviews with the herbolarios, is sometimes strongly associated with superstition. The herbolarios do not have a knowledge of the presence and identity of active principles in plants which are valuable to therapy, as learned from the studies of de Padua et al. (1980) and Gonzales (1981). This is the context in which this study was undertaken.

It is hoped that this work will serve as a potent instrument in disseminating the scientific basis of how plants heal body ailments. Furthermore, it is hoped that this study will help promote the use of herbal medicine and eventually improve the health and economic conditions of the people, especially the poor in the rural areas.

Objectives of the Study

1. To survey, collect, propagate, identify and describe morphologically the commonly used medicinal plants in the province of Bukidnon; and
2. To establish the scientific basis for the medicinal uses of plants by determining the active constituents and their localization within the plant body through histochemical tests

Significance and Limitation of the Study

This study primarily aims to promote the utilization of the common medicinal plants used by herbolarios in some municipalities of Bukidnon and to help establish the scientific basis for the use of these plants. Results of this study might help alleviate health problems, especially among the poor.

Since various surveys on the use of medicinal plants and researches on active principles have been conducted by experts of leading agencies, like the National Science and Technology Authority (NSTA) and the University of the Philippines Los Baños, this study focused on proving the efficacy and acceptability of herbal healing in the province of Bukidnon.

Since it was not possible to conduct a survey of the 22 municipalities of Bukidnon, especially the remote areas or barrios, due to unavailability of transportation and safety considerations, the researcher covered only five towns and six barrios.

MATERIALS AND METHODS

Interviews of herbolarios in several municipalities of Bukidnon were made from May 29, 1986 to January 24, 1987. Data, such as name, age and number of herbolarios interviewed in the municipalities covered, were collected.

The herbolarios were interviewed using guidelines which covered complete information on the uses of medicinal plants. Informal discussions held with herbolarios and data from scientific journals, books and handbooks were used for an assessment of the status of the effectiveness and economic value of medicinal plants of Bukidnon. Likewise, data from periodicals, dictionaries, theses, manuscripts and radio programs were collected.

The medicinal plants commonly used by the herbolarios were: *Coleus blumei* Benth; *Psidium guajava* L.; *Persea americana* Mill.; *Piper betle* L.; *Blumea balsamifera* (L.) D.C.; *Bryophyllum pinnatum* (Lam) Kurz; *Mentha cordifolia* Opiz; *Artemesia vulgaris* L.; *Moringa oleifera* Lam.; *Imperata cylindrica* L.; *Zingiber officinale* (Rosc.); *Chrysanthemum indicum* L.; *Pandanus odoratissimus* L.f.; *Peperomia pellucida* L.; *Andropogon citratus* D.C.; *Jatropha curcas* L., *Carica papaya* L., *Cyperus kyllingia* Rotto, *Hibiscus rosasinensis* L.; and *Mangifera indica* L. Most of these were gathered and planted by the researcher in her residence in Maramag, Bukidnon.

The abundance and availability of these plants in each municipality were the bases of selecting the 20 species of medicinal plants used in this study. Morphological descriptions were made with the use of ruler and meterstick. Photographs of the habitat of the plants were taken.

Free-hand technique was employed on freshly collected young leaves and stems for histochemical tests. Slightly thick sections and the application of minimal appropriate chemical reagents were ideal for the tests.

Photomicrographs were done from suitable specimens which showed the distribution of constituents within the tissues of the plant body. The presence of these constituents was recorded as: 0 = absent; 1 = rare; 2 = abundant; 3 = very abundant.

RESULTS AND DISCUSSION

Histochemical findings showed the presence and the amount of the active constituents which varied in the different tissues of the plant organs (Table 1).

From the foregoing tests, the alkaloids were found to be abundant, followed by oxalic acid, tannin, formic acid, fats and oils, amygdalin, saponin, arbutin and tartaric acid. The alkaloids were the active constituent most common in all species.

The curative values of these medicinal plants for various body ailments were allied and related to one another. In other words, they were associated with the active principles present in each. The findings were similar to the results of the research studies of Quisumbing, Bacalso (1980), Rodriguez (1983), Angeles (1984, Siytangco and Ladion (1985), de Padua et al. (1985) and Ticzon and Baguio 1986.

Herbs have medicinal values. Therefore, they may be as effective or even better than drugs manufactured synthetically, with less side effects or toxic effects on the body.

SUMMARY, CONCLUSION, RECOMMENDATIONS

Morphological descriptions were made and histochemical tests were conducted on 20 species of commonly used plants chosen from among 87 reported medicinal plants.

Studies on plant morphology serve as valuable tools in taxonomic work. Likewise, knowledge of ecological distributions explains variations in the form and structure of plants.

A plant may be considered medicinal if it contains active principles like alkaloids, tannins, oxalic acid, formic acid, fats and oils, amygdalin, saponin and arbutin. Histochemical tests allow one to determine the presence, amount and localization of these constituents in plant tissues.

Knowledge of histochemical analysis in plants is still limited, especially among rural folks and herbolarios. The province of Bukidnon is known for its many medicinal plants and folk medicinal practices.

The following recommendations are put forward to help promote the use of medicinal plants:

1. That massive use and wider acceptance of herbal medicine be carried out through the concerted efforts of researchers, public health personnel, media men, policymakers, school teachers, hilots, herbolarios and household members;
2. That more intensive research and experiments be conducted subjecting other parts of the plant (ie., roots, flowers, fruits and seeds) to histochemical tests; and
3. That government agencies, like the Department of Science and Technology (DOST), National Research Council of the Philippines (NRCP), Department of Health (DOH), World Health Organization (WHO), Philippine Council for Health Research and Development (PCHRD) and chemical companies, invest in herbal medicine research and development.

ACKNOWLEDGMENT

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Table 1. Active constituents and their localization in some commonly used medicinal plants

	Species	*Plant Part*	*ACTIVE PRINCIPLES*									*Medicinal Values*
			Alkaloid	*Tannin*	*Saponin*	*Organic Acids*			*Glucosides*		*Fats & Oils*	
						Formic Acid	*Oxalic Acid*	*Tartaric Acid*	*Arbutin*	*Amygdalin*		
1.	*Mangifera indica* L.	Leaf	vb=2	IL=1	M=1	–	–	–	–	–	ule=1	Anti-inflammatory; Analgesic
2.	*Curica papaya* L.	leaf	ule=1	–	–	–	m=2	–	–	–	ue=1	Anti-inflammatory; laxative; Analgesic
			IL=1	–	–	–	–	–	–	–	M=1	
3.	*Blumea balsamifera* (L.) D.C.	Leaf	e=2	en=1	–	–	en=1	–	–	–	–	Diuretic antispasmodic; Analgesic
			m=2	m=1	–	–	m=1	–	–	–	–	
			vb=2	vb=1	–	–	vb=2	–	–	–	–	
			t=2	–	–	–	–	–	–	–	–	
4.	*Artemesia vulgaris* L.	Stem	–	c=2	–	–	c=2	–	–	vb=2	e=1	Analgesic anti-inflammatory; anti-spasmodic; bactericidal
			–	vb=1	–	–	vb=2	–	–	–	en=1	
		Leaf	e=1	c=1	–	–	m=1	–	–	e=1	e=1	
			vb=2	vb=1	–	–	vb=2	–	–	vb=2	en=1	
5.	*Chrysanthemum indicum* L.	Stem	–	c=1	–	–	e=1	–	–	en=1	e=1	Anti-flatulence; anti spasmodic; bactericidal; hypotensive
			–	–	–	–	c=3	–	–	–	–	
			–	–	–	–	vb=1	–	–	–	–	
		Leaf	e=1	m=1	–	–	ule=3	–	–	–	–	
			m=1	–	–	–	m=2	–	–	–	–	
			vb=2	–	–	–	vb=2	–	–	–	–	
			–	–	–	–	t=2	–	–	–	–	
6.	*Bryophyllum pinnatum* L.	Leaf	en=1	en=1	–	M=1	e=2	–	–	–	UE=1	Analgesic; anti-inflammatory; bactericidal
			m=1	m=2	–	–	en=2	–	–	–	le=2	
			vb=2	vb=2	–	–	m=2	–	–	–	vb=1	

7.	*Cyperus kyllingia* Rotto	Leaf	–	–	–	–	–	–	–	–	–	Anti-pyretic; analgesic; anti- inflammatory
		Stem	vb=2	vb=1	–	–	–	–	–	–	–	
			–	c=1		–	–	–	–	–	–	
8.	*Jatropha curcas* L.	Stem	e=1	–	–	p=1	c=2	–	–	c=1	–	Anti-inflammatory; Analgesic; Anti-rheumatic; anti-flatulence; anti-diarrheal
			c=1	–	–	–	vb=1	–	–	–	–	
			vb=1	–	–	–	p=1	–	–	–	–	
			p=2	–	–	–	–	–	–	–	–	
			–	–	–	–	–	–	–	–	–	
		Leaf	e=1	–	–	le=2	le=1	–	–	–	–	
			m=2	–	–	len=2	m=2	–	–	–	–	
			–	–	–	m=2	–	–	–	–	–	
			–	–	–	vb=1	–	–	–	–	–	
9.	*Imperata citratus* D.C.	Rhizome	vb=2	–	–	e=2	–	–	–	–	e=2	Anti-pyretic; analgesic; antitussive
			Par=1	–	–	Par=3	–	–	–	–	Par=2	
			tra=2	–	–	vb=2	–	–	–	–	–	
10.	*Andropogon citratus* D.C.	Rhizome	vb=2	–	–	e=2	–	–	–	–	e=2	Anti-pyretic; analgesic; antispasmodic; hypotensive; anxiolytic
			Par=1	–	–	Par=3	–	–	–	–	Par=2	
			tra=2	–	–	vb=2	–	–	–	–		
11.	*Coleus blumei* Benth	Leaf	v=1	m=1	–	–	vb=1	–	M=1	–	–	Anti-inflammatory; bacterio-static property
			vb=1	vb=1	–	–	–	–	–	–	–	
			m=1	–	–	–	–	–	–	–	–	
12.	*Mentha cordifolia* Opiz	Stem	P=1	c=1	–	–	–	–	–	–	c=1	Anti-inflmmatory; diuretic; antirheumatic
			c=1	–	–	–	–	–	–	–	-P=1	
			vb=1	–	–	–	–	–	–	–	e=1	
			–	–	–	–	–	–	–	–	–	
		Leaf	ue=1	–	–	–	–	–	–	–	m=1	
13.	*Persea americana* Mill	Stem	e=1	en=1	vb=2	e=2	e=1	–	–	e=1	–	Anti-diarrheal; analgesic; anti-rheumatic; astringent
			c=1	e=2	–	c=1	vb=2	–	–	–	–	
			–	c=1	–	vb=3	c=1	–	–	–	–	
			–	vb=1	–	p=1	–	–	–	–	–	
			–	–	–	–	–	–	–	–	–	
		Leaf	Le=2	m=1	e=1	e=2	–	–	–	e=1	–	
			vb=2	vb=1	vb=2	c=1	–	–	–	–	–	
			M=2	–	P=1	vb=2	–	–	–	–	–	

14.	*Hibiscus rosasinensis* L.	Leaf	ue=1 vb=1 M=1 IL=1	M=1 IL=1 – –	– – – –	– – – –	M=1 IL-1 – –	– – – –	– – – –	– – – –	– – – –	Anti-inflmmatory; antiseptic
15.	*Psidium guajava* L.	Stem Leaf	– – – e=2 M=2 vb=1 – – –	e=1 c=2 vb=1 – e=1 m=3 vb=2 – – –	– – – – – – – – – –	– – – – – – – – – –	e=1 c=2 vb=3 – M=2 vb=1 – – – –	– – – – – – – – – –	– – – – – – – – – –	– – – – – – – – – –	– – – – e=1 – – – – –	Anti-diarrheal; analgesic; anti-inflammatory; astringent; antiseptic
16.	*Moringa oleifera* Lam.	Leaf	ule=1 vb=1 – –	M=1 – – –	– – – –	– – – –	– – – –	– – – –	– – – –	– – – –	e=1 M=1 – –	Anti-inflammatory; astringent; analgesic
17.	*Pandanus odoratissimus* L.	Leaf	vb=2 – –	vb=1 Par=1 –	– – –	– – –	– – –	– – –	– – –	– – –	lc=1 – –	Anti-inflammatory; antivertigo; hypotensive; antidiabetic
18.	*Piper betle* L.	Leaf	e=1 vb=1 –	M=1 – –	– – –	M=2 vb=1 –	M=1 – –	– – –	– – –	– – –	– – –	Antitussive; analgesic; anti-inflammatory; antiseptic
19.	*Peperomia pellucida* L.	Stem Leaf	e=1 vb=1 c=1 – ule=1	en=1 c=1 – – M=1	– – – – –	– – – – M=1	– – – – –	– – – – –	– – – – –	– – – – –	– – – – –	Analgesic; anti-inflammatory; antiseptic; astringent
20.	*Zingiber officinale* Rosc.	Rhizome	Par=1	Par=1	Par=1	–	Par=2	–	–	–	–	Antiseptic; laxative; antitussive; anti-inflammatory; antispasmodic

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BIODECONTAMINATION OF MERCURY, LEAD and CADMIUM BY SELECTED STRAINS OF *RHIZOBIUM*

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ABSTRACT

The plasmid patterns of two *Rhizobium* strains, which were tolerant to Hg and Pb, BJVr7, isolate from *Vigna radiata* Wilczeck (mungbean) and BJL1 30, from *Leucaena leucocephala* Linn. (ipil-ipil) grown in culture media with Hg^{2+}, Pb^{2+}, Cd^{2+}, respectively and without these metals (control), were determined. Two prominent megaplasmid bands, Band 1 and Band 2, were present in both control and test strains. Band 2 became more intense for both strains grown in Pb^{2+} containing media which may indicate that Band 2 (approx. MW, 15 x 10^6 daltons) encodes for Pb tolerance.

Transmission electron micrographs of BL180 grown in 10 ppm Pb^{2+} showed that the metal was concentrated on the cell surface. The X-ray microanalysis spectrum of cells grown in Pb^{2+} showed the absence of Pb^{2+} inside the cell.

The biosorption ability of the fresh cell mass of BJVr7, BJVr12, BJL1 30 and BL1 80 was determined in aqueous solution containing Hg^{2+}, Pb^{2+} and Cd^{2+} in relation to its effectivity for reducing metal ion concentration from solutions. The highest reduction rate for Hg^{2+} (92.4%) and Pb^{2+} (64.7%) was obtained for BJVr12 under the conditions of the experiment. BJVr7 and BJVr12 gave the highest reduction rate for Cd^{2+}.

INTRODUCTION

Rhizobium strains, which are heavy producers of mucilaginous polysaccharides, can find potential application in the bioconcentration of heavy, toxic metals from dilute solutions. Previous studies (Mamaril et al., 1989; Mamaril et al., 1990) have shown that these bacterial strains can sequester and concentrate a variety of heavy metals from dilute aqueous solution to a high degree. The ability of these microorganisms to absorb these toxic metals by extracellular traps helps protect the cells by preventing the entry of toxic amounts of these metals into the cell where they can interfere with the cell's metabolic processes. Biotraps for toxic metals from immobilized rhizobial cells can be developed for use in waste water management.

With the increasing awareness of the increasing release of toxic metals, such as Hg, Pb and Cd by industrial establishments and mining operations, it is imperative that research on finding ways to reduce toxic metal concentration in the environment be done. This study reports on the basis of metal tolerance of selected rhizobial isolates, which are prodigious producers of gummy polysaccharides, and their ability to reduce Hg^{2+}, Pb^{2+} and CD^{2+} from dilute aqueous solutions by biosorption.

REVIEW OF LITERATURE

In the past 200 years of heavy industrialization and mining operations, great changes in the distribution and solubilization of metals have occurred on the earth's surface. Metals, such as Hg, Pb and Cd, are reaching toxic levels which pose dangers to animal and human health. Higher forms of life take millions of years to evolve regulatory mechanisms involving metal ions, while lower forms of life, such as microorganisms, can rapidly evolve regulatory systems to cope with increasing concentrations of toxic metals. Microorganisms have very short generation times which lead to faster evolution rates to enable the organism to develop strategies to maintain low intracelullar concentrations of toxic metals (Wood and Wang, 1983).

One such strategy is to acquire a transferred resistance to these toxic metals by means of extra chromosomal DNA molecules called plasmids (Silver and Misra, 1988; Silver, 1992). These plasmids carry genes which control their replication, segregation and copy number, as well as ancillary phenotypic functions, such as antibiotic resistance, heavy toxic metal resistance and a variety of other activities. Microbial resistance or tolerance to heavy toxic metals can be brought about by four known mechanisms. One mechanism is by altering the membrane transport system to prevent entry of the toxic metal ion into the cell; the second is by intracellular and extracellular sequestration by specific heavy metal ion-binding components (e.g., proteins, polysaccharides, ligands) at the bacterial cell wall; the third is by specific cation or anion efflux systems; and the fourth is by detoxification of the toxic metal ion to a less toxic form (Silver, 1992). Microbial resistance to Hg has been reviewed in detail by Silver and Misra (1988). The seven Hg resistance

systems that have been sequenced contain the gene for mercuric reductase, the enzyme that converts toxic Hg^{2+} ions to less toxic volatile Hg^{o}. Four of these systems also determine the enzyme, organomercurial lyase, which breaks the Hg-C bond and renders toxic compounds, such as methylmercury and phenylmercury, less toxic.

The biochemical transformation of mercury compounds to inert forms by mercury resistant bacteria, which carry plasmids, have been studied by several researchers (Schottel, 1975; Summers and Silver, 1978; and Pan-Hou and Imura, 1981). Mercury resistance of a strain of *Enterobacter aerogenes* was shown to be determined by a plasmid. The resistance appeared to be due to the alteration in cellular permeability to mercury and not to enzymatic volatilization of mercury (Pan-Hou et al. 1981).

Microbial resistance to lead occurs via mechanisms similar to mercury detoxification such as biomethylation (Craig et al., 1981). Sulfide plays a key role in the biological cycle of mercury and lead. Hydrogen sulfide is quite effective at volatilization and precipitation of Hg and Pb through disproportionation chemistry.

Cadmium resistance in microorganisms was also shown to be mediated by a plasmid. Two separate plasmid genes have been shown to be involved in CD^{2+} resistance. Cad-A gene encodes for proteins that are involved in the cellular efflux of CD^{2+} from the inside of the cell. Cad-B gene encodes for the synthesis of a CD^{2+} binding protein which is similar to metallothionen (Silver and Misra, 1980).

Intracellular and extracellular traps that are synthesized by microorganisms help reduce toxicity levels of heavy toxic metal ion concentrations. Metallothionen, a sulfuhydryl containing protein, binds strongly to metallic ions and can reduce metal ion concentration from solutions to a high degree. This bioconcentration of metal ions can also be obtained by biosysnthesis of ligands that bind enzymes which are responsible for precipitating metals extra-cellularly (Wood and Wang, 1983)

Biosorption or bioconcentration of metals by microbial biomass has been observed as early as 1940 (McCall, 1940). The use of microbial biomass to adsorb heavy toxic metals from aqueous industrial effluents and sludges offers an economical and convenient alternative to physicochemical techniques, such as ion exchange, activated carbon adsorption, electrolysis and precipitation by sulfides and carbonates. Several researchers compared chemically and microbiologically digested heavy metal sludges using *Thiobacillus thiooxidans* and *T. ferrioxidans* to remove zinc, cadmium and copper. They found that the cost of sludge treatment in terms of chemicals was decreased by 80% when a microbiological leaching method was used (Tondwalkar et al., 1990).

Biosorption, which describes the capacity of microorganisms to sequester or concentrate a variety of heavy metal ions from dilute aqueous solutions, offers a more economical method for metal removal and recovery, especially when the initial concentration is in the range of 10-100 ppm. Microbial biomass could be used effectively to decontaminate waste effluents from mining operations, as well as in

refining, electroplating and nuclear fuel processing plants (Tsezos et al., 1983). Bioconcentration of heavy metals by microbial biomass can be several thousand times or more over their concentration in the environment (Touvinen and Kelly, 1974).

MATERIALS AND METHODS

Microbial Strains and Culture Media

The microbial strains used were *Rhizobium* strains that were observed to produce large amounts of mucilaginous polysaccharides and were tolerant to mercury and lead. They are listed in Table 1.

The rhizobial strains were cultured in yeast extract mannitol broth (YEMB) and in YEMB containing varying concentrations of Hg^{2+}, Pb^{2+} and CD^{2+}. The strains were maintained in yeast extract mannitol agar (YEMA) kept under refrigeration (5°-10°C)

Plasmid Patterns of BJVr 7 and BJL1 30

Growth Media. Aqueous tryptone yeast extract (TY) medium contained 0.5% (w/v) Bactotryptone, 0.3% Bactoyeast Extract and 7mM $CaCl_2$. Aqueous peptone (PA) medium contained 0.4% (w/v) Bactopeptone and 2mM $MgSO_4$. This medium was found to give good lysis and plasmid preparation.

Buffers. The buffer was 50mM-Tris-HCl with 20mM EDTA pH 8.0 (TE). Dialysis buffer was 10mM-Tris-HCl with H 1mM EDTA Ph 8.0. Tris-borate electrophoresis buffer contained per liter 10.8 g Tris, 0.93 g EDTA and 5.5 g boric acid, pH 8.3.

Isolation of Megaplasmids

The inoculum was cultured in TY medium containing no heavy metals for the control, 50 ppm Hg^{2+} for Hg tolerance, 30 ppm Pb^{2+} for Pb tolerance and 10 ppm CD^{2+} for Cd tolerance.

About 200 ml of PA medium was inoculated with the prepared inoculum and shaken at ambient room temperature (30-33°C) for three days. The cells were harvested and adjusted to a final concentration of about 10^8 cells per ml, washed in TE buffer and resuspended in 16 ml TE buffer and pre-digested one hour at 37°C. Some 2 ml sodium dodecyl sulfate (10% SDS w/v in TE buffer) was added and the mixture was incubated at 37°C with gentle shaking for one hour or until lysis was complete.

The lyzate was adjusted to pH 12.4 by addition of 2M NaOH (about 0.5 ml) with gentle but thorough stirring (plastic or glass rod). After standing for 30 min at room temperature, the lyzate was adjusted to pH 8.5 with 2M Tris HCl, pH 7.1

(about 1.5 ml), and transferred to a 40 ml centrifuge tube. Then, 5M NaCl was added (4.8 ml) to give a final concentration of 1M. The contents of the tube were mixed by gentle inversion and left on ice for at least four hours. The SDS/NaCl precipitate complex was brought down by centrifugation at 10,000 rpm for 20 min at 4°C. The supernatant was carefully decanted to a fresh tube and 50% (w/v) polyethylene glycol (PEG) was added to give a final concentration of 10%. The contents of the tube were mixed by gentle inversion and left on ice overnight. The DNA precipitate was brought down by centrifuging at 7,000 rpm at 4°C for 15 min. The supernatant was discarded and the pellet was resuspended in 0.5 ml TE buffer containing 0.1% (v/v) diethylpyrocarbonate to inhibit nuclease activity. The samples were stored at 4°C.

Agarose Gel Electrophoresis of Megaplasmids

A horizontal perspex (plastic) apparatus giving a gel slab 13.3 cm wide, 14 cm long and 0.6 cm thick was used. Sample cells were formed using a perspex comb with nine teeth. Samples of crude preparations were centrifuged for 2 min in an Eppendorf 5412 centrifuge and 40 ul of the supernatant was added to 10 ul loading dye (20% W/V agarose in Tris/borate buffer) at 45 mA(180V) and 40°C for six hours. The gel slab was then removed from the apparatus and stained for 20 min in Tris/borate buffer containing ethidium bromide (0.5 ug/ml), removed and stored at 4°C for four hours before visualizing the plasmid bonds to reduce the background fluorescence. The gel was illuminated under a UV light (366 nm) to visualize the plasmid bands and photographed using a Polaroid Land Camera with Polaroid Type 55 film.

Electron Microscopy Studies

Rhizobium BL180 strain was grown in YEMB containing 50 ppm Hg^{2+} in 10 ppm, Pb^{2+} and in 5 and 10 ppm CD^{2+} for five days.

The cells were harvested by centrifugation. The cell pellet was fixed with 2.5% glutaraldehyde for one hour. Then the cells were suspended and washed in phosphate buffer pH 7.2 and cetrifuged again. The pellet was dehydrated using an ethanol series (i.e., 0%, 70%, 80%, 90%, 100%). Exposure time per ethanol concentration was 30-40 min. After dehydration, a 50:50 polyprolylene (PPO) treatment was followed. The PPO was gradually replaced with analdite (3:1, 2:2, 1:3) to inbed the sample. Sectioning was done (blue-green interference color) with Ultra cut E. Transmission electromicrographs were then taken of the prepared samples.

Prepared samples of Pb^{2+} and Dc^{2+} grown *Rhizobium* were sent to Ms. Carole Winters, University of Cardiff, Wales, United Kingdom for X-ray microanalyses (X-RMA) examination. X-RMA was done using Philips 300-TEM operated at 80 KV equipped with LINK-860 Series II-Multichannel Analyser and Energy Dispenser Detector.

Biosorption of Mercury, Lead and Cadmium

Mercury, HG^{2+}. A ratio of 0.i 1 g of fresh cell mass to 10 ml solution containing 50 ppm Hg^{2+} was used. The cell suspension was agitated at 120 strokes per min at ambient room temperature which ranged from 30°C to 33°C. Two agitation times were used, 30 min and 60 min under the same experimental parameters of agitation and temperture.

The cell-Hg^{2+} suspension was centrifuged at 5,000 rpm for 5 min after shaking for 30 min or 60 min. The supernatant was analyzed for Hg^{2+} chemically by the colorimetric dithizone method (Sandell, 1959).

The percentage reduction of Hg^{2+} concentration in the aqueous solution was obtained by subtracting the Hg^{2+} concentration of the supernatant from the original concentration before treatment with the rhizobial biomass. Absorbance of the samples and YEMB control samples were determined at 540 nm for Hg and 510 nm for Pb.

Lead, Pb^{2+}. A ratio of 0.10 g cell mass to 100 ml solution containing 30 ppm Pb^{2+} was used. One set was agitated for two hours and another set for six hours. The same experimental parameters for Hg^{2+} determination was employed. The cell-Pb^{2+} suspension was centrifuged after two hours or six hours. The supernatant was analyzed for Pb^{2+} concentration by the colorimetric dithizone method. The % reduction of Pb^{2+} concentration from the original solution was then calculated.

Cadmium, Cd^{2+}. An initial concentration of 10 ppm CD^{2+} solution was used in combination with 0.5% *Rhizobium* cell mass and another set with 0.1% *Rhizobium* cell mass. One set was agitated at two hours and another at six hours about 120-150 strokes per min. The cell suspension was centrifuged and the supernatant analyzed for Cd^{2+} concentration by atomic absorption spectrophotometric (AAS) method at the Central Analytical Service Laboratory of BIOTECH. The % reduction of Cd^{2+} concentration from the original concentration was computed.

RESULTS AND DISCUSSION

The plasmid patterns of *Rhizobium* strains BJVr7 and BJL1 30 grown in the presence of Hg^{2+}, Pb^{2+} and Cd^{2+} and in the absence of the toxic metals (control) are shown in Figure 1. Three distinct bands of Hind lII digest of Lambda phage are noted: first band of phage DNA approximates 15 X 10^6 daltons; second band approximates 6 X 10^6 daltons; and third band, 4.26 x 10^6 daltons.

Both strains grown in the presence of toxic metal ions and the control strains exhibited two distinct megaplasmid bands, Band 1 and Band 2. However, BJVr7 control exhibited a relatively more intense Band 2 than that for BJL1 30. Band 2 has an approximate molecular weight of over 15 X 10^6 daltons. This megaplasmid band became more intense for both strains grown in the presence of 30 ppm Pb^{2+} which may indicate increased synthesis of plasmid DNA encoding for Pb^{2+} resistance.

However, strains grown in Hg^{2+} and Cd^{2+} showed very weak megaplasmid bands which may indicate low survival of the strains in the medium containing 50 ppm Hg^{2+} and 10 ppm Cd^{2+}

Based on the intensity of the plasmid bands of the Hg^{2+} and Cd^{2+} grown strains, BJVr7 relatively has more intrinsic tolerance than BJL1 30 for these toxic metals. The megaplasmid profile of BJL1 30 offered more convincing evidence of the role of Band 2 in determining lead tolerance. The relatively weaker intensity of Bands 1 and 2 of BJL1 30 as compared to that of BJVr7 may indicate lower survival of BJL1 30 cells at these higher concentrations of Hg and Cd. However, it is surmised that if surviving cells at these concentrations should be cultured again in the same media, a more intense Band 2 would be predicted due to more biosynthesis of Band 2 plasmid DNA to cope with the higher concentration of the toxic metals.

Results of the electron microscopy studies for *Rhizobium* BL1 80 grown in 10 ppm Pb^{2+} and the X-RMA spectrum of the prepared samples (single cell) are shown in Figure 2. The dense black areas are concentrated on the outside of the cells. The X-RMA spectrum of BL1 80 cultured in the presence of lead did not show the presence of Pb^{2+} inside the cells. The dense spots found intracellularly do not contain Pb^{2+}. The X-RMA spectrum of the extracellular bacterial cell deposit is shown in Figure 3 which also shows Pb present extracellularly.

BL1 80 grown in 5 ppm and 10 ppm CD^{2+} did not reveal the presence of Cd in the cell. It is surmised that the detection limits of CD at these levels has been reached by the methods employed or that Cd^{2+} was pumped out of the cells by intracellular efflux pumps.

Results of the adsorption of Hg^{2+} by selected *Rhizobium* strains from aqueous solution after agitation for 30 min are given in Table 2A and after 60 min in Table 2B. The *Rhizobium* strains exhibited quite high % reduction of Hg^{2+} from solution from 90.6% for BJVr 7 to 92.4% for BJVr12. Increasing agitation time to 60 min did not increase reduction of Hg^{2+} substantially. Adsorption of more than 90% occurred during the first 30 min, therefore the adsorption parameter to be optimized would be the ratio of cell mass to Hg^{2+} concentration after 30 min. The contact time between cells and Hg^{2+} solution could be decreased tremendously if microbial mass to Hg^{2+} solution ratio would be increased as is occurring in processes involving adsorption columns.

Table 3A gives the results for the % reduction of Pb^{2+} of a rhizobial cell suspension after two hours of agitation and agitation rate of 120 strokes per min at a temperature range of 30-33°C. A ratio of 0.1 g rhizobial mass to 100 ml of solution containing 30 ppm Pb^{2+} was used. The % reduction ranged from 62% for BL1 80 to 69% for BJVr 12. Increasing the agitation time to six hours (Table 3B) did not increase adsorption substantially. About 60% reduction was obtained for the first two hours. These results suggest that higher adsorption rates may be obtained by increasing cell mass ratio to Pb^{2+} concentration than by increasing time of agitation.

Adsorption of CD^{2+} by the same rhizobial strains showed similar results (Table 4A) as that obtained from Hg^{2+} and Pb^{2+}. A % reduction of CD^{2+} ranging from 58.75% for BJVr 12 to 39.30% for BJL1 23 was shown for a microbial cell mass of 0.1 g to 100 ml of 10 ppm Cd^{2+} solution (120 strokes per min for two hours at room temperature). Increasing agitation time to six hours did not increase % reduction of Cd^{2+} substantially. However, in the case of BJL1 30, % reduction of Cd^{2+} decreased from 40.15% to 36.25%; 39.30% to 35.66% for BJL1 23; 54.70% to 51.90% for BJL1 80; and for BJVr 12 from 58.75% to 45.25%. This may indicate that expulsion of Cd^{2+} from the cells was taking place during this length of time of six hours either by cell lysis or intracellular efflux pumps. The results obtained from increasing the microbial cell mass ratio from 0.1 g to 0.5 g to 100 ml of 10 ppm Cd^{2+} solution are shown in Table 4B. Increased % reduction of Cd^{2+} in solution is shown in Table 4B. Increased % reduction of Cd^{2+} in solution from 58.75% to 85.60% for BJVr 12, and from 40.15% to 86.60% for BJL1 30 after two hours agitation at 30-33°C was noted. Increasing agitation time from two hours to six hours decreased % reduction of 86.60% to 82.10% for BJL1 30. A slight increase of 1.66% was obtained for BJVr 12.

SUMMARY/CONCLUSIONS/RECOMMENDATIONS

Plasmid profiles of two rhizobial strains 1445 BJVr 7 and 1461 BJL1 30 showed that Pb tolerance was genetically determined by a megaplasmid of over 15 X 16^6 daltons which was designated as Band 2 in the plasmid profile. Based on the intensity of the plasmid bands, one can surmise the tolerance of the rhizobial strain to Hg, Cd and Pb. Strain 1145 BJVr 7 is more tolerant to the presence of Hg and Cd than 1461 BJL1 30. Both strains can grow very well in the presence of 30 ppm Pb^{2+}.

Transmission electron microscopy and X-ray microanalysis examination of 1347 BL1 80 grown in 10 ppm Pb^{2+} revealed the absence of Pb inside the bacterial cell. The Pb was shown to be outside the cell.

Rhizobial strains 1347 BL1 80, 1445 BJVr 7, 1458 BJL1 23, 1461 BJL1 30 and 1469 BJVr 12 were tested for their capacity to adsorb Hg^{2+}, Pb^{2+} and CD^{2+}. 1347 BL1 80 was not included in the Hg tests. All strains were able to reduce 50 ppm Hg^{2+} solutions to a range of 3.8 to 4.7 ppm Hg^{2+}, a reduction of about 92% at a ratio of 0.11 g cell mass to 10 ml of 50 ppm Hg^{2+}, agitation rate of 120-150 strokes per minute, agitation time of 30 min at ambient room temperature (30-33°C). Increasing agitation time to 60 min (one hour) did not significantly increase reduction rate. 1469 BJVr 12 gave the highest reduction of 92.4% (30 min) and 1445 BJVr 7, 93.6% (one hour) under these conditions.

For Pb reduction, 1469 BJVr 12 gave the highest % reduction of 69.0% followed by 1445 BJVr 7 and 1461 BJL1 30 of 64.7% at a ratio of 0.1 g cell mass to 100 ml 30 ppm Pb^{2+}, agitation rate of 120-150 strokes per min, agitation time of two hours at ambient room temperature (30°-33°C). Increasing agitation rate of time to six hours did not increase reduction rate significantly.

For Cd reduction, 1469 BJVr 12 gave the highest % reduction of 58.75% followed by 1445 BJVr 7 of 56.00% at a ratio of 0.1 g cell mass to 100 ml of 20 ppm CD^{2+} solution, agitation rate of 120-150 strokes per min. agitation time of two hours at ambient room temperature. Increasing agitation time to six hours did not increase reduction rate significantly. Instead, a decrease in reduction capacity is shown for all strains except 1445 BJVr 7. Increasing the ratio of cell mass from 0.1 g to 0.5 g to 100 ml of 10 ppm CD^{2+} increased adsorption capacity of 1461 BJL1 30 from 40.15% to 86.60%.

From these studies, the best microbial strains for reducing Hg, Pb and Cd are 1469 BJVr 12, 1445 BJVr 7 and 1461 BJL1 30. All the other strains were effective for Hg reduction under the conditions of the experiment.

Increasing cell mass ratio to metal concentration is a more effective way of increasing reduction of heavy toxic metal ions from solution than increasing agitation time. Further studies will be pursued on increasing the ratio of cell mass to metal ion concentration. One means by which this high ratio will be achieved is by the use of immobilized rhizobial cells in columns as biotraps.

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Special thanks are extended to Ms. Carole Winters of the University of Cardiff, Wales, United Kingdom for helping us in the electron microscopy studies, particularly the X-ray Microanalysis of the *Rhizobium* BL1 80 grown in 100 ppm Pb^{2+}.

Table 1. Selected *Rhizobium* strains in reduction of heavy metals in aqueous solution

Accession No.	*Strain Designation*	*Host plant and place of origin*
1349	BL1 80	Ipil-ipil/Calumpit, Bulacan
1445	BJVr 7	Mungbean/Calaca, Batangas
1458	BJL1 23	Ipil-ipil/Calumpit, Bulacan
1461	BJL1 30	Ipil-ipil/Calumpit, Bulacan
1469	BJVr 7	Mungbean/Davao City, Davao

Table 2. Average absorbance and percentage reduction of mercury (Hg^{2+}) of the supernatant of a rhizobial cell suspension (10 ml of 50 ppm Hg^{2+}: 0.11 g cell mass, agitation rate: 120 strokes/min, temperature: 30-33°C.

Rhizobium strain		*Ave. Abs. at 540 nm after agitation*	*Residual concentration ppm, Hg^{2+}*	*% Reduction in Hg^{2+} concentration*
A. Agitation time: 30 min.				
1145	BJVr 7	0.77	4.7	90.6
1458	BJL1 23	0.71	4.2	91.6
1461	BJL1 30	0.63	4.0	92.0
1469	BJVr 12	0.66	3.8	92.4
B. Agitation time: 60 min.				
1145	BJVr 7	0.59	3.2	93.6
1458	BJL1 23	0.65	3.7	92.6
1461	BJL1 30	0.72	4.2	92.0
1469	BJVr 12	0.64	3.6	92.8

Table 3. Lead (Pb^{2+}) content of supernatant of a rhizobial cell suspension at a cell ratio of 0.1 g cell mass to 100 ml 30 ppm Pb^{2+} (agitation time: two hours; agitation rate: 120-150 strokes per min.; temperature: 30-33°C)

Rhizobium strain	*Ave. Abs. at 510 nm after agitation*	*Residual concentration ppm, Pb^{2+}*	*% Reduction in Pb^{2+} concentration*
A. Agitation time: 2 h			
1347 BL1 80	0.62	11.5	62.0
1445 BJVr 7	0.54	9.6	64.7
1458 BJL1 23	0.59	10.0	64.0
1461 BL1 30	0.54	9.6	64.7
1469 BJVr 12	0.53	9.3	69.0
B. Agitation time: 6 h			
1347 BL1 80	0.50	8.6	71.0
1445 BJVr 7	0.54	9.6	64.7
1458 BJL1 23	0.50	8.6	71.0
1461 BL1 30	0.50	8.6	71.0
1469 BJVr 12	0.52	9.1	70.0

Table 4. Cadmium (Cd^{2+}) content of the supernatant of rhizobial cell suspension at 2 cell ratios and 2 agitation times (two hours and six hours)

		Agitation Time			
		2 h		6 h	
	Rhizobium strain	Residual conc'n ppm CD^{2+}	% Reduction in Cd^{2+} conc'n, ppm	Residual conc'n ppm Cd^{2+}	% Reduction in Cd^{2+} conc'n, ppm
A.	Cell ratio: 0.1 g cell mass: 100 ml of 10 ppm CD^{2+} solution				
	1347 BJL1 80	4.53	54.70	4.81	51.90
	1445 BJVr 7	4.40	56.00	4.29	57.05
	1458 BJL1 23	6.07	39.30	6.43	35.65
	1461 BLl 30	5.93	40.15	6.37	36.25
	1469 BJVr 12	4.12	58.75	5.47	45.25
B.	Cell ratio: 0.1 g cell mass: 100 ml of 10 ppm Cd^{2+} solution				
	1347 BLl 80	3.34	66.60	3.06	69.40
	1445 BJVr 7	5.04	49.55	4.64	53.55
	1458 BJL1 23	6.27	37.25	8.00	44.60
	1461 BLl 30	1.34	86.60	1.79	82.10
	1469 BJVr 12	1.44	85.60	1.27	87.26

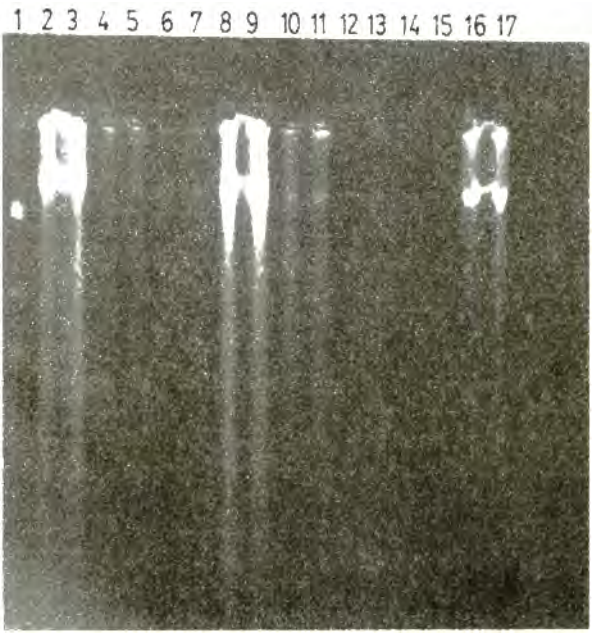
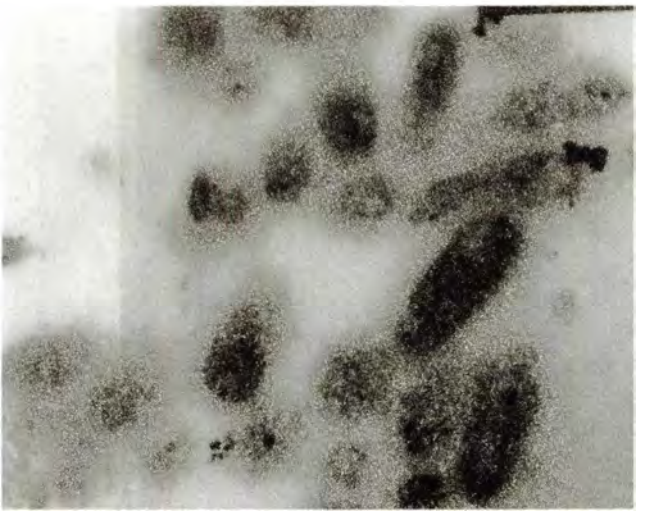
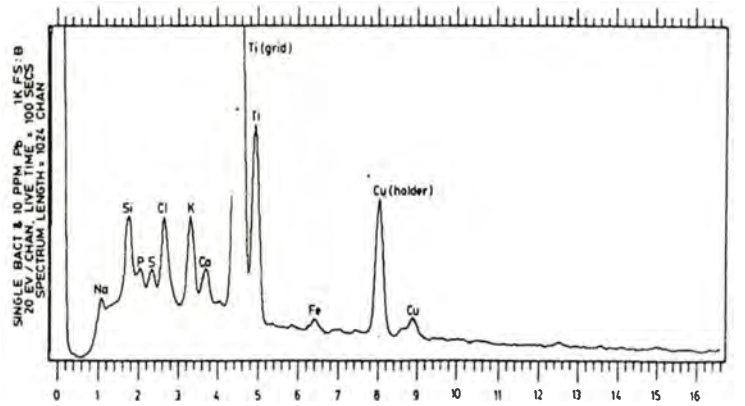


Figure 1. Electrophoretic patterns of plasmid of BJVr 7 (Mungbean isolate) and BJL1 30 (Ipil-ipil isolate) grown in TY medium at lower concentration (micro wells)

Lane	1	Lambda phage DNA
	2	BJVr 7, control
	3	BJVr 7, control
	4 & 5	50 ppm Hg^{2+} in medium
	6 & 7	BJVr 7 10 ppm CD^{2+} in medium
	8 & 9	BJVr 7 30 ppm Pb^{2+} in medium
	10 & 11	BJL1 30, control
	12 & 13	BJL1 30, 50 ppm Hg^{2+} in medium
	14 & 15	BJL1 30, 10 ppm CD^{2+}
	16 & 17	BJL1 30, 30 ppm Pb^{2+}



(a)



(b)

Figure 2. **Transmission electron micrograph of *Rhizobium* BL1 80 grown in YEMB containing 10 ppm Pb^{2+}, (a) and X-ray microanalysis spectrum (intracellular), (b)**

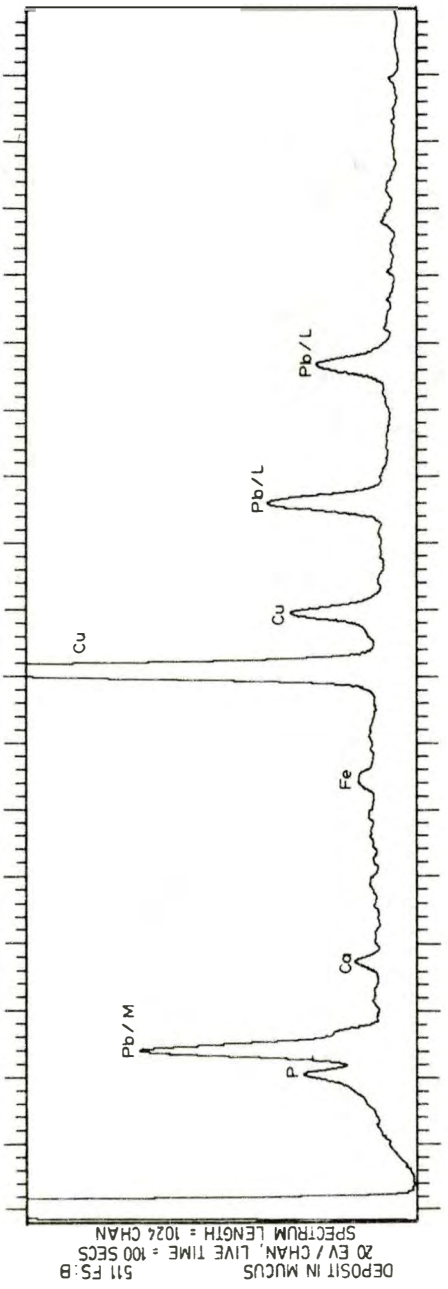


Figure 3. X-Ray microanalysis spectrum of *Rhizobium* BL1 80 grown in YEMB containing 10 ppm Pb (extracellular deposit)

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THE PARACALE MINES AND ITS ENVIRONMENT AN ASSESSMENT OF ITS BIOLOGICAL AND PHYSICOCHEMICAL CHARACTERS

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ABSTRACT

This is a report on monitoring of toxic heavy metals in coastal waters near a mining site in Paracale, Camarines Norte. One of the oldest mining sites in the country, it is over four centuries old. Cd, Cu, Pb, Au, Ag, Hg and Zn were analyzed from water samples and soft tissues of gastropods *Terebralia sulcatus* and *Telescopium telescopium,* bivalve *Crassostrea rhizophorae* and in some selected organs of white mullet *Mugil curema* Valenciennes. Data show the high concentration of Au and Ag making them important metals for mining. Cd, Cu and Pb were found in considerable amounts in water samples and were consistently present in tissues of mollusks and fish. Zinc was below the allowable level in water but was bioaccumulated in *Crassostrea* tissues.

The different environmental parameters like water pH, salinity, temperature, water column height and turbidity were recorded. Plankton, fish and mollusks were collected for classification and identification. At least 34 known commercial species of fish were caught in the area. Of the many gastropod and bivalve species collected both from the rocky shoreline and muddy, brackishwater mangrove area, only *Terebralia* and *Nerita* are commercially important. T. *telescopium* is abundant though not commercially important.

Regular monthly sampling of water, sediment and commercially important species of gastropods, bivalves and fish is being done. The monthly fluctuation of metals is monitored to determine possible biotransfer of metals from sediment to organisms.

INTRODUCTION

Paracale is one of the oldest mining sites in the country. It was first discovered by the Spaniards in 1571 during the height of their quest for more land (PS Journal, 1990). Even before the Spaniards' discovery of the gold town, the natives had been digging and constructing canals in search of gold. Thus Juan de Salcedo named the village "Paracale" which literally means digger of canal or *"Para calle."*

Location and Accessibility

Paracale is a seashore town, about 190 km southeast of Manila (335 road km). It lies in the northeastern portion of the province of Camarines Norte, between 140°15'00" N latitude and 122°45'00" E longitude. Bounded by natural barriers, ranging from hills and mountains to seas, it has the ocean to the north, Mt. Bagacay to the east and hilly terrain to the south and west. It shares a border with the municipality of Vinzons on the east, with Labo on the south and with Jose Panganiban on the west (Bureau of Soils and Water Management Topographical Map; PS Journal, 1990) (See Fig. 1.).

The Paracale gold mines are now leased to the United Paragon Mining Corporation, a joint venture of Filipino and Australian investors that started in August 1988. Full operation started with an estimated production of 500 kg of gold per month (PS Journal, 1990).

The mining area is over four centuries old, yet, no studies have been conducted on the impact of mining on the environment of Paracale and surrounding towns. The effect of the mining activity on marine and other aquatic organisms has not been assessed.

A few studies have been conducted locally which are related to the researchers' investigative effort in Paracale, Camarines Norte. Theede, Alino and Rosito (1987) have a preliminary report on the heavy metal content of marine bivalves from shallow areas near the Cebu harbor area. A reforestation and management study of mangrove swamp was done in Guimaras Island (Baldevarona, 1990).

This report is on the heavy metal load of the coastal waters of Paracale, a mining town with both large- (United Paragon) and small-scale miners who discharge effluents into the water systems of Paracale and Malaguit. Shellfish sample collections in the area served as indicator species on the presence of heavy metals and were correlated with the heavy metals detected in the aquatic environment.

MATERIALS AND METHODS

Selection of Sampling Stations

The sampling areas and collection sites were categorized into three: estuarine, bay and residential areas. Estuarine areas included the mangrove area of

Casalugan/Padanlan (Station 1), and Mangkasay and Nakulo points (Stations 3 and 4, respectively).

The bay areas included three Stations: (1) the vicinity of the mining area; (2) 500 meters away from the mining area; and (3) Pulandaga point. The residential area was covered by the Maligaya River system (Appendix 1).

Sampling of water and collection of specimens were done regularly in these stations except during unfavorable weather.

Water Sampling Method

Collection was done once every month in each sampling point using an improvised water sampler. Samples collected were analyzed for Ph, temperature and salinity on site and immediately acidified with conc. HNO_3 for preservation.

Fish, Shellfish and Plankton Sampling Method

Both fish and shellfish were collected in the designated stations. For classification and identification purposes, fish and mollusks were preserved in 10% buffered formalin and brought to the laboratory. Live specimens were maintained alive in fresh seawater. Some of the fish were obtained through the kindness of local fishermen. Fish from the river was caught by net or by hook and line. Mollusks were collected by hand. A maximum of 50 representatives per species were collected. Some were preserved while the rest were weighed and used for heavy metal analysis. Plankton samples were collected with a plankton net and immediately preserved with 10% formalin and 4 mL of glacial acetic acid in seawater for microscopic identification.

Water Analysis

Water analysis was done following "Methods for Chemical Analysis of Water and Wastes" (EPA, 1973). The water digests were analyzed by flame spectrography (Shimadzu AS-600).

Biological Samples

The fish organs and the soft tissues of gastropods and bivalves were preweighed to 1 g per sample in 2-3 replicates. Digestion of samples was done using conc. HNO_3 and 50 % H_2O_2 (AR) in a hot plate with hood. Samples were filtered and ultrapure water was added before spectrography (furnace or flame, Shimadzu-AS-600).

Preparation of Standard

Metal standards were prepared separately with analytical grade Cu, Cd, Ag, Au, Hg, Pb and Zn (Prepared Standards, Merck). Mercury analysis was done using cold vapor technique.

RESULTS AND DISCUSSION

Ocular Observation and Interviews

The collection of fish in the mangrove areas, in the river system and in the rocky shoreline of Paracale introduces one to several species of commercial fishes. The town alone has a frequency of fish catch reaching an average of three tons per fisherman per year. Although fishermen experienced some problems regarding fishing methods, fish caught was not directly affected in terms of volume. This was true for those who fished outside Paracale bay several miles away from the town toward the Pacific.

The volume of fish caught along the bay, however, had been reduced to almost nothing. This was attributed to the use of destructive methods of fishing like poisoning through sodium cyanide and plant extracts (*buli-buli*), dynamite fishing (*bumbong*) and use of fine mesh nets which caught even the fry of large fish species.

By observing the present condition of the seabed and the shallow waters of the coastal area, the researchers discovered that even the corals were heavily affected by siltation from the mining area. The wave actions hitting the dump site of the mine caused erosion which was very evident at Station 7B of UPMC. The silt mixed with the seawater to produce a very turbid water interphase with the clear water of the sea. It was not possible to find crawling mollusks in the vicinity.

Aside from the point sources mentioned, there were other non-point sources such as leaching and human waste. In addition to these prooblems, fish kills happened once a year. The cause of the fish kills is still not clear to the people and officials of the municipality. However, they suspect it could be due to cyanide leak from the mine tailings pond located at the peak of the mountain where the mining area is also located.

To safeguard the fishing grounds of Paracale, "Operasyon Bantay-Dagat" was established to prevent illegal methods of fishing near shore and off-shore. The existence of this safeguarding has been questioned as dynamite fishing and use of fine mesh nets continue. In the river system of Paracale alone, people reported use of sodium cyanide or "susa" to catch fry of the greasy grouper (*Epinephelus*) which are sold at very low cost and transported alive to Manila or exported.

Fish caught in Paracale, Camarines Norte, are listed with their common names and scientific nomenclature in Appendix 2. This was the result of a half-year inventory of fish in the area. The collection is not yet complete. The most abundant

fish in the river systems of Paracale was found to be the white mullet, *Mugil curema* Valenciennes.

Gastropods and bivalves were also collected identified and classified (Appendix 3). This is also not a complete list. Some of the collected shells have not yet been classified. In the mangrove areas, *Terebralia sulcatus,* "sihi" and *Crassostrea rhizophorae,* "tihim", a gastropod and bivalve, respectively, were the most abundant. The *sihi* were found embedded in the muddy substratum of the muddy estuarine. Usually, these were not easily recognizable because of the mud covering the shell. According to the people's reports, this species, including the mangrove species of *Nassarius, Telescopium* and *Nerita* were formerly found in commercial quantities. At present, they are not viable as food sources.

Biochemical Analyses

Environmental Parameters. The pH and salinity of the water in the different stations are summed up in Table 1.

The mean pH of water from different stations reached the NPCC standard which was between 6.5 and 8.5 (NPCC, 1977). Except for those collected from Station 5 (Maligaya area) with an average of 5.5, the reason for this very low pH is yet to be determined (Fig. 3). Perhaps observation of the activities of the people in the area could provide a background on the reasons for this.

The mean salinity during three sampling periods in 1992 varied (Fig. 4). Lowest salinity readings were in Stations 2 and 3 which were both in river system of Malaguit. This made it suitable to some growing and developing fish species, as the lower the salinity, the greater the solubility of oxygen (Coastal Ecosystem Management, 1983 from Milo C. Bell, 1973). Salinity also has a direct relationship with the chlorinity of the water which determines the amount of sodium chloride in water. This could also be from leaching salt deposits. The amount of chloride reached exceeded the maximum in seawater (1000 mg/L). In the samples, chlorine concentration ranged from 500 to 3850 mg/L. The highest reading obtained was from Station 4 in Pulandaga Point. The Office of the Governor, Austin, Texas (Coastal Ecosystem Management, 1983) presented in 1973 a water quality measure and the environmental events in land use, such as mining. The data identified predicted interactions of dissolved salts and water surrounding the area with which pH could also be interacting thus making it a necessary parameter to consider.

Water Samples. Analysis of water collected during different seasons in Paracale, which included dry (May), wet (September) and cold, windy (December), are recorded in Table 2. Two standards were used for acceptable metal contents of the water samples, one from NPCC and the other from the US-EPA. There are significant differences in the standards set for various heavy metals but the researchers confined themselves to standards set by the NPCC (1977).

A summary of the results of analysis of water samples collected from nine stations in Paracale, Camarines Norte is given in Table 2. Seven metals were analyzed

which were relevant in studying the ecology of the mining area (Figs. 5 and 6). Each metal analyzed was of significant function in the toxicity of the environment. Gold (Au) was not considered toxic as there was no given acceptable concentration in aquatic system in both standards. The remaining six metals (Cd, Cu, Pb, Hg, Ag and Zn) variedly affected the life histories of some indicator organisms. Cd, for instance, does not play any important physiological function in many mollusks and can even substitute Ca in some shells. Cu and Zn are two elements required by organisms for enzymatic and physiologic functions. Pb, Hg and Ag in high concentration caused various histological, biochemical and physiological damage to some fish and mollusks.

The data show that Paracale is indeed a gold mine town. There was a high concentration of gold in water sampled from all nine stations. This high concentration could be due to leaching processes in the sediment and the soils. Cu, which is usually associated with gold, ranked second to gold reaching up to 0.44 mg/L. This exceeds the allowable level set by NPCC which is 0.02 (EPA has 0.05 mg/L) and is consistent in all the stations. The fact that Cu is associated with gold in mineral-rich rocks collected beneath the Paracale mountains might give a clue to the presence of gold and determine the distribution of the atomic form of this metal in the water. Zn, on the other hand, was way below maximum permissible levels except in the vicinity of the mining area (0.13-0.23 mg/L). The latter, however, was still within the allowable level of 0.1-0.2 mg/L (NPCC, 1977).

Cadmium concentration was almost uniform in the different sampling stations. It remained within the allowable level except during the May sampling when it reached 0.09 mg/L. Lead was way above its tolerable limit in seawater (0.05 mg/L, NPCC and EPA) with an almost consistent reading of 0.2 mg/L with a little increase in the December sample in Station 7A. Mercury, with allowable concentration of 0.002 mg/L (NPCC 1977), was found in trace amounts or almost undetectable level except in a December sample from Station 7C (0.001 mg/L). This quantity overshoots the EPA's 0.0005 mg/L maximum permissible level. Silver was also very high, 0.03-0.06 mg/L, while NPCC allows only 0.01 mg/L in seawater in the Philippines. Silver was highest in Station 6 in December (Figs. 4 and 5). Lead, cadmium, mercury and copper are considered micropollutants (Bondon et al., 1988) and are filtered by mollusks like oysters and clams.

The least increase in the metal content of waters was usually observed in the vicinity of the mining area. The heavy metals were carried presumably by the water flow to the estuarine area in the closed portion of the river system which now serves as a sink or basin storing the mining effluents.

Biological Samples. The brackishwater fish, white mullet *Mugil curema* Valenciennes, the gastropods *Terebralia sulcatus* and *Telescopium telescopium* and bivalve *Crassostrea rhizophorae* were chosen as indicator species in this study. Although *Telescopium* is not important commercially, it was found to be one of the most abundant species in the mangrove area. This could well serve as indicator of pollution because of its sensitivity to change in environment and its

availability throughout the year. These were the preferred indicators because they stayed in place and were sensitive to disturbances as per the Coastal Ecosystem Management (1983) recommendation.

Analysis of biological samples showed consistent concentration of four metals (Ag, Au, Cd and Pb) in the various tissues of the fish and mollusks (Table 3). Only Cu and Zn showed variable concentrations in the different tissues of the indicator species. Ag, Au, Cd and Pb readings were 0.05, 1, 0.5 and 1 mg/kg (ppm), respectively, in mollusks. Cu was highest in *Terebralia sulcatus* (16.5 mg/kg) and lowest in selected organs of *Mugil curema* (2 mg/kg). However, a spectacular bioaccumulative response to Zn was exhibited by the *Crassostrea* with 772 mg/kg, followed by the digestive gland of *Telescopium* (17 mg/kg), then by the intestine and stomach (10.8 mg/kg) and the gills and liver of *M. curema* (12 and 9.75 mg/kg, respectively) (Figs. 7A and 7B).

Crassostrea appears to accommodate the presence of excessive amounts of zinc by accumulating the metal in the soft tissues. This could have greatly diminished the size and weight of the whole bivalve; and the size and weight of *T. Telescopium*. These findings suggest that people have been discouraged from consuming the shellfish in the area because of the heavy metal pollution in the area that has greatly reduced shellfish size (Molvear and Skei, 1988).

The deleterious effects of these heavy metals have been assessed in many types of filter-feeding mollusks (Norton and Murray, 1983; Harbo et al., 1983; Theede et al., 1987; Molvaer and Skei, 1988; Bondon et al., 1988; Devi, 1989) and in fish collected from a marine environment. It has been noted by the Coastal Ecosystem Management (1983) that concentration of heavy metals in the marine or coastal environment should not exceed safe levels to avoid deleterious effects on organisms. The lowest of these are in the μg concentration with highest in mg level. For instance, Cd should be present at only 0.2 μg/L (ppb). Below this concentration, there is minimal risk of deleterious effects (Coastal Ecosystem Management, 1983). In this analysis, Cd level and those of the rest of the heavy metals had exceeded the minimum risk threshold.

SUMMARY/CONCLUSIONS/RECOMMENDATIONS

The findings of this study showed that:

1. Paracale is still primarily a fishing town with abundant fish available. However, problems existed regarding methods of fishing.
2. Shellfish abundance is endangered. The common edible gastropod, *Terebralia sulcatus*, was heavily affected.
3. Heavy metals suspended in the aqueous environment were of considerable concentration with Au having the highest level, due to active mining in the area. Zinc did not exhibit any threat to the organisms.

4. *Crassostrea rhizophorae*, a bivalve, showed bioaccumulation of zinc which reached 772 μg/g. Shell size and weight were affected.

5. Since the heavy metal concentrations exceeded the minimum risk threshold, there could be certain deleterious effects which may be detected in the histological structures of the organisms.

It is further recommended that further studies be conducted on the histology and physiology of the organisms exposed to the total dissolved metals in Paracale waters. A regular twice a month sampling should be considered as well as additional environmental parameters like speed of water flow and DO in the study areas.

ACKNOWLEDGMENT

We would like to thank the following for the support granted to our research work in Paracale:

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The people of Paracale and officials of the town for their cooperation and kindness in providing necessary information; and

The DENR and Mines Sector of Region V in Legazpi City for making available some literature, the Bureau of Soils and Water Management (Quezon City) for the maps.

DESCRIPTION OF SELECTED STATIONS

1. Padanlan/Casalugan area is a closed river system which ends at Casalugan (NW of Paracale). It receives seawater every day from 9 am to 12 nn and from 5 pm to 9 pm. Freshwater of the surrounding barangays of Padanlan and Bagumbayan drain into the river. It is a good site for fishponds and nurseries. Some fishermen have constructed fishpens and fish traps which they use during high tide.

2. Mangkasay area (Malaguit river) is a close branch of the Malaguit river receiving seawater at the same time as Casalugan/Padanlan. Fewer fishponds are located here. The banks are lined with abundant mangroves, *Rhizophora sp.*, with attached bivalves and gastropods.

3. Nakulo area (Malaguit river) is an area with many small-scale miners along the river banks and crossing the area of Calaburnay. There is occasional fish traping activity using permanently constructed structures blocking the water flow during high and low tides.

4. Pulandaga point is a bay* lined with sand at the central portion and flat corals at two sides. Rocks and boulders, which could be remains of old fish port or pier, serve as habitat for some gastropods and bivalves, while flat corals are for the

*Bays which are typically quite open to the sea and receiving strong tidal flow, are well flushed through tidal exchange and they often receive considerable additional circulation driving power from freshwater inflow (Coastal Ecosystem Management, 1983).

crustaceans, coelenterates and fishes. There are patches of sea grasses and several species of macroalga and corals.

5. Maligaya area is a residential area near the mouth of the Paracale river. The fish port is located 25 meters away. Most of the families are in small-scale mining business with gold extraction by amalgamation. Several temporary ponds for gold panning contribute wastes to the river via small ditches.

6. Some 500 meters away from the mining area is the outer portion of Paracale bay with unstable water column due to wave action. This serves as a fishing ground for small fishermen who mostly use nets. The substratum is originally sandy but at present overlayed with yellowish to brownish silt, may be due to dumping by the mining activity along the coastline of Longos point.

7. The Mining Area (A, B, C). Three stations were established in the vicinity of the mining area for possible variations in metal loads depending on the structures located in each area. Station 7A is near the cyanide treatment plant. Station 7B is the opening of the underground water disposal with soil similar to that collected under water 500 meters away in Paracale bay. Station 7C is the portion of the Longos point which was originally a sandy beach but at present not suitable for swimming because of heavy siltation covering the coralline shores.

Table 1. Physico-Chemical analysis of water samples from Paracale (On-Site)

Month of Collection	*May*	*Sept*	*Dec*		*May*	*Sept*	*Dec*	
Parameter	*pH*	*pH*	*pH*	*Ave*	*Sal %*	*Sal %*	*Sal %*	*Ave*
Site								
1. Padanlan Pt	8.1	–	–	8.1	5	–	4	4.5
2. Mangkasay Pt[x]	–	7	7.4	7.2	–	1	1	1
3. Nakulo Pt[x]	–	7	7.5	7.25	–	3	1	1
4. Pulandaga Pt	8.1	7	8.6	7.9	5	5	5	5
5. 300 m away UPMC[x]	5.5	–	–	5.5	4	–	–	4
6. Maligaya Pt	8.1	7	8.1	7.73	3	3	2	2.5
7. UPMC Proper								
a. Pt. 1[x]	–	–	7.4	7.4	–	4	4	4
b. Pt. 2[x]	–	–	6.9	6.9	–	2	2	2
c. Pt. 3[x]	–	–	8.3	8.3	–	2	2	2
NPCC STANDARD Ave.	7.5				0.5			

[x]Final Sampling Points (Additional sites which started September 1992)
– No analysis was done (due to unfriendly weather or equipment malfunctions).

Table 2. Heavy metal loads of the Paracale waters May to December sampling periods

(PPM) Analytes		Stations I	II	III	IV	V	VI	VIIA	VIIB	VIIC	Permissible level NPCC	US-EPA
(Cd)	May x	0.09			0.09		0.09	0.09				
	Sept xx	0.0145	0.0115	0.0115	0.07	0.01	0.01	0.01	0.014	0.01		
	Dec xxx	0.01	0.01	0.01	0.01	0.01		0.01	0.01	0.01		
AVE		0.038	0.01075	0.011	0.057	0.01	0.05	0.037	0.012	0.01	0.01	0.01
(Cu)	x	0.25			0.25		0.25	0.25				
	xx	0.07	0.055	0.05	0.065	0.075	0.08	0.91	0.75	0.185		
	xxx	0.03	0.01	0.01	0.02	0.04		0.16	0.05	0.02		
AVE		0.117	0.032	0.03	0.112	0.058	0.165	0.44	0.4	0.102	0.02	0.05
(Au)	x	0.5			0.5		0.5	0.5				
	xx	1	1	1	1	1	1	1	1	1		
	xxx	1	1	1	1	1		1	1	1		
AVE		0.833	1	1	0.833	1	0.75	0.833	1	1	NA	NA
(Pb)	xx	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2		
	xxx	0.2	0.2	0.2	0.2	0.2		0.34	0.2	0.2		
AVE		0.2	0.2	0.2	0.2	0.2	0.2	0.27	0.2	0.2	0.05	0.05
(Hg)	x	0.0002			0.0002		0.0002	0.0002				
	xx	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005		
	xxx	0.0005	0.0005	0.0005	0.0005	0.0005	--	0.0005	0.0005	0.001		
AVE		0.0004	0.0005	0.0005	0.0004	0.0005	0.0004	0.0004	0.0005	0.0008	0.002	0.0001
(Ag)	x	0.02			0.02		0.02	0.02				
	xx	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04		
	xxx	0.04	0.04	0.04	0.04	0.04		0.04	0.04	0.04		
AVE		0.033	0.04	0.04	0.033	0.04	0.06	0.033	0.04	0.04	0.01	0.00005
(Zn)	x	0.06			0.22		0.02	0.04				
	xx	0,095	0.07	0.14	0.055	0.095	0.075	0.3	0.175	0.2		
	xxx	0.09	0.08	0.08	0.14	0.15		0.35	0.14	0.06		
AVE		0.082	0.075	0.11	0.138	0.122	0.048	0.23	0.158	0.13	2	0.1

National Pollution Control Commission, 1977
US-Environmental Protection Agency, 1973

Table 3. Heavy metal concentrations in the soft tissues of gastropods (*Telescopium, Terebralia*) and bivalve (*Crassostrea*) and selected organs of white mullet, *Mugil curema*

SAMPLES	ANALYTES(mg/kg) Silver	Gold	Cadmium	Copper	Mercury	Lead	Zinc
Crassostrea	0.05	1	0.5	5		1	772
T. telescopium ST	0.05	1	0.5	9.5		1	10.8
T. telescopium OG	0.05	1	0.5	14.2		1	17
Terebralia sulcatus	0.05	1	0.5	16.5		1	9.25
Average	0.05	1	0.5	7.175		1	199.95
White Mullet							
Mugil curema (Valenciennes)							
Gills	0.05	1	0.5	2		1	12
Liver	0.05	1	0.5	2		1	9.75
Gonad	0.05	1	0.5	2		1	4
St + Intestine	0.05	1	0.5	2		1	4
Kidney	0.05	1	0.5	2		1	4.5
Average	0.05	1	0.5	2		1	6.85
Minimum Risk Threshold[x]	NA	NA	0.2 µg/L	0.01mg/L	0.01 mg/L	0.02	mg/L

[x]National Academy of Science, 1973. Coastal Ecosystem Management, 1983.

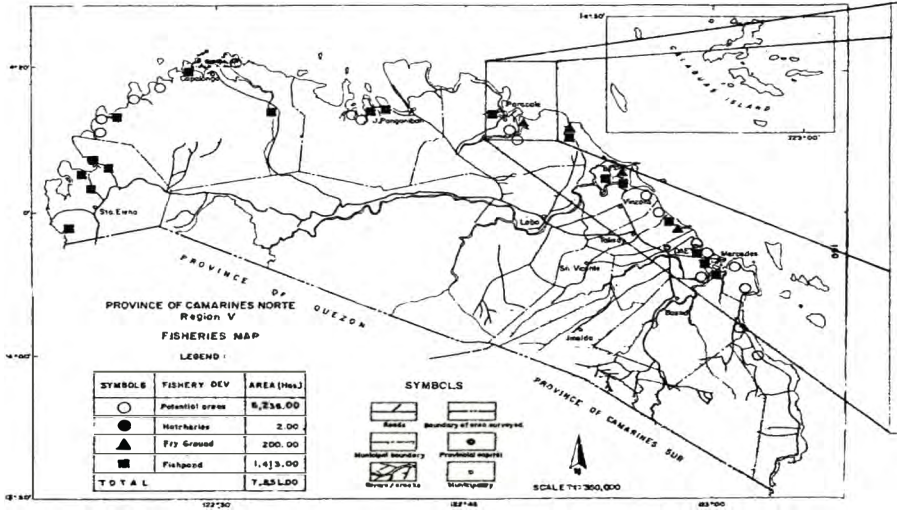

Figure 1. **Potential areas for fisheries development and the topographical location of Paracale**

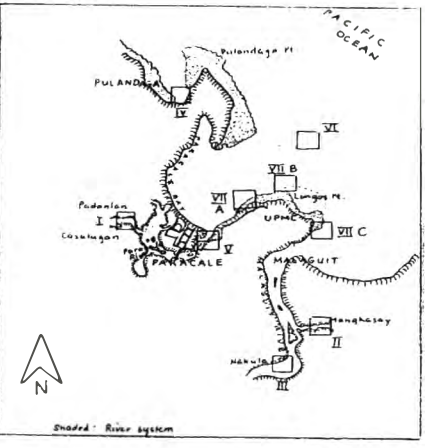

Figure 2.

Collection sites in Paracale, Camarines Norte (Stations I, II, III, IV, V, VI, VIIA, B and C)

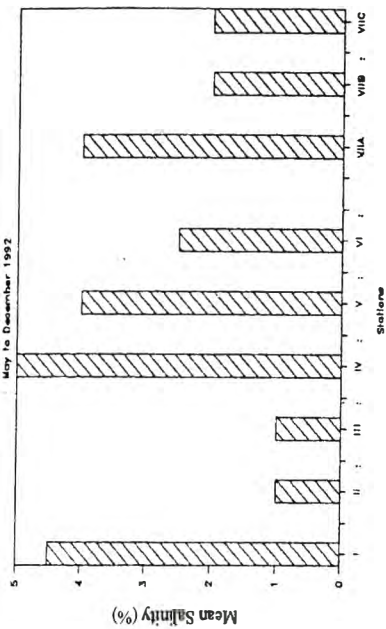

Figure 4. Mean Salinity of Paracale Waters

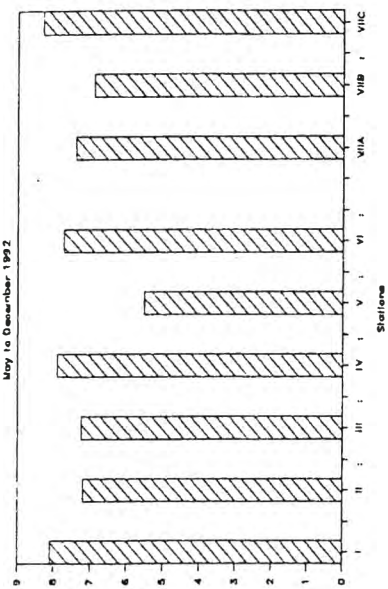

Figure 3. Mean pH of the Paracale Waters

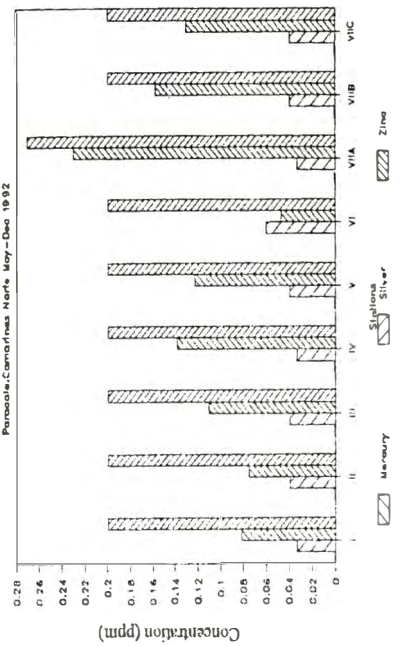

Figure 6. Average metal concentrations of water

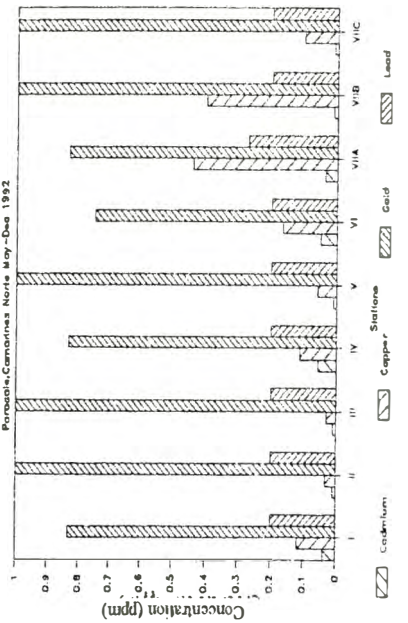

Figure 5. Average metal concentrations of water

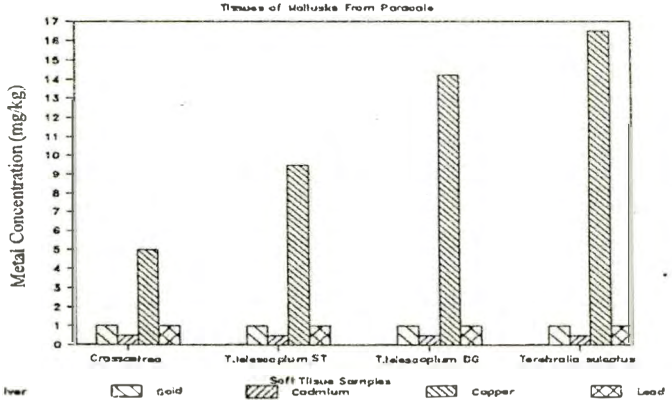

Figure 7A. Average metal concentrations in soft tissues of mollusks

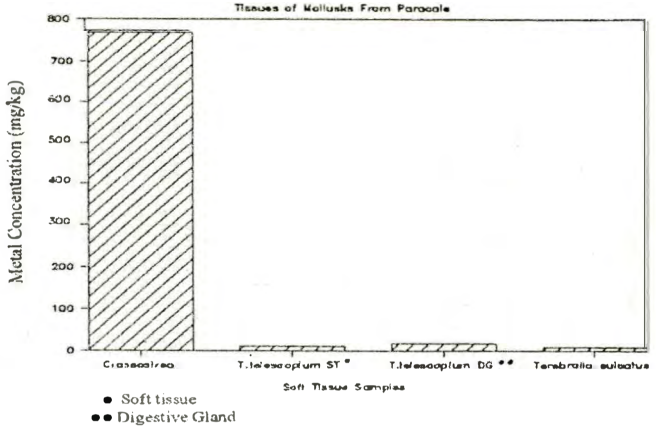

Figure 7B. Average zinc concentrations in soft tissues of mollusks

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IMPACT OF ACUTE COPPER INTOXICATION OF SOME ORGANS OF *CLARIAS MACROCEPHALUS* GUNTHER

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ABSTRACT

Effects of acute copper exposure to *Clarias macrocephalus* at 1.2 mg/L were determined over a period of 48 hours. The gill filaments, kidney and liver were examined for any pathological effects. Histological analysis showed extensive changes in each organ. The gill filaments exhibited hyperplasia of lamellar epithelium, cell proliferation between secondary lamellae and a corresponding reduction of the interlamellar space. Increased vacuolation, widespread necrosis and degeneration of epithelial cells were likewise evident. The tail kidney was characterized by irregularly-shaped cells. Dilation of the Bowman's space with accompanying hypertrophy of the glomerulus was evident. Extrusion of cellular materials characterized the lumen of the tubules. The liver tissue showed general destruction. It was marked by the absence of sinusoids, increased vacuolation and the presence of partially coagulated blood in the central vein.

INTRODUCTION

Copper, like zinc and iron, is considered an essential element for animals. Being a micronutrient, it is present in *Clarias batrachus* at the level of 6.888 + 0.17 ug/g (Daramola and Oladimeji, 1989). However, at high concentrations it may cause poisoning or changes in the organoleptic qualities of food, owing mainly to its role as catalyst in many degradation processes (Galindo et al., 1986).

Copper is a metal water pollutant that may be derived from anthropogenic activities, especially from industrial and agricultural wastes that drain into bodies of water. It tends to accumulate in bottom sediments from which it may be released by various processes of remobilization. Among the heavy metals, copper constitutes an important section of the nonferrous metal industry (Daramola and Oladimeji, 1989). The waste from such industries contains traces of copper which when

discharged into bodies of water are made available to aquatic organisms, particularly fish. These trace amounts may be concentrated in harmful levels (Uthe and Bligh, 1981; Segner, 1987). They can thus move up the food chain in several forms, thereby reaching man in whom they could produce chronic and acute ailments.

Copper accumulation is a function of exposure time and concentration in water. The greater the concentration of copper and the longer the time of exposure, the larger the amount of copper residue found in the fish. The rate of uptake is initially very high and at lower concentrations, although this decreases with time. An increased rate of accumulation over a longer period of exposure implies an impaired capacity to eliminate the heavy metal cupric ions from the fishes' system. This may result in high body burden that may be passed on to consumers (Daramola and Oladimeji, 1989). Investigations of acute effects of copper on fish are therefore an important aspect of environmental pollution control, most especially since human activities progressively increase the concentrations of heavy metals in the aquatic system.

In this study, *Clarias macrocephalus* Gunther are exposed to acute copper contamination. This species has been selected for factors such as its local importance, size, availability, food value and its suitability in the laboratory. Histopathological effects of the metals in the fish are analyzed in vital organs such as the gills, kidney and liver.

The introduction of small amounts of copper ions from natural and enthropogenic sources into the aquatic environment causes multiple changes in freshwater organisms, even at non lethal levels (Khangarot and Ray, 1987a). For instance, exposure of mammalian test animals to heavy metals, even at moderate levels of contact, has been shown to alter immunological responses (Koller, 1980). Sublethal exposure of freshwater fishes to copper on the other hand, have more so reduced their immune responses (Viale and Calamari, 1984). This sublethal toxic effect of heavy metals on the immune system of fish has been pointed out as modification to the outbreak of infections leading to bacterial or viral diseases (Sindermann, 1979). It can be postulated then that the histology of tissues has been altered by exposure to copper.

Vernberg et al. (1974) reported a decrease in metabolic rate in Uca pugilator as induced by heavy metals. Likewise, cupric ions were shown to cause respiratory and cardiovascular depression in *Mytilus edulis* and the effect was attributed to the passive binding of cupric ions with organic ligands (Scott and Major, 1972).

The minimum requirement and maximum tolerable levels for dietary copper remained to be determined for most species of fish. In rainbow trout, the highest admissible concentration is less than 0.005 mg, and for carp less than 0.007 mg. Long-term action of low $CuSO_4 . 5H_2O$ concentration causes extensive pathological changes in the histological structure of parenchymatous organs and in the copper content in the gills and liver of these experimental fish (Svobodova et al., 1982). Murai et al. (1981) observed a toxic effect of supplemental copper at 32 mg Cu/mg

diet in channel catfish. Toxicity was characterized by a reduction in growth, altered blood parameters, an increased feed gain ratio and food refusal.

MATERIALS AND METHODS

A. Test Specimen

The catfish *Clarias macrocephalus* was obtained from a hatchery and used as the test specimen. The juvenile catfish measuring 6.0-8.0 in and weighing about 10.0-20.0 g was acclimated to laboratory conditions three days prior to the experiment.

B. Preparation of Copper Metal

A stock solution of copper was prepared from copper sulfate ($CuSO_4$.20) by dissolution in deionized water and acidification with concentrated HNO_3. The test concentration of 1.2 mg/L was prepared using the stock solution.

C. Copper Treatment

Six aquaria with capacity of 5 l were used in the study. These were divided to make three duplicate set-ups for acute concentrations of copper and one for control. Dechlorinated tap water, which was previously allowed to stand for three days in a tank, was used in all tests.

Three juvenile fish were placed in each aquarium. The fish were fed to satiation twice a day using commercially avialable fish flakes.

D. Paraffin Method

The gill filaments, liver and kidney were processed for histological analysis using the standard paraffin method.

RESULTS

Juvenile catfish exposed to copper were observed to be initially hyperactive following exposure. After approximately three hours, the fishes surfaced, floated for a few minutes and gulped air. At the time of loss of equilibrium, they appeared slanting at the bottom with slight opercular movements. Swimming was sluggish and in abnormal positions. Finally, at the time of death the fishes were found lying on the floor of the aquaria exhibiting feeble opercular movements and labored breathing. Fifty percent mortality was observed over a period of 48 hours.

A distinct response to the physiological stress of copper exposure is the secretion of mucous. Copper induced appreciable mucous secretions, especially at the time of death when the fish were coated with excessive mucous on the body surface. This was evidenced by the change in the clarity and color of water to a murky yellow-brown hue. Other copper-induced responses included reddening of the region surrounding the pectoral fins and barbels, and the limping of barbels.

The fish in the control set-up exhibited normal movements, good reflexes and immediate responses to physical disturbances. A considerable low amount of mucous secretion and less cloudy water were observed.

Histopathology

Gills. Figure 1 shows the untreated gills with normal histological architecture.

Histological alterations of the gill filament were observed in copper-induced *Clarias macrocephalus*. Hyperplasia of the lamellar epithelium was pronounced (Fig. 2). Cell proliferation between the secondary lamellae was apparent. This eventually caused interlamellar space reduction. Loss of interlamellar space was accompanied by sloughing off of the lamellar epithelium.

The secondary lamellae also exhibited a reduction in height and a thickening of cell epithelium. This proliferation of the secondary lamellar epithelia produced a short and stubby appearance (Fig. 1).

A curve characterized the arrangement of pillar cells, as compared to the normal straight orientation (Fig. 1).

Degeneration of pillar cells also occurred.

Cells in the gill filaments exhibited widespread necrosis. Degeneration of cells was evident in the irregularly-shaped, shrunken and unhealthy appearance of cells in the secondary lamellae. Vacuolation (Fig. 4) was noticed.

Degeneration of acidophilic chloride cells near the secondary lamellar base between lamellae was also noted (Fig. 3).

Tail Kidney. Figure 5 is the normal histoarchitecture of the tail kidney. Histological changes characteristic of deformation of cells were observed in the renal capsule (Fig. 6). Irregularly-shaped cells replaced formerly cuboidal Bowman cells. Dilation of the Bowman's space was marked. This was associated with hypertrophy of the glomerulus.

Vacuolation and disintegration of the cells in the epithelium of the renal tubule were observed. Cells of the renal tubule and corpuscle appeared indistinct and largely undemarcated by cell membrane boundaries (Fig. 7). Hydropic degeneration was also manifested in the epithelial cells of the proximal segments of the renal tubule, largely occluding the lumen. The extrusion of cellular materials into the tubular lumen forming cellular casts further gave the lumen a cloudy appearance. Due to hyperplastic growth of epithelial cells and subsequent degeneration (shrinking), sinusoidal spaces were created within the nephron tissue.

Liver. Copper-induced effects disrupted the normal architectural patterns of the liver (Fig. 8). The integrity of the liver tissue was destroyed leaving large gaps and an appearance of torn tissue. Absence of sinusoidal spaces was marked, emphasizing the swelling of the cytoplasm. The plates of liver tissue exhibited many cellular spaces devoid of nuclear content or with significantly reduced nuclei, indicative of edematous hepatocytes (Fig. 9).

The tail kidney exhibited several histopathological changes. Defense mechanisms similar to the gills were apparent. These included dilation and hypertrophy of the Bowman's cells as well as vacuolation.

Degeneration was evident in different forms, the most pronounced of which was exsanguination. Loss of blood was due to the destruction of the red blood cells by hydropic distension due to water uptake, leaving the cells anemic (Khangarot et al., 1987b). Hydropic degeneration of the cells in the renal tubule contributed largely to blood loss. Extrusion of cellular casts in the tubular lumen was also observed by Chang and Spreacher (1976). These cellular masses are believed to be discharged by the kidney during cell injury, thereby occluding the lumen.

Sinusoidal spaces were created within the nephron tissue due to the initial hyperplastic growth of epithelial cells and subsequent degeneration or shrinking.

The liver is the organ responsible for the detoxification proccesses. Fish liver contains enzyme systems necessary for the detoxification of toxic materials such as heavy metals, pesticides and petroleum hydrocarbons (Haensly et al., 1982). It is therefore the main organ for accumulation of the toxicant copper. Several studies serve as evidence that the liver accumulates the greatest amount of copper residues as compared to muscle, gills, brain and blood (El-Domiaty, 1987; Radhakrishnaiah, 1988).

Extensive histopathological changes in the liver were observed due to increased copper content (Svobodova et al., 1985). Among these are extensive vacuolations, destruction of the integrity of the hepatocyte, absence of sinusoids, presence of cellular spaces without nuclear material and proliferation of partially coagulated blood in the central vein.

Studies by Lanno et al. (1987) show that rainbow trout has the ability to sequester dietary copper in discrete granules in the cytoplasm of the hepatocytes. The toxicant is acted upon by liver superoxide dismutase which stabilizes the system by binding to radicals (Gaitlin and Wilson, 1986). However, increased copper intake reduces the enzyme activity, thereby allowing prevalence of copper toxicity in the hepatocytes. The advanced effect of copper toxicity is evidenced by destruction of the integrity of liver tissue.

Water intake brings about the swelling of cytoplasm thus narrowing eventually closing sinusoidal space. Swelling of the cytoplasm of hepatocyte leads to its subsequent destruction (Sultan and Khan, 1981). Edema, which refers to the increase in the volume of tissue fluid (Leeson, 1985), also results in the presence of cellular spaces with greatly reduced nuclei.

Partial coagulation of the central vein blood is also indicative of copper toxicity as seen in the liver of *Mollinenesia* (Sultan and Khan, 1981).

Extensive vacuolation is a prominent feature of hepatocytes induced by copper. This indicates an increase in fat and glycogen content (Grizzle and Rogers, 1976). Lipid accumulation is a common hepatic response to toxic agents such as heavy metals, carbon tetrachloride, phosphorous and chlorinated hydrocarbon insecticides (Anthony et al., 1986).

SUMMARY

Histopathological effects of 1.2 mg/L copper exposure on *Clarias macrocephalus* over a period of 48 hours were analyzed. The gill filaments, tail kidney and liver showed disruption of the normal histological pattern. Copper at 1.2 mg/L is toxic to *Clarias macrocephalus* G.

Figure 1. The control gills with normal histological pattern (x 100)

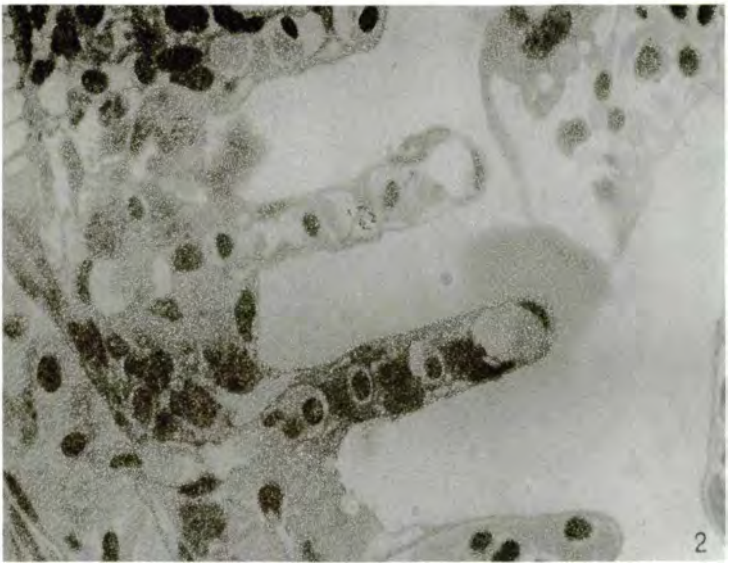

Figure 2. Copper-treated gills have pronounced hyperplasia of the lamellar epithelium (x 100).

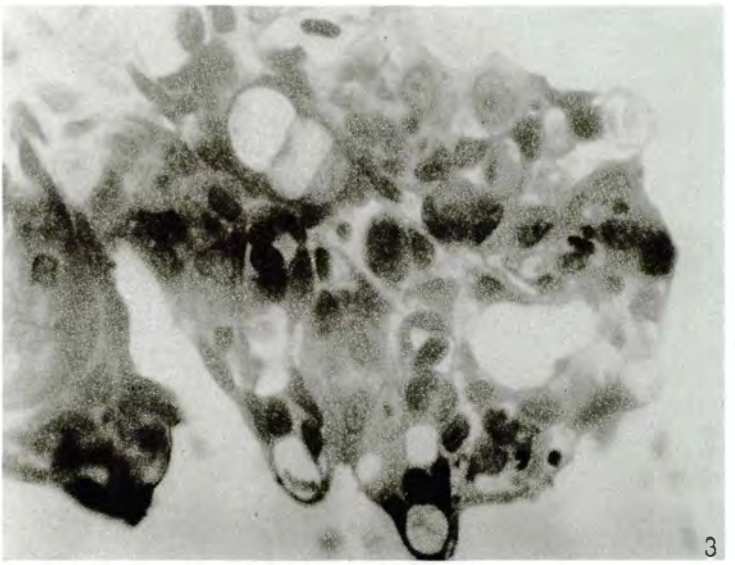

Figure 3. Some lamellae of the treated gills have short stubby appearance (x 100).

Figure 4. Vacuolation of cells is evident in the treated gills (x 100).

Figure 5. The normal histoarchitecture of the tail kidney

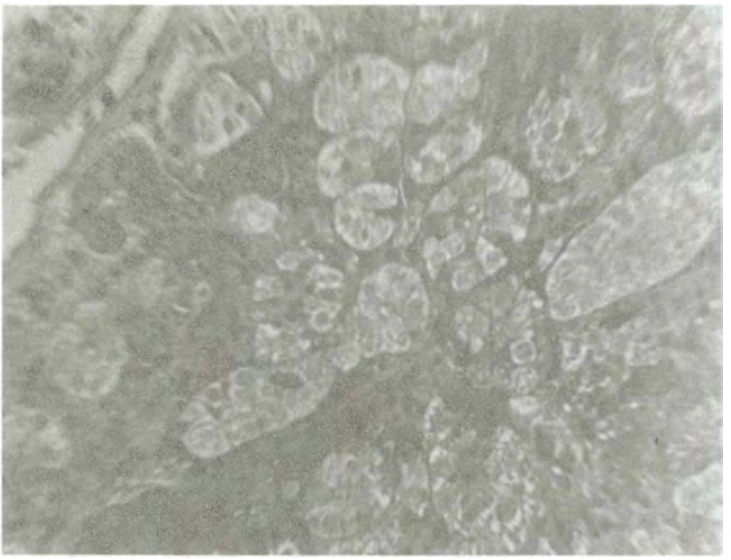

Figure 6. Irregularly shaped cells appear in the treated kidney.

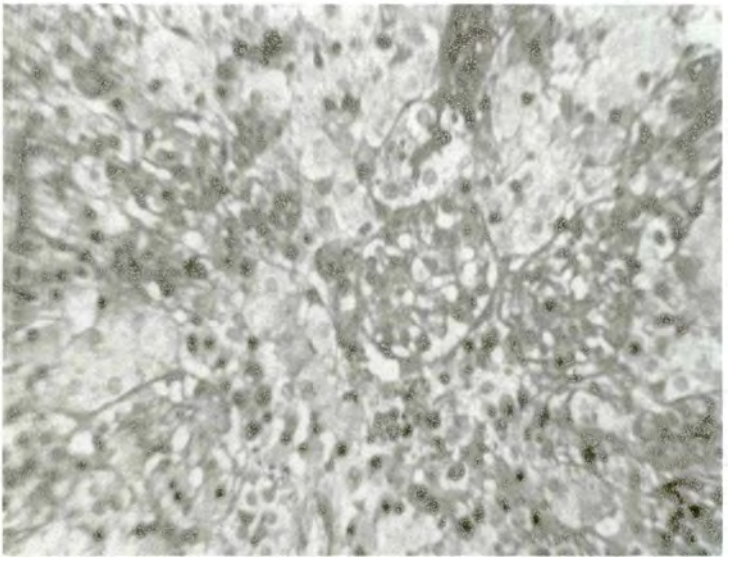

Figure 7. Cell membrane boundaries are not distinct in the renal tubules of treated fish (x 400).

Figure 8. The normal architectural pattern of the control liver (x 400)

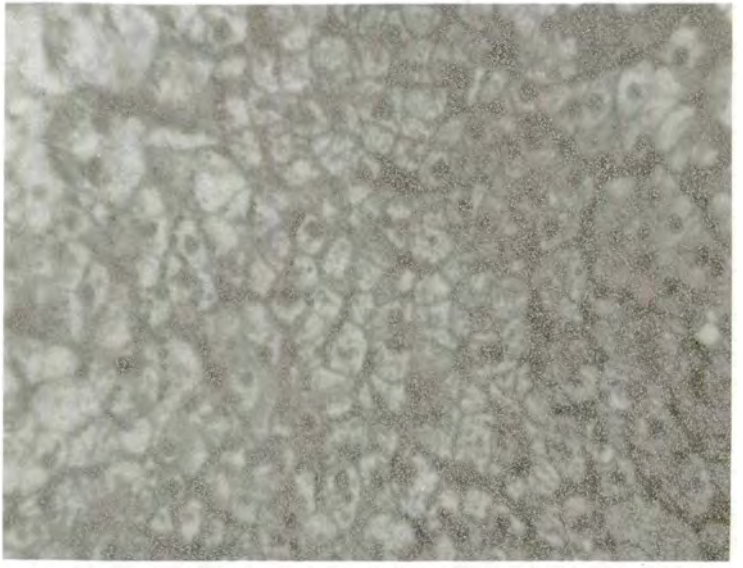

Figure 9. Edematous hepatocytes are formed in the treated liver (x 400).

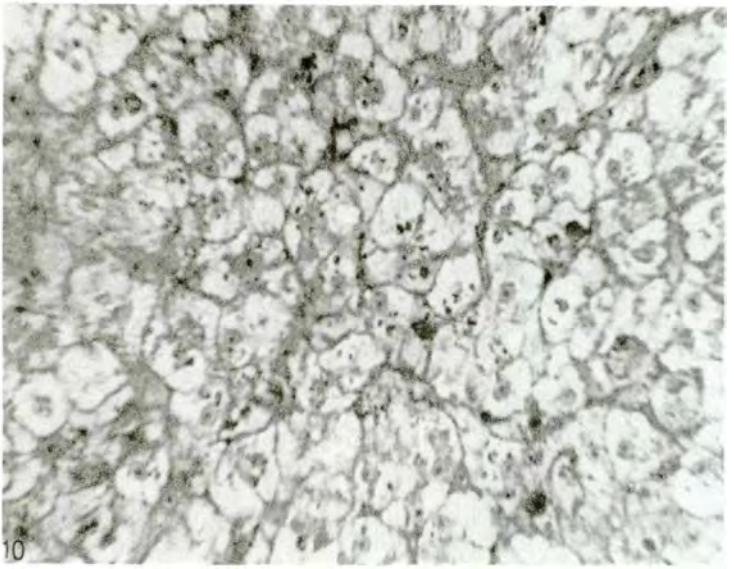

Figure 10. Widespread vacuolation of hypertrophied hepatocytes is evident in treated fish (x 400).

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OCCURRENCE OF ROOT KNOT NEMATODES IN PERENNIAL AND UNUSUAL PLANTS AND THEIR CONTROL USING BACTERIA

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ABSTRACT

Two species were identified from 35 root-knot nematode populations established from galled roots of infected plants, namely, *Meloidogyne incognita* and *Meloidogyne javanica*. Eighty-nine percent of the population was *M. incognita* while only 11 percent was *M. javanica*. Four races of *M. incognita* were determined through differential host tests, with races 1, 2 and 3 equally common.

Meloidogyne incognita populations showed two EST phenotypes in starch-gel electrophoresis: 2-bands for 22 isolates and 1-band for three isolates. On the other hand, all the populations exhibited two G6-PDH bands.

No bands were resolved for *M. javanica* populations regardless of the enzymes studied.

Antibodies and antigens from related root-knot nematode species and races elicited precipitin lines in agar-gel immunodiffusion tests.

Ornamentals and plantation crops were more prone to infection than the tree species. *Meloidogyne javanica* was dominant in hardwoods while *M. incognita*, race 1 was common in ornamentals and in Los Baños.

The infected plants represented 24 plant species and 17 families. Twenty-four of the infected plants were new hosts.

Bacterial isolate SFE was promising among several strains screened for nematicidal activity. Hatchability of treated root-knot nematode egg masses was significantly less. Plants drenched with treated egg masses were tall and had less root galls and heavier fresh weights. The isolate, which was identified as *Bacillus stearothermophilus*, was comparable to *Bacillus thuringiensis* strains tested.

INTRODUCTION

The most devastating nematodes today are undoubtedly the root-knot nematodes, *Meloidogyne* spp., which have been linked by experimental evidence to yield decline in cultivated plants (Ducusin and Davide, 1971; Madamba et al., 1971; Castillo et al., 1977). Together with other nematodes, they have caused severe losses to agriculture worth P6.5 billion in 1985.

Despite a long host list, most research has focused on the more economically important annuals. Little attention has been given to perennials and unusual plants, which may also be hosts of the nematode.

In addition, not much information is available on taxonomic identities, which is crucial in developing control strategies. On the basis mainly of the perineal patterns of adult root-knot nematode females and differential host response, the species *Meloidogyne incognita, M. javanica, M. arenaria, M. thamsi, M. hapla and M. megatyla* have been reported in the country (Valdez, 1968; Madamba, 1981; Davide, 1981).

The variability of root-knot nematode perineal patterns makes it difficult to identify them solely on this basis unless sufficient specimens are available (Franklin, 1972; Thorne, 1961). A reliable, rapid method for identifying root-knot nematode species and races is therefore needed to augment conventional methods which make use of perineal patterns.

Problems and limitations associated with various control methods, such as chemical nematicides which are expensive, dangerous if used improperly and damaging to the environment, have led to the search for options which may complement existing methods within the context of an integrated control program. Research on biological control began early this century, but the technology became commercially available only recently. It used to focus mainly on fungi, although a parasitic bacterium, *Pasteuria penetrans,* has attracted much interest in recent years. The study intends to fill this need by screening for potential biocontrol agents.

The aim of this research is to obtain information on the identity, distribution and host-plant occurrence of root-knot nematodes, study their biochemical and physiological variabilities as means of identifying among and within species, as well as controlling them biologically by means of bacteria.

MATERIALS AND METHODS

Survey and Collection

Galled roots from root-knot nematode-infected plants were collected from Laguna, Batangas and Quezon. Nematode cultures, which provided materials for identification and subsequent studies, were initiated from the infected roots used as inoculum on a susceptible tomato variety.

Pure cultures were established from a single egg mass obtained from each of the initial cultures and maintained by successive monthly transfers into new tomato seedlings.

Identification

Root-knot nematode identifications were based on the variability in the morphology of the adult female, response to a set of host differentials and protein profiles of the successfully established populations.

The perineal patterns of adult nematode females were processed according to standard procedures (Taylor and Netscher, 1974). The females were extracted from infected roots softened by enzyme preparations. The differential host test used by the International Meloidogyne Project was adopted with the following plant varieties used: tobacco NC 95, cotton Delta Pine, pepper California Wonder, watermelon Charleston Grey, peanut Florrunner and tomato VC 11.

Esterase (EST) and glucose 6, P dehydrogenase (G-6 PDH) enzyme activity were determined from homogenates obtained by macerating 45-day-old root-knot nematode females of 28 selected root-knot nematode populations. In addition, EST enzyme profiles of egg, juvenile and adult development stages of the nematode were compared.

Electrophoresis was carried out using Mupid 2 mini-gel electrophoresis unit. The starch-gel was prepared using 10% hydrolyzed starch and buffer appropriate for the EST and G-6, PDH enzymes investigated. Bromophenol blue solution, which was used as tracking dye, and the protein extracts from nematode samples were introduced into sample wells soon after the gel had solidified and cooled.

The electrophoretic apparatus was placed inside a refrigerator where the run was carried out for two hours. Subsequently, the gels were stained with alpha naphthyl acetate and incubated at 37°C for 40-45 minutes until distinct bands appeared. The relative electrophoretic mobility of each isoenzyme was calculated as the ratio of the movement of the band to that of the tracking dye.

Studies on protein profiles were also conducted using antigen-antibody reaction in immunodiffusion tests. Antisera were produced in rabbits through intravenous injection of antigen preparations consisting of supernatants from homogenates of macerated root-knot nematode females. The immunization schedule consisted of three intravenous injections with five-day rest period in between, followed by another three consecutive shots administered by the same route.

Serum containing the antibody was aspirated from blood collected from the rabbit's right marginal ear. Antibodies were isolated from the serum preparations by repeated resuspension and precipitation using distilled water and anhydrous Na_2SO_4. The colorless alpha-globulin fractions were dialyzed against 0.01 phosphate buffer with pH 7.5.

Agar gel plates were prepared using Noble agar. A centrally-located well and four peripheral wells were made in each dish. A few drops of the antisera were

placed in the center wells while antigen preparations from known root-knot nematode species were placed in the peripheral wells. Plates were incubated at 22°C in a moist chamber and examined after 48-96 hours. Reciprocal serological tests were conducted using the same gel patterns.

Biocontrol

Samples which were obtained from cultivated soil around Los Baños were placed in test tubes containing tryptone-glucose-yeast extract broth (TGYB) and incubated for 48 hours to allow sporulation. Afterwards, the test tubes were heated to 70°C to kill all non-spore formers. Aliquots were transferred to other te t tubes, and the process was repeated several times to ensure that only non-spore formers remained.

Hatchability and greenhouse tests were undertaken to determine nematicidal activity. Ten milliliters of the bacterial suspension grown overnight by shaking in 20% coconut water was prepared and introduced into an equal volume of distilled water containing root-knot nematode egg masses. For the control, an equal volume of 20% coconut water was added to the distilled water with the egg masses. The number of juveniles hatched after 72 hours was counted, and percentage reduction in egg hatch was computed.

One hundred milliliters of the bacterial isolate grown overnight in 20% coconut water was drenched into the soil planted previously to a root-knot nematode-susceptible tomato variety VC 11. Soon after drenching, root-knot nematode eggs were introduced into four depressions at the base of each plant. Seedlings watered with the same volume of tap water and 20% coconut water served as controls. Initial and final heights of the plants were taken, and the number of galls counted. Per cent change in height and galling were also noted.

The potential bacterial isolates were identified on the basis of their morphological and physiological characteristics.

RESULTS AND DISCUSSION

Survey and Collection

The survey, involving cereals, fruit and tree species, plantation crops and ornamental plants, resulted in the collection of 32 root-knot nematode-infected plants from which about 35 nematode populations were successfully established.

Identification

Investigation based on the morphology of the perineal patterns of adult root-knot nematode females augmented by differential host response tests and

protein profile studies through electrophoresis and immunodiffusion tests has resulted in the identification of two root-knot nematode species, namely *Meloidogyne incognita* and *Meloidogyne javanica*.

The majority of the root-knot nematodes identified were *Meloidogyne incognita* (89%) while a few (11%) were *Meloidogyne javanica* (Table 1 and Figure 1). Four races of *M. incognita* were identified by the differential reaction of tobacco NC-95 and cotton Deltapine 16. *Meloidogyne incognita* races 1, 2 and 3 were equally common (Table 2).

Meloidogyne incognita populations showed two EST phenotypes: 2-bands for 22 isolates (79%) and 1-band for three isolates (11%) (Figure 2). On the other hand, all the *M. incognita* populations exhibited two G 6-PDH bands (Table 3). These studies confirm previous investigations by Dickson et al. (1971).

The identification of *Meloidogyne incognita*, race 2, which differential host tests could not distinguish from *M. arenaria* variants that did not infect peanuts, was confirmed when these populations exhibited two EST bands with R_f values similar to that of the other *M. incognita* isolates (Figure 3). They also showed two G6-PDH bands. On the other hand, Dickson et al. (1971) were unable to detect G6-PDH bands(s) in *M. arenaria*.

All *M. javanica* populations did not show any band, regardless of the enzyme studied. Esbenshade and Triantaphyllou (1987) reported that the rate of migration in a gel is highly dependent on various factors in addition to the amount of protein. Myers (1965) observed that protein concentration in nematode homogenates will determine the amount of enzymes detected so that the absence of band(s) may indicate insufficient number of nematodes in the sample. Ishibashi (1970) cited several workers who observed that variation of protein complement depended upon the developmental stage of the nematode in the sample. His studies showed that the stainability of the moving bands became intensive as nematode development proceeded. In these studies, highly staining EST and G6-PDH bands were obtained using nematode concentrations of 70-100 / 0.2 ml solution of sample. Samples with 45-day-old females also produced dark-staining bands in the gels (Figures 4 and 5).

In this study, esterase (EST) and glucose-6 dehydrogenase (G6-PDH) enzyme profiles, which were resolved on starch-gel electrophoresis with Mupid apparatus, were species specific. In previous studies, Pableo (1979) identified three races of *M. incognita* by disc-gel electrophoresis of soluble proteins from nematode homogenates.

Antibodies and antigens from related root-knot nematode species / races elicited precipitin lines in agar gel immunodiffusion tests. Figure 6 showed *M. incognita*, race-1 antibody-containing anteserum in center well reacted sharply against antigen preparations from the same species but not to antigens from *M. javanica* and *M. arenaria* populations. Similarly, precipitin lines were detected in *M. incognita*, race-2 containing peripheral wells (Figure 6). Identical results were obtained in reciprocal tests involving *M. javanica* and *M. arenaria*.

Hussey (1971) and Dickson et al. (1971) and other investigators obtained varied results from their research. This may be attributed to the differential extraction procedures employed. In this study, inadequate amounts of antigens and low-titer prepared antisera were noted. It is likely that the low-antibody response of rabbits to intravenous immunization is due to other weakly immunogenic antigens or inefficient route in the administering of antigens in eliciting antibody response. Consequently, undiluted antigens and antisera preparations were used in the tests. In addition, extended immunization schedules with greater quantities of antigens were resorted to.

Occurrence and Distribution

More root-knot nematodes were observed in both ornamental plants (75%) and plantation crops (57%) while tree species were less infected (Table 4). No infections were observed in mahogany, apitong, tanguile, lanzones and several other tree species. *Meloidogyne incognita* race-1 is common to ornamentals which were collected in Los Baños (Table 5). On the other hand, *M. javanica* infections were found in hardwood species: narra, lumbang [*Aleurites moluccana* (L.) Willd.] ipil-ipil and guyabano.

No perineal patterns were observed on adult root-knot nematodes isolated from upland rice but they responded to the standard differential host response tests. Two populations from Cuenca, Batangas showed reactions similar to those of *M. incognita*, race-1 while two others from Alitagtag and Tanauan, Batangas reacted in the same manner as *M. incognita*, race-2.

A host index of root-knot nematode-infected plants in the Southern Tagalog Region is shown in Table 6. The host plants represented a total of 24 plant species and 17 families. Infection in 24 host plants was a new observation.

Biological Control

Twenty-six out of 120 bacterial isolates recovered from cultivated soils, forest soils and animal manure around Los Baños have been found to possess nematicidal activity. Isolate SFE, identified as *Bacillus stearothermophilus* based on morphological and physiological tests appeared outstanding among the strains screened so far. The hatchability of root-knot nematode egg masses treated with the isolate was significantly less (Table 7). In addition, plants drenched with treated egg masses had less galls and were heavier and taller (Tables 8 and 9). The performance of the isolates was also comparable to APP 27 strains of *Bacillus thuringiensis* (Table 10).

One noteworthy characteristic of *Bacillus* species is that it forms spores that produce metabolites which may possess potent biological activity. In *Bacillus thuringiensis*, the spores exhibit powerful endotoxin effective against many insects and nematodes. The danger of biological magnification in the food chain, pollution

and the accumulation of toxic residues are less likely in bacteria produced by a living system that can maintain itself in nature. Moreover, the bacterium is an ideal biocontrol agent because of its ability to sporulate and maintain its resistant state indefinitely, so that once it is in the soil, it remains for a long time ready to act when needed without being reapplied. It is non-toxic to vertebrates (particularly the toxin in *B. thuringiensis*) so that it is unlikely to harm man or domestic animals while exhibiting results against a wide variety of plant pests.

IMPLICATIONS

The occurrence of root-knot nematodes in this country is indeed more extensive than originally thought. It is likely that many host plants remain to be discovered.

Meloidogyne incognita appears to be the predominant species. It is polytypic, occurring in many races.

A "micro" system that is sensitive enough to allow the electrophoretic analysis of minute quantities of protein characteristic of plant-parasitic nematodes because of their size can enhance the ability to identify protein profiles in *Meloidogyne* spp. With further refinements, electrophoresis and immunodiffusion techniques could be potential tools in the quick and accurate identification of root-knot nematode species and races.

The potential of bacteria as biocontrol agents against root-knot nematodes should be vigorously studied.

Table 1. Identification of root-knot nematodes from cereals and perennial plants in three Southern Luzon provinces

Root-Knot Nematode Species	Number of Populations	Occurrence (%)
Meloidogyne incognita	31	89
Meloidogyne javanica	4	11
Total	35	100

Table 2. Identification of *Meloidogyne incognita* races from cereals and perennial plants

Root-Knot Nematode Species/Races	Number of Populations	Occurrence (%)
M. incognita		
Race 1	10	32
Race 2	9	29
Race 3	11	36
Race 4	1	
Total	31	100

Table 3. Migration values (Rf) of GG PDH Isozyme in root-knot nematodes

Root-Knot Nematode Spp	Band A	Band B
Meloidogyne incognita, r-1		
11 – UPLB Campus (acalypha green)	0.50	0.46
15 – UPLB Campus (cat's tail)	0.50	0.46
16 – UPLB Campus (San Francisco)	0.50	0.46
17 – UPLB Campus (poinsettia)	0.50	0.46
Meloidogyne incognita, r-2		
1 – Jamboree (cacao)	0.48	0.40
7 – CES (mango)	0.48	0.40
8 – CES (papaya)	0.48	0.40
11 – Alitagtag (rice)	0.48	0.40
24 – San Pablo City (guayabano)	0.48	0.40
Meloidogyne incognita, r-3		
4 – BPI Economic Garden (corn)	0.48	0.40
5 – CES (sugarcane)	0.48	0.40
12 – Cuenca [jackfruit (2x)]	0.48	0.40
18 – UPLB Campus	0.50	0.46
19 – BPI Economic Garden (ipil-ipil)	0.50	0.46
22 – San Pablo City (cacao)		
Meloidogyne javanica		
2 – Jamboree (narra)	–[a]	–
3 – Jamboree (lumbang)	–	–
6 – CES [ipil-ipil (2x)]	–	–
30 – Lucena (guayabano)	–	–

a – sign indicates absence of band(s)

Table 4. Occurrence and distribution of root-knot nematodes from cereals and perennial plants

Host Plant	Total Number of Plants	Infection (%)
Fruit Crops	16	50
Ornamentals	8	75
Plantation Crops	7	57
Commercial Tree Species	7	43
Ordinary Tree Species	5	20
Cereals	4	50
TOTAL	47	

Table 5. Occurrence and distribution of *Meloidogyne* species from cereals and perennial plants

Host Plant	*M. incognita*				*M. javanica*	Total
	Race 1	Race 2	Race 3	Race 4		
Fruit Crops	2	5	3	1	1	12
Ornamentals	5	–	1	–	–	6
Plantation Crops	1	2	4	–	–	7
Comm. Tree Sp.	–	–	1	–	3	4
Ordinary. Tree Sp.	–	–	1	–	–	1
Cereals	2	2	1	–	–	5
Total	10	9	11	1	4	35

Table 6. Host index of root-knot nematodes, *Meloidogyne* spp. in perennial and unusual host plants in the Southern Tagalog Region

Host Plant	Family	Root-Knot Species	Locality
Acalypha hispida Burm. f., cat's tail	Euphorbiaceae	*M. incognita*, r-1[a]	3
Acalypha wilkesiana Muell. Arg., copper leaf	Euphorbiaceae	*M. incognita*, r-1	3
Aleurites moluccana (L.) Willd., Lumbang	Euphorbiaceae	*M. javanica*[a]	1
Ananas comosus (L.) Merr., pineapple	Bromeliaceae	*M. incognita*, r-1	2
Annona muricata L. guyabano, soursop	Annonaceae	*M. incognita*, r-2[a] *M. javanica*[a]	6 15
Artocarpus heterophyllus Lam. Lmk., jackfruit, langka	Moraceae	*M. incognita*, r-3[a]	12
Carica papaya L. papaya	Caricaceae	*M. incognita*, r-2	2
Chrysophyllum caimito L. starapple, caimito	Sapotaceae	*M. incognita*, r-1[a] *M. incognita*, r-2[a]	15 14
Citrus grandis L. Osbeck, pomelo, lukban	Rutaceae	*M. incognita*, r-3[a]	6
Cordiaeum variegatum (L.) Bl., San Francisco croton	Euphorbiaceae	*M. incognita*, r-1[a]	3
Coffea sp. coffee, kape	Rubiaceae	*M. incognita*, r-2[a] *M. incognita*, r-3[a]	7 13
Clerodendron intermedium kasupangil	Verbenaceae	*M. intermedium*, r-3[a]	5
Euphorbia pulcherrima Willd. ex Kl. poinsettia, pascua	Euphorbiaceae	*M. incognita*, r-1	3

Lxora spp. santan	Rubiaceae	*M. incognita*, r-3[a]	3
Leucaena leucocephala de Wit, ipil-ipil	Mimosaceae	*M. incognita*, r-3[a]	4
		M. Javanica[a]	2
Mangifera indica L., mango, mangga	Anacardiaceae	*M. incognita*, r-2[a]	2
		M. incognita, r-4[a]	14
Oryza sativa L., upland rice	Graminae	*M. incognita*, r-1[a]	10, 11
		M. incognita, r-2[a]	8, 9
Naphelium lappaceum L. rambutan	Sapindaceae	*M. incognita*, r-1[a]	14
Persea americana Mill. avocado	Lauraceae	*M. incognita*, r-2[a]	16
		M. incognita, r-3[a]	14
Plumeria sp. kalachuchi	Apocynaceae	*M. incognita*, r-1[a]	3
Pterocarpus indicus Willd., narra	Papilionaceae	*M. javanica*[a]	1
Saccharum officinarum L., sugarcane	Graminae	*M. incognita*, r-3	2
Theobroma cacao L. cacao	Steruliaceae	*M. incognita*, r-2	1
		M. incognita, r-3	6
Zea mays L. corn	Graminae	*M. incognita*, r-3	4

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[a]New reports for the Philippines
[b]Each number refers to a specific location where nematode species were collected:

1. Boy Scouts Jamboree, Los Baños, Laguna
2. Central Experiment Station (CES), UPLB, Los Baños
3. UPLB Campus, Los Baños
4. BPI Economic Garden, Los Baños
5. Looc, Calamba
6. Biuyan, San Pablo City
7. Lawaguin, Nagcarlan
8. Bilog-bilog, Tanauan, Batangas
9. Dominador, Alitagtag
10. Dalipit, Cuenca
11. San Felipe, Cuenca
12. Ibabaw, Cuenca
13. Sariaya, Quezon
14. Tiaong, Quezon
15. Lucena City
16. San Pablo City

Table 7. Effect of different *Bacillus* isolates on hatchability of root-knot nematode eggs

Treatment	Mean Count of Juveniles[1]	Reduction in Hatchability Relative To C1 (%)	C2 (%)
SFE	49.0 c	65.5	67.5
BCN	84.0 b	40.9	44.4
C1 (tap water)	142.0 a		
C2 (coco water)	151.0 a		

[1]Means followed by a common letter are not significantly different (DMRT, P=0.05).

Table 8. Effect of different *Bacillus* isolates on height, top and root weights of root-knot nematode-infected tomato

Isolate Applied	Height Increment (cm)		Top Weight		Root Weight	
	% Increase[1]		% Increase[1]		% Increase[1]	
SFE	41.1	74.8	80.9	136.2	80.0	88.4
BCN	6.9	32.4	38.3	80.6	24.4	30.2
C1						
C2						

[1]Left column refers to percentage increase in relation to C1 (control 1, tap water); right column refers to percentage increase in relation to C2 (control 2, coconut water).

Table 9. Effect of different concentrates of *Bacillus* sp. (SFE isolate) on plant height and gall formation

Treatment	Mean Height Increment (cm)	Increase In Height	Mean Count	Reduction In Galls
15%	44.3 b	6.2	59.2	16.6
30%	47.5 b	13.9	55.2	22.2
50%	56.2 a	34.8	31.7	55.4
Control	41.7 b		71.0	

Table 10. Effect of a local *Bacillus* sp. isolate and selected *Bacillus thuringiensis* strains on the hatchability of nematode eggs

Treatment	Meant Count of Juveniles[1]	Reduction in Hatchability Relative To C1 (%)	C2 (%)
AP	44.0 c	69.0	70.9
APP-6	78.0 b	45.1	48.3
APP-27	53.3 c	62.5	64.7
SFE	49.0 c	65.5	67.5
BCN	84.0 b	40.9	44.4
C1 (tap water)	142.0 a	-	-
C2 (coco water)	151.0 a	-	-

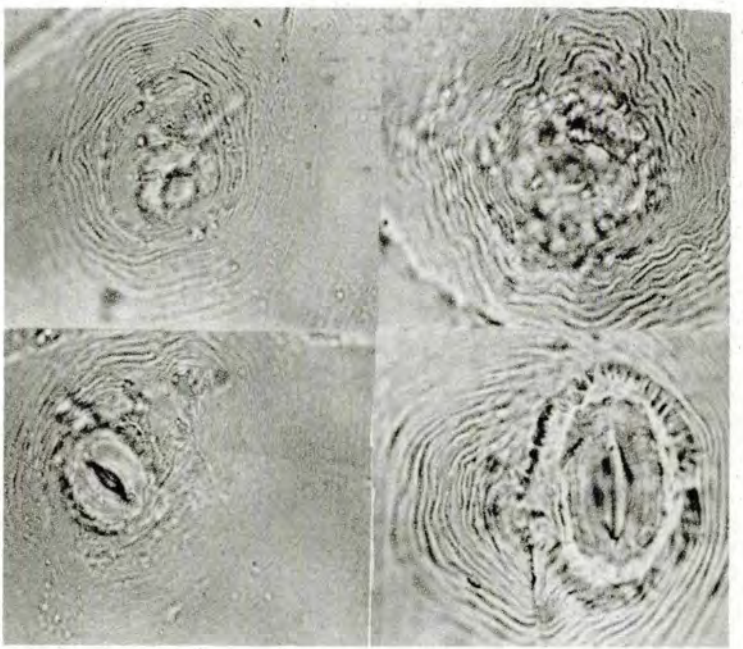

Figure 1. Perineal patterns of *Meloidogyne* spp.: ***Meloidogyne incognita,*** **race 2 from upland rice, Bilog-bilog, Tanauan, Batangas;** *M. incognita*, **race-1 from San Francisco, UPLB campus, Los Baños (top pictures, left to right);** ***M. incognita,*** **race-3 from corn, BPI, Economic Garden, Los Baños,** ***M. javanica*** **from ipil-ipil, Central Experiment Station, UPLB (bottom pictures, left to right) (photomicrographs, 640 x)**

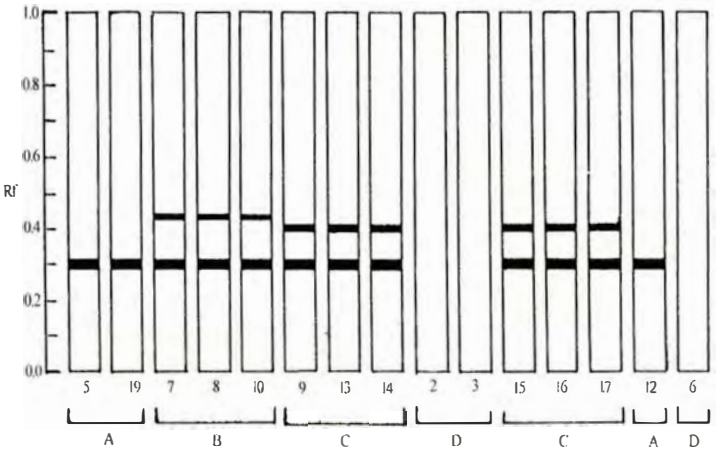

Figure 2. Esterase phenotypes observed in a study of 28 root-knot nematode populations. Each phenotype is designated by letter suggestive of the species it specifies (A = *M. incognita,* race-3; *B=M. inc.* r-2 / *M. are.* r-2; C = *M. incognita,* race-1; D = *M. javanica*).

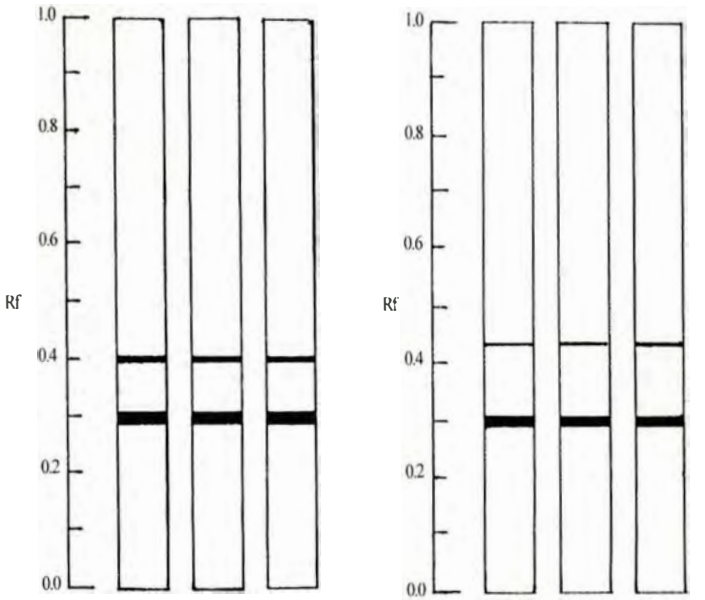

Figure 3. (left): Esterase phenotypes of *Meloidogyne incognita,* race-1; (right): Esterase phenotypes of *Meloidogyne incognita,* race-2 /*M. arenaria,* race-2.

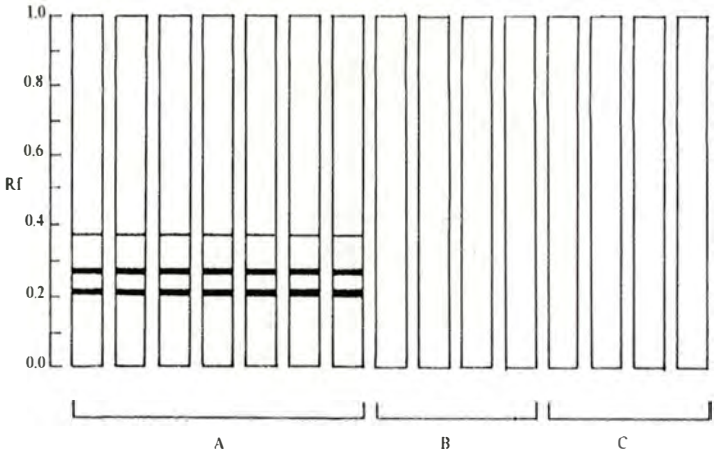

Figure 4. Electrophoretic protein patterns of *Meloidogyne* sp. showing variations dependent on the developmental stages of root-knot nematodes. A = 45-day-old root-knot nematode (mixed samples); b = larvae; C = egg.

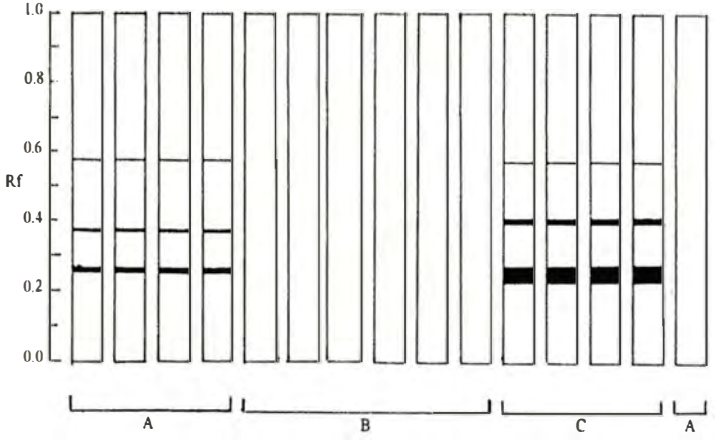

Figure 5. Electrophoretic protein patterns of mixed samples showing variations dependent on the stages of maturity of female nematodes. A = 30-day-old; B = 60 day-old; C = 45-day-old root-knot nematodes (mixed samples)

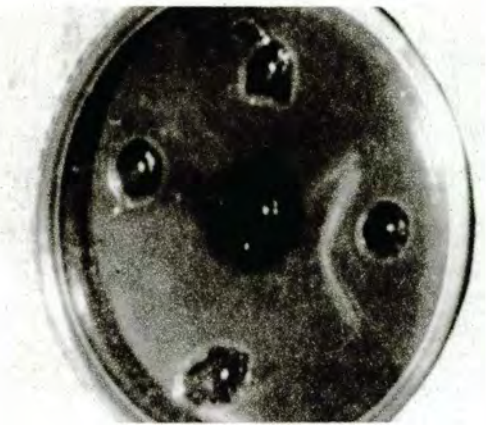

Figure 6. Antibody-antigen reactions eliciting precipitin lines. Central well contains antiserum from *Meloidogyne incognita,* race 1. Clockwise, from top, peripheral wells containing *M. arenaria,* race 2; *M. arenaria,* race 1; *M. incognita,* race

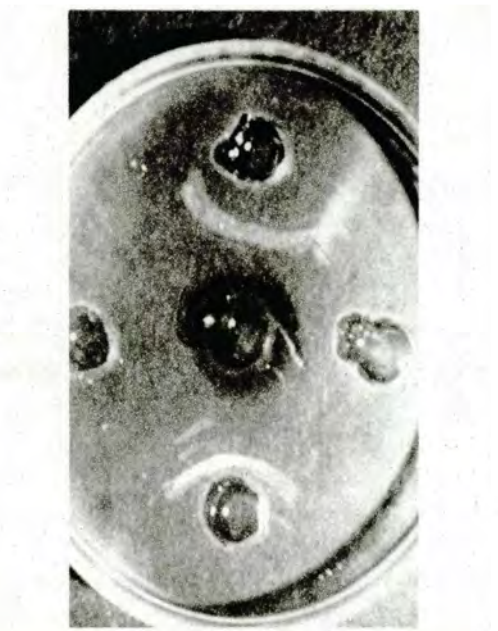

Figure 7. Antibody-antigen reactions showing precipitin lines. Center well contains antiserum from *Meloidogyne incognita* race 2. Clockwise, from top, peripheral wells containing *M. incognita,* race 2; *M. incognita,* race 1; *M. incognita,* race 2 and *M. javanica.*

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GENETIC ANALYSIS OF *ACANTHAMOEBA* SPP. ISOLATED FROM DIFFERENT GEOGRAPHIC REGIONS OF THE PHILIPPINES

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ABSTRACT

Acanthamoeba spp. have been isolated from water and soil samples collected all over the Philippines. The amoebae were grown on 1.5% NNA lawned with *Escherichia coli*, then cloned using a Skerman™ micromanipulator and axenized prior to mass production in PPYG medium.

Morphological study of *Acanthamoeba* cysts by PATAg r staining method showed three distinct groups: polygonal, rounded and stellate cysts. Phase contrast microscopy of wet mount preparations (Carl Zeiss Axiovert™) and toluidine-stained trophozoites revealed distinguishing features of *Acanthamoeba:* centrally located nucleolus, numerous dense vacuoles and acanthopodia. It is, however, very difficult to distinguish among strains based on trophozoite morphology.

Soluble proteins were extracted by freeze-thawing technique and total protein concentrations were determined by Bradford method. Protein samples (25 µg-100 µg) were loaded on an IEF gel (pH gradient 3.5-9.5) followed by isoelectrofocusing at 400V-1200V for six hours on a Biorad™ horizontal electrophoresis system. Specific substrates for acid phosphatase (Acph), esterase (Est) and alkaline phosphatase (Alph) isozymes coupled with either formazan or tetrazolium stain were reacted with the protein samples.

Analysis of genetic distances (D) calculated from similarity indices (I) of isoenzyme profiles indicates that groupings are not necessarily consistent with cyst morphology but they correlate strongly with geographic distribution. This suggests that further biochemical characterization is necessary to be able to classify Philippine strains. Moreover, pathogenic character of some environmental isolates that show close genetic relationship with pathogenic reference strains needs to be studied.

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INTRODUCTION

Acanthamoeba species are among those free-living amoebae that have been recognized to be pathogenic to humans and experimental animals causing a fatal granulomatous amebic encephalitis (GAE) and a debilitating corneal keratitis (Fig. 1-B). They are very ubiquitous and highly resistant to harsh environmental conditions probably owing to their cyst-forming characteristic and their relatively simple nutritional requirements. Although their pathogenic potential has been reputedly limited to a few species, it is believed that the problem lies more deeply on the difficulty of distinguishing among strains which appear so homogenous with respect to their oftentimes unstable morphologic, behavioral and physiological characters (Griffiths et al., 1978). Indeed, conventional criteria for classification schemes in protozoans are so limited. For these reasons, interpretations on the taxonomic position of *Acanthamoeba* have been quite difficult, especially at the species level (Martinez, 1983; Willaert, 1976; and Costas et al., 1983).

In protozoans, isoenzyme pattern analysis has been employed making use of isoelectrofocusing techniques in polyacrylamide and agarose gels (Daggett et al., 1983; De Jonckheere et al., 1984; Pernin et al. 1985; Pernin, 1984; and Adams et al., 1989). This biochemical method may be particularly useful for *Acanthamoeba* in which the availability of stable characters for classification schemes is limited.

More than a hundred strains obtained from soil and water samples from different localities in the Philippines and from clinical specimens have already been cloned (Matias et al., n.d.). In this study, some of these representative *Acanthamoeba* strains were subjected to isoenzyme analysis using acid phosphatase, alkaline phosphatase and esterase in an attempt to show interstrain genetic variability. The resulting zymograms were compared and **similarity indices** (*I*) as well as **genetic distances** (*D*) were calculated. *D* values were subjected to cluster analysis by UPGMA method (Yagita and Endo, 1990), and correlated with geographic distribution and cyst morphology. Moreover, *D* values were used to determine quantitative relationships between known pathogenic *Acanthamoeba* and Philippine isolates whose pathogenic character is yet unknown.

MATERIALS AND METHODS

Isolation and Propagation

Collection of Samples. Soil and water samples as well as clinical specimens from suspected *Acanthamoeba* infections were collected from various sites, namely: Camarines Norte (**Bc**), Cotabato (**Cot**), Davao (**Dav**), Iloilo (**Ilo**), Mindoro Occidental (**Mocc**), Novaliches (**W**), Diliman (**C**) and **Hl** (a clinical isolate). Reference strains include *Acanthamoeba lenticulata, A. quinalugdunensis, A. mauritanensis, A. castellanii* and Renk (a German isolate). Water and soil samples [the latter was resuspended in sterile 0.15M phosphate-buffered saline (PBS), pH 7.2] were vacuum-filtered on glass fiber filters. These glass filters with the trapped particulates were

inverted on petri dishes overlaid with 1.5% non-nutrient agar (NNA) lawned with 24-hour culture of *E. coli* and incubated at 37°C. The bacterized agar plates were observed for amoebal growth after 48-72 hours.

Cloning. Agar plates showing positive growth were harvested by flooding the plates with 10 mL sterile 0.15 M PBS and scraping the agar surface. The cell suspension was pipetted out, pooled together into a centrifuge tube and spun at 1000 rpm for 5 min. The supernatant was discarded and the pellet was washed thrice with PBS by repeated centrifugations. After washing, the pellet was resuspended in 0.5 mL sterile PBS and a small drop was put onto one end of a previously prepared NNA-overlaid slide. The slide was observed under 16X objective. Floating live cysts (indicated by somewhat granular interior) were dragged to the opposite end of the slide using a Skerman™ micromanipulator. Thereupon, the cyst was transferred to a bacterized NNA plate by slicing out the agar overlay from the slide. About 10 cysts were selected per region or clinical sample. The plates were incubated at 37°C and observed daily for amoebal growth.

Axenic Cultivation. Axenization was done by initially growing the cloned cells in *E. coli*-lawned agar plates. Upon reaching confluence and with 90% of the cells in trophozoite stage, the plates were flooded with 10 mL proteose-peptone-yeast extract-glucose (PPYG) liquid medium containing 500 I.U. penicillin and 500 μg/mL streptomycin. The liquid medium was supplemented with 5% calf serum (Gibco™). Cultures that were cleared of bacteria were aseptically transferred to sterile plates without agar overlay. Thereafter, cells reaching exponential growth were subcultured using PPYG to a final cell concentration of 10^6/mL. Aseptic condition was maintained throughout the mass cultivation process. Once the cell concentration reached 10^9/mL (around 3 days of culturing), harvesting was done by pooling the cells into a centrifuge tube and by repeated washing with amoeba saline (AS) (Matias, 1991) at 1,000 rpm for 5 min each washing. Final washing was in 0.01M Tris-HCl (pH 7.2); afterwhich, cells were stored at -20°C until use. The same procedure was followed for the propagation of the reference strains.

Morphological Study of Cysts and Trophozoites

PATAg r Staining of Cysts. Morphological study of cysts was done for each isolate using periodic acid-thicarbohydrazide-silver reduced (PATAg r) staining technique by Matias et al. (1991) with slight modifications. Morphological study of trophozoites was done by washing fresh cultures with PBS and dropping onto slides with and without fixation. For the fixed preparations, 25% glutaraldehyde 0.05 M cacodylate buffer, pH 7.2 was used as fixative followed by toluidine blue staining for 10 min. Cells that were not fixed were immediately observed and photographed.

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Preparation of Protein Samples

Extraction of Soluble Proteins. Soluble proteins from *Acanthamoeba* cells were extracted by resuspending the stored cells in one-half volume of extraction buffer (0.15M Tris-HC1, pH 7.2) containing 1 mM protease inhibitors in Eppendorf tubes. The tubes were immersed in acetone containing dry ice for rapid freezing of cells and then transferred to 37°C water bath for immediate thawing. This was done several times until 100% lysis was achieved as monitored under the phase contrast microscope. Lysates were then spun for one hour at 32,000 rpm in a refrigerated Beckman™ ultracentrifuge (4°C) to remove membranous components. Extracts were stored in 100 µl aliquots at –70°C until use.

Determination of Total Protein Concentration. Analysis of total protein concentration according to the method of Bradford (1976) was done for each isolate. Protein solutions of bovine serum albumin containing 1 µg – 10 µg of the protein were pipetted into 12 x 100 mm test tubes in triplicates and adjusted to 0.1 mL with 0.15M PBS, pH 7.2. One mL of Bradford reagent was then added to the test tube and the contents mixed by vortexing. Absorbance at 595 nm was measured after 2 min in 3 mL quartz cuvettes using a Beckman™ DU-60 Spectrophotometer calibrated against a reagent blank prepared from 0.1 mL of the PBS and 1 mL of Bradford reagent. The weight of protein was plotted against the corresponding absorbance resulting in a standard curve which was used for the determination of total protein in the unknown samples. For the unknown samples, 1:100 dilution of extract was made to react with 1 mL Bradford reagent. The resulting absorbance readings were recorded and inputted into a Lotus 1-2-3 regression analysis. Total protein yield was determined by extrapolation of computer-generated regression output of the BSA standards.

Isoenzyme Analysis

Isoelectric Focusing in Polyacrylamide Gel. Gel casting glass plates (10 cm X 12.5 cm X 0.2 cm) pre-treated with commercially available bind saline (LKB™) were used for the preparation of 6% polyacrylamide gel with 0.6 mL Sigma™ ampholine solution (pH 3.5-9.5) (Matias, 1991). The solution was degassed using a vacuum pump for at least 5 min; afterwhich, 0.16 mL riboflavin stock solution was added. The gel mixture was then carefully pipetted in between a plastic gel mould (pre-treated with repel silane, LKB™) and the glass plate, avoiding air bubbles from being trapped. The gel mixture was left to polymerize for one hour under a fluorescent bulb. Electrode strips soaked in respective electrode solutions were then placed on the opposite ends of the gel. Electrode solutions consisted of 1M H_3PO_4 (anode-electrode solution) and 0.1M NaOH (cathode-electrode solution). The gel was prefocused at constant 400 V for 30 min before loading the samples (absorbed in 10 mm x 6 mm filter paper). Differing amounts of protein depending on the isoenzymes to be detected (Table 1) were loaded by putting them 3 cm from cathodic end of

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the gel. The time of running was about 2.5 hours at an initial voltage of 400 V increasing the voltage by 200 V after every 30 min up to 1200 V. After electrofocusing, the glass plate (with the gel still anchored on it) was carefully removed from the IEF cooling plate and transferred to ceramic trays.

Detection of Isoenzyme Activity. Gels were incubated with buffered substrate solutions specific for one of the abovementioned isoenzymes (Table 1) at 37°C (Pernin, 1985; Matias, 1991). Substrates (Sigma™) in appropriate buffers with coenzymes and dye complexes include alpha-naphtholphosphate, alpha-naphthylacetate and naphthyl-ASBI-phosphate.

Analysis of Genetic Distances

Construction of Dendrograms. Matrices of genetic distance (*D*) values were calculated from indices of similarity (*I*) among isozyme profiles and then subjected to computer-aided UPGMA cluster analysis for construction of dendrograms where (Nei, 1972):

$$I = \frac{[(Jx)^2 \cdot (Jy)^2]\, Bxy}{\sqrt{(Jx) \cdot (Jy)}} \qquad (1)$$

Jx = band frequency of strain X
Jy = band frequency of strain Y
Bxy = no. of monomorphic bands bet. strains X & Y

and;

$$D = -\log I \qquad (2)$$

RESULTS AND DISCUSSION

Three major groups of *Acanthamoeba* were recognized based on cyst morphology (Figs. 1a-1c). Groupings were made according to the classification scheme by Pussard and Pons (1977). Most C strains exhibited round-shaped cysts including **Ilo1** and **Mocc2** strains and the reference strain, *A. lenticulata*. Polygonal cysts were also observed in some **C** strains (**C**-11, **C**-4) which are the characteristic cystic shape of *A. castellanii* (a pathogenic strain), *A. mauritanensis* and *A. quinalugdunensis*. A clinical isolate, **H**-1, and two **W** strains were also found to be polygonal in shape. The third group consisting of **W**-3, **Bc3**, **Dav4** and **Cot4** showed stellate-shaped cysts.

Geographically isolated strains showed morphological homogeneity based on cyst structure suggesting the wide distribution and adaptability of these organisms. Stellate-shaped cysts appear to be the most widely distributed. Round-shaped cysts seem to be more localized in the central archipelago. An interesting observation

may be noted in the polygonal group to which two pathogenic strains, **H1** and *A. castellanii*, belong.

Figure 2a shows *Acanthamoeba* in trophozoite stage. Numerous dense vacuoles are visible in this wet mount preparation with the diameter varying from 15-45 μm. Stained preparations (Fig. 2b) showed the centrally located, dense karyosome (nucleolus) surrounded by a clear nuclear halo. This has been recognized as one of the distinguishing characteristics of *Acanthamoeba* (Matias et al., 1991).

Soluble protein extracts of *Acanthamoeba* strains showed isoenzyme activities for acid phosphatase, alkaline phosphatase and esterase except for **C**-13 which did not show alkaline phosphatase activity (Figs. 3a-3b). Zymogram patterns of all 20 strains are shown diagrammatically in Figures 4a-4c. Computed Rf values for each band were used to calculate the frequencies of monomorphic or co-migrating bands between any two strains (designated as Bxy) (Table 2). The latter were then used for estimating Table 3 showing similarity index (*I*) and genetic distance using equations (1) and (2) in Materials and Methods. Cluster analysis of genetic distances (*D*) showed two major clusters, clusters A and B, which are 0.615 *D* units apart (Figure 5). Cluster A may be further split into two clusters, A_1 and A_2, with a genetic distance of 0.589. Similarly, cluster B may be subdivided into two clusters, B_1 and B_2, showing a genetic distance of 0.522.

It appears that the distributional patterns of the different *Acanthamoeba* isolates were consistent with the clustering pattern of their respective zymograms. Cluster A comprises the different species collected from the central Philippine archipelago while Cluster B are those collected from the southern part of the Philippine archipelago. However, pathogenic strains *A. castellanii* and **H1** appeared in separate clusters, suggesting that isoenzyme activities for phosphatases and esterase do not reflect pathogenic character. This does not support earlier isoenzyme studies in other closely related groups where common pattern for strains of known pathogenicity has been observed (Pernin, 1984; De Jonckheere, 1982). Isoenzyme analysis of pathogenic and nonpathogenic thermophilic *Naegleria* by Pernin (1984) for seven enzymatic activities including acid phosphatase revealed a common pattern for three pathogenic strains although nonpathogenic strains showed more heterogeneity. Recently, a group of geographically isolated Philippine *Naegleria* strains isolated from a heated swimming pool and a geothermal power plant correlates strongly with a human brain isolate and with *N. fowleri* (a known pathogen) based on isoenzyme pattern and antigenic analyses (Matias, 1991; Yap et al., 1991).

There are seven known pathogenic species of *Acanthamoeba*, namely: *A. astronyxis, A. castellanii, A. polyphaga, A. rhysodes, A culbertsoni, A. palestinensis* and *A. hatchetti* (Warhurst, 1985; Ma et al., 1990). Isoenzyme markers for pathogenicity-related character have not been identified. Since there was only one pathogenic reference strain available for this study, the clinical isolate may belong to any one of the other six species mentioned above. In clusters A_2 and B_2, it would be interesting to test whether the **W** strains, **D4**, **Cot4** and **Mocc2**, are pathogenic or not by means of experimental infection in animals in order to elucidate

more conclusively the possible correlation between pathogenic strains, **H1** and *A. castellanii*, and the abovementioned strains. This, in turn, may give insights on the relationship between isoenzyme activity and pathogenic character.

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Table 1. Staining conditions of enzymes assayed*

Enzyme	Substrate	Dye	Other Reagents	Buffer	Time	Amt. Protein
Acid phosphatase	Alpha-naphthyl phosphate, 100 mg	Fast Garnet BBC 200 mg		Acetate buffer 0.05M, pH 5.0	5 min	25 μg
Esterase	Alpha-naphthyl acetate, 100 mg	Fast Red TR Salt 100 mg	PVP, 500 mg	Phosphate buffer 0.1M, pH 7.4	30 min	100 μg
Alkaline phosphatase	Alpha-naphthyl ASBI-phosphate 300 mg	Fast Red TR Salt 100 mg	PVP, 300 mg NaCl, 1.7 g MgC12.6H20, 20 mg	Tris-HCl buffer 0.2M, pH 9.0	1h	300 μg

*Amounts given are for 100-ml buffer solution.

Table 2. Matrix of Bxy values (number of co-migrating or monomorphic bands)

	C1	C3	C4	C5	C11	C13	W3	W4	W6	Mocc 2	Cot4	H1	Dav4	Ho1	Bc3	*A. lent.*	*A. ql.*	*A. maur.*	Renk	*A. Castel.*
C1		13	10	11	4	4	5	7	5	7	4	5	7	9	11	8	5	7	3	8
C3			10	10	6	4	8	7	5	11	7	6	4	4	7	8	5	8	4	5
C4				7	6	2	6	5	5	11	8	7	4	7	9	10	5	7	2	5
C5					4	3	4	8	6	9	7	8	8	9	11	11	5	7	4	10
C11						9	5	3	6	6	4	4	3	3	6	4	5	3	7	4
C13							5	3	4	6	4	3	4	4	3	3	5	3	7	3
W3								12	10	9	5	3	2	3	3	4	5	8	6	6
W4									13	10	7	4	5	4	4	5	5	9	6	9
W6										7	3	4	4	5	4	3	7	6	8	9
Mocc2											13	12	11	9	9	13	6	12	7	4
Cot4												12	7	6	5	9	3	7	4	3
H1													8	6	7	11	2	6	2	3
Dav4														6	9	9	2	8	4	3
Ho1															9	9	4	6	2	5
Bc3																12	6	5	3	6
A. lent.																	5	9	2	3
A. ql.																		3	8	6
A. maurit.																			5	8
Renk																				4

Table 3. Nei's Genetic Distance (D) calculated from similarity indices of zymogram profiles

	C1	C3	C4	C5	C11	C13	W3	W4	W6	Mocc 2	Cot4	H1	Dav4	Ilo1	Bc3	*A. lent*	*A ql.*	*A maur.*	Renk	*A. Castel.*
C1		0.208	0 352	0 311	0.642	0 626	0 600	0.465	0.600	0 565	0 697	0.623	0.428	0.367	0.301	0 439	0.612	0.516	0.319	0.449
C3			0 331	0 331	0 445	0.605	0.375	0.445	0 579	0.348	0.433	0.523	0.651	0.699	0 477	0.419	0 591	0 438	0.651	0 632
C4				0 517	0.476	0.937	0 530	0.621	0 610	0 378	0 405	0 486	0 681	0 486	0.398	0 352	0.621	0.526	0.982	0 663
C5					0.652	0 761	0.706	0.417	0 530	0.465	0 463	0 428	0.380	0.377	0 311	0 311	0 621	0.526	0.681	0.362
C11						0.176	0.502	0 735	0.423	0.533	0.599	0.622	0.698	0.746	0.466	0.642	0.513	0.786	0.330	0.652
C13							0.486	0.719	0.538	0.517	0.583	0.730	0.557	0.605	0.751	0.751	0 497	0.770	0.314	0.761
W3								0.188	0.255	0 412	0.556	0.801	0 929	0.801	0.822	0 697	0.568	0 415	0 452	0.530
W4									0.153	0.378	0.422	0.688	0.542	0 688	0.709	0 612	0 580	0 375	0.463	0.366
W6										0 521	0.778	0 676	0.628	0 579	0.697	0.822	0.422	0.540	0.327	0.354
Mocc2											0.252	0.310	0.299	0.435	0.456	0 296	0.600	0.349	0.496	0.817
Cot4												0.199	0.385	0.500	0.600	0 345	0.790	0 473	0.628	0.831
H1													0.349	0.523	0 477	0.280	0.989	0.562	0.952	0.854
Dav4														0.474	0.319	0.319	0.940	0.389	0.602	0.806
Ilo1															0.367	0 367	0 688	0 562	0 952	0 632
Bc3																0.263	0.532	0.662	0.796	0.574
A. lent.																	0.612	0 407	0.972	0.875
A. ql.																		0.852	0.338	0.542
A. maurit																			0.593	0 469
Renk																				0.681

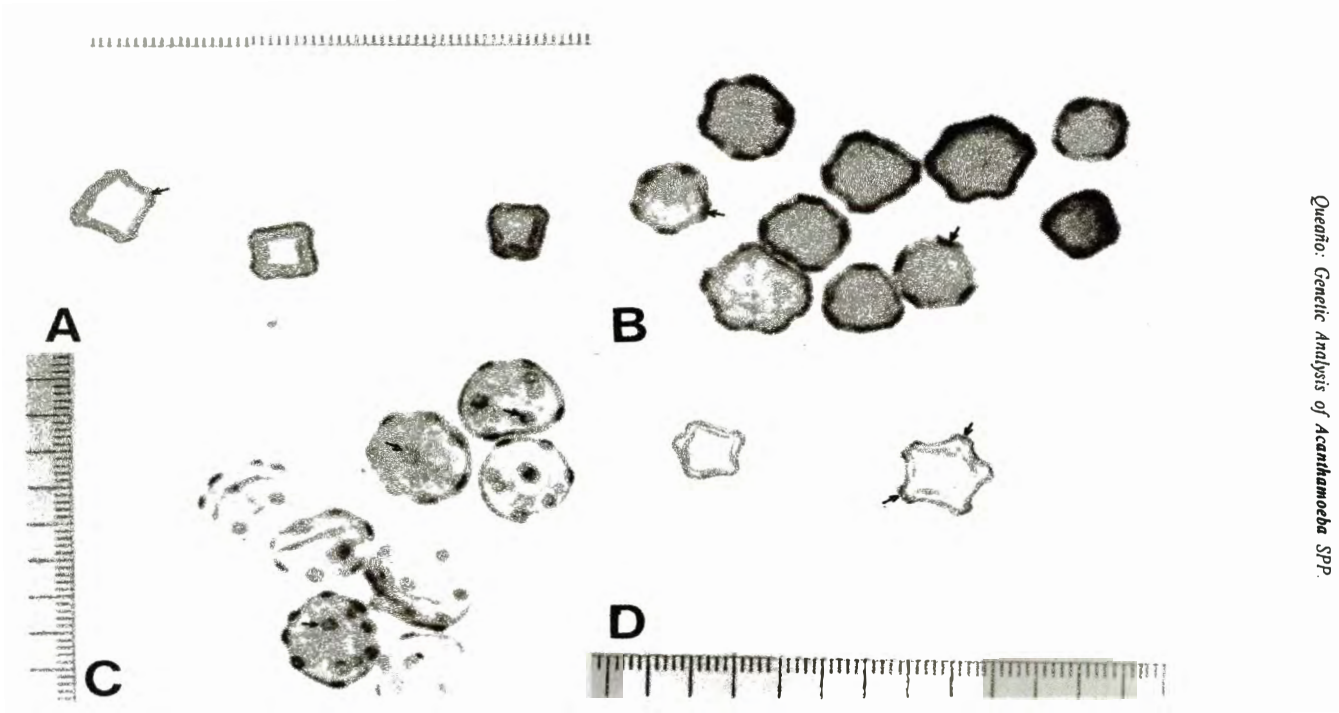

Figure 1. *Acanthamoeba* cysts stained with PATAg r: (a) polygonal cysts with 4-5 sides; (b) polygonal cysts with 6 or more sides; (c) rounded cysts: (d) stellate cysts. Arrows indicate cyst pores. Scale divisions (short bars): 1.19 μm.

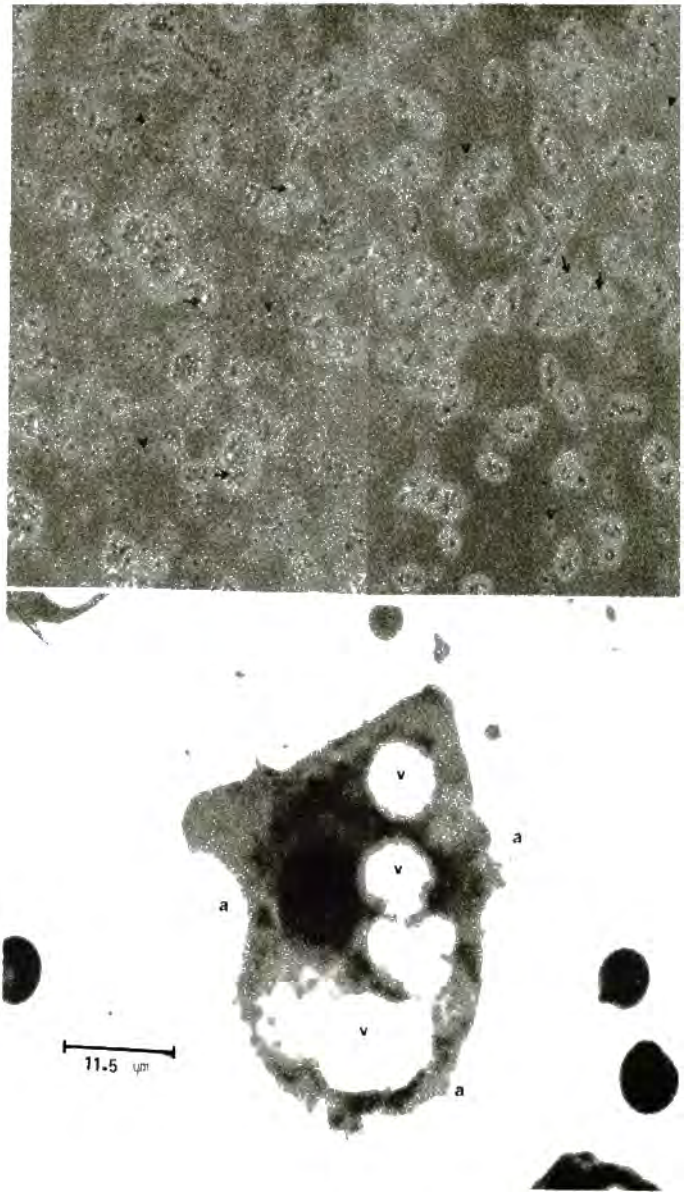

Figure 2. (a) Wet mount preparation of *Acanthamoeba* trophozoites showing dense vacuoles (arrow). Smaller, spherical cells are human RBCs (arrowheads) for size comparisons. Bar: 20 µm. (b) Stained preparation (toluidine blue) of glutaraldehyde-fixed trophozoites. nu – nucleus; nul – nucleolus; ac – acanthopodia; v – vacuoles.

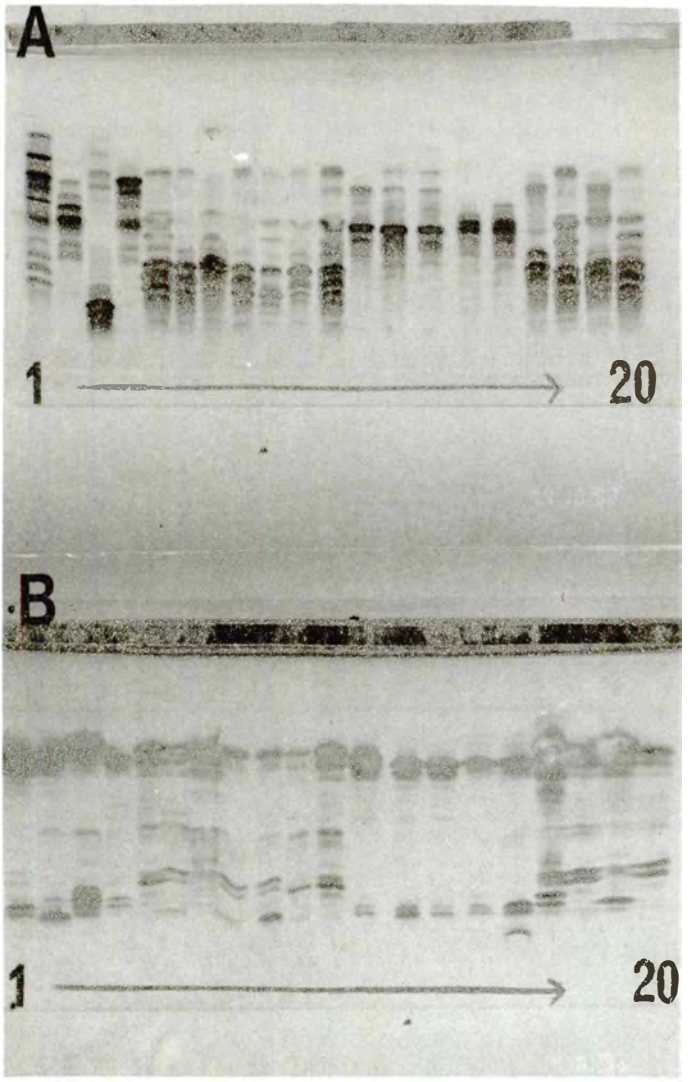

Figure 3. Polyacrylamide gel stained for various isoenzyme activities of soluble protein extracts of *Acanthamoeba* strains isoelectrofocused at 400V-1200V (pH 3.5-9.5). Reference strains: (1) *A. castellanii;* (2) Renk; (3) *A. mauritanensis;* (4) *A. quinalugdunensis;* (6) *A. lenticulata.* Philippine strains: (5) Bc3; (7) Ilo1; (8) Dav4; (9)H1; (10) Cot4; (11) Mocc2; (12) W-6; (13) W-4; (14) W-3; (15) C-13; (16) C-11; (17) C-5; (18) C-4; (19) C-3; (20) C-1. (A) Acid phosphatase activity of soluble protein extracts. (B) Esterase activity of soluble protein extracts. Alkaline phosphatase IEF gel is not shown.

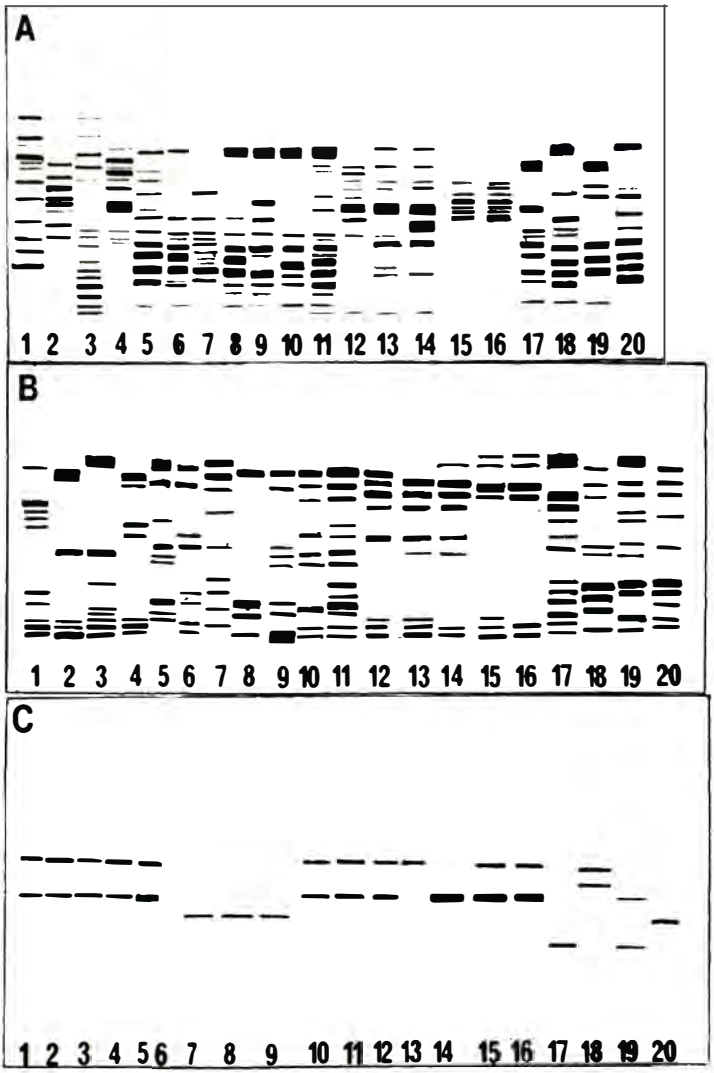

Figure 4. Zymogram patterns of *Acanthamoeba* isolates shown diagrammatically as thin and thick bands. Reference strains: (1) *A. castellanii;* (2) Renk; (3) *A. mauritanensis;* (4) *A. quinalugdunensis;* (5) *A. lenticulata.* Philippine strains: (5) Bc3; (7) Ilo1; (8) Dav4: (9) H1; (10) Cot4: (11) Mocc2: (12) W-6; (13) W-4; (14) W-3; (15) C-13; (16) C-11; (17) C-5; (18) C-4; (19) C-3; (20) C-1. (A) Acid phosphatase zymogram. (B) Esterase zymogram. (C) Alkaline phosphatase zymogram.

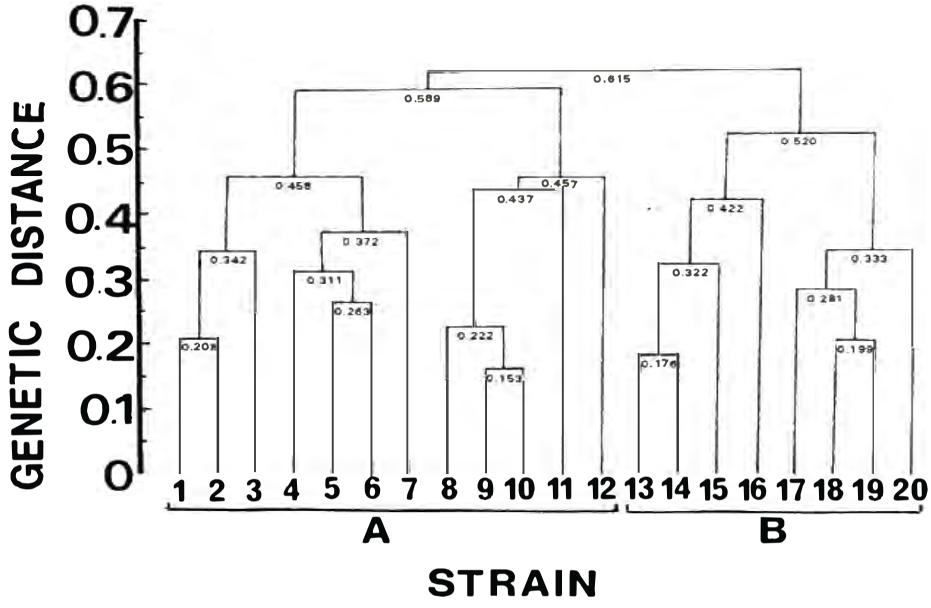

Figure 5. **A dendrogram showing Nei's genetic relationships between major clusters, A and B, and subclusters. Cluster A: (1) C-1; (2) C-3; (3) C-4; (4) C-5; (5) Bc3; (6) *A. lenticulata;* (7) Ilo1; (8) W-3; (9) W-4; (10) W-6; (11) *A. mauritanensis;* (12) *A. castellanii.* Cluster B: (13) C-11; (14) C-13; (15) Renk; (16) *A. quinalugdunensis;* (17) Mocc2; (18) Cot4; (19) H1; (20) Dav4. Cluter A_1: Strains (1) to (7). Cluster A_2: Strains (8) to (12). Cluster B_1: (13) to (16). Cluster B_2: Strains (17) to (20).**

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INVESTIGATIONS OF ENERGY DYNAMICS IN CORAL REEF ECOSYSTEMS

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ABSTRACT

Understanding the processes, quantities and rates of energy fluxes in coral reefs is essential in the rational management of these ecosystems. With the application of certain methods and conceptual approaches, progress has been made in the investigation of the energy dynamics of a reef flat in northwestern Philippines. *In situ* enclosures are employed to study the contributions to productivity of major ecological components of the reef flat. The use of closed chambers ensures that measurements may be referred to a specific entity, and enables proper replication on both spatial and temporal scales. Some aspects of physiology characterized at this level may be extrapolated to the whole community. Open flow respirometry is a technique used to study processes at the community level where closed system measurements are inadequate to encompass the range of interactions and ensuing complexity that emerge on that scale. Concomitant measurements of critical environmental parameters allow the interpretation of metabolic trends. Experiments conducted in the laboratory serve to verify field observations, particularly on the effects of environmental factors on organismal physiology. Data obtained are used in determining spatial and temporal patterns of productivity, as well as the status or "health" of the ecosystem.

INTRODUCTION

This review paper highlights research efforts in the field of coral reef energy dynamics that started in 1989 with initiatives taken at the Marine Science Institute of the University of the Philippines. Studies of energy dynamics find their greatest application in questions dealing with productivity – what critical processes underlie net production of community biomass, which environmental factors (or combinations of these) are key regulators of function and what levels of biomass yield a coral reef can sustain under both normal and stressed conditions.

At the Marine Science Institute, investigations on environmental effects have focused on the factors light and water movement. Experiments are designed around aspects of organismal physiology as a response to variations in these parameters.

It is hoped that certain trends in individual behavior may be extrapolated to the community level to infer underlying mechanisms of patterns reflected at that level.

In the study of the ecosystem, a reductionist approach was adopted initially wherein a reef flat (Lucero in Bolinao, Pangasinan) was conceptually broken down into its major substrate components. The metabolism of each component over an annual cycle was measured using closed respirometry techniques. The basic premise of such approach is that energy is divided up among the different ecological units or compartments, starting with the solar input and ending with the harvested biomass, and as such can be quantified. Periodic measurements over an annual cycle or longer provide indications as to the stability of the system, which is helpful in interpreting or even predicting the effects of environmental perturbations (Yap, 1991a).

The justification for studies on productivity is that a firmer understanding of the biological as well as physico-chemical processes driving it (Hatcher, 1990; Kinsey, 1983) could lead to better management of the harvestable resources of a reef. For example, if long term measurements provide an indication of the amount of energy normally allocated by a community for routine maintenance, this information should serve as a guide as to limits of biomass extraction that the system could tolerate. Beyond such limits, a system may collapse, leading to a series of succession stages that would culminate in a different community which is not necessarily useful for man.

METHODS IN REEF ENERGETICS

A detailed description of methods used in the study of energy flow in coral reef ecosystems is given in Yap (1991b). Energetics studies on reef components may be carried out in the field or in the laboratory. Whole system experiments are usually performed *in situ,* unless adequately-sized and representative microcosms for such investigations are constructed in the laboratory. In all cases, monitoring of the relevant environmental parameters should closely accompany measurements of metabolism in both time and space.

Field measurements of energy flow traditionally make use of either closed system or open-flow techniques (Kinsey, 1985b; Yap, 1991b). In the Philippines, both approaches have been applied on a reef flat (Lucero in Bolinao, Pangasinan). Closed system measurements make use of a Plexiglas cylinder enclosing an organism or a patch of substrate in a watertight manner. A stirrer or similar device is installed to ensure that the enclosed water is well-mixed or homogeneous. A dissolved oxygen probe is attached to the chamber to measure increases or decreases in dissolved oxygen with photosynthesis or respiration, respectively. Respiration is partitioned from photosynthesis by covering the metabolic chamber with a black material to exclude light. Photosynthesis is then measured in the light. Gross and net primary production and respiration are computed for using the widely used conventional equations (Lederman, 1983).

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Open flow respirometry has been carried out following the techniques of Kinsey (1985b). A dissolved oxygen probe is employed to monitor dissolved oxygen changes in a water mass as it flows over the bottom. From such changes, primary production and respiration are computed for, and assumed mainly to represent activity of the benthic community. The metabolism of the plankton is usually insignificant compared to the benthos. Atmospheric diffusion is corrected for.

Laboratory measurements of metabolism essentially make use of the same equipment, like dissolved oxygen probes, incubation chambers and other support equipment such as water baths to maintain temperature, a light source, stirrers. Measurement set-ups may be either closed or flow-through systems (von Oertzen, 1984). The latter are more ideal, as the accumulation of toxic wastes in the metabolic chamber is prevented, and a constant and adequate supply of oxygen, nutrients and essential trace elements can be assured. Flow-through systems are, however, more difficult to construct. One such set-up has been developed by A. Rex F. Montebon of the Marine Science Institute.

Measurements of environmental factors that are known to influence metabolism are essential to studies on productivity. These must be made with sufficient frequency and replication so that the data may be properly used to help explain metabolic trends. Environmental factors of interest include light, temperature, salinity, water motion, the various nutrient species (particularly of phosphorous and nitrogen) and particulate loading of the water column.

RESEARCH THEMES AND APPLICATIONS

Energy Budget of a Coral Reef

A recently completed study (Yap, Montebon and Dizon, 1992b; 1993) involved the investigation of the energy production and consumption of three reef flat substrate components, namely, coral (of the family Fungiidae), rubble and sand. Of these substrates, the coral had the highest production-to-respiration ratio ($P/R>1$) over a diurnal period. The rubble component had a P/R ratio approximating 1, while sand was heterotrophic ($P/R<1$).

This result confirms previous findings that corals in general sustain a net positive primary production (Gladfelter, 1985). In contrast, combined sand and rubble zones in reef flats tend to be heterotrophic, and probably consume the organic carbon produced in the more productive parts of a reef (Kinsey, 1985a). The preliminary picture that emerges is one of energy flow from the autotrophic to the net energy-consuming compartments of a reef, effectively maintaining the whole system. The flow of energy constitutes a number of trophic and detrital pathways, which remain subjects for future study in the Philippines.

Temporal Patterns of Energy Flow

In an investigation of seasonality in metabolism and its stability over time, the three substrates mentioned above were monitored at monthly intervals over an approximately two-year period (The exact duration of monitoring varied for each substrate.) (Yap, Montebon and Dizon, 1992a; 1993). The production and respiration rates of coral, rubble and sand fluctuated only within narrow ranges over an annual cycle, displaying a kind of constancy or stability. A certain stability in function is expected at the ecosystem level (Pomeroy et al., 1988) and it constitutes an emergent property.

Within the relatively narrow ranges of variation in metabolic rates, however, significant effects of the environmental factors light, temperature and salinity were detected. These parameters exhibited significant variations over the seasons into which a year was arbitrarily divided based on temperature and salinity data, namely, dry-cool (December-February), dry-warm (March-April) and wet (May-November). Despite its location at a low latitude, the reef flat studied still underwent a certain seasonality in productivity (Hatcher, 1990).

Environmental Effects on the Physiology of Reef Organisms

One of the most important controlling influences on coral productivity is still that of light (Gladfelter, 1985). In addition to light being important on its own, its effects are also manifested in the influence of an environmental disturbance such as siltation. In such a case, silt in the water column reduces the intensity of light penetrating to the benthos, thus causing a reduction in photosynthesis. This is in addition to physical impacts such as smothering. Light in relation to productivity is thus one of the major points of investigation in studies of coral reef energetics in the Philippines (Montebon and Yap, 1992b; Yap and Montebon, 1992; Yap et al., 1993).

Diurnal averages of light plotted over more than an annual period indicate a range of variation of from 500 to over 2000 microEinsteins m^{-2} s^{-1} (Fig. 1). A laboratory experiment in which corals of the species *Fungia (Danafungia) horrida* in the size range 5-9 cm (average diameter) were exposed to stepwise increments in light intensity, showed significant increases in gross photosynthetic rates (Fig. 2; Yap et al., 1993). The values obtained were used to generate a light saturation curve (Fig. 3) based on the hyperbolic tangent function following the method of Chalker (1981).

A light saturation curve yields useful insights into the ecology of the species. For example, saturation of the coral's photosynthetic machinery by light is shown to occur at an irradiance of approximately 800 microEinsteins m^{-2} s^{-1} (Yap et al., 1993). This is a relatively high value when compared to results of other investigators (e.g., Chalker 1981; Chalker et al., 1983). This is probably due to the relatively shallow depths at which the species is situated and the consequent high light

intensities to which it is normally exposed. If this finding is applicable to other coral species in the tropics, it could be used, for example, in predicting rates of productivity that could be expected in shallow, well-lit waters at various times of the day.

Another environmental factor that has been identified as being an important determinant of coral reef function is hydrodynamics (Hatcher, 1990). Experiments at the Marine Science Institute have started at the organismal level, and a look at the effects of water motion on the photosynthesis, respiration and calcification of a hermatypic coral species, *Porites cylindrica* (Montebon and Yap 1992a). Water motion can exert significant influences on both photosynthetic and respiration rate (Dennison and Barnes, 1988; Montebon and Yap, 1992a). The degree of turbulence on particular reef zones can thus serve as a predictor of relative levels of activity of the primary producers.

The Reductionist versus the Systems Approach

Rates of productivity as they are measured may vary depending on the spatial scale encompassed by the experimental protocol (Hatcher, 1990). The reason is that more numerous and complex (as well as interacting) processes come into play as the space of interest is scaled up. This involves both biotic (e.g., competition) and abiotic (physico-chemical, hydrodynamic) factors. This is why metabolic measurements of organisms or communities within chambers do not necessarily yield data representative of total system function.

For purposes of comparison, *in situ* chamber metabolic runs were immediately followed by open system flow respirometry measurements of the same community in one field experiment (Yap, Dizon, and Montebon, 1992). Trends in photosynthesis and respiration, as well as the flux of the major nutrients, were assessed on both scales. The work is currently ongoing, and it takes into account possible seasonal patterns. Thus, measurements are made during the dry-warm and wet seasons.

DISCUSSION AND CONCLUSIONS

The more fundamental questions in coral reef energy dynamics, such as with respect to the effects of environmental factors on metabolism, are most effectively addressed at the level of the individual organism. It is the basic physiological processes at this scale, as they respond to environmental forcing, that ultimately underlie the complex of phenomena that occur at the level of the community or ecosystem. However, as the level of organization goes up the ecological hierarchy, interactive processes involving both biotic and abiotic factors become more important, and system behavior becomes more complex. One result is in the form of so-called "emergent phenomena" (Pomeroy et al., 1988) that cannot be satisfactorily understood using an entirely reductionist approach.

This is the reason why current research efforts in the Philippines address both levels simultaneously: that of the individual, and that of the whole system. It is hoped that the interpretation of processes observed at each of the above scales would coverge into a rational picture of productivity.

At the level of the organism, the effects of important environmental factors, such as light and water turbulence, are clearly elucidated. Results could be used to explain trends in community behavior on larger scales, such as why net community production might vary among different zones of a reef, or with time (i.e., diurnally, seasonally). Many other processes at the organismal level could be extrapolated to the larger system in this way.

At the level of the ecosystem, broad patterns in productivity in both space and time emerge. As discussed, some of these could be explained by invoking processes at lower levels. This includes energy budgets in reefs, where quantities of materials and energy can be compartmentalized among functionally defined ecological components.

Other system processes are clearly emergent, and they result from interactions at levels higher than the organism. An example is the appearance of system stability in terms of both energy production and consumption over an annual cycle. Such a result provides a clear message about reef function. Quantitative assessments, in addition, give an indication of the amount of energy produced and consumed by the system for maintenance.

All topics treated in this paper have ultimate relevance with respect to the management of coral reef resources. As mentioned earlier, the basic natural process that underlies the usefulness of reefs to man in terms of products, physical protection of the coast and amenities (i.e., recreational activities based on the aesthetic value of these habitats) is productivity. The studies discussed in this paper have as a unifying objective the understanding of the dynamics of productivity of reef ecosystems. The field is ripe for future studies on a diversity of themes, particularly in the tropics.

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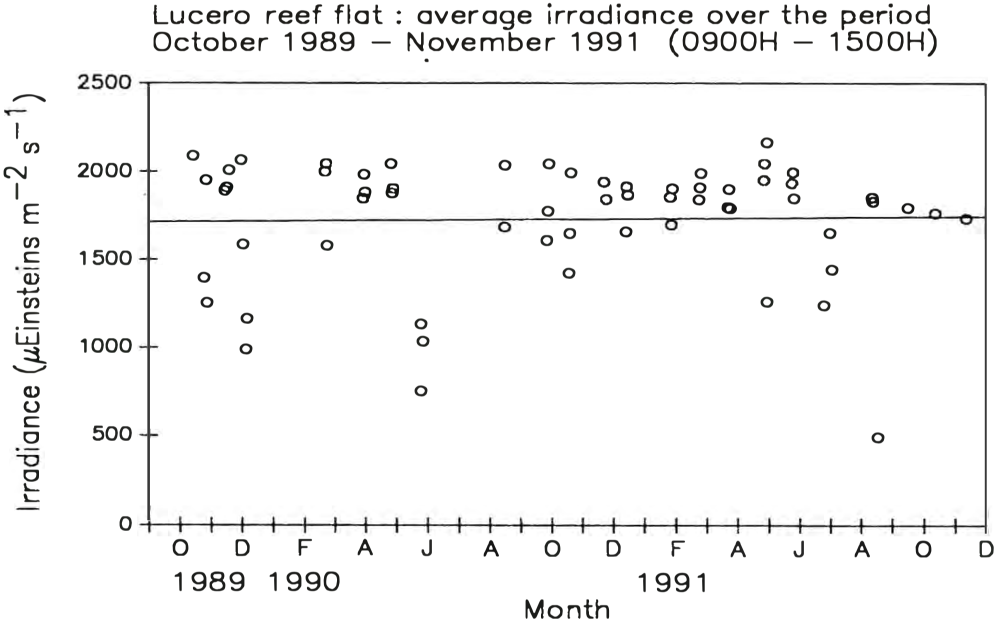

Figure 1. **Variation in light intensity over an annual cycle in a reef flat (Lucero) in Bolinao, Pangasinan at a water depth of about 1 m. Values in microEinsteins m^{-2} s^{-1} are averages of daily values taken between 0900-1500H during monthly visits to the site from October 1989-November 1991 (See Yap, Montebon and Dizon, 1993.).**

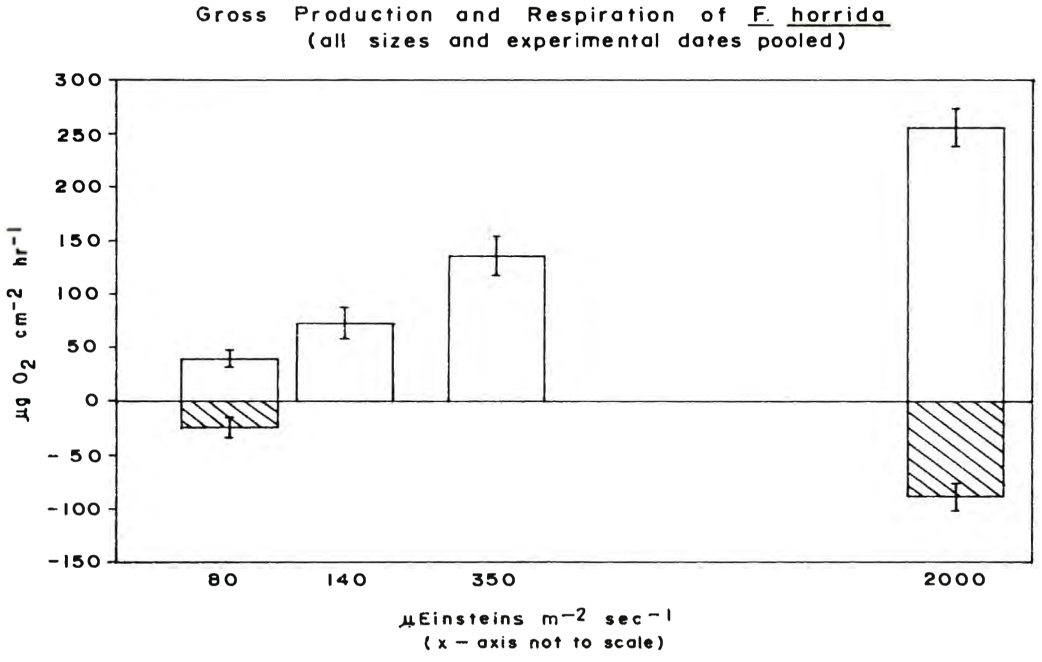

Figure 2. **Gross photosynthesis and respiration of *Fungia (Danafungia) horrida* in response to increasing light intensities from 80 to 2000 microEinstens m^{-2} s^{-1} (See Yap et al., 1993.)**

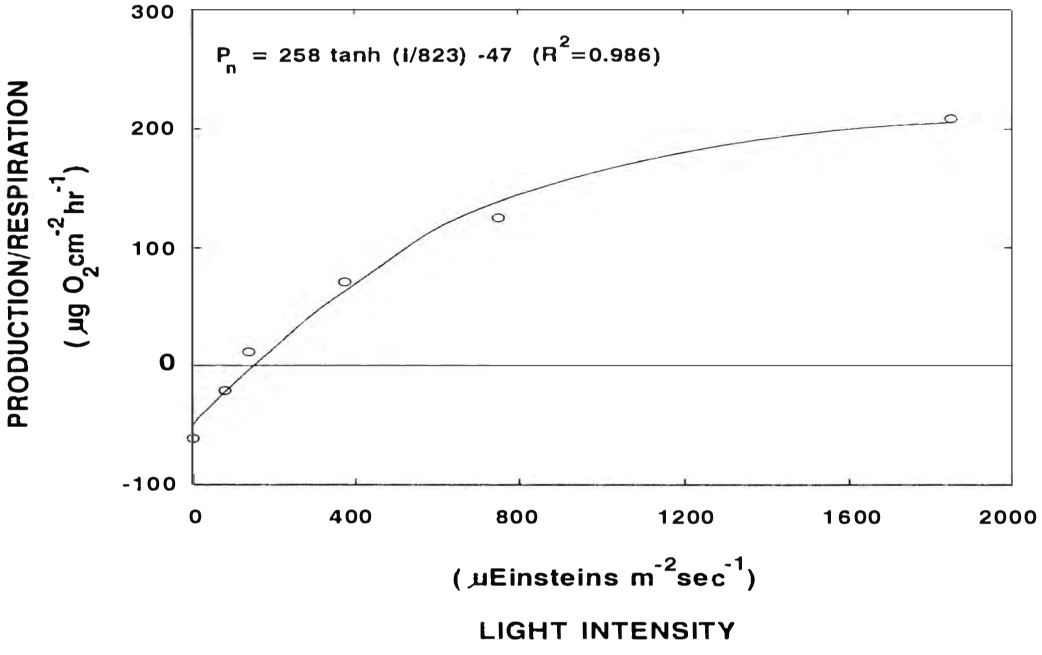

Figure 3. **Light saturation curve constructed on the basis of data in Fig. 2 (from Yap et al., 1993.)**

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LANGUAGE USE AND TRANSFER IN MATHEMATICAL PROBLEM SOLVING: REVISITING THE WHORFIAN HYPOTHESIS AND APPRISING THE MEDIUM OF INSTRUCTION ISSUE

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ABSTRACT

A research approach is suggested to investigate the issue of medium of instruction in teaching math. The approach is based on the weak version of Whorf's linguistic determinism which states that variations in lexical, semantic, syntactic and pragmatic aspects of language influence thought. The approach is used to study the observation that people have a difficult time expressing in Filipino, material that they have learned in English.

This paper looks into the role of language use in the transfer of information in solving mathematical problems. Earlier studies have shown that language-based representations of problems affect performance in math word problem; and that problem-specific information plays an important function in transfer. Based on these studies, it was predicted that transfer of information would be better when the language used to study the problems is the same as that used in the transfer test. The results of one experiment support the hypothesis and are discussed in terms of the Whorfian hypothesis and the issue of medium of instruction used.

INTRODUCTION

It would be an understatement to say that there is currently a disagreement about which language to use as the medium of instruction in our country. Del Pilar (1990) pointed out that this disagreement is readily seen in the divergent language policies of two important educational institutions – the DECS and the University of the Philippines. While the DECS maintains a National Bilingual Policy (English as medium for Science and Math), the UP is now deep in the implementation of its Filipino language Policy (Filipino as medium for all subjects at all levels of education).

Pascasio (1992), however, observed that while there have been a lot of opinion and debate on the language issue, these are often not supported by data. She pressed for the need for more research that will bear on the issue.

This paper is one attempt to present data that will hopefully bear on one aspect of the issue. The study reported in this paper addresses the phenomenon of transfer of problem solving information in mathematics within and across languages. The cognitive psychological approach to the Whorfian hyphothesis is used as the general theoretical frame, and a "representational" model of transfer in math word problem solving is used as the specific frame.

The Whorfian Hypothesis: Linguistic Determinism

Based on anthropological and cross-linguistic studies in the 1920s and 1930s, Benjamin Whorf (1956) proposed the hypothesis that the language we use exerts some amount of control over our thoughts. Whorf proposed that the way we perceive the world and the way we think about it are largely dependent on the structures of the language we use. In this sense, he claimed that language determines thought. The view that language structures control the structures of thought has been labelled the "strong version" of Whorf's hypothesis. The strong version has long been abandoned.

But recently, a "weak version" of Whorf's hypothesis has been revived on the basis of experimental studies of cognition. The weaker hypothesis states that specific cognitive functions can be influenced by variations in the lexical, syntactical, semantic and pragmatic aspects of language. Hunt and Agnoli (1991) recently reviewed the experimental data that strongly support this hypothesis.

Linguistic Determinism and Language of Instruction

Research on the medium of instruction issue in the Philippines has been largely based on studies on the relative effectiveness of Filipino (or a vernacular) and of English as media for instruction (see del Pilar, 1990; Gonzales and Sibayan, 1988). While affirming the value of such studies, this researcher suggests a complementary approach to addressing the medium of instruction issue based on the assumption of linguistic determinism. It is proposed that another track can be used to study the effects of language on specific aspects of the different cognitive skills that students are expected to learn. This approach involves several components.

First, it has to be ascertained whether language use has any role in the operation of specific cognitive skills. Second, if language use has a role, there is a need to specify that role and demonstrate its function in the operation of the cognitive skill. Third, there is a need to determine the effects of varying the particular language used (e.g., first or second language of bilinguals) in order to know whether the use of one language will have predictable costs or benefits relative to the operation of the skill.

The Problem of Transfer

A common observation among Filipino bilinguals who were schooled in English is their difficulty in expressing in Filipino things that they have learned in English. This is particularly true when it comes to concepts related to science and math. From this observation, people have often incorrectly concluded that science and math are not expressible in Filipino and that learning science and math is only possible in English.

One aspect of this problem that is readily identifiable, is the lack of corresponding terminologies in the Filipino language. But aside from this surmountable problem (Miranda, 1991) there is still a general difficulty of transfer from English to Filipino. Others have proposed that this difficulty is mainly due to attitudinal and socio-cultural factors (Acuna, 1993). This researcher proposes that another factor that leads to this difficulty is related to the role of language in the transfer of information. In this research, the process of transfer of information between analogous word problems in math was studied. The languages used in the learning phase and in the transfer test phase were varied to test a specific hypothesis about the role of language in transfer.

The Role of Linguistic Input in Transfer

The model proposed in this study is based on two separate but related bodies of psychological findings. First, several studies have shown that analogical transfer is greatly dependent on similarities in the superficial, problem-specific (i.e., as opposed to the abstract, structural) features of the analogous problems (e.g., Gick and Holyoak, 1980; Holyoak and Koh, 1987), even in highly abstract problems in math (e.g., Bassok, 1990; Ross, 1987, 1989). It could be argued that the language used in the problem text is one of the superficial elements of the problem that is important in transfer.

This argument is related to the second body of findings that shows the importance of semantic representations of the text of word problems in solving these problems. Several studies have shown that variations in the wording, ordering of sentences result in variations in performance on such problems (e.g., Cummins, 1991; De Corte and Verschaffel, 1987; Riley and Greeno, 1988). These studies show that some semantic representations allow for easier inferences needed to answer the questions in the problem. Their findings suggest that to a great extent, linguistic input in word problems affects the understanding of the problem texts and also, therefore, the solutions.

Based on these two sets of findings, the researcher proposed that: (1) in solving word problems, people construct a semantic representation of the information in the text; (2) these semantic representations be couched in terms of the language used; (3) in analogical transfer, people create mappings between semantic representations of the problems; and (4) mapping between semantic representations

should be easier if the representations are in the same language. Points (1) and (3) have already been supported by earlier studies. Point (2) will be indirectly tested in the attempt to explicitly test point (4).

Overview of Experiment and Hypotheses

In this study, subjects were given a set of study problems in basic probability. The study problems included a statement of the problem, a description of the quantitative relations in the problem, and of the relevant principles, concepts and equations; and a worked out solution for the problem. After accomplishing the set of study problems, the subjects were given a set of analogous problems that were very similar in surface content to the corresponding study problems. The subjects were tasked to solve these problems without directly referring to the study problems.

Subjects were given study problems which were either in Filipino or in English. For the transfer test phase, all subjects were given half of the problems in Filipino and the other half in English. Subjects' performance in the test problems was coded in terms of whether there was transfer of information from the corresponding study problem to the test problem. It was predicted that transfer would be better if the test problem was in the same language as the study problem.

METHOD

Subjects

Fifty-three Introductory Psychology students at the University of the Philippines, Diliman participated in this experiment as part of a class requirement. Data from five subjects were not analyzed because their first language was not Filipino (Bicolano, Bisaya, Pampango, English).

Data from another four subjects were not included because they either did not transfer in any of the problems (n=3, they just scribbled numbers) or they solved all the test problems correctly (n=1, suggesting prior experience in the domain). Consequently, only data from 44 subjects were analyzed.

Twenty-two subjects were randomly given Filipino study problems. The other half were randomly given their English study problems. All subjects were bilingual reporting Filipino as the languge used at home and English as the main language used in math education. The subjects were asked to rate in a scale of 1 to 7 (1=not at all proficient; 7=very proficient) their proficiency in speaking and reading in Filipino and in English. The scores for both subject groups show no reliable difference in their proficiency in either language. For those given study problems in Filipino mean proficiency in Filipino was 5.20, and in English, 5.80 [$t(22)=1.91$, stderr=.31, n.s.]. For those given study problems in English, the means were 5.11 and 5.30 [$t(22)=0.62$, stderr=.31, n.s.] for Filipino and English, respectively.

Materials

Word problems in the following problem types in probability were created: conjunction problem with independent events, conjunction problem with dependent events, disjunction problems with exclusive events, disjunction problems with intersecting events. Probability problems were chosen to ensure that subjects had no prior experience in the problem domain; at the same time the problems were easy enough for those with a background in algebra. Four versions of each problem type were created. Each version of a problem type had different surface content. One version of each of the four problem types were combined to form a study set making four sets of four study problems. All study problems were first written in English. These were then translated into Filipino by a student who was proficient in both languages. To ensure that the Filipino and English versions were equivalent, the Filipino translations were translated back into English by another student.

For the transfer test problems, a superficially similar analogous problem was created for each of the study problems. Some of the test problems were written in Filipino and some in English. Using a counterbalancing procedure it was ensured that across the four sets of study problems, each problem type had an equal probability of having a transfer test problem in Filipino or in English.

Each study problem was typed in a separate sheet of paper. Each transfer test was also typed in a separate sheet, with the problem on the top portion, and the rest of the page left blank for the solutions. The study problems and the test problems were arranged in random order and combined to form a test booklet. The test booklet included instructions for the study and test phases, and a questionnaire on subjects' language and math backgrounds. The instructions and questionnaire were written in Filipino for the Filipino study condition, and in English for the English study condition.

Procedure

The subjects were run in groups not larger than eight. Subjects in one session were randomly assigned to either the Filipino or English study condition and given the appropriate test booklet. They were instructed to read each study problem and to try to learn the material as best as they can. They were encouraged to work through the problems by themselves. They were given three minutes to study each problem and they were told when they had 30 seconds left for each problem. After studying the four study problems, the subjects were then tasked to solve the test problems without directly referring to the study problems. They were again given three minutes for each problem and informed when they had 30 seconds left. After completing the test problems, they filled out the background questionnaire.

RESULTS

Answers in the test phase were scored as showing transfer (1.0), partial transfer (0.5) and no transfer (0.0). A complete transfer was scored if the solution indicated that the subjects transferred the correct solution equation and instantiated it correctly for the test problem. A complete transfer was scored even if the final answer was incorrect due to computation errors or incomplete computations. A partial transfer was scored if the solution showed the correct equation but the equation was not correctly instantiated for the test problem. No transfer was scored if the correct equation was not transferred. The mean transfer scores are summarized in Table 1.

The scores were analyzed using a 2 x 2 ANOVA for mixed designs. There was no main effect of either the language of the study problems, $F(1,42) = 0.74$, $\underline{MSe} = .14$, $\underline{p} >, 10$, or the language of the test problems, $F(1,42) = 1.26$, $\underline{MSe} = .11$, $\underline{p} > .10$. These results are consistent with the view that there are no overall costs or benefits of using either English or Filipino as the language of study or test.

Also as predicted there was a reliable interaction between language of study and language of test, $F(1, 42) = 4.35$, $\underline{MSe} = .11$, $\underline{p} < .05$, suggesting that test performance is better if the language used in the test matches that used in the study.

To further test the reliable interaction effect, the scores were analyzed using $\underline{t}$-test for paired comparisons. The analysis showed that subjects who studied problems written in Filipino performed better with the test problems in Filipino than in English, $\underline{t}(21)=2.24$, stderr = .10, $p<.04$. But, for subjects who studied problems written in English, there was no reliable difference in performance for the Filipino or English problems, $\underline{t}(21) = 0.69$, stderr = 10, $\underline{p} > .10$.

This researcher did some data snooping to find out why the apparent advantage in solving test problems written in English among subjects given English study problems was not statistically reliable. Analyzing the proportion of solutions that showed complete transfer, this researcher in effect, used a stricter criterion for scoring transfer. It is possible that subjects who got credit for partial transfer might have only remembered the equation without understanding its structural meaning. By requiring subjects to instantiate the equation correctly, the researcher made sure that subjects who are scored for transfer have indeed made the appropriate structural mappings between the study and test problems. The proportions of solutions that showed complete transfer are summarized in Table 2.

These proportions were then analyzed using a X^2-test for independence. Similar to the results of the criterion scoring, the analysis showed no reliable main effect for the language used for study, $X^2(1, N=176) = 0.82$, $\underline{p} > .10$, and for the language used in the test, $X^2 (1, \underline{N}=176)=0.09$, $\underline{p} > .10$. As with the earlier scores, subjects given study problems written in Filipino were better at transferring information to problems written in Filipino than to those written in English $X^2(1, N=88) = 2.91$, $\underline{p} < .05$, one-tailed. Unlike the earlier scores, however, subjects given study

problems in English, were better at transferring information to problems written in English than to Filipino ones, $X^2(1, N=88) = 3.27, P < .05$, one-tailed.

This reliable difference suggests that subjects who were given study problems written in English were probably equally able to recall the equation with Filipino and English test problems and that, using the scoring criterion, this ability to recall the equations masked the difference in actual transfer.

DISCUSSION

The results support the hypothesis that transfer is better if the language in which a problem was studied is the same as the language of the test. By implication, the data also support the position that some aspects of the semantic representation of word problems are described in the language used during the problem solving episode.

Before discussing the implications of such findings, this researcher wishes to emphasize that this study focuses on only one very specific aspect of math problem solving: the transfer of information from one word problem to a superficially similar analogous one. The effects associated with language use could be different for other aspects of word problem solving. For example, the results do not in any way show the role of either language in developing a semantic representation for either the study problem or the test problem.

Further; the results do not indicate the role of language in understanding the abstract, structural information in the study problem. The transfer effects are also observed under conditions in which the problem solver's conceptual knowledge about the domain is poor. The role of language in transfer when the problem solver has more experience in the domain might be different from what is shown.

The results regarding the role of language on transfer could also be considered rather general. One could still ask the question: Where among the specific components of transfer does language play the particular function demonstrated? Consider the following. First, it is possible that subjects were able to transfer the correct information only in cases where they understood the study problem. This possibility could only be true if those who were given study problems in Filipino better understood the problem types that were subsequently tested in Filipino, and those given study problems in English better understood the problem types that were subsequently tested in English. While the data cannot provide information about which study problems were understood, this possibility was controlled for by using counterbalancing procedures that ensured that it will be equally likely that a problem type will be tested in Filipino and in English.

Furthermore, there is no main effect of transfer performance due to language of study suggesting that there is no overall difficulty associated with understanding the study problems in either language. Hence the possibility raised is not consistent with the experimental data nor with the control procedures used.

There are other possible explanations for the effects that relate to the actual process of transfer. For one, it was earlier posited that studying a problem using one language leads the problem solver to develop a semantic representation of the problem that is described in terms of that language. It is possible then, that the language effects are obtained because this semantic representation is used to guide the understanding of the transfer test problem. Better understanding of the target problem then leads to better test performance. While it is likely that an earlier problem representation can be used to guide the understanding of a new problem, there is the problem of determining which earlier problem to use as guide. The language used cannot specify which earlier problem should be used to guide the processing of a new problem.

Determining which earlier problem episode to use is addressed in another possible explanation of the results. As mentioned earlier, other research have shown that problem solvers use superficial similarity between problems to determine sources of analogous information. It is possible that having the study and test problems in the same language makes the similarities between them more salient. This then allows the problem solver to more easily access the information from the appropriate study problem.

The last possible explanation considered assumes that problem solvers have no difficulty in accessing information from the correct study problem. Differences in transfer lie instead in the ability to instantiate the information correctly for the new problem. It is possible that having the study and test problems in the same language leads to a better grasp of the structural similarities between the problems that are necessary for the correct instantiation of analogous problem information.

It should be noted that the last three proposed explanations are not mutually exclusive. It is possible that similarity in language use operates in all three ways. Fortunately, all three explanations could be studied experimentally by manipulating variables relevant to access and use of problem information in transfer. So like any "good" experiment, the one described in this report raises a lot of useful questions that need to be further studied.

The experiment, however, does provide some answers. For one, by showing how similarity in language use can facilitate analogical transfer, another means by which language can influence thought has been specified. This influence is related to the pragmatic (not to the semantic or syntactic) aspects of language. The influence is not such that one language is structurally better for processing information in math; instead, language is the medium in which information is carried or by which mapping of mathematical concepts is facilitated – a pragmatic function.

The results also provide an explanation for the observed difficulty in expressing in Filipino what one had learned in English. The findings suggest that this difficulty is not due to some limitation of the Filipino language. Instead, it might be due to the possibility that information is represented in the language in which it was learned and transfer is not always easy across languages.

Finally, the results of the experiment suggest that there is another approach to addressing the medium of instruction issue – looking at the specific role of language in the cognitive skills that students are expected to learn. This approach has at least one important implication: language can have different functions in particular aspects of a cognitive skill. Since cognitive skills can be broken down into smaller components, it is possible that language will play different specific roles for each component. If so, then there is no unitary relationship between medium of instruction and learning. That is, depending on the particular role of language in the operation of specific components, using one language might be more effective in terms of one component, but less effective in another.

The conclusions that can be derived from studies of this approach would then be "partial" in nature. More experiments testing a wider range of variables need to be done and more specific models need to be tested before this approach can lead to far-reaching recommendations relevant to the medium of instruction issue. Fortunately such experiments can be done without requiring so much time and expense. Hopefully, a body of similar research findings can soon be developed to complement current research efforts toward resolving the medium of instruction issue.

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Table 1. Mean transfer score (and standard error) as a function of language of study and language of test

	Filipino test	English test
Filipino study	.66 (.06)	.43 (.08)
English study	.58 (.06)	.65 (.08)

Table 2. Proportion of solutions showing complete transfer as a function of language of study and language of test

	Filipino test	English test
Filipino study	.59	.41
English study	.50	.64

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SOCIAL CATEGORIZATION AND IDENTITY IN THE PHILIPPINES

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ABSTRACT

Using a social cognitive theoretical and methodological approach, this research looks into the concept of social categorization, an assumed precondition for the development of social identity, within the Philippine context. The study was conducted among students at the University of the Philippines in Diliman, Quezon City. Three sets of questionnaires were administered to tap into various social category perceptions. Results showed that national category membership is deemed important but is not nearly as salient as one's family, gender or religious group memberships. Perceptions of own ethnic group were seen as more positive than those for the national group and very little overlap in features was noted between the two social categories. Data trends indicate a possible weakness in our concept of national category membership which could provide the possible underpinnings for earlier findings by other researchers on the tenuousness of Philippine national identity.

INTRODUCTION

Bruner, Goodnow and Austin (1956) said that "to categorize is to render discriminably different things equivalent, to group the objects and events and people around us into classes, and to respond to them in terms of their class membership rather than their uniqueness." By grouping objects and events together that, to our minds, have commonalities on certain dimensions, we are able to think about and respond to them in familiar learned ways. Categorization thus serves as an anchor in a complex and potentially overwhelming environment (Lingle, Altom and Medin, 1984).

Nowhere is the value of categorization more apparent than in dealing with our social world. Without categorization, every person and every social situation would have to be processed as new, leaving the social perceiver confused and overwhelmed by the volume of information that needs to be processed prior to engaging in any action.

Tajfel (1981) referred to social categorization as one of our tools for structuring our environment and establishing our own "construction of any particular social reality." Many social processes arise from categorization. Among these are stereotypes (Allport, 1954) and social identity (Tajfel, 1981).

This study investigates social categorization among Filipinos, focusing on ethnic and national categories, their salience and use with the goal of understanding better the underpinnings of national identity. It aims to look at the relevant social categories identified and processed by the Filipino social perceiver, the place of ethnic and national categories within this matrix and the relationship of the ethnic to the national category.

There is no doubt that stereotypes and social identity, particularly national and ethnic identities, have their origins in history and cultural traditions related to one's experiences within a particular socio-political context. Looking into these origins has been the popular approach used by various Philippine researchers (Constantino, 1974; Corpuz, 1990; Doronila, 1989, 1992) in analyzing the stunted growth of our Filipino national identity.

The social cognitive theoretical and methodological framework is suggested in this research as a dynamic alternative to standard approaches. Rather than focusing on historical and contextual antecedents, this approach would treat group memberships and their dynamics as information to be processed and their social outcomes as resultant by-products of such information processing.

METHODOLOGY

Subjects

At the outset, this researcher was interested in obtaining a sample that would give a fair distribution of the different ethnic groups in the country. Since a large national sample was not possible for the research, it was decided to pilot the research with the Social Science classes at the College of Social Sciences and Philosophy of the University of the Philippines in Diliman. The Social Science courses are general education courses required of all students at the university in their first two years of college. It was thus reasonable to expect the students to be fairly varied in their backgrounds. Three Social Science classes were approached with a total of 106 respondents ultimately participating in the study.

The respondents' ages ranged from 16 to 26 years with 46.2% falling in the 18-year-old bracket. Majority (79.2%) were Catholics. More than half of the respondents (65.1%) graduated from a private high school and only 34.9% came from public high schools.

Majority (59.4%) of the respondents listed a home address situated in Metro Manila with the rest listing provincial addresses from all over the country. Only 35.8% of the respondents, however, mentioned the National Capital Region as their

region of origin. The rest mentioned various regions all over the country, although regions in Luzon (Central Luzon and Southern Tagalog) were over-represented (13.2% and 15.1%, respectively).

Respondents categorized themselves as belonging to different ethnic groups. Table 1 shows the distribution of the self-ascribed ethnicity of the respondents. It should be noted that although Manileno is not, strictly speaking, an ethnic group, a small percentage of the respondents gave that as their ethnic affiliation.

Parental ethnicity of the respondents was also varied. Approximately one-third of the respondents (33.96%) had parents who came from different ethnic groups. The rest had parents belonging to the same ethnic group.

Across the different ethnic groups, 65.09% claimed Tagalog or Pilipino as the dominant language used. Of the remaining 34.91%, 13.21% said their dominant language was English. Thus, only a total of 21.7% actually used the language of the ethnic group they belonged to as a major means of communication.

Questions about other languages used by the respondent showed that 22% of those who did not mention Tagalog or Pilipino as their dominant language mentioned it later as another language also used.

Procedure

Three questionnaires were administered at three-to four-week intervals to the study participants. The sequence in questionnaire administration was randomly varied with approximately one-third of the subjects receiving a different sequence each. This was to rule out the influence of response set on subject's performance.

One questionnaire looked into the respondents' perceptions of being Filipino; another looked into perceptions of one's ethnic group; and a third questionnaire focused on social category memberships in general. Additional demographic data, as well as data on parents' ethnicity, region of origin, languages used at home and religion, were also obtained. Data were analyzed using mainly descriptive statistics.

RESULTS AND DISCUSSION

Social Categorization in the Philippines

The first question of interest in the research was the matrix of social categories in which both ethnic and national group memberships may be embedded. To tap into group memberships viewed as relevant and important, respondents were simply asked to list down all the social categories or groups which they felt they belonged to and to rate each one in terms of its personal importance. Order of listing was used as an indicator of category information accessibility and group salience.

The maximum number of social categories listed was 13 with a mean of 7.58 groups mentioned. Among the mentioned categories, family, gender and religion, in

that order, were mentioned first by 91.9% of the respondents. Family was particularly salient with 27.4% mentioning it first. This finding is not surprising and is consistent with previous research on the marked significance of the family to the Filipino (Doronila, 1989, 1992; Medina, 1991; Torres, n.d.).

National group came in a poor fourth among the first mentions with only four respondents mentioning it before all other groups named. Across mentions, however, national group was identified as a relevant social category by 67.92% of the respondents. It would appear then that nationality, though viewed as a relevant social category, is not as salient (i.e., not the first thing that comes to mind) as apparently more basic group memberships.

How did the ethnicity-based social category fare? Many of those who criticize the Filipino's lack of nationalist sentiment often blame heightened sense of regionalism or strong sense of ethnicity as the social cognitive approach that would trace to an overly salient and highly accessible ethnic category. Doronila (1989), citing the literature on Philippine ethnic groups, reported that "ethnic boundaries have not yet been transcended, for which reason it is also reported that Philippine society remains primarily familistic and secondarily regionalistic in orientation."

Data collected showed that ethnicity was mentioned in second place, at the earliest, and this by only 2 out of 106 respondents. Taken all together, however, ethnicity was mentioned as relevant by only 38.68% of the respondents. It appears that more respondents were mindful of the national compared to the ethnic group category. The mean salience rating (based on the category list position) for nationality was 3.24 compared to 2.68 for ethnicity. Though caution in interpretation is advised given the skewness of the sample, the results appear to be consistent with a nationalist mind-shift that Doronila (1992) noted in her research.

Consistent with the data on category salience, the importance ratings given to the national group were also higher compared to those for the ethnic group (mean rating for nationality is 3.08, whereas that for ethnicity is 2.68, given a 4-point rating scale where 4 = very important).

Five types of respondents may be identified based on the category salience data: (1) those who seem to find no relevance to either nationality or ethnicity as evidenced by their failure to mention either national or ethnic social categories; (2) those for whom ethnic group seems dominant, mentioning only this category but not the national group; (3) those for whom ethnicity is primary but do not forget the national group; (4) those who mention or consider the national group before thinking of their ethnicity; and (5) those who consider only the national group. Further research should be done to look into the impact of these five cognitive sets on the definition of national identity.

Ethnic Versus National Group Perceptions

A second question of interest was the comparative assessment of ethnic and national categories. This looked into the possible outcomes of category processing. Aside from a listing and rating of relevant social categories, respondents were also asked to list down 10 features and characteristics which they felt defined their own ethnic group and the national group. The proportion of positive features to the total mentioned was computed and used as an indicator of positivity of one's perception toward that particular social category. Table 2 shows the proportion means for each ethnic group.

The data show a clear trend of subjects favoring their own group over the national group. The literature often refers to this as the in-group bias (Turner, 1981) except that in this case, both ethnic and national groups could be considered as in-groups. The greatest difference in positivity proportions is shown by the Mindanao groups and the least difference is shown by the Chinese/mestizo group. Of particular note is the rather low means for own group perception among the Muslim and the Chinese/mestizo groups compared to the other ethnic groups.

A t-test for correlated means was done on the overall means and the results showed a significant difference between the proportion of positivity toward one's ethnic group and the national group [$t\,(105) = -6.902$, $p < .0001$], indicating that own ethnic group is seen in a more positive light than the national group.

A related issue explored was the perceived relationship between own group and the national group. To do this, the researcher reviewed each subject's feature lists for own ethnic and national groups and counted the number of category overlaps, mentioned features which were common to both groups (Table 3). In the social cognitive literature, common and distinctive features between two categories are used to represent similarity relations (Tversky, 1977; Lingle et al., 1984).

Data trends follow the patterns of the positivity index with the Mindanao groups showing the least similarity with the national group. It would appear that, aside from seeing themselves in a more positive light, the Davao and Muslim groups also perceive minimal similarity between themselves and the national group. One may be tempted to hypothesize cultural alienation. However, due to the limitations of the sample, care needs to be exercised in drawing any conclusions. Further research is recommended to follow up on these questions.

What seems puzzling about the data configuration of pronounced disparity between views about own group and the national group is its typicality for an in-group/out-group relation. It is typical for members of the in-group to exhibit in-group bias, a marked preference for one's own group, and out-group discrimination, heightened negative perceptions of other groups (Turner, 1981).

Ethnic and national groups, however, are not supposed to be in this type of relationship pattern. One may even possibly conceive of a category hierarchy where national group is the superordinate category encompassing ethnic group categorizations. Category overlaps are thus expected to be high and the valence of

perceptions relatively consistent. Further analysis of the social perceiver's category structures could provide some answers but that is beyond the scope of this particular study.

Social Category Features and Stereotypes

In addition to a rudimentary look at the structure of our respondents' social categories, the content of their ethnic and national category schemata were also explored. Three intriguing patterns were noted upon reviewing the content of the respondents' stereotypes of the ethnic and national groups.

First, there were traits that were mentioned by almost all of the respondents when describing the Filipino. These consensus traits included hospitality, religiosity and having close family ties. The regularity of their retrieval indicates the strength of their association with our national group stereotype.

Second, there was also some degree of consensus regarding what the Filipino is not. These features were being industrious, thrifty and modern.

However, it is in comparing ethnic stereotype content with the national group stereotype that we see the extent of our ethnic group-centeredness. Own ethnic group was usually described with positive traits like industrious/"masipag," hardworking, disciplined, thrifty, cooperative, helpful/"matulungin," clean/neat, patient, practical, not traditional, not superstitious.

The opposite negative traits were attributed to the national group, however. Thus the Filipino was described as lazy, always late, undisciplined, extravagant/ showy, having a talangka-mentality, traditional, superstitious, impractical, litterbug, impatient.

Only the Ilocano group assigned themselves some negative traits – pessimistic and stingy – while ascribing the opposite positive traits to the Filipino in general.

A good question to raise after seeing the data trends is why the positivity of ethnic perceptions does not generalize to national group perceptions. Given the awareness majority of the respondents had of the importance of being Filipino (even beyond being a member of one's own ethnic group), one fails to see why there is a denigration of such an important social category.

Tajfel (1981) posited that social categorization is often done in the service of a need for positive social identity. If the group does not satisfy this requirement, the individual can either quit being part of the group or, in the event such an option is unavailable, he may modify his interpretation of the group's unwelcome features to make them more acceptable. Which option our respondents take would make an interesting subject for further study.

CONCLUSIONS

In summary, three major findings are underscored. First, national and ethnic groups are identified as relevant social categories but they are not as salient nor as easily accessible as categories like family, gender or religious groups. Second, being Filipino was more salient and rated as more important than being a member of one's ethnic group. Third, despite the cognitive awareness of the importance of the national category, category content was less positive compared to the ethnic group category.

What are the implications of the findings? It would appear that, on the cognitive level, we acknowledge our Filipino-ness and the importance of being a member of this particular social category. Yet, it seems apparent that we have a less clear picture of what the Filipino is and how being Filipino relates to our other social identities. We see little overlap between our definition of our own group and the Filipino.

On the affective level, we still favor our own group over the national group. We ascribe more positive features to our ethnic group than we do to the Filipino seemingly ignoring the fact that one identity is subordinate to the other.

This research certainly raises more questions with its results than it had intended to answer. Clearly, more research needs to be undertaken if we are to more fully understand the Filipinos' processing of social categories and their social identity.

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Table 1. Distribution of respondents' ethnicity (N = 106)

	Percentage
Tagalog	41.51
Ilocano	13.21
Manileno	10.38
Bicolano	9.43
Cebuano Visayan	8.50
Chinese/mestizo	4.72
Ilonggo	4.72
Pampango	3.77
Davaoeno	2.83
Muslim	.94

Table 2. Mean proportions of positivity of respondents' perception of ethnic compared to national group

	Ethnic	*National*	*Difference*
Ilocano	.891	.669	.222
Tagalog	.845	.713	.132
Bicolano	.915	.677	.238
Pampango	.813	.750	.063
Manileno	.715	.665	.050
Cebuano Visayan	.819	.667	.152
Davaoeno	1.000	.467	.533
Muslim	.667	.143	.524
Chinese/mestizo	.611	.600	.011
Ilonggo	.800	.720	.080
Combined gorups	.830	.679	.151

Table 3. Mean number of overlapping features for ethnic and national groups

	Overlaps with National Category
Ilocano	2.357
Tagalog	3.227
Bicolano	2.400
Pampango	2.500
Manileno	2.455
Cebuano Visayan	3.444
Davaoeno	2.000
Muslim	1.000
Chinese/mestizo	2.200
Ilonggo	2.800
Combined groups	2.821

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GROWTH RESPONSES OF *GMELINA ARBOREA* WITH VA MYCORRHIZA INOCULATION AND NITROGEN FERTILIZER IN VOLCANIC ASH

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ABSTRACT

Growth of *Gmelina arborea* with VA mycorrhiza in infertile volcanic ash was studied using a complete randomized design with four replications per treatment. The treatments were: T1 = *Gmelina* in pure volcanic ash, T2 = Gmelina + VAM, T3 = *Gmelina* + VAM + 10 g N, T4 = Gmelina + 30 g N, T5 = *Gmelina* in Macolod soil (Check treatment).

Plant growth of *Gmelina* in volcanic ash was statistically similar to that of the *Gmelina* in Macolod soil. Gmelina with high N fertilization showed poor growth. Dry weight of *Gmelina* was high in the Macolod soil and in VAM treatments. Soil pH of volcanic ash decreased with N fertilizations, particularly with high dosage. Percent organic matter of volcanic ash increased, but a relatively higher difference was noted in treatments with N. Percentage of nitrogen and potassium was not significantly affected with the treatments. Phosphorus, however, was decreased in volcanic ash, but not much in the Macolod soil. *Gmelina* with high N fertilization showed poor growth. Dry weight of Gmelina was high in the Macolod soil and in VAM treatments. Soil pH of volcanic ash decreased with N fertilizations, particularly with high dosage. Percent organic matter of volcanic ash increased, but a relatively higher difference was noted in treatments with N. Percentage of nitrogen and potassium was not significantly affected with the treatments. Phosphorus, however, was decreased in volcanic ash, but not much in the Macolod soil.

Introduction

The recent eruption of Mt. Pinatubo Volcano in Zambales, Philippines covered huge areas in the nearby provinces with tons of volcanic ash or lahar. The deposition of the volcanic ash into the surface soils in this particular part of the archipelago adversely affected the growth and productivity of ground vegetation. The problem was aggravated by the lahar-covered areas which are infertile, with low organic matter, low nitrogen and low water holding capacity.

Plant growth in the lahar -covered areas could be enhanced through inorganic fertilizer applications. However, the use of such alternative is extremely impossible because these chemical inputs are expensive. One alternative to chemical fertilizer is the use of mycorrhiza.

Mycorrhizal fungi were found to increase absorption of both water and nutrients within plants. Likewise, they help improve soil aggregation and promote nutrient cycling. Besides, they also develop growth-promoting hormones and help in the biological control of pathogens within the plants. The growth promoting effect with VA mycorrhizal fungi, Rhizobium alone or dual inoculation with both symbionts is well documented (de la Cruz et al., 1988). Today, this phenomenon has become more important because nitrogen and phosphorus are limiting plant growth in the uplands worldwide (Mangui at et al., 1989).

Although mycorrhiza has beneficial contribution to improved plant growth, no definitive investigation has been done particularly in lahar or volcanic ash covered areas. It is therefore interesting to find out if VAM fungus is effective in providing the mycorrhizal effect to improve the growth of fast growing species like *Gmelina arborea* in volcanic ash. Likewise, application of nitrogen fertilizer to *Gmelina* in volcanic ash is also worth considering to compare the growth performance of this species with VAM.

METHODOLOGY

A greenhouse experiment was conducted in the Department of Silviculture and Forest Influences, College of Forestry, UPLB, College, Laguna from July to December 1991, one month after the eruption of Mt. Pinatubo. For this study, volcanic ash was collected from San Fernando, Pampanga, an area which experienced heavy deposits of volcanic ash.

A complete randomized design (CRD) with four replications per treatment was used in this study. The treatments were as follows:

T1 = *Gmelina* in Pure Volcanic Ash, Control
T2 = *Gmelina* + VA Mycorrhiza Inoculation
T3 = *Gmelina* + VA Mycorrhiza + l0g N
T4 = *Gmelina* + 30g N
T5 = *Gmelina* in Macolod Soil (Check Treatment)

The VAM fungus *(Glomus macrocarpum)* used in this experiment was secured from the BIOTECH, UPLB, Philippines. A total of 20 clay pots, each with a diameter of 8 inches, were prepared and placed in screen-formed beds inside the greenhouse. Two-month old *Gmelina* seedlings with the same height were selected. The seedlings were thoroughly washed with tap water to completely remove the soils and foreign objects from the roots. Clean seedlings were planted into the prepared pots filled with volcanic ash.

Application of the treatments was done one week after the seedlings were transplanted in the pots to allow adoption of seedlings into the new soil type. For VAM treatments, holes were dug in the pots with volcanic ash enough for the transfer of the seedlings. Before the *Gmelina* seedlings were transferred into the prepared pot, 1 g of VAM inoculant was first added into each pot. One gram VAM inoculant has an equivalent population of 100 fungi. The placement of the inoculants in the volcanic ash was done in such a way that upon planting, the inoculants were about 1-2 inches below the roots of the *Gmelina* seedlings. For Treatment 4, N (urea) was applied in basal method at 15 g per application. The first application was done one week after transplanting, while the last application was done one month after transplanting. A blanket application was done one month after transplanting. A blanket application of 10 g KCl was done in the four volcanic ash treatments since K of volcanic ash was not yet available when the experiment started. Fertilizer was applied by placing it in bond, around the seedlings, about 6 inches radius. Treatment 5 (Macolod soil), being a check treatment, did not use any fertilizer.

The plants were periodically watered to counter the dryness of the volcanic ash and the Macolod soil. Weeds were removed from the pots throughout the duration of the experiment. Observations on the growth and development, and other physiological factors on *G. arborea* were periodically monitored per treatment. Pests and other destructive plant agents were manually removed upon occurrence.

Samples of volcanic ash and Macolod soil before and after the experiment were brought to the Soils Laboratory at UPLB, College, Laguna for proper analyses. Analyses on pH, percent organic matter, percent nitrogen, phosphorus (ppm), and potassium (ppm) were done. Height and diameter were measured at monthly intervals, while the dry weights were measured upon termination.

All the data gathered were statistically analyzed and the results presented in tabular forms.

RESULTS AND DISCUSSION

Generally, *Gmelina arborea* grown in volcanic ash responded significantly with the VA mycorrhiza inoculation. The growth was statistically similar to that of the *Gmelina* planted in the Macolod soil.

Monthly Plant Height and Diameter

Monthly plant height of *G. arborea* grown with VA mycorrhiza inoculation and N fertilizations grown in volcanic ash from Mt. Pinatubo is presented in Table 1. The results indicate that during the first month, *Gmelina* with VAM was the tallest. This was followed by the treatment with 30 g N fertilization, with Macolod soil and with VAM + 10 g N fertilization, respectively. The shortest growth was noted in the plants on pure volcanic ash treatment.

Apparently, there were variations in growth between treatments, but the difference was not statistically significant. The same growth trend was observed statistically in the second month after transplanting. In the third month, *Gmelina* grown in volcanic ash with VAM inoculation was the tallest, but it did not differ significantly with the *Gmelina* grown in N fertilization. The shortest *Gmelina* was noted in VAM + 10 g N treatment. However, the difference was statistically similar to that of the control and 30 g N fertilization. In the fourth month, *Gmelina* with VAM was the tallest, followed by the *Gmelina* in Macolod soil. The difference however, was not statistically significant. The shortest growth was noted in the 30 g N fertilization, but this did not differ statistically with that of the control and VAM + 10 g N fertilization. The same growth trend was noted in the final month of the study, however, in this particular period, growth of *Gmelina* in Macolod soil did not differ significantly with that of the control and the VAM + 10 g N fertilization. The shortest growth of *Gmelina* was noted in 30 g N fertilization, but it was statistically similar to that of the control and the VAM + 10 g N fertilization.

Monthly diameter of *Gmelina* grown in volcanic ash with VAM and N fertilizations is presented in Table 2. During the first month, diameter of *Gmelina* did not differ statistically between treatments. In the second month, a significant difference was observed. Diameter of *Gmelina* was higher when grown in Macolod soil. The difference was statistically similar to that of the VAM, control and VAM + 10 g N fertilization. The smallest diameter was noted in 30 g N fertilization but it was not statistically different from VAM + 10 g N fertilization. The same trend was observed in the third month after the application of the treatments. In the fourth month, the biggest diameter of *Gmelina* was observed in Macolod soil, followed by VAM and the control; however, the difference was not statistically significant. The smallest diameter was noted in 30 g N fertilization and it was statistically smaller in diameter than the *Gmelina* grown in VAM + 10 g N and the control. Diameters of *Gmelina* in volcanic ash with VAM and in Macolod soil in the final month were statistically bigger than the other treatments. The smallest diameter was noted in 30 g N fertilization. It was not significantly different from 10 g N fertilization, but was statistically smaller than the control.

The results indicate that growth of *Gmelina* in volcanic ash with VAM was statistically similar to that of the Macolod soil. On the other hand, growth

of *Gmelina* with N fertilizations, particularly on the higher dosage treatment, was significantly poor. It could be noted that in these treatments (with N), the *Gmelina* plants developed small leaves and had brown spots, and unusually shed off leaves. Likewise, the roots of the plants at termination showed signs of rotting. Such attributes were distinctly observed in higher N fertilization. The rotting of roots and the irregular shedding off of leaves may be attributed to the toxicity of the chemical fertilizer. It was noted that under N fertilizations, especially under high N dosage, soil pH was extremely reduced (Table 4). Furthermore, it was observed that two months after the application of treatments, one *Gmelina* under 30 g N fertilization died. Chemical toxicity due to acidity could have caused the mortality of the *Gmelina* plants.

Likewise, *Gmelina* grown in volcanic ash and Macolod soil showed superior growth rate in terms of monthly diameter compared to the other treatments. *Gmelina* in these treatments could have efficiently metabolized the energy more than the other treatments, hence, the high growth rate. It was observed that *Gmelina* grown in volcanic ash with VAM and in Macolod soil showed good growth and developed more broad leaves. The VA mycorrhiza fungus in volcanic ash might have effectively infected the roots, thereby contributing to high growth rate.

Likewise, the Macolod soil might have the sufficient nutrients to support good growth of *Gmelina*. Although *Gmelina* grew better in these two treatments, the growth was statistically comparable to the pure volcanic ash treatment. This finding is complemented by the results of the study of Gonzal (1991) on the growth of narra and raintree in volcanic ash and in Macolod soil with or without fertilizer.

Dry Weight

Dry weight of the shoot, root and the whole *Gmelina* plant is shown in Table 3. Dry weight of the shoot was higher in the Macolod soil, but it was not statistically different from that of the treatment with VAM, while the shoot dry weight of the control was statistically comparable to that of VAM. The lower shoot dry weight was noted in the 30 g N fertilization, but it was significantly similar to that of the 10 g N and the control. Moreover, root dry weight of *Gmelina* was highest in the Macolod soil, followed by the VAM and the control. The difference, however, was not statistically significant. The lowest dry weight was noted in the 30 g N, but it was statistically similar to that of the 10 g N, VAM and the control. The same trend was observed on the total dry weight of *Gmelina* with that of the shoot.

Analyses of Volcanic Ash and Macolod Soil

The results of chemical analyses of volcanic ash and Macolod soil before and after the experiment are given in Table 4. Volcanic ash and Macolod soil

before the experiment showed slight differences in soil pH. Greater variations were evident after the termination of the experiment. There was a slight decrease in the soil pH in Macolod soil, while a slight increase was noted in the pure volcanic ash and VAM treatments. A tremendous decrease occurred particularly in 30 g N fertilization. This decrease in the soil pH adversely affected the growth of *Gmelina*. Moreover, percent organic matter in the Macolod soil decreased, while percent organic matter in all the volcanic ash treatments increased.

The addition of water might have contributed to the increased percent organic matter. Tap water that was added into the experimental pots might have contained organisms, hence, the increased percent organic matter. It was observed also that some portions of the pots in the top were colored green, an indication that organisms, algae, were present. Apparently, percent nitrogen and potassium before and after the experiment did not differ statistically. However, in terms of phosphorus content, a tremendous decrease occurred after the experiment in all volcanic ash treatments, compared to that of the Macolod soil. Presence of scavenger organisms from the added water and other sources might have used phosphorus, hence, the abrupt reduction.

CONCLUSION AND RECOMMENDATIONS

Growth of *Gmelina* in volcanic ash with VAM inoculation was statistically comparable with that of the Macolod soil. Therefore, the species may be grown in volcanic or lahar-covered areas. Application of N fertilizer into the plants may be done but it must follow the required amount for plant growth in a given soil fertility. Too much chemical fertilizer application promotes soil acidity that may adversely affect the growth of the plants, as observed in this study.

If *Gmelina* will be used as reforestation species in lahar-affected areas, it is recommended that VA mycorrhiza inoculation be done to attain high survival and good growth. VA mycorrhiza can effectively improve the growth of *Gmelina* even under pure volcanic ash conditions. However, if the same experiment will be conducted, determination of appropriate fertilizer requirement per plant in a given volcanic ash fertility is also suggested.

Table 1. Monthly plant height of *Gmelina arborea* with mycorrhiza in volcanic ash

	Monthly Plant Height (Cm)				
Treatments	*1st*	*2nd*	*3rd*	*4th*	*5th*
Control	14.80	19.98	23.25 ab	31.65 bc	32.00 bc
VA Mycorrhiza	17.45	23.53	26.40 a	40.05 a	40.97 a
VA Mycorrhiza + 10g N	14.85	18.20	18.78 b	28.18 c	32.78 bc
30g N	17.28	22.50	23.07 ab	27.37 c	28.83 c
Macolod Soil	16.50	24.20	26.10 a	37.40 ab	39.75 ab

Means followed by a common letter are not significantly different at 5% level using DMRT.

Table2. Monthly diameter of *G. arborea* with mycorrhiza in volcanic ash

	Monthly Plant Height (Cm)				
Treatments	*1st*	*2nd*	*3rd*	*4th*	*5th*
Control	0.25	0.37 a	0.44 a	0.60 ab	0.67 b
VA Mycorrhiza	0.27	0.39 a	0.47 a	0.71 a	0.89 a
VA Mycorrhiza + 10g N	0.27	0.35 ab	0.40 ab	0.51 b	0.49 c
30g N	0.26	0.27 b	0.32 b	0.36 c	0.37 c
Macolod Soil	0.26	0.40 a	0.50 a	0.76 a	0.93 a

Means followed by a common letter are not significantly different at 5% level using DMRT.

Table3. Dry weight of *G. arborea* at termination

	Dry Weight (Grams)		
Treatments	*Shoot*	*Root*	*Total*
Control	2.40	1.40 ab	3.80 bc
VA Mycorrhiza	3.97 ab	2.05 ab	6.03 ab
VA Mycorrhiza + 10g N	1.65 c	0.80 b	2.45 c
30g N	0.67 c	0.80 b	1.20 c
Macolod Soil	4.43 a	2.75 a	7.18 a

Means followed by a common letter are not significantly different at 5% level using DMRT.

Table4. Analysis of volcanic ash and Macolod soil before and after the experiment

	pH	*OM*	*N*	*P*	*K*
Soils		*(%)*	*(%)*	*(ppm)*	*(ppm)*
Before Planting					
Volcanic Ash	7.0	.015	.008	81.87	40
Macolod Soil	7.5	1.41	.051	19.70	36
After Termination					
Control	7.1	.71	.021	16.40	41
VA Mycorrhiza	7.3	.28	.042	16.40	38
VA Mycorrhiza + 10g N	6.8	1.24	.034	21.31	55
30g N	4.5	1.32	.025	18.25	24
Macolod Soil	7.2	.45	.037	16.20	35

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BIOLOGICAL CONTROL OF GOOSEWEED (*SPHENOCLEA ZEYLANICA* GAERTN.) WITH AN *ALTERNARIA* SP.

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ABSTRACT

Greenhouse and field experiments at the International Rice Research Institute (IIRI) evaluated the potential of a leaf blight pathogen (*Alternaria* sp.) for the control of gooseweed, *Sphenoclea zeylanica* Gaertn. In greenhouse experiments, *S. zeylanica* plants at all growth stages, from seedlings to flowering, were killed by the pathogen when applied as conidial suspensions of 6.3 x 10^3 to 1.4 x 10^6 conidia ml^{-1} over a range of dew period durations. Field trials confirmed the effectiveness of the *Alternaria* sp. to control *S. zeylanica* under varying environmental conditions. Concentrations of 10^5-10^6 conidia ml^{-1} applied at 50 ml 0.25 m^{-2} gave good control of gooseweed, reducing weed density by 80-99% and weed biomass by over 90% in all trials. The *Alternaria* sp. did not affect rice or other non-target species present in the field plots.

Keywords: *Alternaria;* biocontrol; mycoherbicide; rice; *Sphenoclea zeylanica;* weed control

INTRODUCTION

Sphenoclea zeylanica Gaertn. (Gooseweed) is a common, annual herbaceous weed of wetland rice (*Oryza sativa* L.) in Southeast Asia, the United States, the Caribbean area, India, Pakistan and West Africa (Holm *et al.*, 1977). Holm *et al.* (1977) found that *S. zeylanica* was never reported as a weed in any crop other than rice, but Sanders (1990) described it as one of the most common weeds in cotton (*Gossypium hirsutum* L.) in Louisiana. At densities of 20 plants m^{-2}, *S. zeylanica* causes significant yield reduction in transplanted rice (IRRI, 1989). *Sphenoclea zeylanica* competes with rice due to efficient nitrogen uptake (Biswas and Sattar, 1991) and it can also interfere with harvesting (Migo, Mercado and De Datta, 1986).

Several herbicides, such as 2,4-D (2,4-dichlorophenoxy acetic acid), provide good control of *S. zeylanica* (Migo *et al.*, 1986), but there are several problems associated with herbicide use. Application safety, environmental pollution, weed population shifts to more noxious weeds and the development of herbicide resistant forms are some of the concerns associated with the widespread use and misuse of herbicides (Watson, 1992b). The development of tolerant forms of *S. zeylanica,* due to the continuous postmergent application of 2,4-D, has been reported (Mercado *et al.*, 1990; Migo *et al.*, 1986; Sy and Mercado, 1983).

Biological control can offer viable, economic and effective alternatives to chemical herbicide for control of major weeds in rice and other crops in the tropics (Watson, 1992b). Biological control is the deliberate use of natural enemies to control a weed population and plant pathogens have been effectively deployed in the classical (inoculative) approach and in the mycoherbicide (inundative) approach in various parts of the world (Watson, 1992a). The mycoherbicide approach involves the augmentation of indigenous fungal weed pathogens to control or suppress the growth of a problem weed. It attempts to overcome disease constraints (such as low inoculum levels and poor inoculum dispersal) by supplying abundant, virulent inoculum at a time most conducive to disease development. The indigenous fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. f. sp. *aeschynomene* is being used effectively in the Mississipi Delta area of the Untied States on a commercial basis to control northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.] in rice and soybeans [*Glycine max* (L.) Merr.] (Charudattan, 1991).

Few pathogens have been reported to occur on *Sphenoclea zeylanica*. *Cercosporidium helleri* Earle, a leaf mold, was recorded on *S. zeylanica* in India (Ponnappa, 1967) and this pathogen and a leaf blight are commonly observed on *S. zeylanica* in rice fields in the Philippines (Bayot, Watson and Moody, 1992). Bayot *et al.* (1992) suggested that the leaf blight pathogen (*Alternaria* sp.) had potential as biocontrol agent for *S. zeylanica.* Various *Alternaria* spp. have been examined as biocontrol agents of some weed species (Park *et al.*, 1992; Walker, 1981; Walker and Boyette, 1985; Walker and Riley, 1982; Yang, Johnson and Dowler, 1990). The objective of this study was to evaluate the potential of *Alternaria* sp. for control of *S. zeylanica* in rice.

Materials and Methods

Inoculum Production

Stock cultures of *Alternaria* were maintained on 10 ml half-strength potato dextrose agar (1/2 PDA) slants at 4°C. Cultures for inoculum production were started from stock cultures. The pathogen was grown either on 1/2 PDA or on sorghum [*Sorghum bicolor* (L.) Moench] seeds. For growth on 1/2 PDA, two agar slants with the fungus were macerated in 10 ml sterilized, distilled water and added to 250-300 ml acidified cooled 1/2 PDA, the mixture was poured in 90 mm diameter Petri dishes and the dishes sealed with parafilm. For growth on sorghum, 20 g boiled and twice autoclaved sorghum seeds in 250 ml Erlenmeyer flasks were seeded with three mycelia disks (5 mm diameter) from the leading edge of the pathogen growing on 1/2 PDA for one week. Plate cultures and seeded flasks were incubated on the laboratory bench with continuous fluorescent light or in a dark incubator at 30°C for 7-21 days. This *Alternaria* isolate will sporulate under both light and dark conditions. Conidia were collected by flooding plates with distilled water and scraping the surface of the colonies with a glass slide. Conidia from the flask cultures were harvested by adding 50 ml distilled water to each flask and stirring the contents with a spatula. The resulting suspensions were filtered through two layers of nylon cloth and conidial density was determined with a haemacytometer. One drop of Tween 20 per 50 ml was added to the final conidial suspension.

Greenhouse Tests

Six greenhouse trials conducted from October 1992 to February 1993 with conidia concentrations of 10^3-10^6 ml^{-1}, dew period durations of 0-24 h and initial plant heights of 5-25 cm. The experiments were arranged in a completely randomized design with four replications/treatment. *Sphenoclea zeylanica* plants were either grown from seeds or seedlings, collected from paddy fields, were transplanted into saturated soil (Maahas clay, Haplustic suborder) in 10 cm diameter pots and maintained in the greenhouse. Plants were inoculated with the pathogen using a hand sprayer with a volume of 20-25 ml per four pots. Control plants were sprayed with distilled water only. After inoculation, plants were placed in dark dew chambers for 0-24 hours at 25°C and then transferred to the mist room (Yeh and Bonman, 1986) for disease development. In the 0 hour dew period treatment, inoculated plants were placed directly in a corner of the mist room and one set was covered with cardboard for six hours to simulate a dark period. Symptom development on treated plants was observed over a two-week period.

Field Tests

Four field trials were conducted at the IRRI experimental farm from September to November 1992 under varying rainfall and temperature conditions. Rainfall and temperature data were provided by the Climate Unit of Agronomy, Plant Physiology and Agroecology Division, IRRI. Field research plots with naturally occurring high populations of *S. zeylanica* (173-380 plants m^{-2}) were selected as test sites. The experimental areas were puddled, levelled and weeds were allowed to grow. The experimental areas were occasionally flooded because of heavy rain. The treatments were arranged in a randomized complete block design with three or four replications. Plot size was 0.5 m x 0.5 m. The soil was saturated at inoculation. Plants were inoculated using a hand sprayer with a volume of 50 ml per plot except in experiments where spray volume was a treatment. Experiments were repeated in time; there were two experiments (1 and 2) on the effect of conidia concentration and two experiments (3 and 4) on the effect of spray volume. Treatments were applied between 4 and 6 pm; plants were 2-10, 9-20, 11-30 and 16-38 cm in height; relative humidities between 5 pm and 12 midnight were 70-90, 75-90, 80-88 and 72-88%; and the first rain occurred 6-7 hours, 2 days, 1 day and 4 days after inoculation for the first, second, third and fourth experiments, respectively. The number of living plants and the dry weight of above-ground biomass were determined two weeks after inoculation. Plants were cut at the soil level, dead tissues were discarded and living tissues dried in paper bags at 80°C for 3-4 days. Data were analyzed by the analysis of variance and treatment means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Greenhouse Tests

At high inoculum concentration (10^5 conidia ml^{-1} and higher), symptom development on inoculated plants was evident within 24 hours as wilting and leaf cupping. The fungus causes necrosis of the host tissue, initially killing the leaves, then the stems and eventually the entire plant. With lower inoculum concentrations (10^4 conidia ml^{-1} and less) leaf lesions were initially pinhead in size which later expanded and coalesced forming irregular shaped necrotic lesions. Lesions also developed on the stems. Infected leaves were deformed, abscised and eventually the entire plant died. Three to five days after inoculation, most plants were dead and within two weeks all treated plants were dead (Table 1). With concentrations of 10^4 conidia ml^{-1} and higher, 100% kill was achieved within one week. At 10^3 conidia ml^{-1}, two weeks were required for 100% mortality.

Dew periods were not necessary to obtain 100% mortality when plants were subsequently maintained in a mist room in the greenhouse. In the treatment where

no additional free-moisture was provided (0-h-dew duration), all treated plants were killed. These results are quite different from those reported for other *Alternaria* spp. being examined as biocontrol agents of various weeds. Walker and Riley (1982) report at least eight hours of free moisture are required for *Alternaria cassiae* Jurair & Khan to cause severe disease on *Cassia obtusifolia* L., *Alternaria macrospora* Zimm. requires a 24-hour-dew period to incite over 80% mortality of *Anoda cristata* (L.) Schlecht. (Walker, 1981). Park *et al.* (1992) report a requirement of 24-hour-dew period for the *Alternaria* sp. on *Scripus planiculmis* Fr. Schm., and *Alternaria angustiovoidea* Simmons requires at least a 48-hour-dew period to control *Euphorbia esula* L. (Yang *et al.*, 1990).

Under controlled conditions, the *Alternaria* sp. was virulent on development stages of *S. zeylanica* from seedlings to flowering plants. With the other *Alternaria* spp. being evaluated as biological weed control agents, high levels of mortality are obtained only on seedlings to one-leaf stage plants (Walker, 1981; Walker and Riley, 1982). The rapid appearance of symptoms suggests that a phytotoxin(s) may be involved as reported for other *Alternaria* spp. (Stierle, Cardellina and Strobel, 1988; Walker and Riley, 1982; Yang *et al.*, 1990).

Field Tests

As in the greenhouse experiments, symptom development in the field occurred within 24 hours after inoculation. In experiment 1, aside from the usual leaf cupping and wilting observed the day after inoculation, lesion development and necrosis were also evident. The accelerated appearance of symptoms may have resulted from elevated moisture levels due to heavy rain that commenced 6-7 hours after inoculation (Figure 1). Conidia of this pathogen germinate within six hours and penetrate leaf tissues 12-16 hours after inoculation (Bayot *et al.*, 1992). It appears that conidia and germlings of this *Alternaria* sp. are not washed off by heavy rain (i.e., they are 'rainfast'). One week after treatment, most of the *S. zeylanica* plants were either dead or leafless. Two weeks after treatment, excellent control of *S. zeylanica* was achieved in plots treated with the *Alternaria* sp. based on significantly ($p < 0.05$) lower dry weight and number of living plants compared to plants in untreated plots (Table 2).

In experiment 2, 90% kill of plants occurred one week after inoculation in plots treated with 10^5 conidia ml^{-1}. There was no mortality after one week at lower concentrations, but lesions appeared on leaves causing deformation and stunting of *S. zeylanica*. The number of living plants was significantly ($p < 0.05$) reduced when 10^5 spores ml^{-1} were used, but not with lower concentrations (Table 2). Two weeks after inoculation, weed biomass was significantly ($p < 0.05$) reduced in plots receiving 10^4 and 10^5 conida ml^{-1}. Dry weight of *S. zeylanica* decreased with increasing concentration of the *Alternaria* sp. with the lowest dry weight obtained using 10^5 conidia ml^{-1}. Dry weight of plants treated with 10^3 conidia $ml^{-1}$was not significantly ($p < 0.05$) different from control plants. The effect of inoculum concen-

tration with this *Alternaria* sp. was similar to that reported for other *Alternaria* spp. (Walker, 1981; Walker and Boyette, 1985; Walker and Riley, 1982; Yang *et al.*, 1990), but not only seedlings were susceptible as all *S. zeylanica* growth stages including flowering plants were controlled in the field.

In experiment 3, application of 50 ml of the conidial suspension 0.25 m^{-2} gave superior control of *S. zeylanica* (Table 3). However, this was not significantly different from using 25 ml 0.25 m^{-1}. Good control of *S. zeylanica* was also achieved when 12.5 ml was applied and when 50 ml of a three-week-old suspension was used. Thus, conidia stored in water might still be usable for application depending on length and condition of storage.

In experiment 4, *S. zeylanica* plants inoculated with 4.8 x 10^5 conidia ml^{-1} had significantly ($p < 0.05$) lower dry weight and significantly ($p < 0.05$) lower dry weight and significantly ($p < 0.05$) fewer living plants compared to plants in the control plots after two weeks (Table 3). Dry weight, however, was not significantly ($p < 0.05$) affected by spray volumes used but a significant ($p < 0.05$) reduction in numbers of living plants occurred when 50 ml was used compared to 12.5 ml 0.25 m^{-2}. Higher volumes of spray solution may contribute to an extended free moisture period on the plant surface and subsequent increased disease expression.

Echinochloa spp., *Fimbristylis miliacea* (L.) Vahl, *Cyperus difformis* L., *Cyperus iria* L., *Eclipta prostrata* L., *Leptochloa chinensis* (L.) Nees *Ludwigia octovalvis* (Jacq.) Raven, *Monochoria vaginalis* (Burm. f.) Presl, *Ammannia* sp., *Sesbania* sp. and volunteer rice were not affected by the *Alternaria* sp. in these field trials.

These results demonstrate the potential of this pathogen as a biological control agent for *S. zeylanica*. The pathogen gave excellent control of the weed during field trials under varying environmental conditions. Further studies are underway to identify the pathogen to species, determine its host range, determine the possible involvement of phytotoxins, evaluate aspects of virulence and efficacy and optimize inoculum production and formulation. Additional testing of the pathogen in field trials is in progress.

This *Alternaria* sp. has major advantages over other *Alternaria* spp. being evaluated as potential bioherbicides. It does not require extended periods of leaf wetness and it is effective on all stages of plant growth, from seedlings to flowering plants. *Alternaria* spp. do not sporulate in liquid fermentation and must be mass produced using solid fermentation techniques. The inability to economically adapt solid fermentation on an industrial scale is a major reason why the commercialization of *Alternaria* cassiae (CASST ^{R}) has not proceeded. The relatively small market niche for *S. zeylanica* will not likely warrant development of this *Alternaria* sp. as a "commercial product." Therefore, studies are underway to develop methodologies for "onfarm" or "cottage industry" production of inoculum to satisfy local needs and to be cost effective on a small scale.

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Table 1. Effects of dew period duration, conidia concentration and plant height at inoculation on the efficacy of an *Alternaria* sp. to control *Sphenoclea zeylanica* 2 weeks after inoculation

Dew Period duration[a] (h)	Inoculum concentration range (conidia ml^{-1})	Height[b] (cm)	Mortality[c] (%)
24	6.3×10^3-1.4×10^6	5-25	100
7	6.3×10^5	5-25	100
6	2.0×10^4-1.4×10^6	5-25	100
3	4.8×10^4	20-25	100
0*	4.8×10^4	20-25	100
0**	4.8×10^4	20-25	100

[a]The 0-h-dew period duration consisted of two treatments; two sets of inoculated plants (0* and 0**) were placed directly in the mist room wherein one set (0*) was covered with a cardboard carton for 6 h.

[b]The 20-25 cm height represents the pre-flowering to flowering stage of growth.

[c]Determined 2 weeks after inoculation. At concentrations greater than 1×10^4 plants died within 3-7 days afer inoculation.

Table 2. Effect of conidia concentration of an *Alternaria* sp. on dry weight and number of *Sphenoclea zeylanica* 2 weeks after application[a]

Concentration[b] (conidia ml^{-1})	Dry weight (g 0.25 m^{-2})	Living plants (No. 0.25 m^{-2})
September 1992 (Exp. 1)		
Control	11.2b	201.8b
7.0×10^5	0.2a	16.3a
1.8×10^6	0.04a	5.3a
CV(%)	53.2	33.2
October 1992 (Exp. 2)		
Control	17.1c	81.5b
8.0×10^3	13.7c	93.0b
3.0×10^4	8.0b	79.5b
2.6×10^5	0.2a	4.3a
CV(%)	29.3	25.4

[a]Values are means of four replications. In a column, means followed by a common letter within a date are not significantly different at 5% level according to Duncan's multiple range test.

[b]Volume of spray = 50 ml 0.25 m^{-2}

Table 3. Effect of spray volume on dry weight and number of *Sphenoclea zeylanica* 2 weeks after application of a conidial suspension of an Alternaria sp[a]

Volume of spray (ml 0.25 m^{-2})	Dry weight (g 0.25 m^{-2})	Living plants (No. 0.25 m^2)
September 1992 (Exp. 3)		
Control	17.6d	145.0c
12.5	3.0b	40.7ab
25	0.8ab	9.3a
50	0.2a	1.3a
50 (3-week-old)[b]	5.5c	74.7b
CV(%)	23.6	45.0
November 1992 (Exp. 4)		
Control	28.2b	169.3c
12.5	10.5a	89.7b
50	2.1a	33.3a
CV(%)	35.2	17.7

[a]Conidia concentration of September experiment was 1.6×10^6 and 4.8×10^5 in November. Values are means of three replications. In a column, means followed by a common letter within a date are not significantly different at 5% level according to Duncan's Multiple Range Test.

[b]Conidia harvested previously and stored in water at 4°C for 3 weeks prior to use in this experiment.

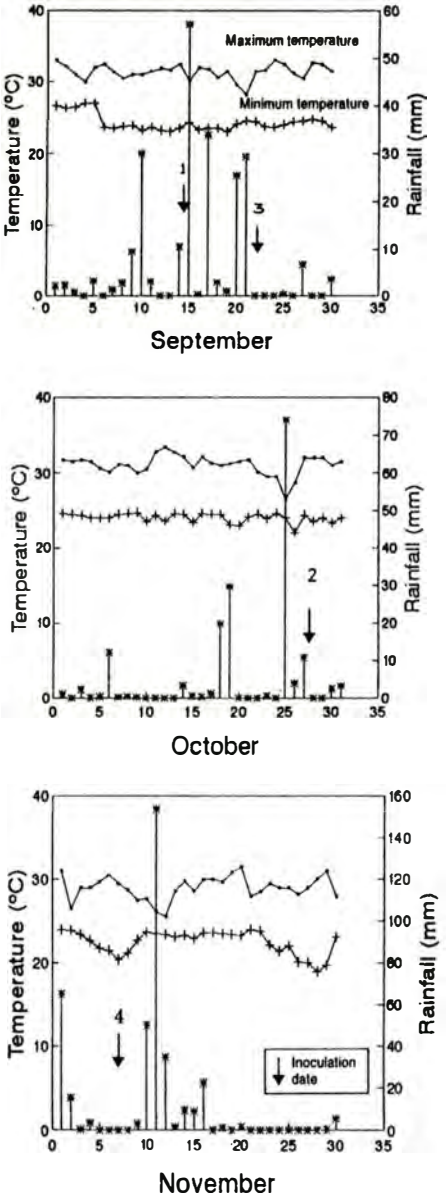

Figure 1. Daily rainfall (mm) and maximum and minimum temperatures (°C) at the International Rice Research Institute, Los Baños, Laguna, Philippines, during September-November, 1992. Arrows marked 1, 2, 3 and 4 indicate the date of inoculation of *Sphenoclea zeylanica* with conidia of an *Alternaria* sp. in the four field trials.

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TRANSFORMATION OF INDICA AND JAPONICA RICES AT IRRI USING MARKER AND AGRONOMICALLY-IMPORTANT GENES[1]

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ABSTRACT

The novel technique of direct gene transfer, which allows the incorporation and expression of foreign genes into crops such as rice, a method not possible through conventional breeding, is an alternative for crop improvement.

PEG-mediated cotransformation of protoplasts of several japonica and indica cultivars was performed using gene constructs containing either the β-glucoronidase gene (pCAL1GC) and hygromycin-resistance gene (pTRA132), or the soybean trypsin inhibitor gene (pSBT1) and the hygromycin-resistance gene. More than 300 putative transformants were regenerated from six varieties.

Histological and molecular analyses were performed either at the callus stage or whole plant level. Plants of IR58 stained with X-gluc solution showed positive GUS reaction. On the other hand, Southern analysis for hygromycin B of 81 Zhonghua 10 plants indicated that 13 contained the gene for antibiotic resistance. All the transformed Zhonghua 10 plants set seeds. Integration of selectable markers into the plants demonstrates the possibility of introducing agronomically-important genes from sources other than rice.

The agronomically-important gene from soybean, soybean trypsin inhibitor, which confers resistance to yellow stemborers, is being used to transform the japonica cultivar Zhonghua 6. Molecular analysis of the transformants demonstrated the incorporation of the gene.

Keywords: Rice, transformation, polyethylene glycol, protoplasts, plasmid, transgenic plants

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important food crop in the world, with more than two billion people, predominantly in developing countries, depending on it. Rice improvement through conventional breeding methods has had considerable success. However, development of novel techniques for the genetic manipulation of this crop offers rapid production and improvement of genotypes to meet the even faster increase in the world's population (Swaminathan, 1982; Khush, 1984; Toenniessen, 1990). The introduction of foreign genes into crop plants promises to overcome some of the substantial agronomic and environmental problems that have not been solved using genes currently available in plant breeders' germplasms.

Several techniques have been employed for rice genetic transformaiton such as the PEG-mediated method, electroporation, biolistic method, pollen tube pathway and *Agrobacterium*-mediated gene transfer system. Despite the availability of various methods, the production of transgenic plants is still considered inefficient due to low recovery and poor fertility (Hodges et al., 1991).

Foreign genes are exploited in the plant transformation process. Since very few cells in a target population become transformed, selection of transformants demands the use of selectable markers. Typically, selectable marker genes encode enzymes which detoxify antibiotics such as hygromycin and thus permit only transformed cells to survive and grow on media containing the antibiotic.

This study reports the production of transgenic plants of the japonica cultivars Zhonghua 6, Zhonghua 10, Taipei 309 and the indica cultivars IR58, IR64 and IR57311-95-2-3 carrying single or two genes of either hygromycin phosphotransferase (hph), β-glucoronidase (GUS) or soybean trypsin inhibitor (SBTI). Evidence of integration of the genes is also presented.

MATERIALS AND METHODS

Plant Materials

The cultivars used in this study were: the japonica cultivars Taipei 309, a variety considered a model system in rice, Zhonghua 6 and Zhonghua 10 (which are high-yielding Chinese varieties developed through another culture); and the indica cultivars IR58, IR64 and the promising line IR57311-95-2-3.

Explant Sterilization

Immature embryos or mature seeds were used as explants. Immature embryos were collected seven days after pollination. The explants were sterilized with 2.6% sodium hypochlorite for 45-60 min with stirring under vacuum, then washed three times with sterile distilled water. These were then placed onto sterile filter papers to absorb excess water.

Callus Induction

Explants were plated in petri plates or 50-ml flasks containing MS (Murashige and Skoog, 1962) medium with 30 g/l of sucrose or maltose, 2 mg/l 2,4-D and solidified with 0.8% agar. Cultures were kept in the dark for 3-4 weeks at 25 ± 1°C. For direct isolation of protoplasts from primary calli, the cultures were kept under diffused light for the first 10 days, then transferred to dark conditions.

Initiation and Maintenance of Cell Suspension Cultures

Ttransformation of Zhonghua 6 utilized protoplasts isolated directly from primary calli (Wu and Zapata, 1992). Suspension cultures were established in the other varieties.

Three- to four-week-old primary calli were used for the initiation of cell suspensions. Embryogenic regions of primary calli were selected and placed into a 50-ml Erlenmeyer flask containing 15 ml liquid R2 medium (Ohira et al., 1973) with 20 g/l sucrose and 2 mg/l 2,4-D. Approximately 0.5g of embryogenic calli was inoculated per flask. The suspension cultures were placed on a gyratory shaker at 100 rpm and cultured in the dark at 25 ± 1°C. The medium was replaced at intervals of 3-7 days until fast-growing cell suspensions were established.

For maintenance of cell suspension, the cells were transferred to 125-ml Erlenmeyer flasks containing 30-ml medium. The medium was replaced every 5-10 days.

Protoplast Isolation

One gram of 3- to 5-day-old suspension cells or 1-month old embryogenic primary calli was incubated in 20 ml filter-sterilized cell-protoplast washing solution, CPW (Zapata et al., 1977) containing 1-2% (w/v) cellulase RS (Yakult Honsha Co., Tokyo, Japan) and 0.05-0.2% pectolyase Y-23 (Seishin Pharmaceutical Co., Tokyo, Japan). The mixture was kept in the dark at 25-26°C for 2-5 hours. Enzymatic digests were filtered through four layers of 25 μm nylon mesh and the protoplasts were washed three times with CPW by centrifugation at 800 rpm for 5 min each. Protoplast yield was determined using a haemocytometer.

Plasmids Used

Protoplasts were cotransformed with the gene constructs containing either the β-glucoronidase gene (pCAL1GC) and hygromycin-resistance gene (pTRA132), or the soybean trypsin inhibitor gene (pSBTI) and pTRA132. β-glucoronidase is a reporter gene which could be monitored histochemically (Jefferson, 1987). GUS catalyzes the hydrolysis of a wide variety of β-glucoronides and makes it excellent for gene fusion experiments. Hygromycin-β-phosphotransferase gene (hph) is a

selectable marker which is responsible for resistance to hygromycin. It modifies the antibiotic by phosphorylation making it non-toxic (Singh et al., 1979). Soybean trypsin inhibitor is an agronomically-important gene as this confers resistance to yellow stemborer. Protein inhibitors are some of the plant chemicals in storage tissues that limit predation by insects and other herbivores.

Transformation Using PEG

About $8x10^6$ protoplasts were resuspended in 1 ml of CPW solution and incubated with 50 µg each of the plasmids used for cotransformation for 10 min to allow sufficient contact between the DNA and the plasma membrane. One ml of 40% (w/v) PEG 8000 Mannitol-Magnesium solution was added dropwise, with gentle mixing between each addition, to 20% final concentration. The mixture was incubated at 25°C for 5-30 min and then diluted slowly with 30 ml of Krens, F buffer (Krens et al., 1982), followed by centrifugation at 780 rpm for 15 min to pellet the protoplasts. The supernatant was removed, and the protoplasts were washed once more with CPW solution.

Protoplast Culture

Purified protoplasts were gently mixed with modified R2 containing 137 g/l sucrose and 1 mg/l 2,4-D or Kao's modified medium (Kao et al., 1970), KPR-2 (Thompson et al., 1986) medium containing 1.2% Sea Plaque agarose. Density of protoplasts was adjusted to $1x10^6$ per ml. One ml of the mixture was placed into a 60 x 15 plastic petri dish. When the mixture was completely solidified, 100 mg of nurse cells (Oc rice cell line graciously provided by Dr. K. Syono, University of Tokyo) suspended in 5 ml of the protoplast liquid medium was added into each petri dish. The petri dishes were placed on a slow shaker (40 rpm) and incubated in the dark at 25 ± 1°C for one week and then cultured without shaking. The nurse cells were removed at 12-15 days after culture and fresh protoplast medium was added to the agarose beads.

Selection of Transformed Calli

Hygromycin selection for resistant colonies was performed 14 days after protoplast culture. The protoplast-derived colonies embedded in agarose blocks were immersed in protoplast medium containing different concentrations of hygromycin B (25-100 µg/ml) for four weeks. The calli were allowed to proliferate in semisolid medium with 1 mg/l 2, 4-D for 7-10 days. The hygromycin-resistant colonies were counted and transformation efficiency was calculated as the percentage of the number of hygromycin-resistant colonies per total number of protoplasts originally plated in the agarose block.

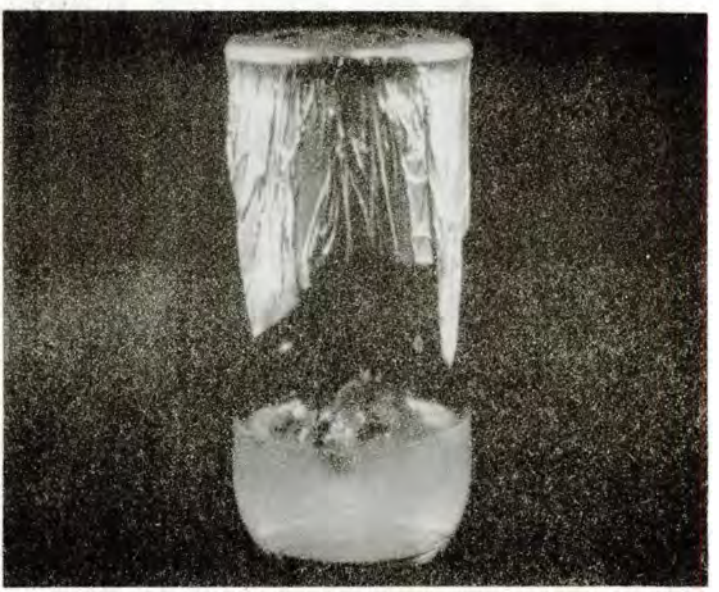

Figure 1. Unirradiated calli (2n) of *N. glauca* 60 days after irradiation

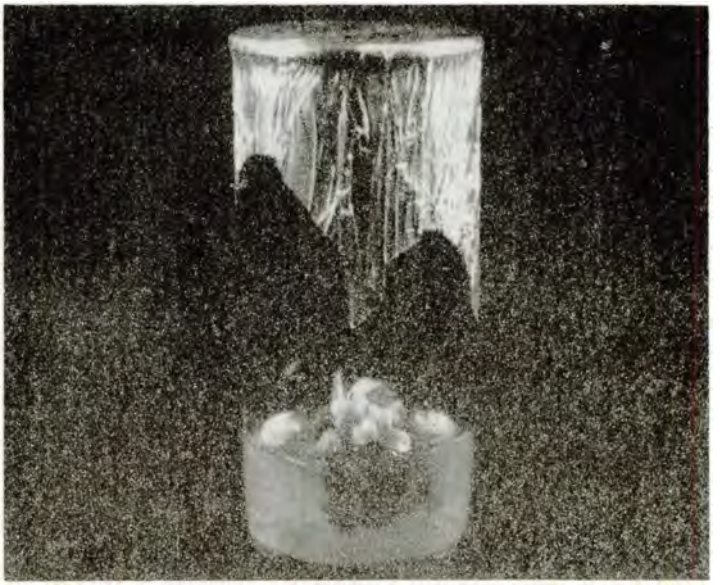

Figure 2. Unirradiated calli (n) of *N. glauca* 60 days after irradiation

Table 6. Plantlet survival of *Nicotiana* species expressed as number of regenerated plantlets per callus with buds 7 days after incubation

Species	Gamma radiation dose (kR)	Ploidy	
		Haploid	Diploid
N. glauca	0	1.60	4.00
	5	0.00	0.20
	10	0.00	0.00
	20	0.00	0.00
N. langsdorfii	0	0.50	0.70
	5	0.33	2.00
	10	0.33	0.00
	20	0.00	0.00
N. tabacum	0		4.00
	5		1.00
	10		0.00
	20		0.00

Table 7. Plantlet survival of *Nicotiana* species expressed as number of regenerated plantlets per callus with buds 10 days after incubation

Species	Gamma radiation dose (kR)	Ploidy	
		Haploid	Diploid
N. glauca	0	1.20	1.70
	5	0.00	0.00
	10	0.00	0.00
	20	0.00	0.00
N. langsdorffii	0	2.20	1.29
	5	0.17	0.00
	10	0.00	0.00
	20	0.00	0.00
N. tabacum	0		1.68
	5		0.00
	10		0.00
	20		0.00

Table4. Number of plantlets regenerated from irradiated calli of *Nicotiana* species 7 days after incubation

Species	*Gamma radiation dose (kR)*	*Ploidy*	
		Haploid	*Diploid*
N. glauca	0	8	12
	5	0	1
	10	0	0
	20	0	0
N. langsdorffii	0	5	7
	5	1	4
	10	0	1
	20	0	0
N. tabacum	0		40
	5		1
	10		0
	20		0

Table5. Number of plantlets regenerated from irradiated calli of *Nicotiana* species 10 days after incubation

Species	*Gamma radiation dose (kR)*	*Ploidy*	
		Haploid	*Diploid*
N. glauca	0	5	12
	5	0	3
	10	0	0
	20	0	0
N. langsdorffii	0	22	45
	5	0	3
	10	0	0
	20	0	0
N. tabacum	0		32
	5		0
	10		0
	20		0

Table2. Effect of gamma radiation on bud regeneration of *Nicotiana* species callus culture 7 days after incubation

Species	*Gamma radiation dose (kR)*	*Calli of haploid origin*		*Calli of haploid origin*	
	buds	*Without buds*	*With buds*	*Without buds*	*With buds*
	0	7	5	9	3
N. glauca	5	12	0	7	5
	10	12	0	12	0
	20	12	0	12	0
	0	2	10	2	10
N. langsdorffii	5	9	3	10	2
	10	9	3	12	0
	20	12	0	12	0
	0			2	10
N. tabacum	5			12	0
	10			12	0
	20			12	0

Table3. Effect of gamma radiation on bud regeneration of *Nicotiana* species callus culture 10 days after incubation

Species	*Gamma radiation dose (kR)*	*Calli of haploid origin*		*Calli of haploid origin*	
	buds	*Without buds*	*With buds*	*Without buds*	*With buds*
	0	4	8	2	10
N. glauca	5	12	0	12	0
	10	12	0	12	0
	20	12	0	12	0
	0	2	10	2	12
N. langsdorffii	5	4	8	6	6
	10	2	10	7	5
	20	12	0	12	0
	0			0	12
N. tabacum	5			7	5
	10			12	0
	20			12	0

reported that regeneration of shoot buds from gamma irradiated haploid tobacco cell suspension was completely inhibited at a dose of 2Krad, while the same phenomenon with UV-irradiated cells was observed at 8,000 ergs/mm^2. Inherent genetic differences and the differences in dose rate and post-irradiation cultural conditions did influence the radiosensitivity of haploid cells of tobacco (cv. Virginia Gold).

Similar results have been obtained in other crops. In the case of the pigeon pea *Cajanus cajan* (L.), only calli exposed to lower dose (5kR) differentiated plantlets (Shama Rao and Narayanaswamy, 1975); in *citrus sinensis,* doses of 28-32kR proved to be lethal to callus culture (Spiegel-Roy and Kochba, 1973).

Hell (1983) reported that a stimulatory effect of gamma radiation on bud morphogenesis was detectable mainly on calli from tissues of diploid origin of tobacco (cv. Wisconsin-8). Radiation treatment decreased the survival of plants with low ploidy. This effect could have been the result of an action at the cellular level in accordance with the reported high sensitivity of cells with low polidy (Galun and Raveh, 1975).

Table1. Effect of regeneration media on the shoot differentiation of calli cultured on media* of different hormonal conditions

NAA (mg/l)		0.01			0.01			0.01		
6BA (mg/l)		A	B	C	A	B	C	A	B	C
3	1	15	13	27	15	15	91	15	0	0
	15	14	22	15	15	156	15	15	80	
	5	15	1	4	15	1	33	15	15	73

A: number of cultured calli
B: number of calli which regenerated shoots
C: number of shoots regenerated
* Regeneration media contained inorganic and organic components of Murashige and Skoog (1962), 3% sucrose and 0.6% agar (pH 5.6-5.7)

dose maintained their original color. Buds regenerated from diploid calli were more profuse than buds regenerated from haploid ones.

The number of regenerated plantlets from irradiated calli of *Nicotiana* species at different ploidy levels is presented in Tables 4 and 5. Plantlets were considered "regenerated" when they produced four or more fully developed leaves (Fig. 13). In unirradiated diploid calli, *N. tabacum* and *N. langsdorffii* yielded more regenerated buds than *N. glauca* (Figs. 1-5). Diploids produced more plantlets than haploids. Diploid calli of *N. langsdorffii* and *N. glauca* irradiated seven days after incubation yielded more regenerated buds than *N. tabacum* (Table 2). Diploid calli of *N. langsdorffii* irradiated seven days after incubation produced the most regenerated buds followed by diploid calli of *N. tabacum* and *N. glauca* (Table 3). High dose of irradiation inhibited bud regeneration in both haploid and diploid calli.

In unirradiated diploid calli, the order of plantlet regeneration was *N. tabacum* > *N. glauca* > *N. langsdorffii* (Table 4). In diploid calli irradiated seven days after incubation, the order of regeneration was *N. langsdorffii* > *N. glauca* = *N. tabacum* (Table 4).

On the other hand, in unirradiated diploid calli, *N. langsdorffii* yielded the most number of regenerated plantlets followed by *N. tabacum* and *N. glauca* (Table 5). The order of plantlet regeneration was *N. langsdorffii* = *N. glauca* > *N. tabacum* in the diploid calli irradiated 10 days after incubation (Table 5).

The survival ability of irradiated calli expressed as the number of regenerated plants per callus with buds is shown in Tables 6 and 7. Diploid calli of *N. tabacum* and *N. langsdorffii* have greater survival ability than the diploid calli of *N. glauca* at seven days after incubation. Results also showed that diploids have greater survival ability than haploids. Moreover, survival ability of irradiated calli continues to decrease as the radiation dose increases.

DISCUSSION

Buds regenerated from irradiated calli showed a remarkable decrease in their survival. The same results were obtained by Hell (1983). These results might be ascribed to radiation which induced damages in the cells of the regenerated buds. These damages could be responsible for injuries at the physiological level during the rooting and subsequent growth of the plants, which ultimately led to an impairment of their chances of survival under the prevailing conditions. It is also possible that the differences in the ploidy level or the genotypic constitution of the cultured calli might produce variations in the endogenous level of growth regulators, or the sensitivity of tissues to the exogenously added regulators (Kerbany et al., 1976).

Furthermore, results showed that the number of plantlets regenerated was dependent on ploidy and radiation dose. Radiation treatment decreased the survival of plantlets with low ploidy (Vendketeswaran and Partanen, 1976). Eapen (1976)

INTRODUCTION

In a previous study (Balito et al., 1989), the effects of gamma-ray irradiation on the growth of calli in *Nicotiana* species were studied. Haploid and diploid calli of *N. glauca* and *N. langsdorffii*, together with diploid calli of *N. tabacum* cv. Bright Yellow taken as control, were exposed to various doses of ^{60}Co gamma irradiation at different times after incubation. When the increasing rate of irradiated calli to the unirradiated calli was used as an indicator of radiosensitivity, *N. tabacum* was more sensitive than *N. glauca* and *N. langsdorffii*. Previous results also showed that haploid calli were more radiosensitive than diploid ones in *N. glauca*, but no significant difference was observed between them in *N. langsdorffii*.

Effects of ionizing radiation on growth and differentiation of plant tissues cultured *in vitro* have been reported in tobacco (Eapen, 1976; Hell, 1978); pigeon pea (Rao and Narayanaswamy, 1975); orange (Spiegel and Kochba, 1973) and avena (Miura et al., 1974). These results have been mainly due to radiation effects on endogenous IAA levels in plant tissues. Gamma-radiation effects could also be related to cytokinins and to other organic substances present in the culture media (Ussof and Nair, 1974).

This study was undertaken to investigate the effect of irradiation on the regeneration of buds and plantlets from haploid and diploid calli of *Nicotiana* species.

The culture medium used in this experiment was Murashige and Skoog's medium (Murashige and Skoog, 1962). Sucrose was used for callus growth at 30 g/liter. The medium was adjusted to pH 5.6-5.7 with NaOH or HCl after it was supplemented with hormones. It was solidified with agar (0.6%). The media were autoclaved for 15 minutes at 121°. All incubation activities were done in a sterile transfer room and the cultures were maintained in the culture room at 28.5° with illumination incident of 200 to 300 lux.

RESULTS

Preliminary observation on the effect of regeneration media on the shoot differentiation of *N. tabacum* (n=24) calli, which served as control, is shown in Table 1. The calli were cultured on media with different hormonal conditions. Among the different hormonal conditions, NAA at 0.10 ppm and 6-BA at 3 ppm gave the highest number of regenerated plantlets. Thus, this hormonal combination was used throughout the study.

The effect of gamma radiation on bud regeneration of calli irradiated at different days after incubation is shown in Tables 2 and 3. Bud regeneration from haploid and diploid calli was significantly inhibited by irradiation (5-20kR) regardless of time duration after incubation (Figs. 6-11). Calli irradiated at higher doses continued to darken and subsequently die (Fig. 12). On the other hand, calli irradiated at lower

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EFFECT OF GAMMA-RAY IRRADIATION ON THE REGENERATION OF BUDS AND PLANTLETS FROM HAPLOID AND DIPLOID CALLI IN *NICOTIANA* SPECIES

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ABSTRACT

Haploid and diploid calli of *Nicotiana glauca* (n=12) and *Nicotiana langsdorffii* (n=9), together with the diploid calli of *Nicotiana tabacum* (n=24) cv. Bright Yellow which were taken as control, were exposed to various doses of ^{60}Co gamma rays at 7 days and 10 days after incubation. Bud regeneration from haploid and diploid calli was significantly inhibited by irradiation (5-20kR) regardless of time duration after incubation. Calli irradiated at higher doses continued to darken and subsequently die.

Meanwhile, calli irradiated at lower dose maintained their original color. Buds regenerated from diploid calli were more profuse than buds regenerated from haploid ones. In unirradiated diploid calli, *Nicotiana tabacum* and *Nicotiana langsdorffii* yielded more buds than *Nicotiana glauca*. Diploids produced more plantlets than haploids. Diploid calli of *N. langsdorffii* and *N. glauca* irradiated seven days after incubation, yielded more regenerated buds than *N. tabacum*. Diploid calli of *N. langsdorffii* irradiated 10 days after incubation produced the most number of regenerated buds followed by diploid calli of *N. tabacum* and *N. glauca*. High dose of irradiation inhibited bud regeneration in both haploid and diploid calli. In unirradiated diploid calli the order of plantlet regeneration ability was *N. tabacum* > *N. glauca* > *N. langsdorffii*. In diploid calli irradiated seven days after incubation, it was *N. langsdorffii* > *N. glauca* = *N. tabacum*.

On the other hand, in unirradiated diploid calli, *N. langsdorffii* yielded the most number of regenerated plantlets followed by *N. tabacum* and *N. glauca*. The order of plantlet regeneration ability was *N. langsdorffii* = *N. glauca* > *N. tabacum* in the diploid calli irradiated 10 days after incubation.

The survival ability of irradiated calli expressed as the number of regenerated plants per callus with buds has shown that diploid calli of *N. tabacum* and *N. langsdorffii* have greater survival ability than the diploid calli of *N. glauca* at seven days after incubation. Results also showed that diploids have greater survival ability than haploids. Moreover, survival ability of irradiated calli continues to decrease as the radiation dose increases.

Key Words: *Nicotiana,* diploid, haploid, callus, radiation sensitivity, survival ability

Figure 7. Southern blot analysis of IR 58 showing the integration of the GUS gene in both the total and digested DNA

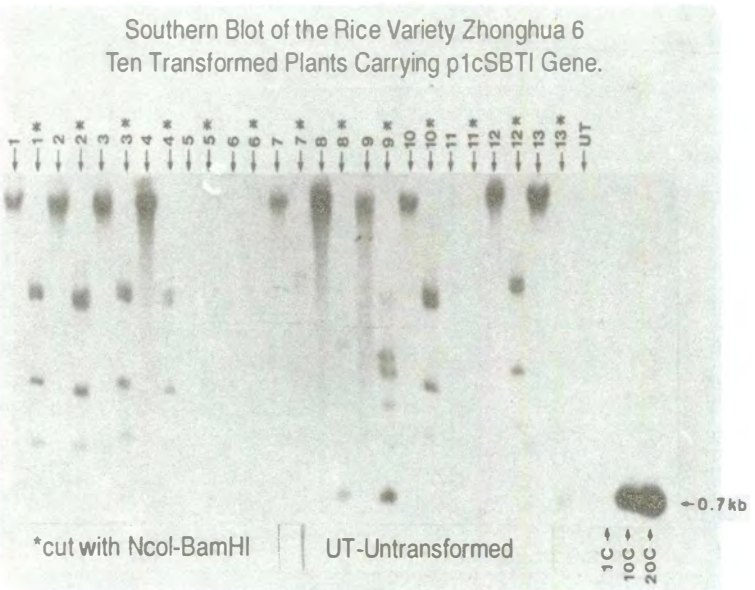

Figure 8. Southern blot analysis of Zhonghua 6 transformants showing the integration of the SBTI gene in both the total and digested DNA

Figure 5. Histochemical analysis for the GUS gene showing its integration into the plant

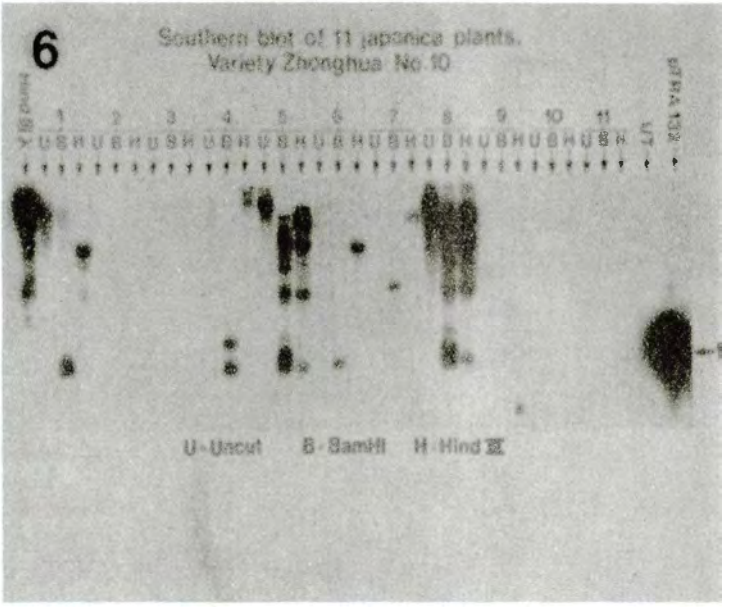

Figure 6. Southern blot analysis of Zhonghua 10 plants showing integration of the hygromycin-resistance gene in some of the plants

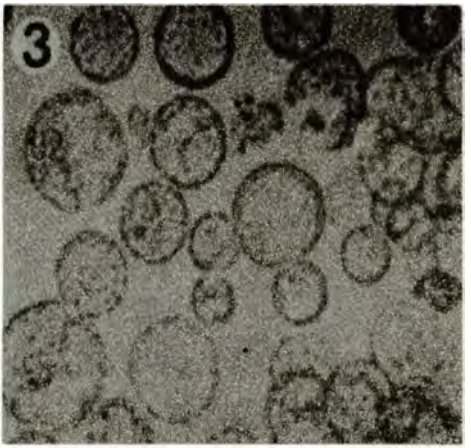

Figure 3. Freshly-isolated rice protoplasts

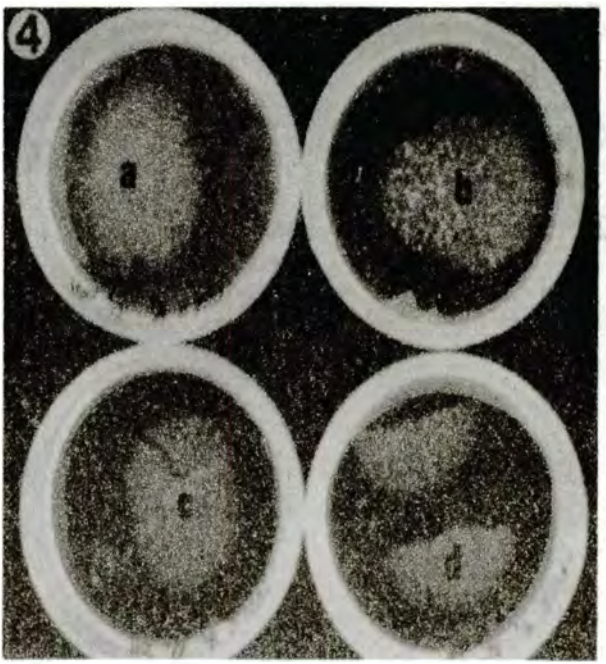

Figure 4. Hygromycin-resistant colonies of Zhonghua 10 calli after 25 days in selection medium with different hygromycin concentrations. a = 100 μg/ml; b = control; c = 75 μg/ml; d = 50 μg/ml.

Figure 1. Embryogenic calli used for the establishment of cell suspension or direct isolation of protoplasts from primary calli

Figure 2. Embryogenic cell suspension used for protoplast isolation

Table 1. Putative transgenic plants of Zhonghua 10 regenerated from hygromycin-resistant colonies

Hygromycin Concentration (μg/ml)	*Calli giving Plants (No.)*	*No. of Plants*		
		Regenerated	*Analyzed*	*With HYG*
25	77	167	60	6
50	16	20	4	1
75	25	27	13	4
100	7	10	4	2

Table 2. Molecular analysis of transgenic plants and putative transformants in six rice varieties

Variety	*Putative transformants (No.)*	*Plants analyzed (No.)*	*Genes for cotransformation*[1]	*Plants positive for Southern (No.)*			
				GUS	*Hyg*	*Kan*	*SBTI*
IR58	1	1	GUS + Kan	1	–	*	–
	2	2	GUS + hph	2	2	–	–
IR64	9	9	GUS + hph	0	0	–	–
Zhonghua 10	224	81	GUS + hph	13	13	–	–
Zhonghua 6	17	0	GUS + hph	*	*	–	–
	39	24	SBTI + hph	–	*	–	> 5
Taipei 309	1	0	SBTI + hph	–	*	–	*
IR57311-95-2-340		0	GUS + hph	*	*	–	–

[1]GUS = β-glucoronidase; Kan = Kanamycin; Hyg = Hygromycin; SBTI = Soybean trypsin inhibitor

– = Not studied; * = Being analyzed

Using the 1.8 Kb GUS fragment of pCAL1GC as a probe, Southern blot analysis of transgenic IR58 plants also confirmed the presence of GUS gene in the high molecular weight region, and in the 1.8 Kb band corresponding to the intact GUS gene coding sequence (Fig. 7).

Twenty of the 24 Zhonghua 6 plants analyzed for the presence of SBTI gene showed positive signals in both total and digested DNA (Fig. 8). The 20 plants were regenerated from seven individual calli. This result demonstrates the enormous possibility of incorporating foreign genes of agronomic importance into rice where the source of resistance is not available in the cultivated rice germplasm. Soybean trypsin inhibitor was found to be inhibitory to the midgut trypsin of the yellow stem borer, the major lepidopteran pest of rice. About 18% of the area devoted to rice in Southeast Asia is affected by this insect pest. Actual screening under proper containment facilities for the resistance of the transgenic plants to yellow stemborer will be done.

Performance of Transgenic Plants

More than 300 putative transformants have been regenerated from these experiments (Table 2). The transformants were generally inferior in morphology with less tiller number than the control. The Zhonghua 6 plants are now flowering in the Phytotron, while all the Zhonghua 10 plants have been harvested and all of them set seeds. The number of seeds produced per plant varied from 1-106.

The success of this technique allows use of other available plasmids which carry genes of agronomic importance such as those conferring resistance to pests and diseases.

ACKNOWLEDGMENT

The authors are grateful to Dr. K. Syono for the Oc cell line, and Dr. N. Murai and Dr. R. Nelson for providing pTRA132 and pCAL1GC plasmids, respectively.

Protoplast Isolation, Culture and Selection

Sufficent amounts of protoplasts for transformation were isolated from primary calli or cell suspensions (Fig. 3). A protoplast population with a 50% survival rate after PEG treatment is considered optimal for transformation studies. In this case, the suitable PEG incubation period was 15-25 min. The absolute transformation efficiency did not increase when PEG incubation was beyond 25 min. Longer periods of incubation decreased protoplast survival rate and colony formation, although transformation efficiency based on total colony number was higher.

Survival of colonies decreased with increasing hygromycin B concentration (Fig. 4). Likewise, the number of regenerated putative transgenic plants was inversely proportional to the concentration of hygromycin in selection medium (Table 1).

Histochemical Analysis for GUS

Histochemical analysis of transgenic plants such as roots, leaves, stems, leaf sheaths and spikelets of IR58 and Zhongua 10 demonstrated the expression of the GUS gene (Fig. 5).

Molecular Analysis of Putative Transformants

Southern blot anlaysis of DNA isolated from leaf blades of 81 plants regenerated from Hygromycin-resistant colonies confirmed the presence of the introduced Hph genes in 13 plants. Untransformed plants did not show any hybridization band.

Integration of the foreign gene was illustrated by the formation of a smear in only the high molecular weight region when undigested genomic DNA was hybridized with the ^{32}P-labelled 1.1 Kb BamHI fragment of pTRA132 (Fig. 6'). Five of six plants (Nos. 1, 4, 5, 6 and 8) showed hybridization signals in the 1.1 Kb region corresponding to the intact Hph coding sequence. Plant No. 7 showed a single hybridization band higher than 1.1 Kb indicating that at least one of the BamH1 restriction sites of pTRA132 was mutated. Plant Nos. 4, 5 and 8 had multiple hybridization bands at molecular weight regions higher than 1.1 Kb indicating multiple insertions of foreign gene and at least one of the BamH1 restriction sites of the plasmid was altered during integration of the gene into the genome. This alteration apparently did not interfere with the hph function since the colonies were selected in the presence of the antibiotic.

It was observed that the introduced hph gene was integrated at various patterns. The presence of multiple bands at molecular weights higher than expected is an indication of multiple insertion events and rearrangements of the integrated gene, both of which have been commonly observed in transformed plant materials (Rhodes et al., 1988; Lyznik et al., 1989; Gordon-Kamm et al., 1990).

Regeneration of Selected Transgenic Calli

Hygromycin-resistant calli 1-2 mm in diameter were transferred to N6 or R2 regeneration medium. Plants 10-20 cm in height were transferred to culture solution (Yoshida et al., 1976) and reared in the Phytotron at 21/29°C night/day temperature under natural light. After 3-4 weeks, the plants were transferred to pots and kept in the Phytotron until maturity. Seeds were collected from the fertile plants and the vegetative portions were burned. The plant height and tiller number relative to control were noted.

Histochemical Analysis for GUS Gene

Histochemical analysis of transgenic plants for the presence of GUS gene was conducted on various tissues such as calli, roots, leaves, stems, leaf sheath and spikelets (Jefferson, 1987).

Molecular Analysis of Putative Transformants

Molecular analysis to confirm the integration of foreign genes was performed on 3 IR58, 81 Zhonghua 10 and 24 Zhonghua 6 putative transformants sampled at random. DNA was extracted from leaf blades collected 1-2 months after transplanting following the procedure of Dellaporta et al. (1983). DNA extracts were subjected to restriction enzyme digestion, electrophoresis and Southern blot analysis according to the protocol of Maniatis et al. (1982). The DNA probes used for hybridization were the 1.1 Kb BamHI fragment of pTRA132 for the hph gene, the 1.8 Kb GUS fragment of pCAL1GC for the GUS gene and the 0.7 Kb fragment of pSBT1 for the SBT1 gene.

RESULTS AND DISCUSSION

Callus Induction and Establishment of Cell Suspension

Production of sufficient embryogenic calli from all the varieties was observed at 3-4 weeks after plating (Fig. 1). Embryogenic cell suspension lines were established in R2 medium within one month after initiation (Fig. 2). Establishment of cell suspension within the shortest time possible is very critical for high efficiency of plant regeneration.

Suspension cultures consisted of spherical cells and the cell fresh weight was doubled in 3-7 days. The clusters, which represented growth centers, differed in size, ranging from a few to 100 or more cells per cluster.

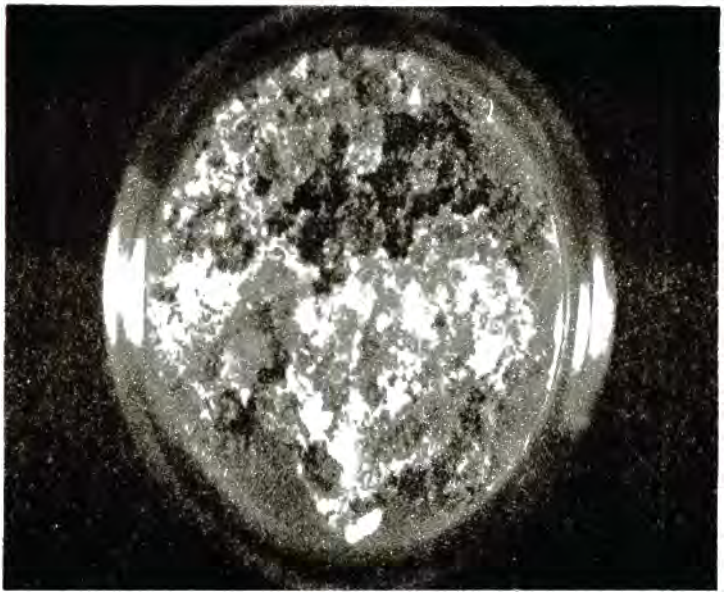

Figure 3. Unirradiated calli (2n) of *N. langsdorffii* 60 days after irradiation

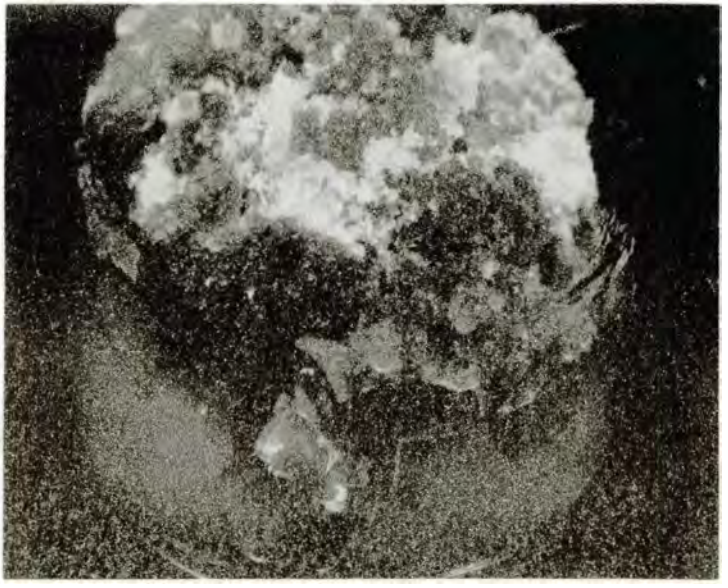

Figure 4. Unirradiated calli (n) of *N. langsdorffii* 60 days after irradiation

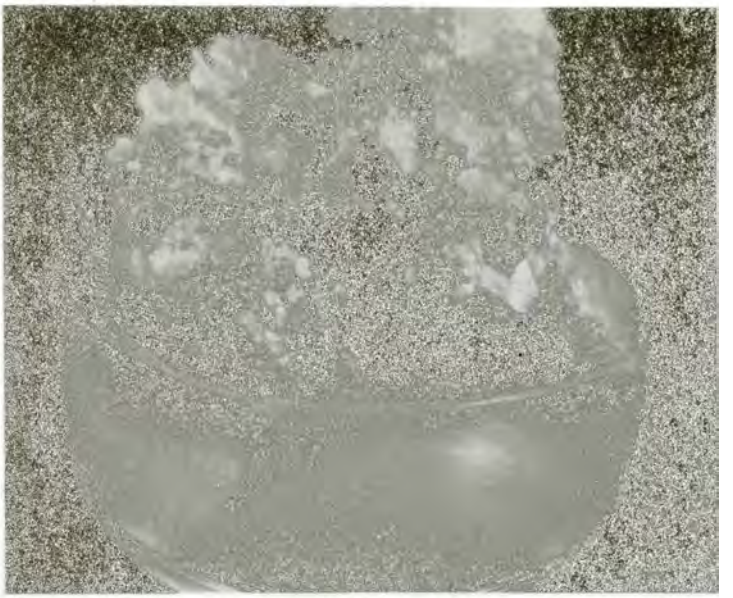

Figure 5. Unirradiated calli (2n) of *N. tabacum* 60 days after irradiation

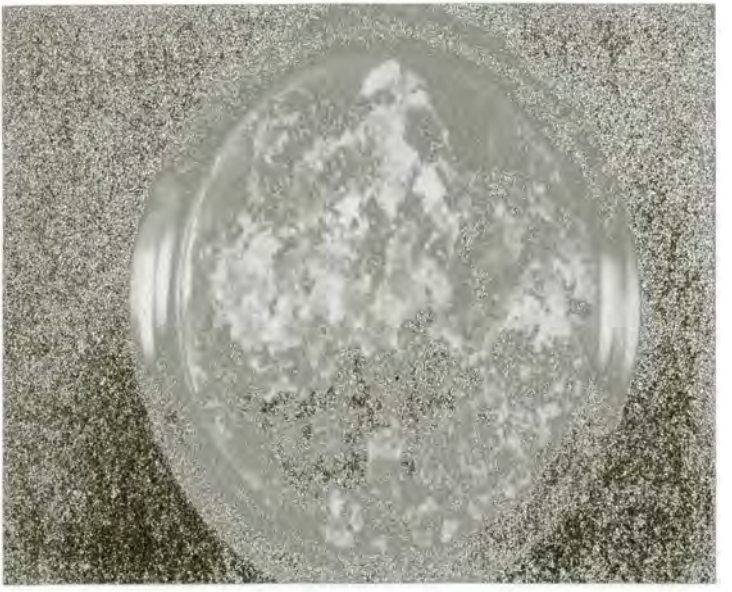

Figure 6. Irradiated calli (2n) of *N. tabacum* 60 days after irradiation

Figure 7. Irradiated calli (2n) of *N. glauca* at 5kR 60 days after irradiation

Figure 8. Irradiated calli (2n) of *N. langsdorffii* at 5kR 60 days after irradiation

Figure 9. Irradiated calli (2n) of *N. glauca* at 10kR 60 days after irradiation

Figure 10. Irradiated calli (2n) of *N. langsdorffii* at 10kR 60 days after irradiation

Figure 13. Regenerated plants from irradiated calli (2n) of (A) *N. langsdorffii* (B) *N. tabacum* and (C) *N. glauca*

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ANALYZING THE MONOCYCLIC PROCESS IN SHEATH BLIGHT OF RICE UNDER SEMI-CONTROLLED CONDITIONS

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ABSTRACT

Rice trap plants, used as probes, were exposed in quadrats that were inoculated with sheath blight (*Rhizoctonia solani* Kuhn) to study the spread of the disease. The effects of leaf wetness regime, leaf contact frequency and strength of inoculum source were quantified using this approach. Environmental variables were manipulated in the quadrats by covering them with plastic cages for different durations (leaf wetness), planting hills at different spacings (leaf contacts) and varying the amount and placement of inoculum in the canopy (source strength). Each experiment involved three successive batches of trap plants.

The infection efficiency increased with the accumulation of wet and dry daily cycles. Incidence and severity on sheaths increased with increased crop density. Increasing the amount of initial inoculum led to increase of disease incidence, leaf severity and number of infection points. Most disease variables were higher in treatments involving placement of initial inoculum at the leaf level compared to placement of the same amount of inoculum at the base of the plants. Disease spread declined with the successive batches of trap plants suggesting a decline in the number of infectious lesions over time.

INTRODUCTION

Sheath blight (ShB) is a fungal disease of rice caused by *Rhizoctonia solani* Kuhn (*Thanatephorous cucumeris*) (Frank) Donk. Under favorable conditions, the disease causes lesions on leaf sheaths, which coalesce, and spread to the upper leaf sheaths and on the leaf blades. In the last three decades, as modern, semidwarf nitrogen-responsive cultivars were introduced, the economic importance of sheath blight increased in many rice growing regions of the world (Teng, 1990). In both lowland and upland rice production areas, 25-50% yield loss could be incurred in rice as the disease develops on the flag leaf (Kannaiyan and Prasad, 1978). Roy (1979) found that 36% yield loss could be incurred at tillering stage and 11.73% at booting stage inoculation (Tsai, 1974). In the Philippines, the range of yield losses in farmers' fields was reported as negligible (0.4% – 23%) depending on the variety and the nitrogen input to the crop (Ou and Bandong, 1976)

Detailed experiments are necessary to study and quantify epidemiological mechanisms. Details of the different environmental factors associated with sheath blight (ShB) epidemiology can be derived from experiments under semi-controlled conditions where the host, the pathogen and some environmental factors can be manipulated. Factors possibly affecting ShB epidemics, such as leaf wetness, crop density and leaf contact frequency, and the strength on inoculum source, have been selected for screenhouse experiments. These factors have therefore been artificially manipulated (stimulus), in order to measure the disease response (Zadoks, 1972). Several biotic and abiotic factors can be manipulated in quadrats of rice hills that represent field plots. The disease response can be quantified by the use of trap plants that can probe the conduciveness of a given environment in a quadrat. Measurements of disease parameters can be derived from both the trap plants and the quadrat. Knowledge on the dynamics of sheath blight can help in developing a simulation model of ShB epidemiology that can be used to formulate methods for successful management of sheath blight.

Most epidemiological studies on sheath blight have been conducted in temperate countries. In Japan, detailed ecological studies on sheath blight have been conducted to develop a computerized forecasting system. Hashiba and Ijiri (1989) developed a computerized forecasting system of yield losses due to sheath blight (BLIGHTAS). However, this cannot be used under tropical conditions where effects of climate on sheath blight epidemiology might strongly differ from those of temperate regions. Some aspects of sheath blight epidemiology in the tropics have already been studied by many researchers. However, information on the effects of leaf wetness duration, the amount of initial inoculum and some cultural practices on sheath blight spread are still lacking.

The experiments were conducted from March to May 1992 in the screenhouse of the International Rice Research Institute, Los Baños, Laguna, Philippines. The objectives of the study were: to develop an epidemiological method for the study of monocyclic processes in sheath blight; to establish a functional relationship

between different leaf wetness durations and sheath blight spread; to establish a functional relationship between different crop density treatments and sheath blight spread; and to establish a functional relationship between different levels of initial inoculum and sheath blight spread.

MATERIALS AND METHODS

Experimental Environment, Land Preparation and Crop Establishment

Experiments (Table 1) were conducted in a screenhouse enclosed with wire mesh, with an average temperature of 31.7/28.5°C, relative humidity of 68.3/72%, and light intensity of 690/1050 watt/m^2, inside and outside of the screenhouse. The experimental area was plowed and harrowed 10 days before transplanting. Basal application of 80 kg/ha urea (45-0-0) was carried out during the final harrowing and levelling, a day before transplanting. A short-culmed rice cultivar, IR72, raised in a dapog seed bed, was used in all experiments. Plants were transplanted 10 days after sowing at 6 seedlings per hill, 20 x 20 cm spacing, except for spacing treatments in experiment 2 (Table 1). At maximum tillering stage, plants were topdressed with 40 kg/ha urea.

Inoculum Preparation and Inoculation

Sheath blight inoculum was prepared following Mew and Rosales (1986). Isolate AG-1-LR-1 of *Rhizoctonia solani* Kuhn was mass cultured in PDA plates for five days. Rice-grain-hull (RGH) was thoroughly mixed at 1:5 ratio. The mixture was soaked in water for two hours. Heat resistant bottles, measuring 8 x 20 cm, were filled with the RGH mixture to 80 percent, covered with aluminum foil and tied with rubber bands. The RGH-filled bottles were autoclaved at 100,000 Pascal at 121°C for two hours. A five-day old culture of *R. solani* in PDA plates was inoculated to RGH mixture at 1:4 (agar plate: RGH bottles) ratio after cooling. The inoculated mixture was used as source of ShB inoculum after 10 days of incubation at room temperature. Source hills were inoculated with ShB at maximum tillering stage, 40 days after transplanting. Except for experiment 3, stems and leaves were inoculated with 5 g of ShB inoculum following the insertion method of Yoshimura and Nishizawa (1954). The inoculum was placed directly at the base of each hill above the water line. In all experiments, leaves in each hill were held together by a rubber band tied at about 15 cm below the uppermost leaves and removed seven days after inoculation. To enhance infection, inoculated plants were sprayed with water three times a day and covered with plastic cages every night for seven consecutive days.

Quadrat and Trap Plant

An experimental unit was composed of a quadrat of 3 x 3 hills: 8 source hills and 1 trap plant. The latter was a disease-free hill that had been grown separately, and then transplanted at the center of the quadrat. The source hills were inoculated plants surrounding the trap plant. The quadrat was used to represent field plots where several biotic and abiotic factors could be manipulated. The trap plant was used to probe the conduciveness of a given environment, i.e., manipulated conditions prevailing in the quadrat, for disease spread. Any change on the trap plant with regard to ShB severity, number of infection points and incidence reflected the conduciveness of the environment to disease in the quadrat. Each quadrat was sprayed with water and covered with plastic cage for 12 hours, every night for three days except for leaf wetness treatments in experiment 1 (Table 1). After exposure, the trap plant was transferred into pots, sprayed with water and covered with plastic cage for another three days.

Treatments

Three experiments were conducted to test the effects of a series of environmental factors on the srpead of sheath blight using the trap plant and the quadrat. The treatments (Table 1) in all experiments were aimed at manipulating the quadrats prior to and during the exposure of the trap plants. Each treatment was represented by quadrats randomly distributed in replicates. Leaf wetness duration in experiment 1 was manipulated by covering the quadrats with plastic cages. In experiment 2, contact frequency between plant tissues (leaves and sheaths) was manipulated by varying the density of hills. In the first two experiments, 5 g of ShB inoculum per hill was used. The amount was either decreased or increased, and placed at different positions on source plants in experiment 3.

Experimental Design

All experiments were laid out in a randomized complete block design. Except for experiment 2, quadrats were separated by a row of border hills, while alleys (30 cm wide) were provided to separate replications. In experiment 2, the quadrats were assigned to 1 m x 1 m plots with hills planted at spacing similar to that of the assigned quadrat. Plots and replications were separated by 40-cm wide alleys. Three batches of trap plants were exposed successively into quadrats at three-day intervals.

Collection and Analysis of Data

Four variables (Table 2) for disease measurement were considered simultaneously because one variable may not sufficiently reflect the factors that contributed to the production of new lesions. A few infection points could for instance result in

a high severity. Infection efficiency was quantified as the ratio of the number of infection points on the trap plant to the number of infection points on the source hills. Infection points on the stems and on the leaves were considered to account for the spread of disease from the source hills to the trap plant by leaf-to-leaf and leaf-to-sheath contacts. Sheath blight severity, count of infection points and incidence on tillers (Table 2) were gathered from the source hills and from the trap plants. The source hills were assessed for ShB prior to exposure of the trap plants in the quadrats. One source hill was randomly chosen and five of its tillers were assessed for ShB. On reach of the trap plants, all the tillers and their leaves were assessed for ShB after the three-day incubation in pots. All variables used in ShB assessment (Table 3) were analyzed using a repeated-measures ANOVA (Madden, 1986). Arc-sine transformation was applied on data gathered from variables 1 and 4 while for variable 3, log transformation was used to normalize the distribution of values (Gomez and Gomez, 1984).

RESULTS

Experiment 1
Effect of Leaf Wetness on the Spread of Sheath Blight

The effects of leaf wetness regimes on sheath blight development on trap plants are presented in Table 4. Significant differences were found among the treatments for all variables, except for severity on stems (Ss). Disease parameters on trap plants were usually highest in treatment E (intermittent wet and dry 12/12 h periods) followed by treatment F (continuous wetness). Treatment E was found signficantly different from other treatments for all variables, except for incidence on tillers (Nit/Nt) and Ss.

Batches of trap plants were observed to strongly differ in sheath blight development as represented by significant ($P<0.01$) variance ratios for all variables. A strong decline among batches was observed for all variables, particularly infection efficiency (IE), which was highest in batch 1 (mean = 0.61), followed by batch 2 (mean = 0.09) and batch 3 (mean = 0.07) (Table 5).

The interaction of batches with treatments (A x B) was not significant for any variable except IE ($F = 3.44$, $P < 0.01$). The strong A x B interaction on IE indicates that treatments were ranked differently among batches (Table 5). Treatment D ranked second in batch 1, fourth in batch 2 and third in batch 3. Treatments E and B consistently ranked first and fifth, respectively, in all batches. Treatment C in batches 1 and 2 ranked third, while treatment F ranked second in batches 2 and 3.

Experiment 2
Effect of Crop Density on the Spread of Sheath Blight

There were no significant differences among crop density treatments with respect to SI, IPs + IPI and IE (Table 6). Nit/Nt, however, significiantly increased with increasing crop density. It was higher in treatment A (mean = 0.53, 15 x 15 cm spacing) than treatments C (mean = 0.17, 20 x 20 cm spacing) and D (mean = 0.15, 25 x 25 cm spacing). A similar effect was observed with respect to Ss, i.e., increase in sheath blight severity on the stem as crop density was increased.

A strong batch effect was observed on severity on leaves (SI), total number of infection points on stem and leaves (IPs + IPI) and IE as indicated by their variance ratios significant at $P < 0.01$. Values of some disease variables decreased among the batches of trap plants. This effect was not noted for Nit/Nt and Ss. No significant interaction of treatments with batches (A x B) was noted for any of the variables.

Experiment 3
Effect of the Strength of Inoculum Source on the Spread of Sheath Blight

Table 7 shows the effect of the strength of the inoculum source on sheath blight spread. Significant treatment effects were found on Nit/Nt ($F = 13.8$, $P < 0.01$), SI ($F = 19.5$, $P < 0.01$) and IPs + IPI ($F = 13.8$, $P < 0.01$). High amount of initial inoculum applied to the source hills accounts for high disease severity on trap plants as indicated by highest means attained for all variables on treatments F and G, where total amounts of initial inoculum were 10 g and 7.5 g per hill, respectively.

The position of inoculum on the leaves had a stronger effect on disease variables than positioning the same amount of inoculum on the stems. This is particularly shown in comparing means for SI and IPs + IPI between treatments D (SI = 0.24, IPs + IPI = 3.09) and E (SI = 0.48, IPs + IPI = 4.24).

There was a significant batch effect ($F = 4.76$, $P < 0.05$) on IPs + IPI. This suggests a decrease of infection points within the source hills of the quadrats with the successive batches of trap plants. The interaction of treatments with batches (A x B) was significant ($F = 1.94$, $P < 0.05$) on SI, i.e., ranking of treatments varied among batches. Treatment F had the highest mean in batches 1 (0.70) and 2 (0.71), but not in batch 3 where it ranked fourth (mean = 0.37). In treatment G, mean values in batches 1 (0.61) and 2 (0.51) ranked third but had the highest mean in batch 3 (0.46). Consistent ranking in all batches was observed for treatments C and H which ranked fifth and eighth, respectively. There was a decline of treatment mean values from the first to the third batch in most of the variables. This decline was strong in SI (Table 8) with mean values across treatments of 0.44 in batch 1, 0.41 in batch 2 and 0.35 in batch 3. Strong block effect was observed in SI ($F = 13.5$, $P < 0.01$) and IPs + IPI ($F = 5.92$, $P < 0.01$).

DISCUSSION

Screenhouse experiments were conducted to understand the dynamics of sheath blight. The effect of leaf wetness duration, crop density and strength of inoculum source on sheath blight spread was quantified using a probe (trap plant) exposed in a manipulated environment (quadrat).

Moisture on crop canopies has been the basis of attempts to develop several disease forecasting methods (Fry, 1982). Quantification of the influences of water-related variables, such as daily rainfall, rainfall intensity and duration, humidity and surface wetness, can help develop prediction rules for disease development. Among the water-related variables, leaf wetness duration often has a direct influence on pathogen activity. In some pathosystems, leaf wetness periods are often regarded as synonymous with infection periods and can account for a large amount of the variability in subsequent disease intensity (Royle and Butler, 1983).

The first experiment conducted, with strong differences between leaf wetness treatments, showed that leaf wetness regimes play an important role in ShB development and spread (Table 4). Comparison of treatment mean values indicated that ShB is particularly enhanced when subjected to intermittent wet and dry periods. Sheath blight severity and incidence were lower on trap plants subjected to continuous wetness (treatment F) than to intermittent wet and dry periods (treatment E). Increased leaf wetness period (treatments A to E) was associated with increased ShB intensity. Similarly, focus expansion of Rhizoctonia aerial blight of soybean is enhanced by prolonged free moisture (Yang et al., 1990). The mild effect of treatments on ShB severity on the stems may be due to the short exposure (3 days) of the trap plants in the quadrats.

The strong decline of ShB infection efficiency from batch 1 to batch 2 and batch 3 suggests that the number of infectious lesions on the source hills has declined during the exposure of the second and third batches of trap plants. This would mean that lesions in this experiment were infectious only for 3-10 days after inoculation. Zadoks and Schein (1979) called this phenomenon in the epidemic process as "removal", the transition from the infectious to the non-infectious state. Lesions that are no longer infectious were removed from the epidemic process.

The interaction between treatments and batches on infection efficiency suggests that treatment effects differed among batches of trap plants. Infection efficiency on trap plants subjected to continuous wetness (treatment F) ranked fourth in batch 1 and second in batches 2 and 3. Continuous wetness therefore appears to slow down the removal process, i.e., contributes in prolonging the infectious period of the inoculum.

Crop and canopy densities influence the microclimate within canopies and haveb een shown to increase ShB incidence and severity (Premalatha Dath, 1990). Results of the second experiment (Table 6) showed that incidence on tillers and severity on stems were significanlty affected by the spacing treatments. Incidence and severity were higher in the 15 x 15 cm (treatment A) and 15 x 20 cm (treatment

B) spacing treatments than in the 20 x 20 cm (C) and 25 x 25 cm (D) spacing treatments. In other words, disease intensity was higher with increased crop density. This result conforms with many reports (Roy, 1978; Srinivasan, 1980; Hori, 1982; Kannaiyan and Prasad, 1983; Ou, 1985). In this experiment, manipulating the plant spacing amounts to altering the contact frequency among healthy (trap plants) and diseased (source hills) plants in constant, favorable climatic conditions. There is high contact probability among the plants in dense planting so that disease is easily spread from plant to plant (Premalatha Dath, 1990).

The effect of crop density was mild on severity on the leaves, total number of infection points and infection efficiency. Although contact frequency was higher among the leaves than on the stems, temperature and relative humidity below the canopy were usually more favorable for ShB development (Ou, 1985). This could explain why the crop density variation had a significant effect on the stems (incidence and severity) and not on the leaves.

Strong differences were found among the batches of trap plants on severity on the leaves, total number of infection points and infection efficiency. For incidence and severity on the stems, batch effect was not significant. This could be attributed to the frequency of contact between the source hills and the trap plant where contact among the leaves was higher than contact between the stems.

The results of the third experiment (Table 7) indicate that the amount of inoculum in the source (strength) affects ShB spread. The effect of amount of initial inoculum was significant on incidence, severity on the leaves and on the total number of infection points. Except for infection efficiency, all variables had highest mean values on either treatments F or G which correspond to an inoculum amount of 10 g and 7.5 g per hill, respectively. All other treatments with low amount of initial inoculum had also low mean values in all variables. For severity on the leaves and the total number of infection points, significant differences were found when treatments F and G were compared to treatments A, D and H. This indicated that increasing the amount of initial inoculum from 2.5 to 7.5 and 10 g induced ShB severity, particularly the incidence on tillers, severity on leaves and the number of infection points. A similar result expressed by using area under the disease progress curve was obtained by Sharma (1989).

Positioning of inoculum on the leaves had a stronger effect on disease intensity than positioning the same amount of inoculum on the stems. The growth habit of transplanted rice (Vergara, 1979) allows contact between hills. During the third experiment, it was observed that leaf-to-leaf and leaf-to-sheath contacts markedly increased from early to maximum tillering stages. The frequency of leaf-to-leaf contacts was higher than the frequency of leaf-to-sheath contacts. This probably explains the higher ShB severity on trap plants exposed in quadrats that had been inoculated at the leaf level.

The interaction between batches and treatments with respect to severity on the leaves was indicative of changed treatment effects in each batch. For instance, treatment F (10 g of inoculum per hill) had the highest mean in batches 1 and 2, but

not in batch 3 where it ranked fourth (Table 8). Treatment G (7.5 g of inoculum per hill) had the third mean severity on leaves in batches 1 and 2, but had the highest mean in batch 3.

The quadrat and the trap plant were used to understand the effects of a series of environmental factors on the dynamics of ShB. This approach revealed that under semi-controlled conditions, intermittent leaf wetness, increased contact frequency, increased amount of initial inoculum and positioning of inoculum at the leaf level favor ShB spread. The methodology can be used to address a number of driving variables of the rice-Shb pathosystem.

Table 1. Screenhouse experiments with their corresponding treatments.

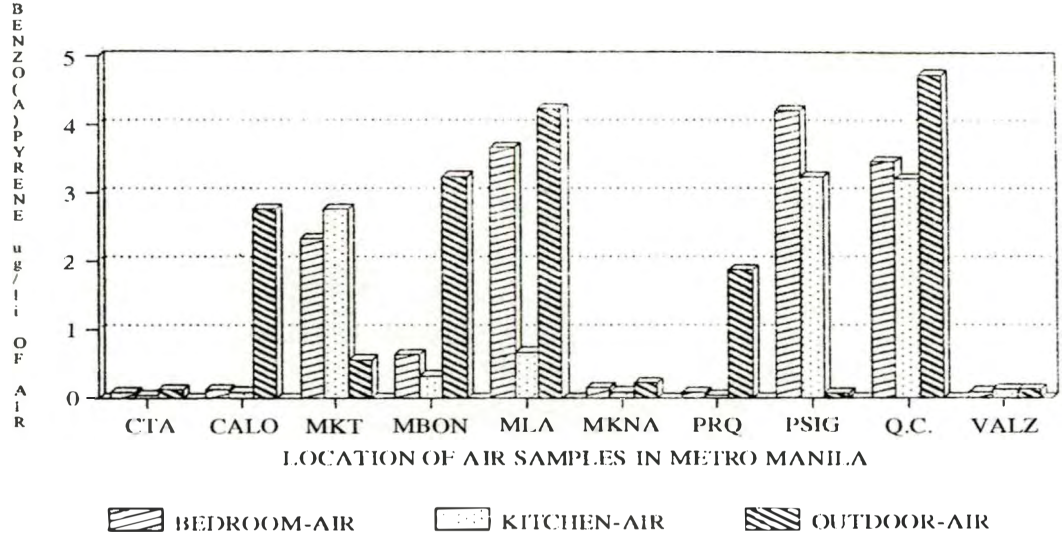
Experiment	*Treatment*
1 Leaf Wetness (5 reps)	A – non-inoculated quadrats; no water spray and no caging
	B – inoculated quadrats; no water spray and no caging
	C – inoculated quadrats; with water spray and 12-hour (1 night) caging
	D – inoculated quadrats; with water spray and 24-hour (2 nights) caging
	E– Inoculated quadrats; with water spray and 36-hour(3 nights) caging
	F – continuous wetness; treatment E plus water spray on day time, every hour for 3 days
2 Crop Density (4 reps.)	A – 15 cm x 15 cm spacing(49hills/m^2) B – 15cm x 20cm spacing(42hills/m^2) C – 20cm x 20cm spacing(35 hills/m^2) D – 25cm x 25cm spacing(25 hills/m^2)
3 Strength of inoculum source (5 reps.)	A – 2.5 g of inoculum on the stems
	B – 2.5 g of inoculum on the leaves
	C – 2.5 g of inoculum on the stems and on leaves (5 g/hill)
	D – 5 g of inoculum on the stems
	E – 5 g of inoculum on the leaves
	F – 5 g of inoculum on the stems and on the leaves (10 g/hill)
	G– 5 g of inoculum on the stems and 2.5 g on leaves
	H – no inoculum

Table 2. Operational definition of the variables used in ShB assessment

	Variable	*Definition*
1.	Incidence	The ratio of the total number of infected tillers over the total number of tillers per hill
2.	Infection point	A typical sheath blight lesion which may or may not expand and coalesce with other lesions
3	Infection efficiency	The ratio of the total number of infection points on the trap plants over the total number of infection points on the source hills
4.	Severity	The percent area covered by sheath blight lesions on the host tissues

Table 3. List of variables used for sheath blight assessment

Acronym	*Meaning*	*Unit*
Nit/Nt	Incidence on tillers	%
Ss	Severity on stem	%
Sl	Severity on leaves	%
IPs + IPI	Total infection points on stems and leaves	number
E	Infection efficiency	



Cta-Cainta; Calo-Caloocan; Mkt-Makati;
Mbon-Malabon; Mla; Q.C.; Prq-Parañaque;
Psig-Pasig; Valz-Valenzuela; Mkna-Marikina

Figure 3. Benzo(a)pyrene content of organic extracts of air particulates from indoor and outdoor air in Metro Manila

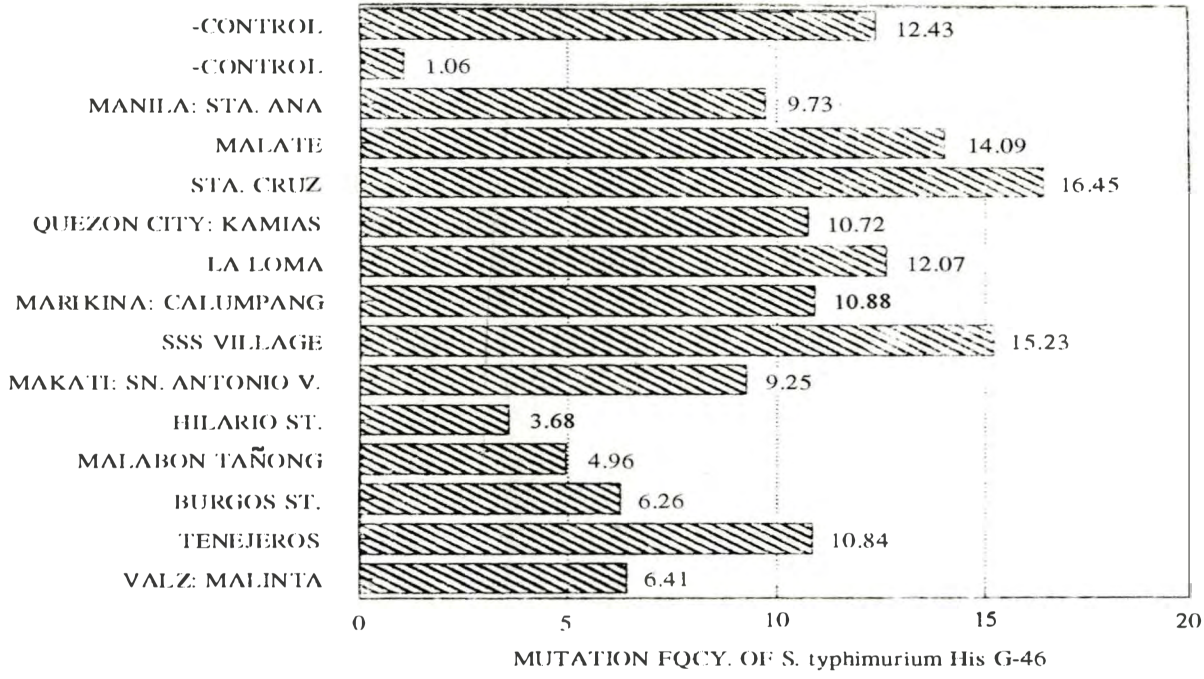
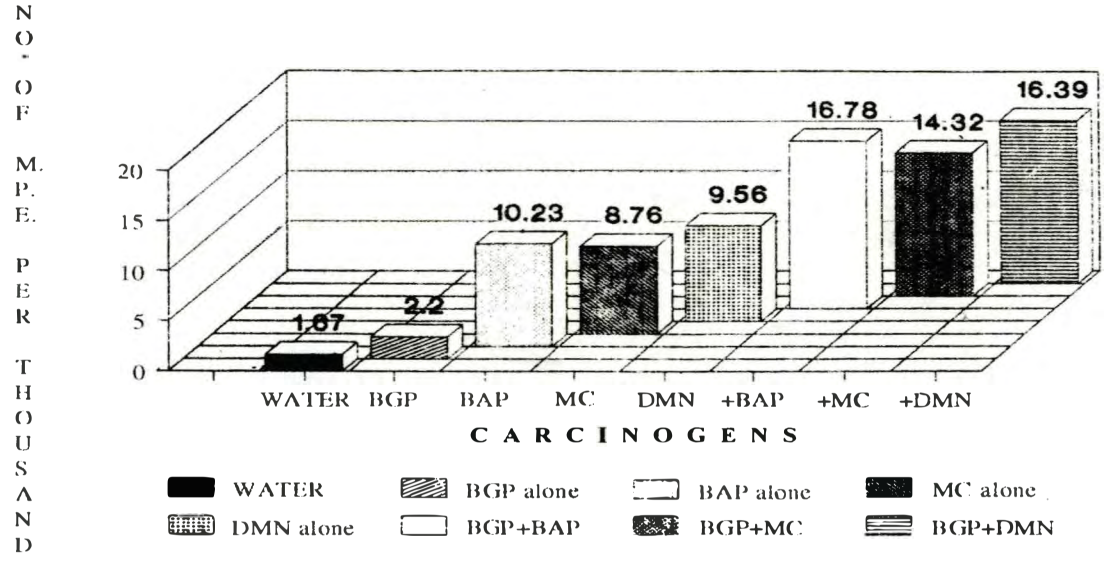
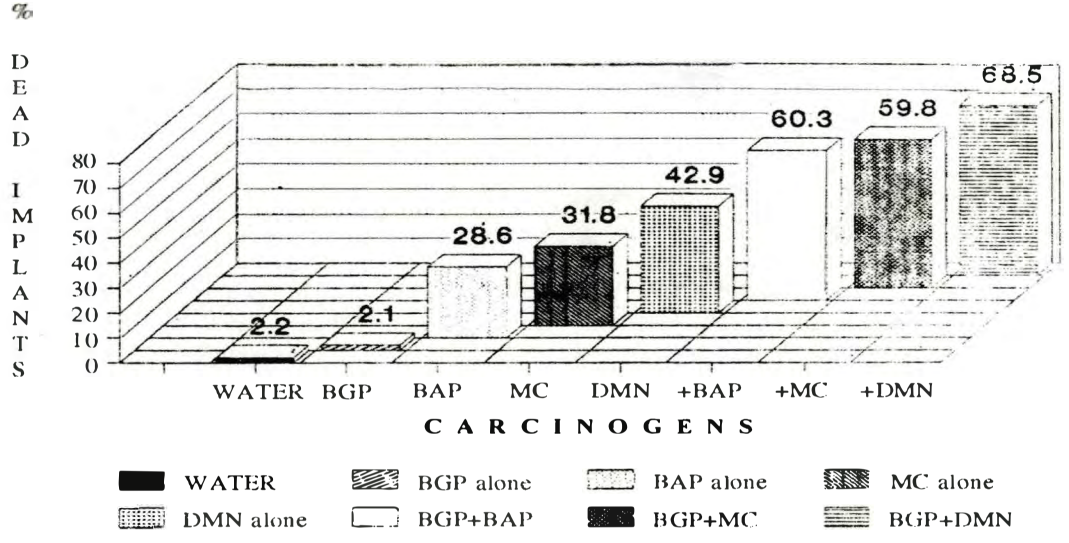


Figure 4. Mutagenicity after metabolic activiation of personal samples from student commuters to Diliman, Q.C.



BAP – Benzo(a)pyrene,
MC – Methylcholanthrene
DMN – Dimethylnitrosamine

Figure 5. Effects of benzo(ghi)perylene on the chromosome breaking effects of BAP, MC and DMN



BAP - Benzo(a)pyrene,
MC - Methylcholanthrene
DMN - Dimethylnitrosamine

Figure 6. Effects of Benzo(ghi)perylene on Germ Cell Genotoxicity of Benzo(a)pyrene, Methylcholanthrene and Dimehtylnitrosamine

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POSTER PAPERS

BIOLOGICAL SCIENCES

UPDATE OF TAXA OF LEGUMES IN THE PHILIPPINES

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Leguminosae (Fabaceae) ranks as third largest family of flowering plants and second to grass family in economic importance. Leguminous species have long been recognized as sources of food, fodder crops, timber, raw materials for industry, soil fertility amelioration and conservation. Countless numbers of species are associated with human activities and tapped for their aesthetic value. Treatment of this large group varies among systematists: the three subfamilies are treated as such or as distinct families.

Updating on taxa was undertaken in preparation for the rewriting of Philippine flora and as baseline information for basic and applied fields of study. The research study is an assessment of the diversity of the resources available after Merill's publication, as a result of plant introduction. Since his last compilation on legumes in 1923, there have been substantial increases in number of genera and species both native and introduced taxa for the three families.

For Caesalpiniaceae, the introduced taxa are mostly woody ornamentals; for Mimosaceae, those that are concerned with reforestation activities; and for Papilionaceae, forage crops, protein sources, those for green manuring and soil amelioration.

Updating of nomenclature based on recent systematic accounts is another focus of the research studies.

SOME ENDEMIC FERNS IN MT. APULANG, KITANGLAD RANGE, BUKIDNON

VICTOR B. AMOROSO, F. M. ACMA and H. P. PAVA. Central Mindanao University, Musuan, Bukidnon

Field collections of pteridophytes and ecological data were conducted in Kitanglad mountains, Bukidnon. Live and herbarium specimens were used for morphological studies and their status was determined: whether endangered, rare, depleted, endemic or economic species. Results of the study revealed the presence of 88 species, 54 genera and these were grouped into 28 families. The description and classification, including local names, habitat and distribution of the species, are presented in the paper.

Ecological parameters were likewise collected such as temperature, relative humidity, altitude, soil organic matter, type of soil, rainfall, slope gradients and forest types.

CRUSTACEAN PARASITE FROM PHILIPPINE FISHES: A REVIEW

NELLIE C. LOPEZ, Institute of Biology, College of Science, University of the Philippines, Diliman Quezon City

Forty-two species of copepods, one species of branchiuran and some species of isopods comprise the crustacean parasites recorded from Philippine fishes. Most of the records are from wild marine fishes and the reports mainly consist of the original taxonomic records. Eight species belonging to five genera have been recorded from cultured or fresh water fish. Mortality of host fish has been recorded for four species. Little is known about the pathogenecity of the other species. There are few reports on the biology and life cycle of these parasites. Chemical treatment is the standard practice in the control of the parasites of cultured fish.

GOLDEN APPLE SNAIL: AN ABSTRACT BIBLIOGRAPHY 1981-1992

MELODIA R. CARIASO, M.D. EBUENGA, A.V. DE LARA and B.F. CAYABYAB, National Crop Protection Center, University of the Philippines Los Baños, College, Laguna

The golden apple snail is an introduced freshwater gastropod in the Philippines that has become a major aquatic pest particularly in lowland rice. At present there seems to be some confusion regarding the identification and nomenclatural position of the golden apple snail. Historically, the earliest known information gathered on the golden apple snail as pest was in 1909.

In spite of the perceived significant role of this snail in agriculture there is a scarcity of information on its bionomics and geographical distribution. Whatever limited information is available comes from widely scattered scientific and popular publications. Thus far, no attempt has been made to compile research results into a meaningful abstract bibliography.

This abstract bibliography on golden apple snail has an international coverage with a compilation of more than 250 entries collected from various sources. The closely related genera referred to here are *Azpullaria, Pomacea* and *Pila*. These information on the related genera are included in this bibliography. The wide array of information collected to date was organized using the micro Computerized Documentation System/Integrated Set of Information System (CDS/ISIS) software. The golden apple snail in particular provides a data base which is available for searching and retrieval at the Surveillance and Forecasting Team Office, National Crop Protection Center, University of the Philippines Los Baños, College, Laguna.

A SURVEY OF THE RHOPALOCERA (LEPIDOPTERA) OF MT. MAKILING

BONIFACIO F. CAYABYAB, C.R. BALTAZAR, F.F. SANCHEZ, N.O. AGUILAR, and A.W. TEJADA, National Crop Protection Center, University of the Philippines Los Baños, College, Laguna

A survey of the Rhopalocera (butterflies: Lepidoptera) at Mt. Makiling, U.P. Los Baños was conducted from March 14, 1990 to April 3, 1992. The modified Pollard's transect technique was utilized.

The 26-month butterfly survey yielded 145 species and subspecies of Rhopalocera comprising 74 genera (butterflies) in 8 families of superfamily Papilionoidea and 16 genera from the solitary family (Hesperiidae) of superfamily Hesperioidae. Fourteen species and subspecies are new records for Luzon.

The range of temperature, where most Rhopalocera (butterflies) were counted, was between 25°C and 28°C. The peak of abundance of the Rhopalocera (butterflies) was recorded between 26°C and 27°C.

Most Rhopalocera (butterflies) on wings were observed from 0800 to 1000 hours. The peak density was observed between 0900 to 1000 hours.

There were two population peaks observed within a year. One is during the dry season in the months of April and May while the rainy season peak is from June to August.

An index of abundance was established for each major species. The combined data from 1990-1992 showed that five species reached the hundred mark of index of abundance. These are *Eurema sarilata aquilo* (Pieridae), *Emma hecabe tamiathis* (Pieridae), *Ypthima sempera sempera* (Satyridae), *Appias albina semperi* (Satyridae) and *Jamides cleodus semperi* (Lycaenidae).

ECTOMYCORRHIZAL FUNGI ASSOCIATED WITH DIPTEROCARPS IN MT. MAKILING

NELSON M. PAMPOLINA, R.E. DE LA CRUZ and M.U. GARCIA, University of the Philippines Los Baños, College, Laguna

Fourteen species of basidiomycetes belonging to the seven genera, i.e., *Russula, Lactarius, Scleroderma, Amanita, Boletus Paxillus,* and *Cantharellus,* were collected under the dipterocarp stand in Makiling Botanic Garden (MBG), University of the Philippines Los Baños College of Forestry. These were characterized morphologically and anatomically, prior to identification to at least genus level. The *Rusulla* species dominated the area representing five different species from *Lactarius* and one each for the other genera. *Lactarius piperatus* was observed to appear all throughout the period of collection. Among the identified dipterocarp host species, *Parahorea malaanonan* revealed a wide range of ectomycorrhizal association.

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Apparently, the development of fruiting bodies under such vegetation was favored with onset of wet season when the relative humidity reached 78% and monthly mean temperature of 28°C. Furthermore, symbiotic association seemed to fit soil which was characterized to be weakly acidic (pH 5.9) with low available phosphorous content (1.6).

External and internal examination of root samples under Scanning Electron Microscope (SEM) verified the fact that dipteroarps were ectomycorrhizal formers. This is exemplified by Hartig net formation in the obliquely elongated epidermal cell walls and fungal mantle on the root surfaces. The absence of vesicles and arbuscules in cleared and stained roots further supports these findings. Moreover, clamp connection and septation in hyphae proved that the mycosymbionts were basidiomycetous fungi.

STUDIES ON THE EFFECT OF METHYL PARATHION ON THE SPERM MORPHOLOGY OF THE ICR STRAIN MOUSE

IMELDA F. PAGULAYAN, Institute of Biology, College of Science, University of the Philippines Diliman and ZENAIDA G. BAOANAN, Natural Sciences Research Institute, University of the Philippines Diliman

Sperm morphology test was used in this study to evaluate the potential testicular toxicity of methyl parathion on sexually mature ICR strain mouse.

Male mice were injected intraperitoneally with sublethal doses of 1, 3 and 6 mg pesticide per kg body weight. Abnormalities on the acrosomal head morphology were analyzed through smear preparation. The incidence of variant abnormal shapes of the acrosome was found to be dose dependent and such were observed in the following forms: amorphous, rhomboid, beak, balloon, distally branched, banana, funnel, sunflower, calyx, branched and double headed. These abnormalities were correlated with changes in the motility and penetrating capacity of the sperms thereby affecting fertilization.

MATHEMATICAL, PHYSICAL AND ENGINEERING SCIENCES

A SIMPLE ASSOCIATIVITY TEST FOR FINITE ALGEBRAIC STRUCTURES

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The defining *structure matrix* S(G) of a finite algebraic system manifests most of its abstract properties through certain unique characteristic patterns of its entries – except the *Associative Postulate* PA. Using S(G), however, three matrices $P(g_r)$, Q and R (r) can be formed by means of which we can show that: If and only if $\langle G;\otimes\rangle$ is a group, then it follows that

$$R(r) = P(g_r) \circ Q = \sum_{x=1}^{n} g_{rx} P(g_x)$$

where r = 1, . . . ,n, $g_{rx} = g_r \otimes g_x$, g_r, $g_x \in G$, $P_{x=1}(g_r)$,$P(g_x)$ are permutation matrices, and o is matrix multiplication. Hence, if $<G; \otimes >$ is a group, then its operation $\otimes$ is associative and its matrix R (r) exhibits unique pattern that is determined by PA: its diagonal entries are all equal to g_{rx} for all r. This pattern can therefore be used as a simple *Associativity Test* for finite systems that are defined in terms of their structure matrices. The test is easy to apply and it involves only the formation and evaluation of n R (r) matrices as against the n^3 pairs of triple products normally required for testing a finite system of order n.

HEALTH SCIENCES

ANTI-FERTILITY EFFECTS OF KANDI-KANDILAAN [*STACHYTARPHETA JAMAICENSIS* (L. VAHL)] LEAF EXTRACTS ON THE FIRST 10 DAYS OF PREGNANCY OF ALBINO RATS *(RATTUS NORVEGICUS)*

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The effect of *Stachytarpheta jamaicensis* (L. Vahl) leaf extracts on the first 10 days of pregnancy in albino rats was investigated using 0%, 15% and 30% concentrations given orally and with five replicates per treatment.

The 30% extract caused a significant decrease in RBC, hematocrit, number of pups, placenta and corpora lutea. However, there was an increase in hemoglobin and WBC values.

The 15% extract caused an increase in RBC and WBC counts, hematocrit and hemoglobin values but had no significant effect on the number of pups, placenta and corporal lutea. There was ova lost between ovulation and implantation for the control group while both the 15% and 30% extract-treated rats had an average of 0.5. Histoanalyses of the liver and lungs showed congestion, necrosis and hemolytic spots. Also, there were scars and mummified fetus in the placenta of the 30% extract-treated rats.

Results indicated that *Stachytarpheta jamaicensis* (L. Vahl) leaf extract at 15% and 30% levels affected the normal reproductive physiology of albino rats on the first 10 days of pregnancy. These results also support earlier reports that it has abortifacient and anti-fertility effects.

AGRICULTURAL SCIENCES

CHEMICAL COMPOSITION AND FEEDING VALUE OF HEDGE LUCERNE [*DESMANTHUS VIRGATUS* (L) WILL.] IN FORMULATED BROILER RATION

ZOSIMO M. BATTAD, N.D. LONGBOY and R.R. SAIR, Mariano Marcos State University, Batac, Ilocos Norte

This study was conducted to: 1) evaluate the chemical composition of Desmanthus leaf meal (DLM); 2) determine the effects of varying levels of DLM in formulated ration (FR) on the feed consumption, feed efficiency, gain in weight, dressing percentage and carcass yield of birds; and 3) determine the cost of production, net income and return on investment of broiler production. Using a randomized complete block design with 3 replications (10 birds/replicate), 150 straight-run day-old Hubbard broiler chicks were given the following dietary treatments: 100% commercial mash-CM, 100% formulated ration-FR, 97% FR with 3% DLM, 94% FR with 6% DLM and 91% FR with 9% DLM.

While the treatment diets significantly affected feed efficiency, final weight, daily gain in weight and total gain weight of birds, these did not influence the eviscerated carcass yield with or without giblets (dressing percentage) and the percent breast, thighs, drumsticks, wings and backs of slaughtered birds. Increasing levels of DLM in FR correspondingly increased the fiber contents of the diets. Feeding commercial mash entailed the highest expense among the diets while the addition of 6% DLM in FR realized the highest net income per bird. Desmanthus leaf meal inclusion in FR was found to be best at 6% level although the growth rate was comparable with DLM inclusion at 3% and 9% levels. The addition of 6% DLM to formulated broiler ration is recommended because it elicited better performance in birds while obtaining the highest profit.

IMPORTANT INSECT PESTS OF MANGIUM *(ACACIA MANGIUM* WILLD.)

RICARDO D. BRAZA, Forest Research Department, Paper Industries Corporation of the Philippines (PICOP), Tabon, Bislig, Surigao del Sur

This paper presents the first comprehensive account about the important insect pests, i.e., insects causing serious damage such as mortality, stunted growth and severe defoliation of mangium, *Acacia mangium* Willd., in the Philippines. The results of a series of monthly surveys conducted from 1990 to 1992 at the forest nursery and plantations of the Paper Industries Corporation of the Philippines (PICOP) in Surigao del Sur provide first-hand information on the identification, morphological features and nature of damage caused by the seven important insect

pests – four defoliators, two stemborers and a root feeder – to trees and seedlings of mangium, Mangium is one of the exotic tree species in the government's National Forestation Program (NFP). This information will guide NFP workers involved in the development of mangium plantations in the country in the prompt detection and correct identification of the insects – the key to effective management of the pests.

INTEGRATED PEST MANAGEMENT OF ASIAN CORN BORER [*OSTRINIA FURNACALIS* (GUENEE)] ON CORN, (*ZEA MAYS* LINN.) INCLUDING THE USE OF MONITORING AND FORECASTING MODEL IN THREE COMPREHENSIVE AGRARIAN REFORM PROGRAM (CARP) AREAS

BONIFACIO F. CAYABYAB, R.G. DAVIDE, E.A. BENIGNO, M.D. EBUENGA, S.M. BATO, P.G. GONZALES and R.G. BAYOT, National Crop Protection Center, University of the Philippines Los Baños, College, Laguna

Three Integrated Pest Management (IPM) techniques, farmers' practice and control on the Asian corn borer, *Ostrinia furnacalis* (Guenee), were studied in three Comprehensive Agrarian Reform Program (CARP) areas in the country from Oct. 15, 1991 to March 4, 1992.

The study aimed to verify the National Crop Protection Center's developed technologies, together with the government's recommended Maisagana corn borer control, and compare these with the farmers' practice and control.

A randomized complete block design (RCBD) using 375-sq. m. per plot replicated four times for each treatment was done.

The results revealed that in terms of yield, the National Crop Protection Center's (NCPC) Technology #1. Spot treatment + detaselling and NCPC Technology #2. Monitoring and simulation + release of Trichogramma were statistically comparable with the government's Maisagana program and better than the farmers' practice and control in reducing the Asian corn borer field population and subsequent damages.

Population and damage data showed that the densities of the Asian corn borer were generally low and damages were below the economic threshold in all the three areas.

DEVELOPMENT OF NATURAL PART SKIM MOZZARELLA-TYPE CHEESE FOR THE PIZZA INDUSTRY

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A natural Mozzarella-type (MZA) cheese was developed from different blends of reconstituted skim milk and fresh whole cow's milk (RSM/WCM) and ana-

lyzed for gross composition, meltability and stretchability in comparison with that simultaneously made from fresh whole cow's milk (WCM). This move was inspired by the ready market for Mozarella cheese in the expanding local pizza industry.

Physico-chemical analysis showed that the replacement of part of the WCM with 10% reconstituted skim milk (RSM) had a highly significant effect on total solids, fat and specific gravity of the cheesemilk, with the higher substitution giving lower total solids and fat but higher specific gravity. Fat content of blends varied from 2.30% to 1.27%. The blend had no significant effect on pH and total protein. However, the substitution tended to increase the titratable acidity.

Cheese analysis revealed that all blends had no significant effect on cheese pH and salt and for moisture at the lower substitution. Higher substitution levels gave a significantly higher moisture but more rigid curd. The blend, however, had a highly significant effect on fat and fat-in-dry-matter (FDM) content of cheese which decreased with increasing levels of RSM. Fat-in-dry-matter varied from 39.28% to 24.81% compared to 52.82-58.41% of the control cheese.

All the blended (RSM/WCM) part skim MZA cheeses exhibited no oiling off and gave lower meltability but better shreds and stretchability with longer strings than the full-fat WCM cheese when baked. Part-skim cheeses of specific blends that had the best shredding, melting and stretching characteristics without oiling off were identified and therefore would be highly acceptable for a pizza topping required by the local pizza industry.

SENSITIVITY OF *COTESIA PLUTELLAE* AND *DIADEGMA SEMICLAUSUM* PARASITOIDS OF DIAMONDBACK MOTH *(PLUTELLA XYLOSTELLA* L.) TO SEVERAL INSECTICIDES

BELEN MORALLO-REJESUS and J.B. OBRA, Department of Entomology, College of Agriculture, University of the Philippines Los Baños, College, Laguna

The toxicity of several insecticides (deltamethrin, fenvalerate, teflubenzuron, malathion, methamidophos, carbaryl, cartap and *Bacillus thuringiensis* var *kurstaki)* to adult *Cotesia plutellae* and *Diadegma semiclausum* was determined by residual film method. Based on LC_{50} and LC_{95}, methamidophos, malathion, cartap and deltamethrin were toxic to *Cotesia* while fenvalerate, carbaryl, teflubenzuron and *B. t. kurstaki* were relatively non-toxic to the parasitoid. *Diadegma* was most susceptible to methamidophos, cartap, fenvalerate and deltamethrin. In contrast carbaryl was non-toxic to this parasitoid.

The effect of teflubenzuron, *B.t. kurstaki* and cartap on the parasitization and development of *Cotesia* and *Diadegma* was determined by spraying the cabbage plant using the LC_{50} of DBM to the insecticides with two methods of exposure: 1) one-day-old parasitized second instar larvae of DBM were sprayed with insecticide; and (2) the larvae were sprayed first before they were exposed for

parasitization. The first method showed that cartap has the least effect with 67.7% parasitism compared to 75% in the control while teflubenzuron and *B.t. kurstaki* reduced survival to 49 and 45.6%, respectively. The second method showed a reduction in parasitization ranging from 59 to 88% especially with *B.t.* and cartap.

PREPARATION AND EVALUATION OF CONTROLLED RELEASE FORMULATIONS OF HERBICIDES USING RADIOCHEMICAL TECHNIQUES

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Two alginate-and two corn-cob-based controlled release formulations of thiobencarb were studied for the release rates of the active ingredient in distilled and paddy water. The release of the active ingredient from the corn cob formulation coincided with the critical period for weed competition.

Likewise, three alginate formulations of thiobencarb and one of propanil were prepared in the laboratory with varying kaolin content. Four alginate-based formulations of butachlor were likewise prepared with the incorporation of rice straw as a substitute carrier. The release rates of the active ingredients of these formulations in water were studied with the use of radiotracer techniques and analysis by gas chromatography.

CYTOLOGY, MORPHOLOGY AND POLLEN FERTILITY OF INTERSPECIFIC HYBRIDS BETWEEN *ORYZA SATIVA* and *O. OFFICINALIS*

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UPL Ri_3 was crossed with *Oryza officinalis,* a source of resistance against BPH, CLH and WBPH. Embryos were cultured *in vitro* resulting in several identical hybrid plants. F_2 seeds were obtained from natural seed setting in F_1 plants. These were germinated *in vitro* and grown in the greenhouse for evaluation. The present study aims to analyze the morphology, pollen fertility and cytology of the F_1 and F_2 plants.

The F_1 hybrids had semi-erect, grassy and vigorous growth. Like the wild parent, they had perennial life cycle, rigid awns, well exerted panicles and purple stigma, auricles and basal leaf sheath. F_2 plants resembled the F_1s but these had soft-textured awns, moderately well-exerted panicles and longer anther, flagleaf, panicles, grain and culm. Variation in tillering ability, panicle type, grain size and color of the leaves, basal leaf sheath, awn, apiculus and internode were noted among the F_2 plants.

Pollen fertility obtained from the F_2s ranged from 0 to 0.45%, similar to 0.49% pollen fertility obtained from the F_1s. A wide array of chromosomal aberrations were noted in the F_1s and F_2s. Among these are the occurrence of univalents, trivalents, laggards, bridges, early disjunction, non-congression, chromosome elimination and extra chromosomes.

POLLINATION STUDIES IN PASSION FRUIT

R.B. PIMENTEL and V.N. VILLEGAS, Institute of Plant Breeding, University of the Philippines, Los Baños, College, Laguna

Pollination studies were conducted on *Passiflora edulis var. flavicarpa* (yellow variety) to determine the extent of self-incompatibility in a selected line. Self incompatibility was assessed by selfing and outcrossing the selected line and determining the percentage of fruit set 7-10 days after pollination. Stigmatic receptivity and pollen fertility were also evaluated.

MOLECULAR TAGGING OF THE GENE FOR BROWN PLATHOPPER RESISTANCE TRANSFERRED FROM WILD SPECIES *ORYZA AUSTRALIENSIS* INTO RICE *O. SATIVA*

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Brown planthopper (BPH) is the major insect pest of rice. Incorporation of genes from diverse sources for pest resistance is an important strategy to develop varieties with durable resistance. Following embryo rescue, hybrid and backcross progenies were produced from the cross of cultivated rice (*O. sativa* 2n = 24 AA) and *O. australiensis* (2n = 24EE). Of the 600 BC2F4 progenies derived, 4 were resistant to BPH. One of the introgresion lines (IR65482-4-136-2-2) resistant to biotype 1, 2, 3 of BPH was used for RFLP analysis. Monosomic alien addition line analysis showed that the gene for BPH resistance is located on chromosome 12 and is monogenically inherited. Of the 14 probes mapped to chromosome 12, only one marker (RG457) detected introgression. Cosegregation between RG457 and BPH resistance was studied in F2 derived from the cross of introgression line and the susceptible recurrent parent. Molecular analysis showed that the gene for BPH resistance is linked with RG457 at a distance of 3.68 ± 1.29 cm. Such close linkage will be useful in marker-based selection while transferring BPH resistance from introgression line into other elite breeding lines. Introgression at molecular level indicates that the mechanism of alien gene transfer is probably genetic recombination through crossing over rather than substitution of whole or large segment of chromosome of wild species.

ESTABLISHING A COUNTRY CORE COLLECTION IN MUNGBEAN

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The Asian Vegetable Research and Development Center is responsible for the global base collection of mungbean. The total number of accessions registered in its collection is 5511. A core collection or a condensed yet representative assembly of accessions from this germplasm collection is being developed.

The objective is to establish groups of relatively homogenous accessions, based on morpho-agronomic descriptions, upon which nomination for inclusion in the core collection could be based. The strategy used is to form country core collections from which a global core could be established.

SOMATIC KARYOTYPE OF SEVEN SPECIES OF *CAPSICUM*

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Capsicum annuum, C. frutescens, C. baccatum, C. chinensis, C. eximium, C. pubescens and *C. chacoense* had distinct karyotypes based on chromosome arm ratios and length. *C. baccatum* and *C. eximium* had very similar karyotype. A secondary constriction producing a satellite was observed in a metacentric chromosome of *C. annuum* and *C. chinense*. This was observed in two metacentric chromosomes of *C. pubescens* and *C. chacoense*. *C. frutescens, C. baccatum* and *C. eximium* did not show any satellite. *C. frutescens* has a very similar karyotype to *C. annuum* except for presence of a SAT-chromosome in the latter.

The banding pattern due to cold treatment was found useful in chromosome identification in pepper. The metacentric chromosomes in all species showed centromeric and tolomeric bands except in *C. pubescens* and *C. chacoense* which in addition showed intercalary bands. Some chromosomes of *C. baccatum* showed darkly stained whole arms. This distinguishes it from *C. eximium* with which it has a very similar karyotype in terms of length and arm ratios. Bands were useful in distinguishing pairs of homologous chromosomes which were difficult to identify based on length and arm ratios alone.

Based on the karyotype analysis, *C. baccatum* is closely related to *C. eximium*. They were the only two species that had very similar karyotype. Even so, banding pattern in the two differed. Variation in the karyotype of the different species suggests structural differences in their chromosomes. The nucleolus organizing region is involved in some cases as shown by differences in the number of SAT-chromosomes.

IMPROVING YIELD POTENTIAL OF WET-SEASON RICE CROP: THE IMPORTANCE OF PANICLE SIZE

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In the wet season, the contribution of panicle number to rice (*Oryza sativa* L.) grain yield is limited by solar radiation. Therefore, panicle size may play an important role in improving yield potential. A study was conducted in 1992 wet season to determine the importance of panicle size on grain yield. Comparison was made between IR72 and IR60819-34-2-1 under four fertilizer regimes and several split applications. Maximum yield was 5.94 t/ha for IR72 and 6.5 t/ha for IR60819-34-2-1. IR60819-34-2-1 outyielded IR72 across N treatments. Twenty-eight percent more spikelet per panicle for IR60819-34-2-1 than IR72 resulted in 15% difference in spikelets/m^2 between the two cultivars. No significant differences were observed in panicles/m^2, filled spikelet percentage and 1000-grain weight between the two cultivars. Although yield of the two cultivars differed significantly, their total biomass was similar so that IR60819-34-2-1 had higher harvest index than IR72. To achieve high yield in the wet season, emphasis should be given to panicle size rather than panicle number.

LATE SEASON N APPLICATION TO IMPROVE HEAD RICE YIELD AND GRAIN QUALITY

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In high-yield irrigated rice *(Oryza sativa L.)* environments, fertilizer recommendations involved split application of N basally and 5-7 days before panicle initiation (PI) which leaves the crop solely dependent on soil N from flowering to maturity. This research was conducted to test whether or not an additional N application at flowering would improve yield and grain quality. Two field experiments were conducted at IRRI during 1992 dry season (DS) using varying rates and timing of N application on three rice cultivars. In one experiment, a new hybrid line (IR64616H) responded to an additional 45 kg ha^{-1} applied at flowering with a yield increase of nearly 1 t ha^{-1}, while the yield increase for IR72 and IR58109-113-3-3-2 was only 0.3 t ha^{-1}. For all cultivars, however, N applied at flowering resulted in 33% increase in the head rice yield, 27% for milled rice protein and 15% for translucency. In a second experiment, N application at flowering did not increase grain yield but resulted in significant increase in head rice yield (+14%), milled rice protein (+16%) and translucency (+13%). Application of 40 kg N ha^{-1} at flowering had a greater effect on grain quality than a comparable increase in the basal of PI N rate. These results emphasize the importance of late season N supply for improving the nutritional status of rice grain and the head rice yield.

INDUCTION AND LONG-TERM MAINTENANCE OF CALLUS FROM ENDOSPERM TISSUE OF CALAMANSI (*X CITRO FORTUNELLA MITIS* J. Ingram and H. Moore)

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Endosperm tissues were successfully excised from 60-64 day old seeds of calamansi. Primary calli were preferentially induced (33-35%) from the mid portion of the endosperm tissue using Murashige and Tucker (MT) medium (1966) supplemented with 1.0 mg/L 2,4-dicholorophenoxyacetic acid (2, 4-D) and 0.1 mg/L 6-benzylaminopurine (BAP).

The primary calli proliferated when subcultured onto MT medium with 2.0 mg/L 2,4-D, 5.0 mg/L BAP and 5% sucrose. Callus growth was better under diffused light (75%) than in complete darkness (0) or under lighted condition (25%). Subsequent monthly subcultures of calli led to the establishment of long-term callus cultures which to this time are one year and seven months old. These long-term callus cultures proliferated and formed green compact meristemoids when subcultured onto Murashige and Skoog (MS) medium with 0.5 mg/L BAP and 11% galactose. The aged calli [(a condition required for shoot regeneration in the case of calamansi (Avenido, et al, 1991)] are currently subjected to conditions for plantlet regeneration with the ultimate aim of producing plants with seedless fruits.

EMBRYOGENIC AND ORGANOGENIC CALLUS INDUCTION, MAINTENANCE AND PLANTLET REGENERATION IN MAIZE (*ZEA MAYS* L.) CV IPB VAR 4 AND INBRED LINE P123

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Media additives, asparagine, L-proline and casein hydrolysate (CH) were tested for embryogenic (E) and organogenic callus induction and maintenance in maize (*Zea mays* L.) using immature embryos of cultivar IPB var 4 and inbred line Pi23. Best results on callus induction were observed on cultures grown on MN_6 medium which consisted of N_6 salts and supplemented with 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2, 4-D), 2.25 mg/L glycine, 1.5 mg/L thiamine HCl, 9.2 mg/L nicotinic acid, 1.5 mg/L pyridoxine HCl, 2% sucrose and the media additives, L-proline (2.3 g/L) and case in hydrolysate (200 mg/L). The primary calli obtained were friable and compact.

Maintenance of E calli was achieved by consistent selection of such calli and transfer every 14 days onto fresh medium with increased concentration of 2, 4-D (1.0 mg/L). Brief exposure (3-6 days) of E. Calli to differentiation medium supple-

mented with 3-4 mg/L 6-benzylaminopurine (BAP) increased the recovery of germinating embryos. Transfer of plantlets to hormone-free medium allowed their continued development.

Initial evaluation of regenerated plants under greenhouse condition showed abnormalities such as dwarfing, tillering and absence of tassel. General 1 (G1) progeny of IPB Var 4 was recovered by pollination of the ear with pollen of cv Yellow Sweet Glutinous from field-growth plants.

"BASIC SEED" PRODUCTION IN POTATO (*SOLANUM TUBEROSUM* L.) CV BANAHAW AND ASN 69.1

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"Basic seeds" or minitubers of potato cv Banahaw and ASN 69.1 were derived from microtubers of "pre-basic seeds" produced *in vitro*. The microtubers ranged in size from $\geq$ 5 mm to $\geq$ 10 mm. Multiplication rate from microtuber to first generation (G1) minituber was 1:4 for both varities. G1 minitubers ranged in size from $\leq$ 10 mm to 40 mm. Generation 2 (G2) minitubers were produced from G1 minitubers at the rate of 1:4 for Banahaw and 1:5 for ASN 69.1. G2 minitubers ranged in size from $\leq$ 10 mm to $\geq$ 45 mm. All microtuber production was conducted at the CMPB laboratory, IPB while all minituber production was conducted at the Bureau of Plant Industry, Baguio City.

This basic seed production is a vital component of the microtuber production technology for the commercial production of certified seed tubers of potato introduced in 1992 by Rasco, Pateña and Barba.

PLANT REGENERATION FROM *INDICA* AND *JAPONICA* RICE PROTOPLASTS AND CYTOLOGICAL AND FIELD STUDIES OF REGENERATED PLANTS

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One of the most significant developments in plant tissue culture has been the isolation and culture of protoplasts. Protoplasts are ideal for gene transfer and generation of variation.

Protocols for protoplast isolation from suspension cultures and direct isolation from primary calli, culture and plant regeneration have been established. More than 5000 plants have been regenerated from different varieties of *indica*, *japonica* and wild rice.

The chromosome behavior and number of 92 IR43 and 35 IR57311-95-2-3 protoplast-derived R_0 plants were determined. There were 62% of the IR 43 and 97% of the IR 57311-95-2-3 regenerants which were normal diploids. Tetraploidy and abnormal chromosome behavior were observed in both cultivars.

Taipei 177 protoclones and the seed-derived control were evaluated and compared on 13 agronomic traits, grain quality characteristics and yield components under field conditions for two seasons. Significant variations both positive (+) and negative (-) relative to control were observed for number of days to flower (-), culm length (-), panicle length (+), flagleaf length (+), flagleaf L-W ratio (+) and primary branch per panicle (+) in the protoplast-derived lines. No significant difference in yield was observed.

MICROENVIRONMENT, DISEASE DEVELOPMENT AND YIELD INTERACTION IN RICE-CORN MONOCROPPING AND INTERCROPPING SYSTEMS

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This study was conducted at Central Mindanao University, Musuan, Bukidnon from May to October, 1991 to characterize the microenvironment of a rice-corn intercropping system, and to determine the relation of some microenvironmental parameters with disease development and disease severity.

Daily field temperatures (soil and air) were reduced by rice + corn intercropping. A lower air temperature profile with higher relative humidity resulted from intercropping, and it is more conservative of soil moisture than the monoculture. Incidence of major diseases like rice blast was lower in magnitude and less severe in the intercrop compared to monoculture on the later stages of crop growth. Downy mildew in corn increased with time with higher incidence in monoculture compared to the intercrop. Planting late in the wet season, with the early onset of disease produced higher disease incidence levels. Incidence and severity of rice blast were found higher with lower day time Rh, air temperature and soil temperature while downy mildew increased with lower air temperature.

There was little variation in yield and yield components in intercropping and monocropping. Increase in rice blast incidence reduced the yield by as much as 17.76% with a delay in planting (June 15). With downy mildew infection, greater plant density in monoculture had yield advantage over intercropping. Intercropping rice with corn in the uplands creates a better system of utilizing space, moisture, light and suitable soil and air temperature.

EVALUATION OF TRADITIONAL PROCESSING METHODS OF NAMI (*DIOSCOREA HISPIDA*) IN TERMS OF CHIP QUALITY, NUTRIENT AND DIOSCORINE CONTENTS

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A comprehensive survey was done in eight provinces in Northern Philippines to evaluate the indigenous processing methods for nami in terms of chip quality, nutrients and nutrient contents. Nutrient and diocorine were analyzed following the processing stages of common brine soaking method in chips produced from other detoxyfying methods.

Weight, quality, nutrient and dioscorine contents of chips varied depending on the preserving, soaking and washing treatments used. Lowest percentage and whitish chip color were obtained from chips treated with 5% w/v of 80% proof gin while the rest were comparable. Poor quality with high dioscorine content was obtained in roasted tuber.

SOMATIC EMBRYOGENESIS OF CALLOIDS FROM IMMATURE COCONUT EMBRYOS

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Seven- to eight-month old embryos from either Catigan or Cat x Bay population produced calloids as early as three weeks from initial culture in initiation medium with high 2, 4-D level. Embryogenesis and plantlet regeneration were attained when the auxin concentration was gradually lowered while the cytokinin level was increased. An attempt was made to establish one of the regenerated complete plantlets to *ex vitro* condition. The plantlet was planted in sterilized fine sand in a humidity chamber but it failed to get established. It died after about four weeks.

DAILY GROWTH RATE MEASUREMENTS DEMONSTRATE OSMOTIC AND IONIC EFFECTS OF HIGH SALINITY TREATMENTS ON RICE

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A nondestructive method of screening for salt tolerance in rice involving measurement of leaf elongation rate (LER) was tested on Nona Bokra (salt-tolerant) and IR28 (salt-susceptible) cultivars. Two-week-old seedlings were grown

at 1, 50 and 100 mol m^{-3} NaCl for 21 days and LER measured daily. The LER of Nona Bokra immediately decreased at 50 and 100 mol m^{-3} NaCl, indicating an osmotic effect of NaCl; that of IR28 slowly but continuously decreased implying an ionic effect. Nona Bokra adapted to the stress by maintaining a constant but reduced growth at high NaCl concentrations while IR28 did not. To estimate the entry of salt into the leaf, this screening method was further improved by clipping 1/3 of n-1 leaf for chemical analysis; this did not affect LER. Based on LER, Nona Bokra recovered two days after salinity was removed whereas IR28 did not. The quick recovery of Nona Bokra is another proof of an osmotic effect of NaCl while the failure of IR28 to recover was related to the large concentration of Na in the shoot and the inability to reduce this after salinity was removed. The results demonstrate that measurement of LER provides a convenient and nondestructive measure of salt tolerance. LER indicates the ability of rice to adapt to salinity and provides information about optimum time for sampling. This method may also be useful for evaluating other environmental stresses involving ion excess or deficiency.

MEASUREMENTS OF ULTRAVIOLET-B-IRRADIANCE UNDER FIELD CONDITIONS

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A major consequence of ozone depletion is an increase in UV-B radiation (280-320 nm) which has profound effects both in plants and in human beings. UV-B irradiance measurement, which could be affected by different factors, is critical in the study of UV-B effects on crops. An experiment was conducted to determine UV-B irradiance at different voltages (160-220V), solar irradiance and film photooxidation. Supplemental UV-B was provided by preaged UV-emitting fluorescent lamps that were enclosed by either cellulose acetate (UV-B treatment or mylar polyster (control treatment) films. UV-B irradiance was measured using an Optronic 752 Spectroradiometer. UV-B output was negatively associated with electrical voltage. There was a 5-35% reduction in UV-B irradiance from 205 to 160 volts. To obtain better UV-B output from the lamps, the voltage must not be lower than 205 volts. At IRRI, Los Baños, Philippines, the ambient UV-B level in dry season is about 6.0kJ/m^2/day on a clear day. Ambient UV-B irradiance is linearly related to total solar radiation (r^2=0.97**). The photooxidation of cellulose acetate can reduce the UV-B output by about 25% after one week of exposure. This suggests that cellulose acetate films must be replaced within seven days to ensure appropriate UV-B output. Uniform distribution of UV-B irradiance under the lamps could be successfully evaluated using a portable UVX radiometer.

RICE DEVELOPMENT AND GROWTH AS AFFECTED BY CARBON DIOXIDE

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The fact that atmospheric carbon dioxide (CO_2) is increasing had been established but how it will affect the world's food supply is not known. Few studies have been done on the effects of CO_2 on different rice (*Oryza sativa* L.) cultivars for the duration of crop growth. A controlled environment experiment was conducted to assess the response of five lowland rice cultivars (IR20, IR30, IR46, IR64 and IR72) to ambient (350 uL L^{-1}) and high (425,500,600 and 750 uL L^{-1}) CO_2. Plants were grown from seeding to flowering in naturally-lit, CO_2-, temperature-, and humidity-controlled growth chambers. Temperature inside the chambers was maintained at 33/25°C day/night and vapor pressure deficit was set at 12 mPa. Plant height for all cultivars was increased by CO_2 enrichment with the greatest increase occurring at 500 uL L^{-1}. Tiller number was also increased by high CO_2. Flowering was delayed at 425-500 uL L^{-1} CO_2. Greatest biomass and leaf area were also observed at 425 uL L^{-1} CO_2 for all cultivars except IR30. For IR30, all the high CO_2 treatments increased biomass but were not significantly different from each other. Significant changes in development and growth of rice can be expected in the future with increased carbon dioxide level.

YIELD POTENTIAL LIMITATIONS: FACTORS AFFECTING YIELD OF DEEPWATER RICE IN RELATION TO NEW PLANT TYPE

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Traditional deepwater rices (DWR) are low yielding (<2 tons/ha). Improvement of plant type is important to increase and stabilize yields. Field studies were conducted in 1992 wet season at IRRI to quantify yield differences as affected by number of main stems, primary and nodal tillers. The effects of genotype versus environment were evaluated by comparing growth of DWR cultivar (HTA 60) with high yielding irrigated cultivar (IR72). HTA 60 and IR72 were grown at 20, 40, 80, 160 and 320 plants/m^2 to vary number of different tiller types.

Increasing density in HTA 60 increased yield from 2 to 5 t/ha, as a result of two to threefold increase in leaf area index (LAI) and number of main stems. The LAI and stems/m^2 were also highly correlated with yield in IR72. In HTA 60, only 20 and 40 plants/m^2 produced nodal tillers which contributed <20% of the yield. Plants at high density (HD) had mainly main and primary tillers which yielded more than twice those at low density (LD). However, HD plants had threefold lower yield/panicle for main and primary tillers. Main tiller contributed >35% of

total yield in HD plants. LD plants had more spikelets/panicle than HD plants. There was no significant difference in panicle/straw ratio, filled grain percentage and plant height in either HTA 60 or IR72 although HD DWR plants elongated faster at the start of flooding. New plant type for DWR may be achieved by increasing the proportion of main stem tillers relative to the proportion of nodal and primary tillers. It would be useful to evaluate this with DWR cultivars with low tillering ability planted at high density.

RICE ROOT SAMPLING AND MEASUREMENT TECHNIQUES WITH EMPHASIS ON DROUGHT

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Root system evaluation is essential to understand crop responses to water deficit. Because roots grow underground, however, they are very difficult to study. Moreover, there is generally a large environment impact on root growth and morphology so root observations tend to have large variations. We described several major root sampling and measurement techniques, including trench profile, auger, monolith, pinboard, core and minirhizotron.

The trench profile method needed the most labor, but required no sophisticated equipment unlike the monolith, auger and core sampling methods which required a Comair root length scanner. All techniques used destructive sampling, except the minirhizotron which measured roots *in situ*. Effects of soil texture, cracking, strength and flooding on the choice of sampling method are also discussed.

EFFECT OF SEED AGING ON CROP ESTABLISHMENT OF RICE CULTIVARS IN ANAEROBIC SEEDING

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High seed vigor is important to obtain good crop establishment in direct seeded rice where anaerobic condition is a constraint. The effect of accelerated aging treatment on the cultivar difference in crop establishment was studied under various anaerobic conditions. Seeds of anaerobic tolerant (ASD1, JC178, Thaotabi, Kuhei) and susceptible (IR36, IR50) cultivars were subjected to aging treatment (43°C and 100% RH) for 3, 6 and 9 days. Then their seedling growth was characterized in 100% N_2 gas in the laboratory, in flooded soil (2.5 cm seeding depth, 2.5 cm water depth) in containers in the screenhouse, and in lowland direct seeded field (1.0 cm seeding depth, water saturated soil).

The aging effect persisted even though the environment for seedling growth was changed from laboratory to the field. The additive effect of aging and anaero-

bic soil condition severely depressed percent emergence, vigor index, seedling height, leaf development, length of coleoptile, mesocotyl and roots, and shoot dry weight. Cultivar ASD1 was found to withstand significantly the cumulative stresses of aging and anaerobic soil conditions. Vigor index in the container and coleoptile elongation in N_2 gas closely correlated with the percent emergence in the field with the coefficient of 0.828** and 0.843**, respectively. The results suggest that ASD1 might be a good material for breeding rice cultivar suitable for direct seeding and that vigor index and coleoptile elongation could be used as selection criteria.

DEFOLIATION AS A METHOD OF SCREENING RICE FOR DROUGHT TOLERANCE AT FLOWERING

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Screening rice (*Oryza sativa* L.) for drought tolerance at flowering is difficult because different rices flower at different times. Previous research showed that yields of plants subjected to flowering are related to accumulation and remobilization of non-structural carbohydrates. We hypothesized that defoliation during flowering would induce the plant to remobilize carbohydrates from the culms to the panicles similar to water stress effects. Defoliation was tested on 34 upland (Cavinti, Laguna) and 31 rainfed lowland (Victoria, Tarlac) lines and cultivars during the 1991 and 1992 wet seasons. All leaves or two leaves (flag leaf and penultimate leaf) were removed when first panicles emerged and a non-defoliated treatment was used as control. IR55549-01-2 and IR60077-24-B were high yielders in both control and defoliated treatments while IR60088-22 and IR47686-18-7-B were low yielders under upland condition. IR55736-31-2-1-2-1, IR33380-7-2-1-3 and IR58823-55-8-3-3-3 were high yielders in defoliated and non-defoliated treatments while IR63497-1-3, IR55008-74-3-1-1, IR55008-74-3-1-1 and IR58926-2-3-1-2-2 gave low yields under rainfed lowland condition. Thus, the defoliation method can be used to screen rice for drought resistance at flowering.

CHEMICAL CHANGES IN ABACA FIBER DERIVED FROM STALKS STORED AT VARIOUS DURATIONS

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Stalks of Makiling Puti abaca cultivar at pre-flagleaf stage of development were stored at various periods of time, namely, 0 (control), 10, 20, 30, 40, 50 and 60 days to determine the changes in chemical characteristics of the fiber during

storage. Fiber tensile strength and alphacellulose content were significantly increased when stalks were stored up to 30 days. Regardless of storage duration, on the other hand, fibers extracted from the inner leafsheath had significantly the lowest values for these parameters. Significant interaction effects between storage duration and leafsheath position were observed for the mean percentage fiber recovery, holocellulose and crude fat contents of abaca fiber. Prolonged storage of stalks (60 days) significantly impaired fiber quality of the outer leafsheaths particularly their holocellulose content.

EFFECT OF SULFATE ON METHANE PRODUCTION IN RICE PADDIES

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Rice paddies are considered to be among the most important sources of atmospheric methane (CH_4). Methane production in rice paddies is controlled by many factors including fertilizer application. Sulfate, a common fertilizer component, has been found to inhibit methanogenesis in aquatic sediments. A greenhouse experiment was conducted to determine the effect of sulfate on methane production in a flooded rice soil. Gypsum at 0, 350, 1000 and 2000 ppm sulfate was added to Maahas clay soil with and without IR72. Soil-entrapped CH_4, Eh and pH were monitored weekly for 13 weeks.

The decrease of entrapped methane with increasing levels of gypsum in the unplanted soil confirmed the inhibitory effect of sulfate on methane production. Soil Eh and pH were not significantly affected by the addition of sulfate. Apparently, the inhibitory effect of sulfate was caused by the competition of methane-producing and sulfate-reducing bacteria for common substates (acetate, CO_2, H_2). The seasonal variation of entrapped methane in the planted soil signified the interactive impact of sulfate and plant activity. The low entrapped methane during the vegetative stage of the rice plant regardless of sulfate levels was mainly due to CH_4 oxidation in the rhizosphere and emission to the atmosphere. The increase of entrapped methane during the reproductive stage was attributed to the enhanced methane production due to root decay and exudation. The magnitude of increase in methanogenesis was greatest for the 350 ppm sulfate treatment. This suggests that increased root excudation because of better plant growth may override the inhibitory effect of sulfate at low gympsum levels.

ESTIMATION OF RICE LEAF NITROGEN CONCENTRATION USING A CHLOROPHYLL METER: THE INFLUENCE OF LEAF THICKNESS

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The chlorophyll meter (SPAD) provides a simple, quick and non-destructive method to estimate rice (*Oryza sativa* L.) leaf N status but the linear relationship between leaf N concentration (N%) and SPAD values differs between growth stages. This study determined if leaf thickness was responsible for the differences. This study was conducted on IR72 in a nitrogen management experiment at IRRI farm during the 1992 dry season. Leaf N status was estimated using a chlorophyll meter (SPAD-502) and also determined directly by microkjeldahl procedure. Measurements were taken on five uppermost fully-expanded leaves from each plot before N topdressing at mid-tillering, panicle initiation and flowering. Specific leaf weight (SLW), a measure of leaf thickness, which is the ratio of dry weight to leaf area, was calculated. There were linear relationships between N% and SPAD values, but regression lines differed significantly between growth stages. Based on pooled data from all stages, the degree of linear fit was poor (r^2 = 0.49). Adjusting SPAD values for SLW (SPAD/SLW) improved the prediction of N% (r^2 = 0.93). These results demonstrate that SLW influences the prediction of leaf N status by SPAD, and adjustment of SPAD values for SLW greatly increases the accuracy of prediction.

ENVIRONMENTAL IMPACT OF NEW RICE GROWING TECHNOLOGIES: SOIL MICROBIAL BIOMASS IN WETLAND RICE SOILS

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This study aimed to quantify and establish a mechanistic understanding of the role of soil microbial biomass in soil fertility under intensively cultivated wetland rice. The specific objectives were: a) to quantify the relationships between soil microbial biomass and soil organic carbon content; b) to relate the process of reduction upon flooding to soil organic matter content; and c) to investigate the relationship of soil microbial biomass and soil nitrogen (N) under field conditions.

Soil microbial biomass of wetland soils under aerobic incubation was linearly related to a labile carbon pool in the plough layer rather than total soil organic carbon. Labile carbon in the Ap horizon reflects organic inputs and part of soil organic matter. The rate of reduction upon flooding was related to the C:N ratio of the enriched organic fraction but not total soil organic carbon.

Soil N status was described on a mass basis relative to N uptake by IR72, where the soil N environment was altered by N fertilizer addition. Soil N was measured as exchangeable, soil solution phase and soil microbial biomass N. Soil microbial biomass increased with increasing soil exchangeable and solution phase ammonium resulting in rapid immobilization of large amounts of added fertilizer N by the soil microbial biomass.

These results emphasize the importance of soil microbial biomass in the regulation of soil and fertilizer N supply in flooded soils, highlighting the apparent contrasts in the behavior of the soil microbial biomass under aerobic and anaerobic condition.

ENVIRONMENTAL IMPACT OF NEW RICE GROWING TECHNOLOGIES: THE ROLE OF AQUATIC INVERTEBRATES ON THE DECOMPOSITION OF CROP RESIDUES IN RICEFIELDS

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The aim of this study was to identify the functional role of aquatic invertebrates in the decomposition of crop residues in ricefields. Rice roots and straw were placed in nylon bags with mesh sizes of 4 mm, 0.6 mm and 64 mm to exclude different groups of invertebrates. They were installed at the soil-floodwater interface and in the soil in experimental ricefields subject to different agrochemical regimes.

At 77 DT, mass losses of rice roots and straw were typically in the 40-50% range and 70-90% range, respectively. During the first 14 DT, significantly more root material was lost from the bags incorporated in the soil than those at the soil-floodwater interface. However, by 77 DT the position was reversed. Mass loss of straw was significantly greater at the interface than in the soil throughout the crop season.

Initial mass loss of rice roots was independent of mesh size. Between 14 and 42 DT mass loss rates were fastest in the coarsest mesh, but by 77 DT more of the material in the finest mesh had been lost than in the two coarser meshes. Conversely, mass loss of rice straw increased signficantly with increasing mesh size.

No significant effects of insecticide applications were found on the decomposition of rice roots. At 14 and 77 DT mass loss of rice straw was significantly decreased by the application of carbofuran. The broadcast application of N fertilizer significantly increased the mass loss of rice roots and rice straw at 14, 37 and 77 DT, respectively.

EFFECT OF BENOMYL AND CARBOFURAN ON *APHELENCHOIDES BESSEYI* ON RICE

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Two experiments were conducted in the greenhouse to test the efficiency of carbofuran 3G (4 kg ai/ha) applied in the soil and benomyl applied as seed treatment (0.3% by seed weight) and sprayed on rice seedlings using manufacturer's rate in controlling *A. besseyi* when introduced into flood water.

Carbofuran applied one day after transplanting (DAT) or 30 DAT did not control the nematode. Benomyl applied once as spray at 1 DAT reduced greater numbers of *A. besseyii* in seeds than when applied as seed treatment. Two sprayings of benomyl at 1 and 15 DAT in addition to seed treatment resulted in absence of nematodes in the seeds. However, benomyl sprayed five days after inoculation did not control the nematode.

A. besseyi parasitized rice plants and produced infested grains when inoculated into flood water at transplanting, maximum tillering and/or panicle initiation (PI). Seed infestation was lowest ($P>0.05$ by DMRT) when the nematode was introduced at PI or at transplanting when seeds were given benomyl seed treatment before sowing.

PLANT PARASITIC NEMATODES ASSOCIATED WITH UPLAND RICE AS AFFECTED BY DIFFERENT DURATIONS OF CULTIVATION IN WEST SUMATRA, INDONESIA

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A survey was conducted in 100 fields in 2 upland rice growing areas in West Sumatra (Situing and Kaumang Kuning), Indonesia to determine the frequency of occurrence of plant parasitic nematodes as affected by the duration of cultivation. A total of 1000 root and soil samples were collected at maturity from fields cultivated for 1, 2, 4, 8 and 16 years. *Pratylenchus zeae* was detected in 100% of the fields cultivated for 4, 8 and 16 years with increasing population density as duration of cultivation increased. The rice root knot nematode, *Meloidogyne spp.*, was found in 87% of the roots sampled from Kaumang Kuning while only 12% was found in Situing. It occurred in high population densities in both areas. *Helicotylenchus* spp., *Criconemella spp.* and *Xiphinema* spp. were found sporadically in fields cultivated from 1 to 4 years but in high population densities. Other genera occurring in low population densities in some of the fields surveyed were *Scutellonema sp., Hoplolaimus sp.* and *Tylenchorhynchus sp.*

Based on the frequency of occurrence, *P. zeae* and *Meloidogyne* spp. are considered potentially important nematode pests associated with upland rice especially in fields planted continuously over a long duration.