AGRICULTURAL SCIENCES

1. DIVERSITY AND FORAGING BEHAVIOR OF INSECT POLLINATORS OF VEGETABLE CROPS IN SELECTED FARMS OF NUEVA ECIJA

ANNIE MELINDA P. ALBERTO, EPIFANIA P. CASTRO, and VIRGINIA C. TUMIBAY Central Luzon State University, Science City of Muñoz, Nueva Ecija

A study on the diversity of insect pollinators of vegetable crops in selected farms in Nueva Ecija was conducted to survey and assess the diversity of insect pollinators and to observe the foraging behavior of pollinators.

The insect pollinators were observed on the flowers of seven species of common vegetable crops such as garlic, red pepper, corn, radish, onion, red bell pepper and string beans in selected farms of Nueva Ecija. Their foraging behavior was observed from 0600H to 1800H for every vegetable crop. Foraging activities of the pollinators were studied according to the direction and duration of pollinators on the individual flower and within and between inflorescence.

Ten (10) species of insect pollinators were identified and classified under three orders, seven families and ten genera. Among these pollinators were identified and classified under three orders, seven families and ten genera. Among these pollinators were identified and classified under three orders, seven families and ten genera. Among these pollinators, *Hymenia recurvalis* recorded the highest value in density, dominance, frequency and importance value followed by *Menochilus sexmaculatus*.

The study found out that all pollinators randomly visited the flowers. Some of them stayed for a longer period of time but some are not. It was also observed that pollinators continue to forage and visit the flowers even though they were already visited by other pollinators. However, the pollinators preferred to stay in flowers which were not yet pollinated due to the presence of nectar and pollen that serve as good sources of food for them. Based on the study conducted, few species of pollinators were present in the different selected farms of Nueva Ecija due to the use of insecticides and other pesticides which could harm the pollinators.

Key words: pollinators, insects, vegetable crops, diversity, foraging, behavior, flowers, inflorescence, importance value.

2. DIVERSITY OF PHILIPPINE DERBY SPIDERS Neoscona SPECIES (ARANEIDAE, ARANEA)

AIMME LYNN A. BARRION¹, ADELINA A. BARRION², and ALBERTO T. BARRION³

¹Department of Entomology, College of Agriculture University of the Philippines Los Baños, College, 4031 Laguna

²Genetics and Molecular Biology Division, Institute of Biological Sciences. University of the Philippines Los Baños, College, 4031 Laguna

³International Rice Research Institute, Los Baños, Laguna

An extremely abundant group of animals constituting a considerable portion of the Philippine fauna are the spiders, the most omnipresent and numerous predators in both agricultural and natural ecosystems. Aside from being predators, *Neoscona* species in particular are popularly utilized as derby spiders or game spiders. Through vial-tapping and net-sweeping, a total of 619 derby spiders, consisting of 80% adults (604) and 20% immatures (15), were collected. Majority of the adult derby spiders were females (98%) and these were the ones used for identification. Based on epigyneal characteristics, 11 different species in the genus *Neoscona* were determined, characterized and described namely: *Neoscona nautica* (L. Koch), *Neoscona punctigera* (Doleschall), *Neoscona rumpfi* (Thorell), *Neoscona theisi* (Walchener), *Neoscona vigilans* (Blackwall), *Neoscona facundoi* Barrion f., sp. nov., Neoscona ampoyae Barrion f., sp. nov., and *Neoscona marauoyi* Barrion f., sp. nov., and *Neoscona lipana* Barrion f., sp. nov., *Neoscona marauoyi* Barrion f., sp. nov., and *Neoscona shereeae* Barrion f., sp. nov. The most frequently encountered derby spider was N. punctigera.

Key words: Araneae, Araneidae, derby spiders, epigyneal characteristics, f. – filia, Neoscona, predators, net-sweeping, vial-tapping

3. LEAF TRICHOMES AS RESISTANCE FACTOR IN EGGPLANT (Solanum melongena L.) AGAINST THE LEAFHOPPER, Amrasca biguttula (ISHIDA)

MERDELYN T. CAASI-LIT¹, VICTOR P. GAPUD², BELEN S. PUNZAL³, GINA E. BALAGOT³, N.S. TALEKAR⁴, and E. RAJOTTE⁵ ¹Entomology Laboratory, Institute of Plant Breeding, UP Los Baños, College, 4031 Laguna

> ²Department of Entomology, College of Agriculture, UP Los Baños,, College, 4031 Laguna

³Crop Protection Division, Philippine Rice Research Institute Muñoz, 3119 Nueva Ecija

⁴Asian Vegetable Research and Development Center Taiwan, China

⁵Department of Entomology, Pennsylvania State University, USA

Seven farmers' and commercial eggplant varieties were screened for field resistance to the leafhopper, *Amrasea biguttula* (Ishida) at the PhilRice Central experiment Station, Maligaya, Muñoz, Nueva Ecija in rice-eggplant cropping systems during the 1999 dry season. SRO2, a farmer's variety from Nueva Ecija, was found resistant while Abar, a farmer's variety from Batac, Ilocos Norte, was tolerant. Most of the commercial varieties were susceptible to t he leafhopper with Jackpot as the most susceptible entry.

To confirm the antixenotic mechanism of field resistance demonstrated by some eggplant varieties, trichome characters were measured. Results showed that SRO2 had the highest number of trichomes per field (40 sq mm) in all three sampling dates. This probably suggests that the trichome density in this variety could be the resistance factor that deters leafhopper feeding and oviposition on both 4th and 5th leaves of eggplant.

Longer trichomes were observed on Abar then followed by Dumaguete Long Purple in all sampling periods. Tolerance of Abar to leafhopper infestation could be due to longer tricbomes of this eggplant variety. IPB GS1 had the shortest trichomes at 45 and 105 days after transplanting (DAT) and Jackpot at 90 DAT. Trichome characters in IPB GS1 probably do not confer tolerance or resistance mechanism as trichome density and length was lowest in this variety in all sampling periods.

The results show that trichome characters can serve as resistance factors in some eggplant varieties and probably a combination of trichomes and chemical factors on the surface of the leaves in other varieties of eggplants.

4. ISOVITEXIN-2" -0-8-[6-0-E-p-COUMAROYLGLUCOPYRANOSIDE| FROM UV-B IRRADIATED RICE LEAVES INHIBITS FERTILITY OF *Helicoverpa armigera* (HUBNER)

MERDELYN T. CAASI-LIT¹, GREGORY J. TANNER², MURALI NAYUDU³, and MALCOLM I. WHITECROSS³

¹Entomology Laboratory, Institute of Plant Breeding University of the Philippines Los Baños, College, 4031 Laguna ²CSIRO Division of Plant Industry, GPO Box 1600, Canberra, 2601, ACT

Australia

³Division of Botany and Zoology, School of Life Sciences The Australian National University, Canberra, 0200 ACT Australia

Isovitexin-2"-0-B-[6-0-E-p-coumaroyiglucopyranoside], a novel acylated cglycosyl flavone isolated from UV-irradiated rice affected the viability of eggs laid by *Helicoverpa armigera* (Hubner) by over 90% when added to an artificial diet at 14 ppm.

The anti-fertility effect at this concentration was specific as the compound did not effect other insect growth parameters such as larval weight, duration and survival, pupal weight, duration and total time to adult emergence. When males that had consumed isovitexin-laced diet were mated with control females, there was still reduction in egg viability. This confirmed that the viability-reducing effect after mating could be male-specific.

The related compound is isovitexin-2"- $0-\beta$ -[6-0-E-feruloylglucopyranoside] was much less effective. Other compounds such as isoorientin-2"- $0-\beta$ -[6-0-E-couraroylglucopyranoside], isoorientin-2"- $0-\beta$ -[6-0-E-feruloylglucopyranoside] had little anti-fertility effect while the vitexin standard used as check flavonoid was significantly effective at higher concentration.

These novel compounds are possible candidates for use in Integrated Pest Management strategies. Many such natural products have been shown to be pestspecific, non-toxic to the environment and to the pest-natural enemies, thus representing an alternative to the use of synthetic pesticides. The low toxicity of the compounds to insects in these studies also suggests they might be appropriate for development as natural protectants for plants. With the advances of biotechnology, gene(s) for isovitexin maybe incorporated in the future into target crop varieties that are also host of this polyphagous and economically important major insect pest *Helicoverpa*.

5. THE NATURAL ENEMIES OF CAPTIVE BRED AND RANGE BUTTERFLIES

BONIFACIO F. CAYABYAB, CARLOS L. PADILLA, JESSAMYN R. ADORADA, and ROLANDO G. BAYOT Plant Quarantine Support Laboratory National Crop Protection Center University of the Philippines Los Baños, College, 4031 Laguna

Range butterflies live freely in their respective habitats i.e., plain meadow, forest and orchard while captive bred butterflies are grown inside enclosures or in butterfly houses. Both group of butterflies are attacked by natural enemies.

Field observations on natural enemies of range butterflies was done in Tarlac, Marinduque, and Laguna. Larvae and pupae of various butterflies were collected and natural enemies were monitored. Captive bred larvae and pupae were likewise monitored. This study was conducted at UP Los Baños from 1998-2000.

The natural enemies of range butterflies are birds, hymenoptera and diptera parasitoids, nuclear polyhedrosis, virus, bacteria, fungi, hemiptera and spider predators. On the other hand, the natural enemies of captive bred butterflies are viruses, bacteria and fungi. Red and black ants are most serious insect natural enemies. The rest of the above mentioned natural enemies cannot attack captive butterflies due to the net that serves as a protective sanctuary.

Experimental data on plain tiger butterfly, *Danaus chryssipus* showed that larvae and pupae of range or free flying butterflies are vulnerable to nuclear polyhedrosis virus, dipteran parasitoids and hemipteran predators. Captive bred larvae and pupae of *D. chryssipus* succumbed occasionally to nuclear polyhedrosis viruses and to ants.

The above results are invaluable to butterfly breeders particularly in producing quality livestocks for trading and other purposes.

Key words: butterflies, captive bred, range butterflies, natural enemies, Danaus chryssipus, ants

6. NEW BUTTERFLY RECORDS FROM MT. MAKILING, LAGUNA, PHILIPPINES

BONIFACIO F. CAYABYAB, ROLANDO G. BAYOT, FREDELINO P. PEÑALBA, PERCIUS GALANG, and GENARO KATIMBANG¹

¹Plant Quarantine Support Laboratory National Crop Protection Center University of the Philippines Los Baños, College, 4031 Laguna

A survey of the butterflies of MI. Makiling was done from March 1990 to April 1992. The modified Pollard's transect technique was used. The surveyor walked along the chosen route, counting all the butterflies seen within an imaginary box 5 m ahead of him. The width varied according to the species samples. A 6 m wide transect was used for species that arc both conspicuous and fly at low densities; a 4 m wide transect was used for other species.

Fourteen species and subspecies are new records for Luzon. These are the following: Eurema simulatrix simulatrix (Staudinger). Eurema hecabe tamiathis Fruhstorfer, Euploea leucosticos Quoy and Gaimard, Athyma kasa epimethis C & R. Felder, Neptis cymela samiola Fruhstorfer, Moduza urdneta urdneta C & R Felder, Cupha erymanthis erymanthis Drurry, Arhopala silhetensis malavica Bethune-Baker, Horaga bilineata Semper, Jamides celeno optimus Rocber, Jamides pura cordeaea Fruhstorfer, Nacaduba sanaya metallica Fruhstorfer, Hasora taminatus padma Fruhstorfer. The above new records were invariably reported to have occurred in the Visayas, Mindanao, Mindoro and Palawan.

Three theories of faunal distribution are discussed related to these new records. Of these three theories on faunal distribution of Philippines insects, Townes (1970) theory on reduction of depth of the seas is the most plausible answer to the presence of the new records.

Key words: new records of butterfly, Mt. Makiling, Laguna, species, subspecies, Townes theory

7. THE DIVERSITY OF ONION ARTHROPOD FAUNA IN NUEVA ECIJA

BONIFACIO F. CAYABYAB, CARLOS L. PADILLA, JESSAMYN R. ADORADA, ROLANDO G. BAYOT, ALICE G. AQUINO, and FREDELINO P. PEÑALBA Plant Quarantine Support Laborotory National Crop Protection Center University of the Philippines Los Baños, College, 4031 Laguna

The diversity of onion arthropod fauna in Nueva Ecija was studied from December 1999 to March 2000 in the towns of Gabaldon, Laur Bongabon, and Pantabangan. A standard sweep net with 50 sweeps per plot replicated three times was done in three farmers field per location. Other arthropods were visually counted and collected. Sorting and identification was done at the Plant Quarantine Support Laboratory, NCPC - UP Los Baños.

A total of 51 species from 34 families, and 8 orders were observed. The American scrpentine leafminer *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) an exotic pest was the most conspicuous and dominant pest throughout the growing period of onion. This pest originated from the Americas. Coccinellid beetle, predators and hymenopterous parasites were the dominant natural enemies.

Knowledge of arthropod fauna diversity is an important step in formulating pest management options in onion.

Key words: Diversity, onion, arthropod fauna, Nueva Ecija, coccinellid, hymenopter, sweep net

8. INSECT PESTS OF BAMBOO SHOOTS IN THE PHILIPPINES AND THEIR NATURAL ENEMIES

IRENEO L. LIT, JR.¹, MERDELYN T. CAASI-LIT², and MA. AMABEL A. APRICHO² ¹Museum of Natural History

²Entomology Laboratory, Institute of Plant Breeding University of the Philippines Los Baños, College, 4031 Laguno

Twelve insects were observed to attack the basal shoots (labong) of several bamboo species in plantation and natural stands. They were collected from the field, further observed in the laboratory and identified. The primary pests include at least two species of bamboo aphids (*Pseudoregma* spp.), the bamboo shoot mealybug (*Palmicultor* sp. aff. *Bambusum* Tang), the bamboo shoot soft scale (Coccus sp.), bamboo pit scale [Bambusaspis bambusae (Boisduval)], an undetermined species of thrips and bamboo planthopper (Purohita sp.). Ants that attend to honeydew-producing pests and which cover the emerging shoots with earthen nests or bivouaes are considered secondary pests and include Dolichoderus sp., Solenopsis geminata geminata, S. g. rufa, Anoplolepis longipes Jerdon and Oecophylla smaragdina Fabricius.

Three species of insects were observed to prey on *Pseudoregma* spp., namely the large ladybird beetle *Synonycha grandis*, lacewing *Chrysopa* sp. and the pyralid *Cryptoblabes aphidivora*. The predatory lycaenid *Spalgis epius* Westwood attacks bamboo shoot mealybugs. An undetermined pathogen infests the bamboo planthopper.

9. DELETING A SPECIES FROM A PEST CHECKLIST IS NOT SIMPLE: THE CASE OF THE CACAO MEALYBUG AND BAMBOO

IRENEO L. LIT, JR.¹, MERDELYN T. CAASI-LIT², MA. AMABEL A. APRICHO², and CHRISTOPHER DC. BERNARDO³ ¹Museum of Natural History, University of the Philippines Los Baños, College, 4031 Laguna ²Entomology Laboratory, Institute of Plant Breeding, UPLB

³YRAP Student Research Apppendice, Summer 2000, UPLB

Erroneous records of pests have wide-ranging implications in plant quarantine, pest management and commerce. Among the negative impacts of these erroneous records are non-acceptance of local produce of export to certain countries and proliferation of misidentifications and "inherited" errors in the literature. These are difficult to rectify and the processes involved like nationwide field surveys, taxonomic studies, rearing and feeding experiments are outlined in this paper.

A specific example, the cacao mealybug *Planococcus lilacinus* (Cockerell), erroneously recorded on Philippine bamboos, is discussed. A 3-year nationwide survey of bamboo pests did not yield any cacao mealybug specimen on any bamboo species. Simulataneously, a taxonomic study of mealybugs attacking Philippine bamboos revealed the existence of 9 species but not *P. lilacinus*. Rearing and feeding experiments showed that *P. lilacinus* would rather starve to death than feed on any bamboo species. Therefore, bamboos are not among the many host plants of *P. lilacinus* and this mealybug species is hereby deleted from the list of bamboo pests in the Philippines.

10. DISCOVERING THE LEAFMINER SPECIES OF THE CORDILLERA ADMINISTRATIVE REGION

ELIZABETH A. VERZOLA¹, RAVINDRA C. JOSHI², SONJA J. SCHEFFER³, and NICASIO S. BAUCAS¹

> ¹Department of Agriculture-Regional Field Unit-Cordillera Administrative Region (DA-RFU-CAR), BPI Compound, Guisad, 2600 Baguio City

²Department of Agriculture-Philippine Rice Research Institute (DA-PhilRice), Maligaya, Science City of Muñoz, 3119 Nueva Ecija

³United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Systematic Entomology Laboratory, Beltsville, MD 20705, USA

Since the first outbreak of leafminer on fields planted to 1,000 hectares of potato crop in 1999 at Loo, Buigas, Benguet Province, they have continued to wreck chaos to the vegetable and ornamental crops in the other locations of the Philippines highlands at the Cordillera. We present activities initiated by PLMTF with leafminer specialists around the world. In collaboration with the United States Department of Agriculture (USDA) and leafminer specialists from France, Japan and United Kingdom, four species of leafminers were discovered in Cordillera Administrative Region (CAR), using most modern molecular technique (DNA sequence data) and all known taxonomic (morphological) keys. Leafminers identified were Liriomyza chinensis (Kato). However, L, huidobrensis was the most abundant, widely distributed, and particularly injurious to many highland vegetables, including cut flowers. Molecular analysis of L. huidobrensis specimens from CAR provinces, showed that they were much more similar to the L. huidobrensis in South and Central America than they were to L. huidobrensis in California and Hawaii. Interestingly, all the four leafminer species are exotic insect pests in CAR.

Currently leafminer management campaign is being strengthened by educating farmers to reduce the misuse and abuse of pesticides, lower cost of cultivation, and minimize exposure to pesticides through networking and other information channels.

Key words: leafminers, vegetables, Cordillera Administrative Region, DNA sequence, genetic analysis, networking

11. POLYMERASE CHAIN REACTION-BASED TECHNIQUE FOR DETECTION AND MONITORING OF MOVEMENT OF MOBILE ELEMENTS IN Raistonia solangcearum

RINA D. BAGSIC-OPULENCIA¹, ASUNCION K. RAYMUNDO¹, and MARK FEGAN²

¹Microbiology Division, Institute of Biological Sciences University of the Philippines Los Baños, College, 4031 Laguna

²Department of Microbiology and Parasitology The University of Queensland, Brisbane, Queensland, Australia 4072

Ralstania solanacearum is the causal agent of bacterial wilt, the devastating plant disease of many important crops such as banana, tomato, potato, tobacco, eggplant, ginger and peanut throughout the world. A repetitive element previously cloned from the bugtok strain of R. solanacearum distinguished Philippine banana strains from the vegetables strains by DNA hubridization and by Polymerase chain reaction (PCR). These primers were based on the flanking regions of this element and were previously shown to specifically detect Philippine banana strains. In the present study, these primers were shown to also detect a peanut strain from Indonesia, Heliconia strain from Costa Rica and banana strains from Honduras, Costa Rica and Panama (RFLP 24), all phylogenetically related to Philippine banana strains. Likewise in this study, the repetitive element was sequenced to determine its significance in the organism and to design primers for a more robust and rapid PCR. Analysis of the sequence revealed the presence of an insertion sequence (IS) with terminal direct and inverted repeats. Iss are mobile genetic elements which can insert multiple sites in a target molecule and cause mutation. They are sometimes associated with pathogenicity and virulence functions in plant pathogens.

Primers based on the internal region of the IS amplified a 217 bp product after 2 hr PCR compared to 8 hr using the other set of primers. The new primer set detected not only the Philippine banana strains and members of RFLP 24 but also *Heliconia* strain from Columbia, *Pothos* strains from Hawaii, anthurium strains, and banana strains from Columbia and Peru (RFLP 25). A distantly related potato strain from Peru was also detected indicating that the Iss are more widely distributed than previously revealed. The PCR primers are useful in monitoring movement of the ISs within population of *R. solanacearum* to gain better understanding of the world-wide epidemiology of this organism.

Key words: bacterial wilt, Ralstonia solanacearum, insertion elements, PCR, primers

12. INFLUENCE OF MYCORRHIZAL FUNGI AND Meloidogyne graminicola INTERACTION ON GROWTH OF ONION (Allium cepa L.)

EVELYN B. GERGON¹, M. BROWN², G. BALAGOT¹, C. ESCANO². RUBEN M. GAPASIN³, and SUSAN A. MILLER⁴

¹Philippine Rice Research Institute, Maligaya, Munoz, 3119 Nueva Ecija ²National Institute of Moleculur Biology and Biotechnology, PULB, College, 4031 Laguna ³Visayas State College of Agriculture, Baybay, Leyte ⁴Ohio State University, U.S.A.

The interaction between vesicular-orbuscular mycorrhizal (VAM) fungi and root-knot nematode *Meloidogyne graminicola* was studied in the greenhouse using two onion varieties, Yellow Granex and Red Creole. The first experiment used P-deficient soil without added fertilizer while in the other, the soil was fertilized with 20 bags of 14-14-14/ha. Onion plants inoculated at seeding and at transplanting with three species at VAM fungi- *Glomus mossage*, *G. fasciculatum*, and *Gigaspora* sp. were planted in sterile soil and in soil infested with *M. graminicola*.

Meloidogyne graminicola significantly retarded the growth of onions in the absence of VAM fungi. The growth of VAM inoculated-plants in P-deficient soil significantly increased although bulbs were not produced. Yellow Granex inoculated with Gigaspora sp. and a mixture of the three VAM fungi were significantly taller than those plants inoculated with Glomus spp. Compared with uninoculated plants, the bulb weight and diameter of Yellow Granex inoculated with mixture of the three VAM fungi increased 54% and 24% in fertilized soil without nematode. Greater increase in bulb weight (262%) and diameter (96.4%) was obtained in nematode-infested soil indicating that the mixture of three VAM fungi increased the tolerance of Yellow Granex to M. graminicola. Bulb weight and diameter of Red Creole increased 23% and 13%, respectively, when inoculated with Glomus mossae only in soil without nematode. None of the VAM fungi used in this study reduced symptoms of root-knot disease in onion as indicated by percentage of galled roots.

Key words: interaction, Meloidogyne graminicola, VAM, root-knot, onion, Yellow Granex, Red Creole

13. DEVELOPMENT OF QUICK AND EFFICIENT SCREENHOUSE TESTING FOR NEMATODE RESISTANCE AND SALT TOLERANCE FOR TRANSGENIC RICE

RHODORA R. ALDEMITA, MARISCHELLE M. ROSARIO, DOMINIQUE GALAM, and EVELYN B. GERGON Plant Breeding and Biotechnology Division Philippine Rice Research Institute, Maligaya, Muñoz, 3119 Nueva Ecija

Crop improvement through genetic engineering requires the generation of a number of transgenic plans containing the gene for resistance, subsequent screening for the presence of the transgene and its correlation to phenotypic expression. Due to the delicate nature of the transgenic plants, screening for the phenotypic expression of the transgene should he conducted with utmost care and should be conducted in a contained screenhouse as per the National Biosafety Committee of the Philippines. At PhilRice, a quick and efficient screenhouse testing was developed for nematode resistance based on the procedures at IRRI and PhilRice. The testing was conducted in non-transgenic hybrid rice for 2 months. In addition, an efficient screenhouse testing for salt tolerance using sandy loam soil was developed using non-transgenic inbred rices in the screenhouse. Results of these evaluations and its application to large scale transgenic rice testing will be presented.

Key words: Oryza sativa, nematode resistance, salt tolerance, screenhouse evaluation

14. MOLECULAR CHARACTERIZATION OF ANTHER-CULTURE DERIVED INDICA RICE (Oryza sativa) VARIANTS

NENITA V. DESAMERO, SHARON S. MACABALE, and MARTHA V. CHICO Philippine Rice Research Institute, Maligaya, Muñoz, 3119 Nueva Ecija

Stable breeding lines were generated from anther culture (AC)-derived variants of indica rice variety Wagwag and IR64. Differences in some agronomic traits were established for some variants. Other variants, however, are morphologically and agronomically similar. To genetically differentiate these variants, we DNA fingerprinted them using RAPD (randomly amplified polymorphic DNA) and SSR (simple sequence repeats) markers. A total of 21 and 30 RAPD primers and 20 and 27 SSR primers were assayed for Wagwag and IR64 variants, respectively. With Wagwag, 16 (76.2%) and 19 (95%) RAPD and SSR primers, respectively, are polymorphic for IR64 variants. Polymorphic bands amplified from polymorphic primers were scored and used in

the cluster analysis. A total of 50 (average of 3 per primer) and 140 (average of 6 per primer) bands were generated from polymorphic RAPD primers for Wagwag and IR64, respectively, of which, 49 (98%) and 70 (50%), respectively, are polymorphic bands. While for SSR, 70 (average of 4 per primer) and 22 (average of 2 per primer) loci were amplified from the polymorphic primers for Wagwag and IR64, respectively. The RAPD and SSR markers effectively detected molecular genetic variation among the variants. The AC-derived variants were genetically differentiated as well from the seed-derived progenitors. The efficiency of genetic differentiation depends on the population. With Wagwag variants, clustering was more discrete with SSR markers, while for IR64, RAPD markers genetically separated the variants better than with SSR markers.

Key words: anther culture, RAPD, SSR, indica rice, variants

15. RESPONSE OF CONVERTED BT CORN HYBRIDS AGAINST Ostrinia furnacalis Guenee INFESTATION UNDER LIMITED FIELD RELEASE CONDITION

EDUARDO C. FERNANDEZ¹ and MANUEL L. LOGROÑO² ¹Entomology Laboratory, Institute of Plant Breeding, College of Agriculture University of the Philippines Los Baños, College, 4031 Laguna

²Agroseed Corp., 7th Flr. Ayala Life-FGU Center, Filinvest Complex, Alabang, Muntinlupa City

Corn borer, Ostinia furnacalis Guenee remains the number one insect pest problem in corn production causing yield loss from 20 to 80 percent and under heavy infestation will result to total crop failure. In this study, three locally adapted corn hybrids converted with crylAb (MON 810) gene expression from Bacillus thuringiensis var. kurstaki were evaluated under limited field release condition in the Agroseed Research Station at Lagao, General Santos City together with their isogenic hybrids and local checks. The objective of the field test was to verify the resistance reaction of these materials against Ostrinia furnacalis Guenee observed under containment condition, and compare their agronomic performance. Natural infestation was augmented by artificially infesting about-to-hatch corn borer eggmass at the late vegetative and silking stage. All safety guidelines required by the National Committee on Biosafety of the Philippines (NCBP) in the conduct of the trial were rigorously followed. The Bt corn hybrids exhibited highly resistant reaction in terms of leaf, stalk, and ear feeding damage rating. Significantly, lower counts in the number of entrance holes and length of tunnel in the stalk, number of entrance holes and length of tunnel in the car and the number of larvae and pupa recovered from plants sampled 90 days after planting was noted on the Bt corn entries compared with the non-Bt entries. The trial also demonstrated the high

specificity of the Bt protein (delta-endotoxin) against the corn borer since many beneficial non-target insects particularly Green lacewing, spiders and coccinelid beetles were abundant both in the Bt and non-Bt plots. Aphid population was also higher on the Bt plants. Bt corn yield of 7.1 to 8.5 Uha was significantly higher compared to the non-Bt corn yield of 4.4 to 5.1 Uha resulting in a yield difference of 1.6 to 3.4 Uha or 30 to 69%. The susceptible check, Supersweet yielded only 1.44 tons. The harvested ears of the Bt corn also had better quality because of lower incidence of Diplodia ear rot and Fusarium ear rot as a result of no corn borer damage on the ears.

Key words: com borer, Ostrinia furnacalis, Bt com, crylAb, Bacillus thuringiensis, NCBP, feeding damage, ear quality, Diplodia ear rot, Fusarium car rot

16. EFFECT OF DIFFERENT LEVELS OF NITROGEN AND PHOSPHORUS APPLIED SINGLY AND COMBINED ON THE GROWTH AND FLOWER YIELD OF GLADIOLUS (*Gladiolus* spp.)

HERMINIGILDA A. GABERTAN and CECILIA N. CALIBO Bureau of Plant Industry-Los Baños National Crop Research and Development Center, Los Baños, Laguna

The study was conducted at the Bureau of Plant Industry-Los Baños National Crop Research and Development Center, Los Baños, Laguna. The study aimed to identify the best rate of nitrogen and phosphorus fertilizers applied singly and combined on the performance of gladiolus (Red Long variety) particularly on flower quality and corn yield and to evaluate the economic profitability of the nitrogen and phosphorus applied at different rates. Plant parameters were statistically analyzed following the Randomized Complete Block Design (RCBD) with three replications. Results of the study showed that for single element, plants that were fertilized with phosphorus (0-60-0) produced the most number of florets with a mean of 9, the longest spike with a mean of 66.95 cm. the biggest corn equatorial with a mean of 4.5 cm and the tallest plant with a mean of 56.04 cm. Plants given phosphorus at a rate of 0-30-0 produced the biggest diameter of floret with amean of 10.53 cm. Treatment combinations of N and P at a rate of 30-90-0 produced the most number of florets with a mean of 9, the biggest floret with a mean of 10.25 cma nd the longest spike with a mean of 65.68 cm, the biggest corn equatorial with a mean of 4.54 cm while the rate of 30-30-0 gave the tallest plant with a mean of 56.87 cm. Plants fertilized at a rate of 30-60-0 yielded the highest number of corns with a mean of 260,000 corns per hectare. The highest computed net benefit was recorded in plants fertilized with N and P at the rate of 30-60-0 with P 386,357.00.

Key words: gladiolus, fertilization, flower yield

17. EFFECT OF TIME AND TYPE OF PINCHING ON THE FLOWER PRODUCTION OF ROSES (Rosa spp.)

HERMINIGILDA A. GABERTAN and CECILIA N. CALIBO Bureau of Plant Industry-Los Baños National Crop Research and Development Center, Los Baños, Laguna

Cutflower production becomes an important industry in Asia and is developing into a major industry in the Philippines. There is an increasing demand both for domestic and export markets all throughout the year. Roses being one of the top major cutflowers in the country have the greatest potentials as additional source of income among farmers. The study was conducted from 1997 to 1999 at the Bureau of Plant Industry-Los Baños National Crop Research and Development Center. Primarily the study aimed to evaluate the response of Bravo variety of two types of pinches and to identify the number of days required for a flower to develop up to "harvest maturity" following a pinch. Plants were arranged in splitplot design with 4 replications with type of pinches as the mainplot and time of pinching as the sub-plot. The plants were regularly pinched (soft and hard) with an interval of 15, 30 and 45 days. Soft pinched plants produced significantly longer flower bud stem length with a mean of 27.42 cm. Hard pinched plants done every 30 days had the fastest flower bud maturity with a mean of 39 days and the earliest shoot was formed 19 days after hard pinched. Diameter of flower ranged from 8.42 to 9.26 cm and most of the flowers lasted from 4-7 days.

Key words: roses, Rosa spp., cutflower, Bravo variety, harvest maturity, hard pinch, soft pinch

18. ACCELERATING DOUBLED HAPLOID LINE GENERATION IN RICE THROUGH ANTHER CULTURE MEDIUM MODIFICATION

MARIA CORAZON N. JULATON, NENITA V. DESAMERO, LAILA B. STA. MARIA, LENIE P. ROMANO, MARTHA V. CHICO, WILHELMINA E. VILLALBA, and SHARON S. MACABALE Philippine Rice Research Institute, Maligaya Science City of Muñoz. 3119 Nueva Ecija

In this study, we evaluated two anther culture callus induction media, 2,4-D (MNK) and phenylacetic acid (PAA)-enriched (P10NB) medium, in ten rice genotypes distributed over three planting seasons. Results indicated that the relative advantage of using either one of the media depends on the genotype. At least 50% of the genotypes formed more calli in PAA-enriched medium. With PAA, plants

can be regenerated from cultured anthers directly, that is, without transferring to regeneration medium, referred to as one-step method. In contrast, the two-step method requires transfer of callused explant from callus induction to regeneration medium. Higher frequency of green plant regeneration was recorded in P10NB compared with MNK. Some genotypes performed better in P10NB one-step, while others in two-step method. More plantlets per anther and more fertile doubled haploid plants were obtained from PAA-enriched medium, with better response in two-step method. Initial assessment indicates that the doubled haploid plants regenerated from P10NB matured earlier than those regenerated from MNK. More so, the doubled haploid plants from P10NB-one step matured earlier than those from two-step method. This resulted in the shortening of the seed to seed cycle period from anther source establishment to R_1 seed harvest of regenerated doubled haploid plants. Although better responses were observed in the two-step, the one-step method has the advantage of economizing the culture medium requirement, its chemical components, and the time and labor spent for medium preparation.

Key words: 2,4-D, phenylacetic acid, anther culture, doubled haploid, direct regeneration

19. IN SEARCH OF EXPLANT SOURCE AND CULTURE MEDIUM FOR GENERATION OF EMBRYOGENIC TISSUES FROM IN VITRO CULTURES OF RICE

SHARON S. MACABALE, NENITA V. DESAMERO, and MARTHA V. CHICO Philippine Rice Research Institute, Maligaya Science City of Muñoz. 3119 Nueva Ecija

Good quality embryogenic tissues are excellent explant materials for transformation work. In this study, we evaluated the *in vitro* response of various rice genotypes in different callus induction media, using different explants (mature seed scutellum and epicotyl and young inflorescence). Embryogenic calli were formed from scutellum and epicotyl of four genotypes (LX 286, IR72, IR64 1-1-4 and IR64) in two induction media (RS and RSM). Genotypic differences in callus formation, ranging from 0 to 50%, was observed. The calli generated were either compact and nodular or soft, wet and nodular. Calli were formed within 11 to 27 days and 23-30 days in RS and RSM medium, respectively. The epicotyl explants were cut into top and bottom parts. Calli were formed in the bottom part of the epicotyl and in RS medium only, which varied with genotype. The sorbitol and mannitol added in the RSM medium inhibited callus formation in scutellum and epicotyl cultures. Embryogenic tissues were also induced in young inflorescence (<1 mm) of six genotypes (IR64, IR64 1-1-4, IR64 1-3-12, IR64 1-4-6, IR64 1-7-7, IR64 1-7-1) in two callus induction media (MNK, P10NB). Embryogenic callus formation in 2-4,D-enriched medium (MNK), ranging from 0 to 68%, varied with genotype. Green plants were regenerated from these calli. In PAA-enriched induction medium (P10NB), tissue enlargement, direct somatic embryogenesis and plant regeneration were obtained. When transferred to regeneration medium, the enlarged tissues and somatic embryos regenerated whole plants. The success in transformation depends largely on the regeneration ability of the explant. Transformation work will be conducted using the embryogenic tissues generated in PAA-enriched medium as explants.

Key words: somatic embryos, scutellum, epicotyl, inflorescence

20. GRAFTED TOMATO FOR OFF-SEASON PRODUCTION

LUN G. MATEO, DENNIS R. CACHO, ANACLETO F. BALA¹, JAMES R. BURLEIGH², and CHRISTIAN ULRICHS² ¹Central Luzon State University, Science City of Muñoz, 3120 Nueva Ecija

Central Luzon State Oniversity, Science City of Munoz, 5120 Nueva Ecija

²Asian Vegetable Research and Develapment Center (AVRDC), Taiwan

An experiment was conducted to determine the efficacy of grafting Apollo and CLN5915, a hybrid tomato onto two different rootstock, EG 203 an eggplant and H7996, a tomato variety. Both rootstocks are resistant to bacterial wilt. The beds for planting were raised 30 cm high and provided with rainshelter using 32mesh plastic net.

One month Apollo and CLN5915 seedlings were grafted to the rootstock using plastic tube and put inside a chamber with approximate relative humidity of 85-90% for a week. The surviving newly grafted plants were transferred into a hardening chamber prior to transplanting.

Results for the two-year experiment on percent survival revealed that both grafted tomato varieties had significantly higher plant survival than the non-grafted plants. Grafted Apollo to EG 203 and H7996 gave 97.2 and 77.8 percent plant survival while non-grafted Apollo had zero survival. All plants were attacked hy bacterial wilt. For CLN5915, grafted plants had 97.2 and 91.7 percent survival while the non-grafted plants had 70.8.

In terms of yield, both tomato varieties when grafted to either EG 203 or H7996 yielded significantly better than the non-grafted plants.

Grafted Apollo to EG 203 yielded 13.1 and those grafted to H7996 yielded 11.7 t/ha, respectively. Non-grafted plants yielded 1.3 t/ha. For CLN5915 grafting to EG 203 and H7996 gave comparable yields with an average yield of 21.3 and

21.7 Uha. However, significantly lower yield was obtained from non-grafted CLN5915 with an average of 10.4 t/ha.

Key words: tomato, grafting

21. PILOT SCALE PRODUCTION OF Agaricus bitorquis IN TUNNEL

ANGELITA P. MEDALLA, PATROCINIO O. MACALINAO, and LAYDA C. AGUALIN Mushroom Technology Project, Research and Development Center Rizal Technological University, Boni Avenue, 1500 Mandaluyong City

The successful result of the laboratory investigation (bench scale production) for growing Agaricus bitorquis (Hot Agaricus) necessitated a larger scale cultivation to determine its economic feasibility. The research was undertaken using hybrid strains of high temperature tolerance. Yield and quality of fruit bodies from selected strains were tested without artificially controlled cooling facilities. The selected strain was grown following the same procedures for spawning, casing, pinning and cropping of A. bisporus; the same media composition was likewise used.

Results showed that during the vegetative stage, a significant mycelial run was exhibited by *Agaricus bitorquis* (Strain 101-Australia). There was a profuse mycelial run during the first week of incubation covering 85% of the compost. Complete ramification within 12 to 14 days was noted; casing was applied oncc ramification was seen to be completed. Pins were observed to develop when the carbon dioxide content of air was recorded at 800 ppm or lower Harvestable mushrooms appeared 18 to 20 days after casing. Harvests were made within 35 to 42 days from the day of first flush and were recorded for as long as 150 days from the first day of flush.

A significant mycelial and case runs were exhibited by the strain indicative of good fruiting yields. After eight flushes, an average net yield of 70% was recorded. A biological conversion efficiency (BCE) ranging from 30% to 35% was obtained.

These findings showed that the Agaricus bitorquis has a great growing adaptability to environment and can be cultivated under conditions with temperature from 28 to 30°C.

Key words: Agaricus bitorquis, semi-temperate mushrooms, tunnel production of mushrooms, high temperature-tolerant mushrooms, urban mushroom growing, hot buttons, tropical mushrooms

22. CULTURAL MANAGEMENT OF EXPORTABLE FOLIAGE PLANTS

AMELIA M. NICDAO and HERMINIGILDA A. GABERTAN Bureau of Plant Industry, Los Baños National Crop Research and Development Center, Los Baños, Laguna

There are inherent advantages of the country for the production of exportable foliage. One is the ideal climate for all year round production. The proximity of the Philippines to major Asian countries and the competitive wages and location costs. The study was conducted from July 1996 to December 1999 at the Bureau of Plant Industry-Los Baños National Crop Research and Development Center and was funded by DOST-PCARRD. The project aimed to collect, propagate and evaluate materials used as cutfoliage, to determine the response of each group of foliage plant varieties to different rates of fertilizer, spacing, pruning and shade and to establish a technology on the production and management of exportable foliage. Height of shoots of Dracaena marginata Lam. "Tricolor" was significantly affected by fertilizer treatments and distance of planting. Plants fertilized with 180-0-0 spaced at 60 cm significantly produced the tallest shoots with a mean of 53.19 cm. Significant differences were observed on the height of Pleomele reflexa (Lam.) N.E. Br. "Song of India" as affected by different levels of N fertilizer (60, 120 and 180 kg/ha) and time of pruning (monthly, bi-monthly and quarterly). The significantly tallest plants (100.06 cm) were from those given with fertilizer rate of 120-0-0 and pruned two months, Plant height of Dracaena sanderiana Hort. Sander ex M.T. Mast "Gold" was significantly affected by the rate of N fertilizer (3, 6 and 9 gm/pot) and percent shade, using calibrated nets (30, 50 and 70% shade). Plants fertilized with 6 gm N/pot at 70% shade significantly yielded the tallest plants with a mean height of 132.92. For Dracaena godseffiana Sander "Florida Beauty" fertilization with 3 gm N/pot and grown at 30% shade produced significantly the tallest plants with a mean height of 48.36 cm. Significant differences were observed on the height of Murraya paniculata (L) Lack "Kamuning" as affected by different rates of N fertilizer (50, 100 and 150 gm/pot) and Ca (10, 20 and 30 gm/pot). Kamuning plants applied with 100 gm N plus 20 gm Ca/pot were significantly the tallest with a mean of 125.29 cm.

Key words: cutfoliage, exportable foliage, Dracaena marginata, tricolor, D. Sanderiana, gold, D. godseffiana, Florida beauty, Pleomele reflexa, song of India, Murraya paniculata, Kamuning

23. ENHANCED EFFECTS OF POTATO EXTRACT AND REDUCED NUTRIENT LEVEL ON ANTHER CULTURE RESPONSE IN RICE

WILHELMINA E. VILLALBA, NENITA V. DESAMERO, LENIE P. ROMANO, MARIA CORAZON N. JULATON, LAILA B. STA. MARIA, and MARTHA V. CHICO Philippine Rice Research Institute, Maligaya Science City of Muñoz, 3119 Nueva Ecija

Reducing the amount of basal salts in in vitro culture medium, and using locally available nutrient supplement, such as potato, will lessen the cost of the technology. We evaluated the effect of potato extract and nutrient level reduction in anther culture response of nine genetically diverse rice genotypes. The treatments included N6 basal salts in full, one-half and one-fourth strength, with the addition of 10% potato extract in each callus induction medium. The full strength N6 salts without potato extract served as control. Results indicated varied genotypic response. Anthers of five of the nine genotypes formed calli. Three of the responding genotypes performed better in potato extract-enriched medium. Further enchancement in callus formation was obtained in potato extract-enriched medium with nutrient level reduced by half. Likewise, plant regeneration was obtained in two genotypes, with higher frequency in potato extract-enriched medium containing half strength N6 salts. More doubled haploid plants were obtained in medium with full strength N6 salts and potato extract. Moreover, reduction in incidence of necrosis was observed in potato extract-enriched medium. Further study will be conducted using higher levels of potato extract in callus induction medium and adding the extract in the regeneration medium for enhanced plant regeneration.

Key words: potato extract, anther culture, callus, plant regeneration

24. EMBRYO AND OVULE CULTURE OF THREE WOODY FRUIT SPECIES: AVOCADO, LANZONES AND RAMBUTAN

PABLITO M. MAGDALITA and LOLITA D. VALENCIA Institute of Plant Breeding, College of Agriculture University of the Philippines Los Baños, College, 4031 Laguna

Embryo and ovule culture of avocado, lanzones and rambutan was done to preserve the important genotypes identified in a mass selection breeding programme. Immature or mature embryos of these woody fruit species were excised from fruits of avocado, lanzones and rambutan and cultured on either Murashige and Skoog'of avocado and lanzones embryos was obtained using half-strength MS supplemented with 2.2 uM benzyladenine (BA). Ovules and embryos of lanzones and rambutan, respectively, germinated on either MS or DF supplemented with 0.5 uM BA and 0.5 uM naphthalene acetic acid (NAA). Germinated embryos were allowed to grow into full plants onto the same germination medium.

Key words: embryo, ovule, avocado, rambutan, lanzones, Murashige and Skoog, De Fossard, breeding, benzyladenine, naphthalene acetic acid

25. PRODUCTION OF VIRUS-FREE AND TRUE-TO-TYPE PLANTING MATERIALS OF GARLIC (Allium sativum L.)

LILIAN F. PATEÑA¹, ALICE L. BARJRING¹, LOLITA M. DOLORES¹. MARISA B. MIRANDA¹, ERHARD BARG², SYLVIA K. GREEN³, and RAMON C. BARBA¹ ¹Institute of Plant Breeding, College of Agriculture University of the Philippines Los Baños, College 4031 Laguna

²BBA, Germany

³Asian Vegetable Research Centre (AVRDC). Shanhua, Taiwan, Republic of China

Garlic (Allium sativum L.), one of the most important vegetable crops in the Philippines, is grown in about 7,674 hectares with an average production of 19,314 MT (BAS, 1998). Average yield is quite low, 2,78 t/ha compared to up to 10.6 t/ ha in Thailand, due to low quality planting materials which is attributed to accumulated diseases (particularly virus diseases) through generations of asexual propagation.

Until 1970, the only recorded virus disease of garlic in the country was the tangle top disease. In March 2000, results of our collaborative work with AVRDC and BBA showed that poty (OYDV - onion yellow dwarf virus, LYSV - leek yellow streak virus), carla (GCLV - garlic common latent virus, SLV - shallot latent virus) and allexi (MbLV - miteborne latent virus, GarVA - garlic virus A, GarVB - garlic virus B, GarVD - garlic virus D) viruses were present in our garlic planting materials.

We have devised a technique for producing virus-free planting materials of garlic through a combination of shoot tip and meristem culture. With this technique, 2 accessions from Indonesia (12G - Lumbukuning, 17G - Kuning) and 3 accessions from the Philippines (16G - Tanauan, 22G - Laguna, 30G - Ilocos White) were tested free of the above viruses using ELISA, PCR and electron microscopy indexing techniques. Field trials showed that *in vitro*-derived bulbs had higher yield in terms of number and size of cloves compared to conventionally propagated

bulbs. These results indicate that yield of garlic can be increased using virus-free planting materials. Initial study also showed that tissue culture did not alter the genetic fidelity of the micropropagated materials.

Key words: garlic, Allium sativum, virus-free, poty, Carla and allexi viruses, genetic fidelity

26. A SIMPLE AND RELIABLE PROTOCOL FOR SOMATIC EMBRYOGENESIS AND PLANTLET REGENERATION IN MANGO (Mangifera indica L.)

LILIAN F. PATEÑA, LUZMINDA R. CARLOS-REFUERZO, and RAMON C. BARBA Institute of Plant Breeding, College of Agriculture University of the Philippines Los Baños, College 4031 Laguna

The 'Carabao or Manila Super' mango, a virtually neglected fruit before the advent of KNO_3 flower induction in the early 1970's, has surged as the 3rd leading Philippine export fruit after banana and pineapple. By 1997, it ranked 1st in value of P10.34 billion overtaking banana and pineapple. Prolonging the shelf life of the fruits to further increase the value of mango can be done through biotechnology. The bottleneck is a reliable embryogenesis and regeneration protocol since after gene transfer, plants must be regenerated from the transformed tissues too complete the process.

In our tissue culture studies, we have developed a simple and reliable protocol for somatic embryogenesis and plantlet regeneration in mango: 8 strains of 'Carabao' and 2 unidentified varieties, PHL 12384 and PHL 12378. Over 40 batches of nucellar explants from immature fruits (0.75-5.0 cm long) were cultured *in vitro* from April 1999 to April 2000. Two media were identified, MMSE, Mango Medium for Somatic Embryo Induction, Proliferation and Germination, and MMPR, Mango Medium for Plantlet Regeneration. These are now routinely used. The protocol is reproducible in 14 other varieties of mango. A new revelation is that shifting the base medium from Gamborg's B5 Medium to our own formulation, R Medium (Barba and Pateña's formulation), browning was effectively controlled, which is very crucial in any transformation work. This breakthrough may pave the way to solving the problem of phenolic exudation in plant tissue culture. Browning has limited the successful *in vitro* culture of many woody species including the mango.

Key words: mango, Mangifera indica L, 'Carabao', 'Manila Super', R Medium, somatic embryogenesis, protocol, nucellus, browning

27. IN VITRO GERMINATION OF FRESHLY HARVESTED AND CONSERVED INDIGENOUS ORCHID SEEDS

LILIAN F. PATEÑA, RACHEL C. SOTTO, NESTOR C. ALTOVEROS, ALICE L. BARIRING, MARY ANN A. DIONEDA, and RAMON C. BARBA Institute of Plant Breeding, College of Agriculture University of the Philippines Los Baños, College 4031 Laguna

This study was conducted to develop a technique for conserving the biodiversity of Philippine indigenous orchids using seeds. Our strategy was to conserve these materials under controlled environment. This method complements other existing conservation efforts which involve maintenance of live plants.

Orchid seeds were excised from collected capsules, dried to d•7% and kept at 0°C in the cold storage facilities of the National Plant Genetic Resources Laboratory, IPB. They varied in size, shape and color. Prior to storage, initial viability and % germination in vitro were determined. A total of 74 accessions representing 32 known species and 22 known genera were tested for viability using the modified topographical tetrazolium test (TTZ). A total of 251 accessions representing 29 priority species were tested in vitro. Dried seeds were stored from 2 to 13 months and rehydrated prior to the TTZ viability and in vitro germination tests. Most of the conserved seeds still had high viability (>90%, e.g. Acanthephippium, Dendrobium amethystoglossum, Dendrobium taurinum, Phalaenopsis amabilis) during the 13-month storage period although viability of some species declined drastically (<10%, e.g. Rhynchostylis retusa). Results on in vitro germination of conserved seeds showed that germination decreased with time, although germination of >80% was still obtained after one year of storage (e.g. Dendrobium heterocarpum, Grammatophyllum scriptum). These findings indicate that different species respond differently to low temperature and low moisture storage and consequently to in vitro germination. The above conservation technique is applicable to specific species of Philippine indigenous orchids.

Key words: Philippine indigenous orchids, low temperature, low moisture storage, in vitro conservation

28. MICROSATELLITE DNA MARKERS FOR GENOTYPE IDENTIFICATION IN PHILIPPINE PAPAYAS

MARIBEL M. ZAPORTEZA, ROBERTA N. GARCIA, AMY EMILIANA N. BERNARDO, ANTONIO S. LAURENA, and EVELYN MAE TECSON-MENDOZA Institute of Plant Breeding, College of Agriculture University of the Philippines Los Baños, College 4031 Laguna

Very little is known on the extent of genetic diversity among the Philippine papayas and their wild progenitors. Hence, this study aimed to provide basic information on their genetic base in order to improve the efficiency of the papaya improvement breeding program. Microsatellite markers or simple sequence repeats (SSRs) are DNA markers that provide high level of certainty in genotype identification.

SSRs were obtained from the papaya sequences in the Genbank database and 8 primers were designed based on their highly conserved flanking regions. A Polymerase chair reaction (PCR) protocol was developed using these primers to amplify the SSRs. The PCR products were electrophoresed in a 3% agarose gel, stained with ethidium bromide and visualized under UV light.

Initial efforts using one primer pair generated SSRs from 32 papaya lines. A total of 21 bands, which translated to 27 banding patterns, were obtained. The observed similarity indices among the different lines ranged from 0.33 to 0.95. The samples clustered into three major groups with 0.5 to 0.6 degree of relatedness.

The high level of polymorphism observed demonstrates the microsatellite's capability to quantify genetic diversity and identify the different papaya genotypes. Six more primers will be used to develop SSR markers for our papayas.

Key words: papaya, genetic diversity, microsatellites, SSRs

29. DEVELOPMENT OF NEW PAPAYA RINGSPOT VIRUS COAT PROTEIN AND REPLICASE GENE CONSTRUCTS FOR PAPAYA TRANSFORMATION

VERMANDO M. AQUINO¹, PIERRIDEN A. PEREZ¹, and STANISLAW FLASINSK1² ¹Institute of Plant Breeding, College of Agriculture University of the Philippines Los Baños, College 4031 Laguna

²Monsanto, 700 Chesterfield Parkway North, St. Louis, MO, USA

Pathogen-derived resistance has been successfully used in the management of virus diseases in crop plants. The CP-mediated or replicase-mediated resistance

has been found to be sequence-specific and effective if the challenging virus has a high degree of sequence identity with transgene. In this study the collection of papaya ringspot virus (PRSV) isolates from infected papaya grown in different geographical areas of Philippines was obtained. Viral RNA was extracted from PRSV-infected leaves and reverse transcriptase was used to synthesize the complementary DNA (cDNA) using oligo dT as a primer. To amplify the 3' part of viral genome, which includes the C-terminus of nuclear inclusion protein a (Nla) gene, whole nuclear inclusion protein b (Nlb), and the coat protein (CP) genes, three sets of primers were made in the conserved regions of the PRSV genome. Sequences of the different isolates were compared and variability between isolates was determined. Based on sequence data, one isolate was chosen to develop vectors for generation of papaya resistant to Philippine isolates of PRSV. Specific primers for the coat protein and replicase were made and used to amplify gene fragments. PCR products for the CP and replicase gene were cloned into plant expression cassettes under the control of enhanced 35S promoter. The vectors will be used in papaya transformation to generate resistance to PRSV.

Key words: papaya ringspot virus (PRSV), pathogen-derived resistance, coat protein-mediated resistance, replicase-mediated resistance, transgenic papaya

30. MECHANISM OF RESISTANCE OF PEANUT, Arachis hypogaea L. AGAINST PEANUT LEAFHOPPER, Empoasca ricei Dworakowska and Pawar

MARIA LUZ J. SISON and CANDIDA B. ADALLA Entomology Laboratory, Institute of Plant Breeding, College of Agriculture University of the Philippines Los Baños, College, 4031 Laguna

Five peanut entries, namely NC Ac 343, Myra (PC), UPL Pn4, UPL Pn6, and IPB Pn83-68-140 were tested to determine the mechanism of resistance to the peanut leafhopper, *Empoasca ricei* Dworakowska and Pawar. These were selected based on different levels of resistance to *E. ricei*. NC Ac 343 was the most resistant entry, which showed both antibiosis and antixenosis effects. This entry had the lowest number of adults that settled on the test plants and had twice the number of trichomes per microscopic field than those of the susceptible cultivars IPB Pn83-68-140 and UPL Pn6. Nymphal development was longer while adult longevity was comparatively shorter on the resistant cultivars NC Ac343 and Myra (PC). A significant negative correlation ws found between various morphological characters and number of adult leafhoppers.

Key words: peanut, Arachis hypogaea, peanut leafhopper, Empoasca ricei, antibiosis, antixenosis

31. ANIMAL MANURE AND MYCORRHIZA APPLIED SINGLY AND IN COMBINATION FOR THE CONTROL OF THE RICE ROOT-KNOT NEMATODE (Meloidogyne graminicola Golden and Birchfield) IN GREEN ONION (Allium fistulosum L.)

MARIA LIMA D. DIONGZON¹ and RUBEN M. GAPASIN² ¹Cebu State College of Science and Technology, Barile Campus, Barile, Cebu City

> ²Department of Plant Protection, Visayas State College of Agriculature (VISCA) Baybay, Leyte 6521-A, Philippines

The studies conducted in pot experiments aimed to: (1) evaluate the effects of different animal manure on the growth of green onion and *Meloidogyne* graminicola population and development and to identify the most effective rates against this nematode (2) determine the suppressive effects of combining vesiculararbuscular mycorrhiza (VAM) and animal mature against the nematode and (3) determine the effects of time of application of mycorrhiza-animal manure combination on onion growth and nematode development and population.

The different animal manure (cow, chicken, goat, sheep and pig) improved the growth of green onion. However, the chicken and cow manure at 5 and 10 tons/ha significantly reduced the number of galls, egg masses and nematode population in roots and in soil compared to the lower rate of 2 tons/ha. Vesicular arbuscular mycorrhiza (VAM) combined with the animal manure increased the number of spores and mycorrbizal infection that resulted in a lower number of galls, egg masses and nematode population in roots and in soil. VAM-chicken manure gave the highest reduction of these parameters that ranged from 69 to 92%. When VAM and the animal manure were applied 2 and 4 weeks before the nematode, the number of VAM spores and percent VAM infection were higher which resulted to 84-89% reduction in galls and egg masses. Likewise, nematode population in roots and in soil was also reduced in these treatments. Top and root weights were higher in plants pre-inoculated with VAM and animal manure.

Key words: manure, VAM, root-knot nematode, Meloidogyne graminicola, green onion, Allium fistulosum

32. BIOEFFICACY OF Metarrhizium anisopliae (Metsch.) Sorokin AGAINST THE MIGRATORY LOCUST, Locusta migratoria manilensis Meyen, ON SUGARCANE

JULIETA D. RECUENCO Sugar Regulatory Administration, Luzon Agricultural Research and Extension Center Floridablanca, 2007 Pampanga

The outbreak of the migratory locust in Central Luzon and other parts of Southern Luzon prompted the need to develop control strategies to effectively reduce the insect's population. One of these methods is the application of environment friendly biotic agents like the entomophathogenic fungus, Metarrhizium anisopliae (Metsch.) Sorokin commonly known as green muscardine fungus. This was tested against the locust using different inoculum densities and sources. Spraying locust nymphs of varying ages with the fungus at 10⁸ conidia/ ml, gave 100% infection and mortality. The older hoppers had faster and more infection with higher percent mortality than the younger ones. Infection of hoppers and adults was significantly greater at higher inoculum density (10⁸ conidia/ml) than at lower inoculum density (10⁴ conidia/ml). M. annisopliae - infected males at different male to female ratios resulted in higher percent mortality on 1:1 male to female ratio (87.5) than those inoculated with 1:5 male to female ratio (68.75). Comparing M_{1} – infected males and M_{2} – infected rice hull, M_{2} – infected males afforded faster infection than $M_{\rm c}$ – infected rice hull. The results indicate that $M_{\rm c}$ anisopliae is an effective and promising biological control agent against the destructive migratory locust, Locusta migratoria manilensis Meyen, that damages sugarcane.

Key words: Metarrhizium anisopliae, Locusta migratoria manilensis, inoculum density, inoculum sources, bio-control

33. MANAGEMENT OF THE GOLDEN APPLE SNAIL MENACE THROUGH ENVIRONMENT-FRIENDLY APPROACHES

MARIO S. DELA CRUZ and RAVINDRA C. JOSHI Crop Protection Division, Department of Agriculture-Philippine Rice Research Institute (DA-PhilRice), Maligaya, Science City of Muñoz, 3119 Nueva Ecija

The golden apple snail is an alien species of freshwater mollusks scientifically known as *Pomacea canaliculata* (Lamarck). Locally, it is referred to as "Golden Kuhol". It was introduced in the Philippines for commercial purposes from South

America in the 1980s. Bred and promoted as a potential alternative source of food protein of low-income Filipino farmers for years, it has become a plague in rice farms all over the country including the Ifugao Rice Terraces.

We present new alternatives to reduce the misuse and abuse of synthetic commercial molluscicides. These are prior to planting scleet Philippine seed board rice varieties which exhibit less golden apple snail damage, and apply basal application of inorganic fertilizers as soil incorporation at the recommended rates. Botanical attractants should be used prior to crop establishment to facilitate easy and swift collection of golden apple snails. A new recipe "Chicharon Kuhol" (Snail crackers) for human consumption was formulated to promote its utilization. Additional information on the right-use technology of commercial synthetic molluscicides by proper timing, application techniques, and lowering the recommended dosage to lower the mortality of native snails (non-destructive snail species), but without reducing the mortality of the golden apple snail is highlighted.

Key words: Golden snail, Pomacea canaliculata, management

34. SCREEN BARRIERS REDUCE INFESTATION OF CABBAGE WEBWORM AND DIAMONDBACK MOTH ON PAK-CHOI

AUREA C. ROXAS¹, MARILYN G. PATRICIO¹, TEOTIMO M. AGANON¹, MANOLITO A. BAYUGAN¹, ROLANDO V. PAGADUAN¹, MARLON A. MACTAL¹, JAMES R. MURLEIGH², CHRISTIAN ULRICHS², and N.S. TALIKAR² ¹Central Luzon State University, Muñoz, 3120 Nueva Ecija

²Asian Vegetable Research and Development Center (AVRDC). Taiwan

Pak-choi (*Brassicu rapa*) grown in screen barriers, $2 \ge 18 \ge 18$ m, constructed from GI pipe and 16-mesh nylon netting, effectively reduced infestations of cabbagc webworm (*Hellula undalis*) and diamondback moth (*Plutella xylostella*), but not stripped flea beetle (*Phyllotreta* sp.). In one trial conducted in January-March 1999, screen barriers reduced the incidences of plants damaged by cabbage webworm and diamondback moth by 98 and 95%, respectively. Mean yield was 1.14 kg m⁻² within the barrier, but nil in the open field. In contrast, during a second trial conducted in May-June 1999, screen barriers reduced the infestation of cabbage webworm by 76%. The screen barrier reduced but did not prevent infestation of pak-choi by the stripped flea beetle. In the open field all plants werc infested by the stripped flea beetle, whereas 77% were infested within the barrier. Diamondback moth was not present in the screen barrier and in open field. Marketable yield were 0.13 and 0.004 kg m⁻² in the screen barrier and open field, respectively. These data indicate that screen barriers reduce infestations by diamondback moth and cabbage webworm and increase yield.

Key words: screen barriers, Hellula undalis, Plutella xylostella, Phyllotreta sp., pak-choi

35. BLOOD PROTEIN POLYMORPHISMS AND ASSOCIATION WITH SOME MORPHOLOGICAL CHARACTERS IN DIFFERENT BREED GROUPS OF GOATS (Capra hircus) IN THE PHILIPPINES

ORVILLE L. BONDOC¹, CHESTER D. SOLIS², BEATRIZ R. GARCIA¹, ELIZABETH D. BELTRAN³, AGAPITA H. SALCES⁴, EMILIO C. CRUZ⁵, IVY AMOR F. LAMBIO¹, and JOHN DANIEL P. TEC¹ ¹Animal Breeding Division, Institute of Animal Science, College of Agriculture University of the Philippines Los Baños (UPLB), College, 4031 Laguna ²College of Veterinary Medicine, UPLB

³Dairy Training and Research Institute, UPLB

⁴Ubay Stock Farm, Department of Agriculture Regional Field Unit VII, Ubay, Bohol

⁵Department of Animal Science, Central Luzon State University

The genetic characteristics of 516 goats from the Philippine Goat Breed Registry were analyzed using the biochemical polymorphic loci controlling blood protein. Blood proteins from heparinized blood samples of goats belonging to different breed groups (i.e. purebred Anglo Nublan, Boeer, Saanen, Toggenburg, Native, and crosses among them) were examined using vertical polyacrylamide gel electrophoresis (PAGE). Mature weights and some body measurements (i.e. body length, wither height, heart girth, midriff girth, flank girth, head length, and head width) of the animals were also determined. The electrophoretic typing revealed variations at the protein level attributable to six polymorphic loci [i.e. albumin (Alb), α 2-microglobulin (S α 2), hemoglobin (Hb), esterase (Est), alkaline phospatase (Alp), and carbolic anhydrase (Ca)] between different breed groups. Assuming autosomal codominant genes, the gene frequencies of the polymorphic protein loci were calculated from which measures of genetic variability and genetic distances were estimated. Genetic distances between goat breeds ranged from 0.007 to 0.076. Signficant differences (P<.01) between breeds were also found for the various morphological characters. The computed least square means of the parameters were used to establish performance standards for each breed group. Using a univariate linear animal model that included the fixed effects of breed and sex and the random effects of farm location and individual additive genetic values, the genotypic effects of blood proteins on morphological measurements both between and within breeds were found to be small to moderate. While information on some biochemical polymorphic markers is found useful to clarify genetic constitution and characteristics of registered goats, more data will be needed to confirm the effects of the genotypes on production and reproduction traits, before blood protein genotypes can be considered in the selection of breeding animals.

Key words: registered goats (Capra hircus), blood proteins, polyacrylamide gel, electrophoresis (PAGE), mature weight, body measurements, genetic distance, polymorphic loci, genotype

36. LOCAL SUCCESS RATES AND COST OF PRODUCTION OF EMBRYO TRANSFER IN CATTLE: MULTIPLE OVULATION, EMBRYO RECOVERY AND VITRIFICATION OF EMBRYOS

ORVILLE L. BONDOC¹, ANNABELLE S. SARABIA², EDWIN O. SANCHEZ³, PRUDENCIO B. PEDRO⁴, GABRIEL EDSEL R. VARGAS¹, DEXTER C. AMADA, and IVY AMOR F. LAMBIO¹ ¹Animal Breeding Division, Institute of Animal Science, College of Agriculture University of the Philippines Los Baños (UPLB), College, 4031 Laguna

²Philippine Carabao Center (PCC) at UPLB, College, 4031 Laguna

³ANSA Genetics Inc., Lipa City, Batangas

⁴PCC Headquarters and Gene Pool, Muñoz, 3120 Nueva Ecija

The practical importance of multiple ovulation and embryo transfer (MOET) technologies to genetically improve and conserve superior cattle genotypes in selected nucleus herds depends on the actual success rates obtained in the field and the cost of production. This study compares the on-farm superovulatory response, non-surgical embryo recovery, and costs of production using two kinds of superovulatory drugs (i.e., ovine follicle stimulating hormone or FSH – Embryo-S® and porcine FSH – Folltropin – V® on four breeds of donor cows (Native, Australian Friesian Sahiwal or AFS, American Brahman and Holstein x Jersey cross). The average response to superovulation based on multiple corpus leteum (CL) at the time of embryo recovery is 89.5% or an average of 7.5 CL per donor (3.9 on left ovary and 3.6 on right ovary). A total of 63 embryos were collected from 12 donor cows. The 7 day-old embryos recovered were mostly in the early blastocyst (38.8%), morula (28.6%), or compact morula stage (22.4%). Most

embryos were classified as good (53.1%) or excellent (24.5%). Twenty-one (21) embryos were transferred on 15 recipient cows, of which 4 were confirmed pregnant by rectal palpation 90 days after transfer (i.e., 19.0% pregnancy rate). Thirty-three (33) embryos were cryopreserved by vitrification using EFS 40 solution. The use of Folltropin-V® resulted into significantly (P<.01) higher CL (12.0) and embryos (8.2) compared with that of Embryo-S (5.1 CL and 2.6 embryos). Greater CL ws found for Holstein x Jersey cross (16.0) and AFS (10.0) than the Brahman (6.2) and Native donor cows (6.3). The highest average number of embryos collected per flush was recorded in the AFS (9,5), followed by Holstein x Jersey cross (5.0), Native (3.7), and Brahman (3.5) donor cows. The lowest cost of production per fresh embryo is estimated when using the Holstein as the sire breed (P 2,209.37), AFS as the dam hreed (P 2,178.82), and Folltropin-V® as the superovulatory drug (P 1,897.18).

Key words: multiple ovulation and embryo transfer (MOET), superovulatory drugs, corpus luteum, non-surgical embryo recovery, donor cows, vitrification, pregnancy rate, cost of production

37. VARIATIONS IN KARYOTYPIC CHARACTERISTICS OF DIFFERENT BREED GROUPS OF WATER BUFFALOES (Bubalus bubalis)

ORVILLE L. BONDOC and MA.CARMELA GRACE T. FLOR Animal Breeding Division, Institute of Animal Science, College of Agriculture University of the Philippines Los Baños (UPLB), College, 4031 Laguna

Karyotype analysis was carried out on blood samples of 106 water buffaloes (45 males and 61 females) belonging to different breed groups (i.e., Philippine Carabao, Murrah, and crosses among them), using the Leucocyte Culture Technique. The modal chromosome numbers of the Carabao, F1 50%Murrah-50%Carabao, 75%Murrah-25%Carabao, and purebred Murrah are 2n = 48, 49, 50, and 50, respectively. Diploid chromosome numbers equal to 48, 49, and 50 were observed in the F2 (i.e. inter se among F1 50%Murrah-50%Carabao). Carabaos have larger metacentric chromosomes than the Murrah. The "4/9 Tandem fusion" was evidenet in the 50%Murrah-50%Carabao. Chromosomal aberrations in the form of gaps and/ or breaks, were mostly noted among the crosses. Preliminary results of the ordinary least square analysis showed considerable genetic variability in karyotypic characteristics (i.e. chromosome number, centromeric index, arm ratio, and relative length) within and between breed groups. The practical use of karyotypic characteristics in the local testing and selection of water buffaloes would, however, require analysis of more individual animal performance data to reveal significant correlation with estimated breeding values (EBVs) for particular production and reproduction traits.

Key words: water buffaloes(Bubalus bubalis), chromosome number, chromosomal aberrations, centromeric index, arm ratio, relative length, genetic variability

38. CONVERTING HOUSEHOLD WASTE INTO A RESOURCE FOR FOOD PRODUCTION AND SOIL REJUVENATION: OPPORTUNITIES AND THREATS

CLARITA P. AGANON¹, ELLEN S. ROMERO¹, ALFONSO MERCADO¹, JAMES R. BURLEIGH², and CHIN-HUA-MA²

¹Central Luzon State University, Science City of Muñoz, 3120 Nueva Ecija ²Asian Vegetable Research and Development Center, Shanhua, Tainan, Taiwan, Republic of China

The burgeoning population in Philippine cities threatens environmental sustainability from waste accumulation and food scarcity. To abate the condition, waste management and food production should be linked. This is the model being developed by the Central Luzon State University in collaboration with the Asian Vegetable Research and Development Center, Techniche Universität München and the Local Government of Marilao, Bulacan. A collection and processing scheme was adopted to turn biodegradable household waste into a valuable resourcecompost. Household waste (HW) is composed of kitchen refuse, backyard litter, and weeds. Studies conducted at CLSU, and at San Leonardo, Nueva Eciia - a peri-urban vegetable area-showed that household waste reduced our dependence on inorganic fertilizers. Trials conducted on four farms showed that pechay with household waste as a replacement for half of the required inorganic fertilizer did not suffer yield reduction. Yield of pechay from plots treated with 3.5 t/ha composted HW plus half the recommended rate (1/2 RR) of inorganic fertilizer and yield from plots treated with the full recommended rate of inorganic fertilizer were not significantly different. Similar results were obtained for tomato grown under rain shelter.

A residual effect from composed HW was noted on kangkong seeded after successive applications to six preceding crops. Yields from plots treated previously with HW alone or with $\frac{1}{2}$ HW + $\frac{1}{2}$ RR were significantly greater than yield from unfertilized plots.

The utilization of HW as a fertilizer may be compromised by the presence of lead. Analysis of the individual components of household waste compost from Marilao, Bulacan showed that some components contain lead, probably because of their tendency to accumulate lead.

Key words: compost, household waste

39. ENGINEERED SIDE PLOWING CAPABILITY OF NEWLY DESIGNED DISC PLOW

BERNARDO D. TADEO and ELMER G. BAUTISTA Philippine Rice Research Institute, Maligaya, Science City of Muñoz, 3119 Nueva Ecija

The conventional double disc plow attached to the two-wheel tractor was redesigned to effectively work for normal and side plowing. For normal plowing, two disc plows 30 cm apart were correctly oriented at 20 tilt and 40 disc angles. For side plowing, side-mounted disc plow 43 cm away from the hitch point was oriented at 20 tilt and 20 disc angles, while the gage/thrust wheel 109 cm away from the hitch point was oriented at 20 tilt and 10 disc angles. With this configuration, the moments of side plowing resistance and thrust force were balanced, thus solving the problem of maneuverability. Work rate on side plowing was 0.063 ha/h while on normat plowing was 0.14 ha/h.

40. CHEMICAL CHARACTERISTICS OF RICE WINE RESIDUE AS AFFECTED BY DRYING AIR TEMPERATURE

MA. CONSOLACION L. VILLAVER¹, NESTOR M. CANDELARIA¹, and JUMA NOVIE B. AYAP²

¹Central Luzon State University, Science City of Muñoz, 3120 Nueva Ecija ²Philippine Rice Research Institute, Maligaya, Science City of Muñoz, 3119 Nueva Ecija

Rice wine has great potential in the Philippine market. However, with it comes the big bulk of residue that is generally discarded; a by-product that may still have some economic uses. This study was conducted to evaluate the chemical composition of the residue, and develop an optimum drying condition for better storage. Three drying temperatures (50, 60 and 70°C) and mass thickness (5, 10 and 15 mm) were evaluated together with their effect on proximate composition, calcium and phosphorous contents. As expected the thinner the mass being dried, the faster was the rate of drying. However, this directly proportional relationship was not distinctly observed between temperature and drying time. The drying temperatures did not significantly affect the proximate composition and phosphorous content. Results showed a mean of 18.33% moisture content, 0.60% crude asb, 3.53% erude fat, 0.17% crude fiber, 16.65% crude protein, 60.72% nitrogen-free extract and 0.12% phosphorous. Calcium content, on the other hand, was significantly higher (2.42%) when 50°C was used for drying. These data will give some baseline information for future studies on the feasibility of using rice wine residue as feeds or a component of feeds.

Key words: rice wine residue, drying temperature, mass thickness, proximate composition, calcium, phosphorous, feeds

41. EFFECT OF STORAGE CONDITIONS ON RICE NOODLE QUALITY

MARISSA V, ROMERO¹ and MICHIHIKO SAITO² ¹Philippine Rice Research Institute, Maligaya, Science City of Muñoz, 3119 Nueva Ecija

²National Food Research Institute, Tsukuba City, Ibaraki, Japan

The quality of rice noodles especially those with high moisture content, deteriorates upon storage. In this study, the effects of key storage conditions on rice noodle quality were determined. The factors investigated were packaging materials, storage temperature, and addition of a mold retardant. The packaging materials evaluated were LDPE 30, HDPE 50, OPP 20/EVA 15/LDPE 40, and OPP/CPP 50 while the storage temperatures were 5°C and 25°C. Negamold, a commercial mold retardant was used. For each packaging material, four samples were prepared; two with Negamold and two without Negamold. One set of each sample was stored at 5°C while the other set at 25°C. The samples were examined for physical appearance and mold growth during storage. The latter was evaluated both visually at 6, 12, and 18 days of storage and by plating in PDA at the initial and final days of storage. All the samples stored at 5°C, regardless of packaging materials and presence or absence of Negamold remained acceptable and free for molds even up to 18 days of storage. At 25°C storage, the best packaging materials were OPP 20/EVA 15/LDPE 40 and OPP/CPP 50, which combined with Negamold kept the rice noodles visually acceptable and mold-free until the last day of observation.

Key words: storage, quality deterioration, packaging, polyethylene, polypropylene, storage temperature, mold retardant, Negamold, visual examination, microbiological examination