

## **AGRICULTURAL SCIENCES**

### **ASD No. 1**

#### **ENDANGERED PLANTS IN THE FOREST LANDSCAPE OF QUEZON PROVINCE, SOUTHERN LUZON: BIODIVERSITY CONSERVATION OPTION**

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Several small stores selling various species of plants abound along the Real-Infanta Quezon highway, southern Luzon. Interviews with the vendors who are also collectors revealed that these plants are regularly collected from the forest landscape of Polillo, Is., Quezon province and vicinities. About 90 percent of the plants are indigenous and many are endemic to the Philippines. There are many species of *Nepenthes*, *Hoya*, *Lycopodium*, palms and orchids. *Platynerium coronarium*, a relative of the endangered *P. grande* per IUCN standards and *Grammatophyllum speciosum*, a very large species of orchid are very prominent owing to their size and beauty but are becoming very few.

Sustained harvesting of these plant biota would critically alter the forest landscape of Quezon. However, stopping the people from collecting without any option would also mean starvation. The study found two practical options: managed harvesting and domestication of wild plants.

**Keywords:** Endangered plants, forest landscape, Quezon province, biodiversity conservation option, managed harvesting, domestication

ASD No. 2

**MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF SEEDLINGS  
PLANTED IN LA MESA DAM WATERSHED, PHILIPPINES<sup>1</sup>**

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Among the terrestrial ecosystems, grasslands and degraded areas are the most disregarded. Therefore, successful reestablishment of forest species is very important in disturbed sites to bring back its original cover. Growth and physiological characteristics of species for planting should be evaluated and studied to understand better its growth dynamics.

About six-month old native species such as *Erythrina orientalis* (Linnaeus) Merrill, *Dracontomelon dao* (Blanco) Merrill et Rolfe, *Pterocarpus indicus* Willd., and *Bischofia javanica* Blume were planted in La Mesa Dam Watershed, Philippines to determine growth and physiological characteristics. The objectives of the study were to compare the early growth performance among the species and determine which is/are suitable in the area, to compare the growth performance of species in between flat and mountain areas, and to determine any relations between growth and physiological characteristics. Randomized block design having two blocks and three replicates were established with 4 x 4-m spacing between seedlings. Growth parameters used were height, diameter, dry weight, leaf area, etc. Physiological characteristics such as net photosynthesis, transpiration rate, stomatal conductance, etc. were determined as well through the use of Licor-6400 Portable Photosynthesis System.

Results and analysis revealed that among the four species, *Erythrina orientalis* showed good growth performance as it grows fast and can fix nitrogen followed by *Pterocarpus indicus*. *Erythrina orientalis* grew very well and has a potential for biomass production and reforestation in many denuded areas in the

country. In terms of physiological characteristics, nitrogen-fixing trees, *Erythrina orientalis* and *P. indicus*, exhibited good performance in the field. Generally, the growth and physiological characteristics of four species in the mountain area which has fertile and moist soil conditions were better than in the flat area with dry soil condition.

**Keywords:** *morphological growth, physiological characteristics*

ASD No. 3

### MODELING SOIL EROSION IN A WATERSHED FOR SUSTAINABLE RESOURCE MANAGEMENT

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Most erosion models have been developed based on a plot scale and has limited application to watershed due to differences in aerial scale and the complex system occurring in a watershed. Address this limitation, a GIS-assisted methodology for modeling soil erosion was developed using PCRASTER to predict the rate of soil erosion at watershed level and identify the location of erosion-prone areas for specific mitigation measures not necessarily the whole watershed, thus maximizing resource utilization. The general methodology of desktop modeling of soil erosion at watershed scale is composed of: (a) model development and structuring; (b) formulation of assumptions; (c) gathering of information; (d) database creation, manipulation and processing and (e) dynamic modeling with PCRASTER.

Validity test of the model using three rainfall events revealed that the predicted run-off heights showed a non-significant difference with the actual observed values and high positive correlation with an average value of 0.94 and coefficient of determination ( $R^2$ ) = 0.931. The predicted sediment concentration was fitted against the range of observed values. Sensitivity analysis showed that the model was most sensitive to Manning's roughness coefficient ( $n$ ) for the run-off peak rate and vegetative cover for soil loss.

Erosion hotspots were predicted in areas with low if not absence of surface cover like the parcels of cropland under land preparation, early crop establishment stages areas without soil conservation measures, roads and footpath, and in areas with steeper slopes. The results demonstrate the predictive ability of the model and the significant influence of the surface cover in reducing the run-off and soil erosion in a watershed. This implies that the model could accurately predict runoff height and soil loss occurring in a watershed. Thus, this model could lead to the formulation of cost-effective and efficient strategies for better if not sustainable watershed management.

**Keywords:** Erosion modeling, geographic information system (GIS), watershed, sustainable resource management, "erosion hot spots"

#### ASD No. 4

### PHYSICO-CHEMICAL-RHEOLOGICAL PROPERTIES AND STRUCTURE ELUCIDATION OF CARRAGEENAN FROM SELECTED RED ALGAE IN NORTHWESTERN LUZON, PHILIPPINES

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Carrageenan were extracted from six (6) species of red algae (*Eucheuma arnoldii*, *E. gelatinae*, *Kappaphycus cottonii*, *Halymenia durvillaei*, *Hypnea charoides* and *H. pannosa* growing abundantly in the intertidal and subtidal zones in Northwestern Luzon, Philippines. Their physico-chemical-rheological properties were characterized by a combination of chemical and spectroscopic techniques. Considerable amounts of carrageenan ranging from 17.46% to 33.24% were obtained. The viscosity and gelling properties are being considered in the food industry. The low heavy metal contents and microbial loads denote the safety of these carrageenans to be used in food formulations. Reductive acid-hydrolysis analysis in combination with different chromatographic separations showed galactose and anhydrogalactose as the major sugar residues present in the carrageenans. The IR and <sup>13</sup>C-NMR provided evidence on the type of carrageenans (*E. arnoldii* - iota carrageenan; *E. gelatinae* - beta-kappa carrageenan; *K. cottonii*, *H. charoides* and *H. pannosa* - kappa carrageenan and *H. durvillaei* - lambda carrageenan). The systematic information derived in this

research opens the way for the development of new food products and commences the commercial production of these algae.

**Keywords:** carrageenan, red algae, intertidal and subtidal zones, food industry

#### ASD No. 5

### DISTRIBUTION AND SEASONALITY, BIOMASS AND YIELD OF CARRAGEENAN PRODUCING SEaweEDS IN NORTHWESTERN PHILIPPINES

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*Fourteen alternative non-traditional sources of carrageenan in Northwestern Luzon consisting of four families of red algae. These are the: (Rhodomelaceae - Acanthopora spicifera (Vahl) Borgesen; Solieriaceae - Eucheuma arnoldii Weber-van Bosse;; E. denticulatum (NL Burman) Collins & Herve; E. gelatinae J Agardh; E. muricatum (Gmel.) Weber-van Bosse; Eucheuma sp.; Kappaphycus cottonii (Weber-van Bosse) Doty; K. striatum (Schmitz) Doty; Cryptonemiaceae - Halymenia dilatata Zanadini; H. durvillaei Bory de Saint-Vincent; H. maculata J. Agardh; and Hypneaceae - Hypnea charoides Sonder; H. esperi Bory; H. pannosa J. Agardh). These carrageenophytes were assessed in terms of distribution, seasonality and biomass and similarity index.*

The carrageenophytes were collected in six stations, i.e. Station 1 – Currima, Ilocos Norte; Station 2 – Pagudpud, Ilocos Norte; Station 3 – Cabugao, Ilocos Sur; Station 4 – Balaoan, La Union; Station 5 – Bacnotan, La Union; and Station 6 – Bolinao, Pangasinan.

*H. durvillaei* had the widest range of distribution and was found in all collection stations. On the other hand, *E. gelatinae* was found only in collection station 2 while *E. denticulatum*, *E. muricatum*, *K. striatum* and *H. esperi* were found in station 3.

More species were collected during the onset of the cold months (October). Except for *K. cottonii*, *E. gelatinae* and *H. durvillaei*, all

carrageenophytes collected exhibited seasonality. The species *E. arnoldii* was found only from the months of July to March. Species occurring from October to June were *E. denticulatum*, *E. muricatum*, *E. striatum*, *Eucheuma* sp. and *Hypnea* species. *Halymenia maculata* was present from April to December, while *H. dilatata* was from April to June and October to December.

The highest biomass during the collection period was registered by *E. gelatinae* followed by *H. pannosa*, while the least was *H. esperi*. Among the sampling areas, station 3 had the most diverse carrageenophytes. The northern collection areas (stations 2 -- Pagudpud, Ilocos Norte and 3 -- Cabugao, Ilocos Sur) were more diverse than the southern areas.

The sampling period was significantly related to the biomass of *E. arnoldii*, *E. gelatinae*, *H. dilatata*, *H. maculata*, *H. charoides* and *H. pannosa*. The pH of the water in the various collection area had no significant relationship to the biomass of all *Eucheuma* species collected. Sechis disk (SD) transparency and collection period (quarter) when combined as factors played a significant role in the biomass of carrageenophytes. The substrates of the sampling areas were generally rocky and rocky sandy and the carrageenophytes characteristically of cling or attach to the rocks. In general, algae growth or biomass was high during sunny days and directly related to the SD transparency.

**Keywords:** Carrageenan, seasonality, distribution

#### ASD No. 6

### **CARRAGEENAN YIELD AND CHARACTERIZATION OF SELECTED RED ALGAE OF NORTHWESTERN PHILIPPINES**

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The average weight and dry biomass and percentage dry biomass of the 14 non-traditional carrageenophytes in Northwestern Philippines as well as recoverable dry matter content of the collected carrageenophytes were determined. The presence of hard thalli contributed to high value of dry/wet ratio. Most of the species belonging to Solieriaceae had hard thalli where dry matter content of the species ranged from a high of 16.12% (*E. arnoldii*) to 12.00% (*K. striatum*). Hypneaceae came next with *Hypnea asperi* having 15.00% recoverable dry matter

content followed by *H. pannosa* (12.49%). The least was *H. charoides* at 9.82% where it also registered to be the least among the collections.

The percent yield follows the trend in the biomass of the carrageenophytes. Results point that variations in carrageenan yield existed among the 14 carrageenophyte species. Solieriaceae gave higher yield than the other families. Hypneaceae followed this, and then Cryptonemiaceae; the least was Rhodomelaceae. Highest yield was noted with *E. gelatinae* (35.20%) followed by *K. cottonii* (27.60%) both collected in Station 2 – Pagudpud, Ilocos Norte. Overall, the average lowest yield was obtained from *Halymenia maculata* (14.33%) taken from Cabugao, Ilocos Sur.

Results of the study show that the 14 species of red algae are potential sources of carrageenan with the following species giving the highest yield: *E. gelatinae* J. Agardh, *E. arnoldii* Weber-van Bosse and *E. denticulatum* Collins and Hervey.

**Keywords:** Carrageenan yield, red algaem, Northwestern Luzon, Philippines

ASD No. 7

# **CAGE CULTURE OF NON-TRADITIONAL SOURCES OF CARRAGEENAN INP AAYAS, BURGOS, ILOCOS NORTE**

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Significant biomass of these non-traditional sources of carrageenan (Soleriaceae: *Betaphycus philippinensis* Doty, *Eucheuma arnoldii* Weber-van Bosse, *E. denticulatum* Collins & Hervey, *E. denticulatum* Collins & Hervey, *E. muricatum* Weber-van Bosse, *Kappaphycus cottonii* Doty, *K. striatum* Doty) have been identified and found to be abundant in the provinces of Ilocos Norte and Ilocos Sur

The culture of these non-traditional sources of carrageenan has not been reported in the Philippines. This study aimed to determine adaptability trials/feasibility of the culture of *Betaphycus* / *Eucheuma* / *Kappaphycus* in the

waters of Paayas, Burgos, Ilocos Norte, Philippines using the cage method, and determine the its economics using cages and hanging method.

Growth experiment was made using a Randomized Complete Block Design with treatments depending on the species of *Eucheuma* available. Each treatment was replicated (not less than three times). Propagules of various species of non-traditionally cultured *Betaphycus* / *Eucheuma* / *Kappaphycus* were secured from the various sampling stations of "Distribution and Seasonality of Carrageenan Producing Seaweeds in Northwestern Philippines" (Santos *et al.*, 2002) and cultured in cages in areas at Paayas, Burgos, Ilocos Norte (lat. 18°29'56.7"N and longi. 120°34'10.8"E) for the culture studies.

In general, red algae cultured in cages decreased in weight after initial stocking. However, the algae increased in weight after a month.

Physico-chemical parameters such as temperature (°C), water movement (cm/t), salinity (ppt) and pH (pH units) were determined. Growth of the algae (biomass) was correlated with the physico-chemical parameters at various sampling periods. Water movement and salinity had highly significant relationships with the growth of the various algae while pH was significantly related to growth. Temperature was not significantly related to the growth of cultured seaweeds.

**Keywords:** seaweed culture, non-traditional sources, Carrageenan

ASD No. 8

# **GROWTH OF MACROPROPAGATED IPIL (*Intsia bijuga* Colebr.) SEEDLINGS IN THE NURSERY**

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The quality of planting stocks is a primary factor in the survival of seedlings during outplanting operation for forest restoration. To attain this objective, nursery culture technique like fertilization need to be undertaken to



assure vigorous and healthy planting stocks. Ipil (*Intsia bijuga* Colebr.) is an endangered and potential species for forest rehabilitation. Rooted stem cuttings of ipil were planted in pots and treated with different soil fertilizer alone or in combination as: Rhizo-N, Mykovam, urea, ammonium phosphate, NPK (14-14-14), and complete slow release (13-13-13). The experiment revealed significant results, obtaining a positive increment in shoot length, stem diameter, and dry weight of macropropagated ipil seedlings. Height of seedlings applied with complete slow release fertilizer was 38.60 cm, while control had 11.62 cm, this gave 235.81% increment. The stem diameter of untreated seedlings was 2.10 mm while seedlings treated with complete slow release had 4.76 mm. Dry weight of seedlings was highest for complete slow release fertilizers with 3.52 g over that of the control seedlings with 0.24 g. There was a very high increase in dry weight of tissues when treated with complete slow release fertilizer. Likewise, dry matter and organic matter production of seedlings between control and those applied with slow release fertilizer was comparable. Thus, application of fertilizers resulted in better growth of macropropagated ipil seedlings, as compared to control. However, application of 0.50 g complete slow release fertilizer (13-13-13) provided optimum growth and, thus, met the objective of producing healthy and vigorous planting stocks for upland restoration. Likewise, it is highly recommended that the next phase of the experiment be conducted in the field for a longer period to verify results.

**Keywords:** macropropagated, cuttings, *Intsia bijuga*, fertilization, fertilizer, Mykovam, urea

#### ASD No. 9

#### NON-CONVENTIONAL PREPARATION OF TOBACCO STALK FIBER

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In an effort to establish a cleaner and greener technology in producing fiber from tobacco stalks, two separate studies on the non-conventional preparation of pulp were carried out. First, is the biomechanical pulping method, which entail a totally chemical-free and zero chemical effluent process. This involved mechanical crushing of the material and subsequent biological treatment using four fungal strains. Pulp yield (67-89% on dry stalk material) obtained was remarkably higher than that of the

conventional pulping processes. Delignification activity is markedly noticeable in *Pleurotus ostreatus* and *Phanerochaete chrysosporium* even at very low concentration affording 6.53–11.13 and 14–20 point decrease after two and four weeks, respectively. Unlike the conventional method, the same process did not show any cellulose degradation. The improvised nutrient medium (CRWNM) for the fungi proved very effective in substituting the expensive commercial potato dextrose broth (PDB) medium, making the technology more economically appealing.

The second study which is the non-conventional pulp purification used biological and indigenous bleaching agents. Both agents proved effective in brightening the tobacco stalk pulp. They did not cause any cellulose degradation but effected considerable delignification. The brightness of the pulp was comparable with the conventionally bleached pulp.

In terms of mechanical strength, non-conventionally bleached and unbeaten tobacco stalk pulp proved superior to the other bleached tobacco pulp by other bleaching processes including the commercial softwood kraft bleached pulp.

**Keywords:** Non-conventional, pulp, bleaching, chemical effluent, chlorine-free bleaching, waste tobacco stalks

ASD No. 10

# UTILIZATION OF *TRICHOGRAMMA* PARASITOID AS BIOLOGICAL CONTROL AGENT AGAINST SUNFLOWER HEADWORM *HELICOVERPA ARMIGERA* HUGNER

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The study aimed to evaluate *Trichogramma parasitois* as a potential biological control agent against sunflower headworm. Specifically it aimed to determine the most efficient *Trichogramma* species in parasitizing sunflower headworm egg, to evaluate the effectiveness of different rates of *Trichogramma chilonis* application and to compare the use of *Trichogramma* and insecticide on the yield of sunflower.

Results show that among the four *Trichogamma* species tested *Trichogamma chilonex* proved to be the most efficient in parasitizing *Helicoverpa armigera* egg. Percentage parasitism was greatly affected by the different rates of application of *Trichogamma chilonex*. The highest percentage parasitization was obtained from those which received 70,000 parasites per hectare per release.

Percentage sunflower seed set was greatly affected by the rate of application of *Trichogamma chilonex*. Sunflower applied with 70,000 parasites per hectare per release of *Trichogamma chilonex* has the higher percentage seed set.

Yield of sunflower was significantly affected by the rate of application of *Trichogamme chilonex*. Furthermore, when compared to insecticide treatment application of *Trichogamma chilonex* was better. This implies that *Trichogamma chilonex* applied at the rate of 70,000 parasites per hectare per release is effective in parasitizing sunflower headworm egg, and thus increases sunflower seed set and consequently yield.

**Keywords:** parasitoid, *Trichogamma*, biological control, sunflower, parasitization, headworm

#### ASD No. 11

### SOME MANAGEMENT PRACTICES AGAINST THE EGGPLANT LEAFHOPPER *AMRASCA BIGUTTULA* *BIGUTTULA ISHIDA* ON EGGPLANT

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The study was carried out to develop an economically and environmentally sound management strategy for the eggplant leafhopper *Amrasca biguttula biguttula Ishida* on eggplant. The study involved bioassay of *Metarhizium anisopliae* and use of rice straw mulch, plastic mulch, and insecticide for the purpose of evaluating them against the population density of eggplant leafhopper *Amrasca biguttula biguttula Ishida*.

No *Metarhizium anisopliae* was isolated from the collected insects. Alternatively, three isolates of *Metarhizium anisopliae* were evaluated for bioassay against the eggplant leafhopper. Results revealed a very low percentage mortality of not more than 10% to the insect, hence *Metarhizium anisopliae* was

found to be ineffective against the eggplant leafhopper and therefore was not evaluated in the field.

Fipronil gave the highest mean reduction of eggplant leafhopper population over the other treatments, as well as the highest yield, but there was no significant difference on the mean yield of all treatments. Moreover, the higher return on investment was obtained from fipronil (44.41%), followed by plastic mulch (36.87%), control treatment (22.47%), and rice straw mulch (15.36%), respectively.

Results indicated that, it would be advisable for a farmer with enough capital for investment to use fipronil and plastic mulch. If a farmer has limited capital, rice straw mulch will be the most ideal.

**Keywords:** leafhopper, eggplant, metarhizium, mortality, fipronil

#### **ASD No. 12**

### **INSECTICIDAL POTENTIAL OF THE FORMULATED TOBACCO SEED, *Nicotiana tabacum* L. OIL**

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The pesticidal potential of seven tobacco seed oil formulations (TSOF) against some serious insect pests were evaluated at the Crops Research Laboratory of Mariano Marcos State University and at the National Tobacco Administration, Batac, Ilocos Norte.

The different concentrations (0.25%, 0.50%, 1.5%, 2.5%, 5.0 %, 7.5 %, 10.0 %) of crude tobacco (*Nicotiana tabacum*) seed oil formulated as emulsifiable concentrate had significant insecticidal effect against stored grain insects (*Sitophilus zeamays* and *Callosobruchus chinensis*), termites (*Macrotermes gilvus*), and bean aphids (*Aphis craccivora*).

Mortality of the test insects sprayed with TSOF increased with increasing concentration.

Generally, mortality and efficacy caused by the different formulations was insect type-dependent. Highest mortality of the most

effective formulation (10% TSO) was observed after 24 h in corn weevil (100% mortality), 24 h in bean weevil (16.67% mortality), 48 h in termites (100% mortality), and 6 h in aphids (100% mortality). Corresponding efficacies were 100 %, 60%, 16, and 91%, respectively.

Except in termites, TSOF proved more effective than karate against *S. zeamays*, *C. chinensis*, and *A. craccivora*.

**Keywords:** tobacco seed oil formulation (TSOF), pesticidal, mortality, efficacy

### ASD No. 13

## ALTERNATIVE METHODS OF CONTROLLING INSECT PESTS AND DISEASES USING BOTANICAL MATERIALS AND ANTAGONISTS

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The effectiveness of promising botanical materials such as *Piper betel*, *Impatiens balsamina*, *Vitex negundo*, *Azadirachta indica*, *Carica papaya*, *Allium sativum* and the two antagonists, *Trichoderma harzianum* and *Trichoderma aureoviridae* were evaluated against garlic pests. Of the first six tested in vitro, *Allium sativum* was the most effective in suppressing the growth of *Alternaria porri* and *Sclerotium rolfsii* while the effect of the rests were not significantly different with the untreated control.

Among the dried *V. negundo*, *C. citratus*, *A. indica*, and *Eucalyptus spp* incorporated into the rice hay mulch of garlic, the *V. negundo* treated plots did not show any root rot infection caused by *Sclerotium rolfsii*. which imply that this material can cause a high degree of suppression of root rot disease, caused by *Sclerotium rolfsii*. The lowest incidence of tangle top diseases was observed from the *Azadirachta* treated plots where degree of control was better than the combinations of Feronosferon and Mancozeb.

Marigold and baraniw used as border crops to garlic significantly controlled *Thrips tabaci* infestation which eventually produced higher yield than those without borders.

The antagonists, *Trichoderma* spp. significantly controlled *Sclerotium rolfsii* and *Fusarium oxysporum*. Between the two *Trichoderma* species used, *T. harzianum* gave greater degree of suppression than the *T. aureoviridae*.

**Keywords:** Alternative method, garlic pests, botanical materials, antagonist

**ASD No. 14**

**TOWARDS APPROPRIATE *Bt*-CORN IRM STRATEGIES FOR  
THE ASIAN CORN BORER, *OSTRINIA FURNACALIS* (GUENEE):  
FIELD SURVEYS AND LABORATORY STUDIES OF  
ALTERNATE HOST PLANTS IN MAJOR CORN GROWING AREAS**

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*Bt*-corn is becoming an acceptable alternative crop for many farmers in the country and it is only necessary to prepare an appropriate Insect Resistance Management plan to delay the expected development of corn borer resistance. Key factor in any IRM strategy against the Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée), is the use of refugia. Refugia are simply blocks or strips of corn that do not contain a *Bt* technology for corn borer. This pioneering study aims to identify and determine the distribution and abundance of possible alternate hosts of ACB in corn and non-corn agriculture systems, to establish the occurrence of ACB on these host plants and to determine the growth and development of this pest on the different alternate host plants.

A survey through designed questionnaire was conducted with the assistance of Monsanto Field Actmen from Isabela, Bukidnon and South Cotabato. This survey involved about 250 respondents, distributed in five municipalities per province, five barangays per municipality, five farmers per barangay. Potential alternate hosts were initially identified based on the presence of eggs and larvae perceived to be those of the ACB. Results showed that the

perceived potential alternate crop hosts are tomato, pepper, sitao, okra, cotton and sugarcane whereas the potential weed hosts are aguingay, napier, tigbi, paragrass and wild sorghum. The abundance of weeds in and out of the cornfield was also evaluated, designating the most abundant species as +++ and the least abundant, +. The most abundant weeds were aguingay, trilobed morning glory and spiny amaranth.

For the biological studies, ACB completes its development from egg to adult on aguingay, napier and tigbi in comparison with corn using fresh stalks under laboratory conditions. In our greenhouse experiment, first instar ACB larvae infested onto 45-day old hosts (aguingay, napier, tigbi, cotton and sitao) were observed to feed initially on the leaves of the different hosts but later transferred to corn when continuously reared on these same hosts. Several experiments in the laboratory and in mini-screencages were conducted to determine larval preference and ovipositional preference. Corn, aguingay and cotton were preferred for larval feeding and female egg laying activities. On the other hand, life history studies of ACB using whole plants revealed that ACB completes its growth and development in all the selected alternate hosts except for tomato and pepper. It may appear, however, based on several studies conducted in the laboratory and greenhouse, that tomato and pepper are transient hosts only for activities other than feeding and oviposition.

Based on insect ecological concepts, a host plant of ACB is one that can support its growth and development. This support may be extended to nectar-feeding, mating and/or oviposition by adults, pupation sites or other biological aspects and behavior vital to survival and reproduction. In as much as only seven alternate hosts were studied, it is recommended that further investigation should focus on other hosts other than the one selected above. Studies are also encouraged on the relationship of these alternate host plants and corn borer in different cropping systems in the country.

**Keywords:** *Bt*-corn, Insect Resistance Management (IRM), Asian corn borer, *Ostrinia furnacalis* (Guenee), alternate host plants, life history, corn, *Zea mays*

ASD No. 15

**BIO-CONTROL POTENTIALS OF *TRICHODERMA HARZIANUM*  
AND *BACILLUS SUBTILIS* AGAINST PURPLE BLOTCH DISEASE  
OF GARLIC UNDER FIELD CONDITION**

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The efficacy of *T. harzianum* and *B. subtilis* in controlling purple blotch disease caused by *Alternaria porri* on garlic was conducted under field condition in Batac, Ilocos Norte. Efficacy was measured in terms of percent disease incidence during vegetative (55 DAP), bulb formation (60 DAP), and maturity (90 DAP) stages, and plant height, bulb size and weight. Two rows of garlic plants inoculated with spore-mycelial suspension of *Alternaria porri* were maintained around the experimental areas as spreader plants.

Purple blotch incidence was significantly lower on plants treated with *T. harzianum* than on the untreated plants and comparatively similar to those treated with fungicide during vegetative and bulb formation stages. Infection rate increase during maturity stage although, differences among treatments were not significant. This increase however, did not affect the reproductive performance of the garlic plants as shown by the bigger bulbs produced in fungicide and *T. harzianum* treatments. Those treated with the fungal antagonist three times (30, 45, and 60 DAP) produced comparable bulb size and weight as those sprayed with fungicide. Likewise, garlic plants treated with *B. subtilis* had significantly lower incidence of purple blotch than the untreated plants during bulb formation and maturity stages. Plants that were applied at 30 and 45 DAP and at 30, 45, and 60 DAP had comparable infection rate, bulb size and weight as those treated with fungicides.

The above results show that more frequent application of either *T. harzianum* or *B. subtilis* on garlic plants provides comparable efficacy in reducing purple blotch incidence and comparable bulb size and weight as those treated with synthetic fungicides. These two biorationals therefore, have high bio-control potentials under field condition thus, could be used in IPM program for garlic and other crops that are hosts to *Alternaria porri*.

**Keywords:** Biocontrol potentials, *T. harzianum*, *B. subtilis*, garlic, purple blotch



**ASD No. 16**

**THE WEEDS IN THE PLANTATION FIELDS OF CORDILLERA**

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Knowledge about weeds growing in association with the high-value crops is of significance to provide basis on the various aspects of integrated crop protection program. As a first step, proper identification of weed species is deemed critical in order to develop environmentally-sound weed control measures. Survey, collection, and identification of the weeds growing in association with ornamental and vegetable crops were done in the plantation fields of Benguet and Mt. Province. Using the quadrat sampling method, 1 x 1 sq. m. quadrats were randomly distributed throughout the plantation fields, the number of total points depending on the field size. A total of sixty-six weed species, distributed in twenty different plant families were identified. The dominant weeds cited in decreasing order of importance values, to name a few, are: *Galinsoga parviflora*, *Solanum nigrum*, *Portulaca oleracea*, *Ageratum conyzoides*, and *Amaranthus viridis*. It was notable that 48% of these weeds were found to have beneficial uses either as food, feed to livestock or possessing medicinal properties. Moreover, few species were noted to act as alternate hosts to some plant pests and diseases. Such knowledge now becomes apparently important on how our vegetable farmers and ornamental growers will deal with these noxious but beneficial plants as well.

**Keywords:** quadrat sampling, beneficial weeds, noxious weeds, alternate hosts, importance values, high-value crops, medicinal properties, Cordillera

ASD No. 17

**GENETIC DIVERSITY IN *Cercospora canescens* ELLIS  
AND MARTIN, THE CAUSE OF LEAF SPOT OF MUNGBEAN  
(*Vigna radiata* (L.) WILCZEK) USING REP-PCR**

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Variability of *Cercospora canescens*, a fungus that causes mungbean leafspot was evaluated using rep-PCR (repetitive sequence-based polymerase chain reaction). A total of 32 isolates were collected from mungbean growing regions of Luzon and Mindanao. This study was conducted to determine and characterize the extent of variation and to delineate the phylogenetic relationships among the isolates.

Genetic diversity was assessed using rep-PCR fingerprinting. Three sets of primers (BOX, ERIC and REP) corresponding to conserved repetitive elements were used to generate genomic fingerprints.

Cluster analysis of composite rep-PCR data revealed 28 haplotypes among 29 isolates that were grouped into 7 clusters at the 50% similarity level. Groupings of the isolates tended to correlate with their geographic origin.

Nei's diversity index of the composite data obtained using rep-PCR primers was high at 0.70 which implies high level of genetic diversity. This considerable variation could lead to the emergence of new resistant strains of *Cercospora canescens*.

These results will aid plant breeders in utilizing diverse pathogen strains for future screening of mungbean cultivars and in developing effective disease management strategies.

**Keywords:** *Cercospora canescens*, mungbean, rep-PCR, genetic diversity

ASD No. 19

**PGPR FORMULATION AS SUBSTITUTE FOR NPK FERTILIZATION  
INCREASES FARMING PROFITABILITY**

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The new PGPR ( plant growth promoting rhizobacteria)) formulation, Vital N™ is a wettable powder containing dried new strains of *Azospirillum* sp., vitamins and minerals. It was developed for seed/seedling inoculation. *Azospirillum* spp are free living bacteria growing around roots and reported to fix atmospheric N, promote plant growth by producing IAA for root proliferation and cytokinins for shoot growth. They can also solubilize soil phosphorus and potassium. Tests have been conducted to compare the growth and yield of corn and rice treated with Vital N™ at varying levels of fertilization. Experiments were conducted in accordance with FPA-approved protocols at the BSWM Station, San Ildefonso, Bulacan. The growth and yield of rice and corn were compared using the following treatments: No inorganic NPK added, Vital N™ inoculated, ½ recommended rate of inorganic NPK, ½ recommended rate of inorganic NPK plus Vital N™ , full recommended rate of inorganic NPK and full recommended rate of inorganic NPK plus Vital N™. Technology demonstration farms were established in 79 farmer-owned ricefields in Region 1 (Pangasinan, La Union, Ilocos Sur and Ilocos Norte) to show farmers the comparative yields of rice fertilized with ½ recommended rate of inorganic NPK plus Vital N™ and full recommended rate of inorganic NPK. Data were analyzed statistically. A random interview with farmers using Vital N™ was conducted to gauge their reaction to the technology.

Rice and corn treated with Vital N™ were more robust with more extensive roots, thicker stems and stayed green even when matured. In corn, the increase in yield due to Vital N™ ranged from 1.3-1.4 ton ears per ha. In rice, the increase in yield due to Vital N™ ranged from 0.82 -1.62 ton grains per ha. Both experiments showed that Vital N can further increase the yield of rice and corn significantly beyond that obtained using the full recommended rate of fertilization. Both experiments also showed that the application of the Vital N™ can substitute for half the recommended fertilizer requirement. The 79 technology demonstration farms in Region 1 clearly showed that by substituting Vital N™ with half the

recommended rate of fertilization, higher profits were realized by farmers. Farmers were happy and claimed that using Vital N™ increased germination rates, produced healthier seedlings of tobacco, rice, and vegetables; heavier grains and higher milling recovery in rice; higher yields and protection from fungal root rot in onions; greater number of larger fruits in water melon; earlier fruiting and longer production span in peppers, tomatoes and eggplants. Banana plantlets treated with Vital N™ were also found to have higher survival rates and were more robust.

Vital N™ was developed with the assistance of the Philippine Rice Research Institute.

**Keywords:** Vital N™, *Azospirillum* sp., BSWM Station, NPK

#### ASD No. 19

### **IMPROVED SYSTEM OF PLANT REGENERATION FROM UNIRRADIATED AND GAMMA-IRRADIATED EMBRYOGENIC CULTURES OF AVOCADO (*Persea americana* Mill.) BY SOMATIC EMBRYOGENESIS**

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Regenerating avocado (*Persia americana* Mill.) through somatic embryogenesis is far from being routine and often genotype-dependent. Locally, only 'RCF Purple' variety has been tissue cultured and regeneration needs optimization (Raviv et al, Plant Tissue Culture and Biotechnology, Vol. IV, No.3-4, pp 196-205, November 1998). This study is being conducted to improve, adapt and utilize the regeneration system to induce genetic variability by somaclonal variation and gamma irradiation in local avocado varieties.

Significant improvements in embryogenic culture (EC) induction from immature zygotic embryos of 'Semil' and 'Mainit' avocados were obtained in MP medium (MS salts and vitamins + 0.1 mg/L picloram). Subsequent transfers to MP, RA3 (MS + 0.1 mg/L TDZ and 0.5 mg/L GA<sub>3</sub>) and BA2 (MS + 2.0 mg/L BA + 1.0 mg/L IBA) media resulted in proliferation of globular, heart and cotyledonary stages of somatic embryos. Maturation of somatic embryos was achieved in MM1 medium (B5 macro, MS micro and vitamins with 100 ml/L coconut water). After three trials from over 2,000 somatic embryos, the highest average regeneration

response of 23.7% plantlet recovery was obtained using AD4 medium (B5-MS + 400 mg/L glutamine, 60 g/L sucrose and 2.0 mg/L BA). To date, there are a total of 28 rooted plantlets and 124 shoot regenerants from 'Semil' and 6 shoot regenerants from 'Mainit'. In a separate study, gamma irradiation of ECs with 20 and 30 Gy significantly reduced % re-growth/proliferation by as much as 76.2 and 86.1% for 'Mainit' and 31.4 and 84.3% for 'Semil', respectively. As for 'Semil', LD<sub>50</sub> based on formation of cotyledonary stage SE was at 20 Gy. Seventy-two regenerants have so far been obtained from irradiated cultures. All these regenerants are being micro-propagated for subsequent variant/mutant screening.

**Keywords:** Avocado, *in vitro* mutation, gamma ray, plant regeneration, tissue culture, somatic embryos

**ASD No. 20**

**PLANT REGENERATION VIA DIRECT SHOOT ORGANOGENESIS  
FROM SEEDLING EXPLANTS OF POLE SITAO *Vigna unguiculata*  
{L.} Walp. var. *sesquipedalis* {L.} Koern.)**

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Complete system for *in vitro* shoot induction, plantlet recovery and *ex vitro* establishment for pole sitao (*Vigna unguiculata* {L.} Walp. var. *sesquipedalis* {L.} Koern.) was established for the first time using the protocol originally developed for mungbean and other Asiatic *Vigna* spp. as model. Cotyledon and cotyledonary node (CN) explants excised from aseptic seedlings were cultured using MS salts and B5 vitamins medium with 1.0 to 2.0 mg/L benzyladenine (BA). On the average, percent shoot formation in cultivars 'UPL PS 1' and 'UPL PS 2' were higher using CN (67.6 and 80.4 %) than that of cotyledons (25.2 and 17.8%). However, higher responses can be obtained from cotyledons if younger seedlings (i.e., 1 to 2-d-old) were used. A test on eight pole sitao genotypes (cultivars and advanced breeding lines) for shoot regeneration efficiency revealed genotypic effects with a range of 43.3 to 100%. Individual shoots were excised and rooted at high frequencies (90-100%) using agar-solidified MS-B5 basal medium with 100 ml/L coconut water. Survival of acclimatized regenerants ranged from 47.4 to 88.9 % when transplanted to a 1:1 mixture of garden soil and coir dust under greenhouse conditions. All tissue culture-derived seedlings are morphologically

normal; they flowered and produced pods filled with seeds under field conditions. The system may be applied in micropropagation of inter-specific hybrids and other biotechnology-assisted manipulations (ex. *in vitro* selection and mutagenesis and genetic engineering) in pole sitao.

**Keywords:** Cotyledon, cotyledonary node, legumes, plant regeneration, tissue culture, yard-long bean

#### ASD No. 21

### DEVELOPING PLANT REGENERATION SYSTEMS FOR *IN VITRO* CONSERVATION OF MANDARIN (*Citrus reticulata* Blanco) AND PUMMELO (*Citrus maxima* {Burm.} Merr.)

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Plant regeneration systems are being developed for *in vitro* conservation of the polyembryonic mandarin (*Citrus reticulata* Blanco) and monoembryonic pummelo (*Citrus maxima* {Burm.} Merr.). Mandarin seeds without the seed coat germinated faster (7 days) and at 100% frequency than those with seed coat (33 days and 12-52%). More vigorous seedlings with stunted roots and stout stems were obtained in G medium (B5-MS basal medium with 1 mg/L benzylaminopurine, BAP). From these seedlings, cotyledonary node (CN) and epicotyl explants were excised and re-cultured onto fresh G medium. Cotyledonary node explants either regenerated 3-5 shoots from each axillary bud three weeks after inoculation or that somatic embryos were induced from the base of the cut portion. Multiple shoots formed directly from the apical cut portion of the epicotyl sections closest to the shoot tips (segment a) when cultured onto B5-MS basal medium with 1-2 mg/L BAP. Conversely, organogenic calli were induced from the basipetal cut portion of all the epicotyl sections when cultured in G medium with 1 mg/L naphthalene acetic acid (NAA) with segment c (that closest to the CN) being the most responsive. Multiple shoots were also obtained from axillary buds of young sprouts and seeds from immature fruits of indexed mandarin trees. In pummelo, somatic embryos were induced from albedo tissues of immature fruits in MS and BP media with 1 mg/l each of BAP and 2,4-D. Removal of these growth regulators allowed germination of the embryos and subsequent shoot growth. Organogenic calli was observed using B5, MS and BP media with 0.5 mg/L 2,4-D. After 3-5 months, shoots were regenerated. These regeneration systems are

being utilized using virus-free indexed materials for slow growth and cryopreservation studies.

**Keywords:** Albedo, cotyledonary node, epicotyl, organogenesis, somatic embryogenesis, tissue culture

**ASD No. 22**

## **EFFECTS OF PHYSICAL, CHEMICAL AND LIGHT TREATMENTS ON GERMINATION AND GROWTH OF TISSUE-CULTURED COCONUTS**

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The acclimatization and *ex vitro* establishment of tissue cultured coconut plantlets regenerated either from zygotic or somatic embryos can result in serious losses. Although high germination rates can be achieved on a culture medium, the survival of zygotic embryo-derived plantlets in soil is very low (0-30%). Hence, treatments that could promote development of good quality seedlings having good shoot and root is needed to increase seedling survival *ex vitro*. The effects of physical, chemical and light treatments on the germination and growth of coconut embryos and the tissue culture-derived seedlings, respectively, were investigated. All experiments were conducted in completely randomized design (CRD) with 3 replications. The germination of coconut embryos was promoted significantly when incubated in a liquid Y<sub>1</sub> medium placed in a rollerdrum. Gibberellic acid (GA<sub>3</sub>) significantly affected growth of seedlings including: shoot length, shoot width, root width, fresh and oven dry weights but not root length. However, GA<sub>3</sub> did not promote percent germination. In addition, light quality significantly affected growth of seedlings including: leaf length, shoot length, fresh weight, oven dry weight, number of roots and leaves but not light quantity. Blue, red and yellow light (400-700 nm) significantly promoted growth (leaf length, shoot length, fresh weight, oven dry weight, number of roots and number of leaves) in comparison with the control. These conditions could be used to improve the growth of tissue culture coconuts *in vitro* before they could be acclimatized.

**Keywords:** coconut, tissue culture, gibberellic acid, light, rollerdrum

ASD No. 23

**CRYOPRESERVATION BY ENCAPSULATION-DEHYDRATION AND  
SHOOT REGENERATION IN LIME (*Citrus aurantifolia* Swing.)**

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Cryopreservation of plant material is the only viable option for the long-term storage of germplasm of vegetatively propagated species and species with recalcitrant seed (Ashmore, 1997). This involves the storage at ultra low temperature (-196°C). At this temperature, cell division and metabolic processes stop and plant material can be stored without modification or alteration for unlimited period of time. Lime (*Citrus aurantifolia* Swing.), one of the valuable citrus species in the Philippines due to its high percentage of acid (citric) in the juice, was used to develop a cryopreservation technique. Good quality somatic embryos were encapsulated using 2.5% or 3.0% sodium alginate and dehydrated for 1, 3 or 9 hr. The dried beads were immersed in liquid nitrogen for 12 and 24 hr, then quickly thawed at 60±2°C for 3-5 mins. and cultured on the recovery medium. Cultures treated with 2.5% sodium alginate and 0.5 M sugar and dehydrated for three hr were found most responsive. Remarkable increase in size was observed in all the samples seven days after inoculation onto the recovery medium. Somatic embryos proliferated with or without liquid nitrogen treatment. Proliferation was higher in Rp medium (BP, Barba and Pateña, medium with 0.5 mg/L 2,4-D, 60 g/L sugar and 100 ml/L coconut water) compared to Mr medium (MS medium with 60 g/L sugar and 200 ml/L coconut water) and in the control compared to the liquid nitrogen-treated samples. The somatic embryos matured and germinated and eventually regenerated shoots. This study shows that lime somatic embryos are amenable to cryopreservation and that the encapsulation-dehydration technique can be used to store lime on a long-term basis.

**Keywords:** somatic embryos, cryopreservation, encapsulation-dehydration, *in vitro* conservation



**ASD No. 24**

**SOMATIC EMBRYOGENESIS AND PLANTLET REGENERATION IN  
CALAMANSI (*X Citrofortunella microcarpa* Bunge.)**

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Calamansi (*X Citrofortunella microcarpa* Bunge.), also called 'calamondin' or 'kalamansi' in Tagalog and 'limonsito' in Cebuano, is the most important citrus fruit crop of the Philippines. A system for somatic embryogenesis and plantlet regeneration of this crop was developed for use in genetic transformation and *in vitro* conservation. Somatic embryos were induced using nucellar tissues from immature seeds cultured onto BCO and MCO media (BP, Barba and Pateña, and MS, Murashige and Skoog, media, respectively, with 20g/L sugar and 100 ml/L coconut water). Highest somatic embryo formation was observed in BCO cultures obtained from immature fruits with diameter of 6.0-9.5 mm. Somatic embryo induction decreased as the fruit diameter increased. Somatic embryos were also induced from nucellar tissues cultured onto LMr medium (MS macro and micro, MT vitamins and amino acids with 1.0 mg/L each of kinetin and NAA, 2.0 mg/L BAP and 50 g/L sugar). Proliferation of somatic embryos was enhanced when subcultured onto Gp, Mp and Rp media (Gamborg's B5, MS and BP media, respectively, with 0.5 mg/L 2,4-D, 60 g/L sugar and 100 ml/L coconut water). Maturation and germination of somatic embryos were observed in BCO and MCO media. Plantlets developed in 3-5 weeks and have been acclimatized for transplant to soil. This system for somatic embryogenesis and plantlet regeneration of calamansi can now be used for genetic transformation and *in vitro* conservation studies.

**Keywords:** nucellus, somatic embryogenesis, plantlet regeneration, transformation, *in vitro* conservation

**ASD No. 25**

**SOMATIC EMBRYOGENESIS AND PLANTLET REGENERATION  
IN LIME (*Citrus aurantifolia* Swing.)**

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Lime (*Citrus aurantifolia* Swing.) is one of the valuable citrus species in the Philippines. It has the highest percentage composition for acid as citric but somewhat lower in ascorbic acid than lemon. It is used as juice and as component of alcoholic drinks, beverages and medicinal preparations. Developing a tissue culture system for somatic embryogenesis and plantlet regeneration of this crop would be a useful tool for its micropropagation and *in vitro* conservation. Somatic embryos were induced using nucellar tissues from immature seeds cultured onto G medium (B5-MS with 1.0 mg/L BAP). Somatic embryos were also induced from undeveloped seeds of mature fruit cultured onto Rr medium (BP, Barba and Pateña, medium with 60g/L sugar and 200 ml/L coconut water). Proliferation of somatic embryos was enhanced when subcultured onto Gp, Mp and Rp media (Gamborg's B5, MS and BP media, respectively, with 0.5 mg/L 2,4-D, 60 g/L sugar and 100 ml/L coconut water). Maturation and germination of somatic embryos were observed in BCO and MCO media (BP, Barba and Pateña, and MS, Murashige and Skoog, media, respectively, with 20 g/L sugar and 100 ml/L coconut water), but remained in cotyledonary stage for 5-7 weeks. When the cultures were aged (one year or older) and subcultured onto LMr medium (MS macro and micro, MT vitamins and amino acids with 1.0 mg/L each of kinetin and NAA, 2.0 mg/L BAP and 50 g/L sugar), the somatic embryos germinated and developed into plantlets.

**Keywords:** nucellus, somatic embryogenesis, plantlet regeneration, micropropagation, *in vitro* conservation

**ASD No. 26**

**CROSS COMPATIBILITY OF ELITE PAPAYA INBRED LINES  
TO AN INTERSPECIFIC HYBRID OF *Carica papaya*  
L. x *Carica quercifolia* (Saint-Hil.) Hieron**

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The cross compatibility of four papaya inbred lines to F<sub>1</sub> interspecific hybrid, *Carica papaya* x *C. quercifolia* line 410 was studied. Parental lines used were characterized morphologically. In addition, resistance to papaya ringspot virus (PRSV-P) and pollen viability of line 410 was evaluated under Philippine conditions.

The genotypic compatibility among four crosses between F<sub>1</sub> interspecific hybrid and papaya inbreds (4108, 4172, 5648, and 5893) are evident. Inbreds 5648 and 5893 produced fruits filled with a large number of seeds. All crosses produced viable embryos that grow easily *in vitro*. Remarkable difference was observed in morphological characters among papaya inbreds as well as between papaya and F<sub>1</sub> interspecific hybrid line 410. Cluster analysis separated parent materials into three sub groups. Papaya inbreds derived from local selections (4108 and 4172) and inbreds that were introduced from other countries (5648 and 5893) were subdivided. Line 410 has a separate cluster.

Symptomatology and serological test by indirect ELISA (Enzyme Linked Immunosorbent Assay) confirmed that interspecific hybrid line 410 plants have resistance against PRSV-P. Tetrazolium test, a pollen viability assay, showed a number of viable pollen under local conditions

**Keywords:** Inbred lines, F<sub>1</sub> interspecific hybrid, cross compatibility, Papaya Ringspot Virus (PRSV), pollen viability

ASD No. 27

**DEVELOPMENT OF THERMOSENSITIVE  
GENETIC MALE STERILE (TGMS) LINES AT PHILRICE**

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Thermosensitive genetic male sterility (TGMS) is controlled by a single recessive nuclear gene which interacts with temperature to express pollen sterility or fertility. Its use can simplify the method of exploiting heterosis or hybrid vigor in rice. Also, it is an alternative to the use of cytoplasmic male sterility (CMS) in developing and producing seeds of hybrids.

TGMS lines are sterile at high temperature and fertile at low temperature, thus, multiplying seed of sterile line is easier since there is no need for a maintainer line. In addition, TGMS lines do not require pollen parent with restorer gene hence a wider array of varieties and elite lines could be utilized in crosses thus increasing the chances of finding heterotic combinations. R&D efforts on the use of the system are therefore being pursued intensively at PhilRice. As early as 1994, TGMS lines from different sources were assembled and evaluated as possible donors of *tgms* gene(s) in crosses in breeding for improved TGMS lines. Recently, four agronomically improved and potentially useful TGMS lines were identified from breeding populations alternately grown and evaluated in so-called male fertile environment (MFE) and male sterile environment (MSE). Results of more than two consecutive years of testing indicate that they have stable fertility/sterility behavior in MFE and MSE and can be useful female parents in the development and production of new hybrids.

**Keywords:** thermosensitive, TGMS, hybrid rice, breeding, male sterility

**ASD No. 28**

**GENETIC FIDELITY TESTING IN CITRUS USING  
ISOZYME MARKERS**

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Isozymes have been extensively studied and utilized to assess genetic variations in plant populations generated via *in vivo* and *in vitro* techniques. In our ACIAR-IPGRI-funded citrus project which commenced in January 2003, we developed five enzyme systems to assess genetic diversity between tissue-cultured and non-tissue cultured calamansi, pummelo, mandarin and lime. These systems included shikimate dehydrogenase (SDH), isocitric dehydrogenase (IDH), acid phosphatase (ACP), malate dehydrogenase (MDH) and glutamate oxaloacetate transaminase (GOT). Results showed two SDH banding patterns, one for calamansi and mandarin and another for pummelo and lime. Calamansi and pummelo exhibited a single IDH band. This band was seen in both the tissue-cultured and non-tissue cultured samples, although faint bands were observed in tissue-cultured calamansi and pummelo as well as in non-tissue cultured pummelo. Two ACP bands and three MDH-2 bands were seen in tissue-cultured and non-tissue cultured calamansi. No difference was observed in ACP and MDH-2 enzyme patterns between the tissue-cultured and non-tissue cultured calamansi. For GOT, two bands of activity were obtained both for calamansi and pummelo. Pummelo showed darkly stained bands compared to the light to faint bands of calamansi. The lightly to faint enzyme patterns observed is generally a result of limited sample for analysis. However, this could still be improved using more leaf tissues. In spite of this limitation, it was clearly seen that the tissue-cultured and non-tissue cultured calamansi and pummelo carried similar IDH, ACP, MDH-2 and GOT patterns. No variations have been observed so far between the tissue-cultured and non-tissue cultured materials. These five enzymes systems that were developed can now be utilized to test the genetic fidelity of tissue-cultured (new and aged cultures) and cryopreserved materials.

**Keywords:** genetic fidelity, isozymes, tissue culture

ASD No. 29

**GENETIC VARIABILITY OF RESTORER LINES  
IN RICE (*Oryza sativa* L.)**

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Estimates of genetic variance are useful in designing breeding programs, as the higher the genetic variability and magnitude of diversity, the greater the probability of heterosis. The genetic variability of restorer germplasm at PhilRice were determined by developing half-sib hybrids following the line x tester mating design involving 20 randomly selected restorers and three commercially usable CMS lines and evaluated in two environments. In addition, these restorers were assayed for molecular analysis using SSR markers to determine the molecular divergence at the DNA level.

Analysis of variance for both locations revealed highly significant ( $P < 0.01$ ) differences among restorer lines, CMS lines and restorer lines x CMS lines for all traits. Estimates of genetic variance of restorer lines ( $s^2L$ ) for traits measured were highly significant based on F-test, except for number of productive tillers. Confidence interval estimates ( $s^2L$ ) for all traits were also significantly different from zero except number of productive tillers. This indicates that estimates obtained were significant and that genetic variability exists for these traits.

The 65 SSR markers were polymorphic. A total of 266 alleles ranging from 2 to 8 alleles locus<sup>-1</sup> and a mean of 4.09 alleles locus<sup>-1</sup> were detected. The Nei and Li genetic similarity coefficients among restorers and CMS ranged from 0.22 to 0.71. Twenty four percent of the total alleles detected were rare (63/266) and these were distributed throughout the entire chromosomes of the rice genome. Combined mean performance across locations revealed that several hybrids out yielded the best checks. Twenty-five percent (15/60) of hybrids evaluated at

UPLB exhibited 16.73 – 44.29% yield advantage over the highest yielding check, PSB Rc72H. At PhilRice CES, three hybrids (5%) out yielded the highest yielding check PSB Rc28.

Overall, the results indicate the presence of a wide range of genetic variability and molecular divergence in the PhilRice restorer germplasm, indicating great potential to generate outstanding hybrids for commercial cultivation.

**Keywords:** Genetic variability, molecular diversity, germplasm, restorer lines, SSR markers

**ASD No. 30**

**MORPHOLOGICAL CHARACTERIZATION AND DNA  
FINGER PRINTING OF CMS LINES BRED AT PHILRICE**

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Morphological characterization of CMS lines bred at PhilRice showed appreciable differences between lines for 1,000 grain weight, grain length and width, culm length and number, date to fifty percent flowering, culm strength, flag leaf angle, and secondary branching. Using microsatellite markers and compared with 37 other CMS lines used in our hybrid rice breeding program, the PhilRice-bred CMS lines appeared to be more distantly related and genetically diverse. Informative microsatellite markers with unique alleles that can only be found in PhilRice CMS lines were also identified. With these results we hope to increase the efficiency of heterotic hybrid identification at the Institute.

**Keywords:** CMS (Cytoplasmic Male Sterile), hybrid rice, microsatellite markers

ASD No. 31

**GLYPHOSATE TOLERANCE AND INSECTICIDE EFFICACY AGAINST  
ASIATIC CORN BORER, *Ostrinia furnacalis* (GUENEE)  
OF TRANSGENIC ROUNDUP READY CORN (NK 603/MON 810)  
UNDER SCREENHOUSE CONDITIONS<sup>1</sup>**

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The glyphosate-tolerance and insecticidal effectiveness of transgenic roundup ready (RupR) corn line NK 603/MON 810 expressing the *Bacillus thuringiensis* gene against the Asiatic corn borer, *Ostrinia furnacalis* (Guenee) was evaluated under screenhouse conditions in comparison with isohybrid DK818.

Roundup® herbicide at two dosage rates: 0.72 and 1.42 kg a.e. ha<sup>-1</sup> was applied at 15, 30 and 15 and 30 days after planting (DAP) on both corn hybrids. To assess susceptibility to corn borer, the plants were artificially infested with neonate larvae at 20 and 40 DAP. Herbicide effect and insect pest damage were determined on the transgenic corn and non-transgenic counterpart in comparison with respective untreated checks.

Agronomic performance indicated comparable seedling emergence and vigor between the transgenic cotton and isohybrid line. Herbicide application at both rates totally killed the treated isohybrid line DK 818, while full growth and development was noted for the transgenic corn until maturity. Extreme susceptibility to corn borer was noted on artificially infested isohybrid line as evidenced by severe occurrence of leaf perforations, petiole or stalk breakage and clumped whorl causing stunted growth. On the other hand, only a few pinpricks on the leaves of transgenic corn were observed that had negligible adverse effect on the plant's growth and development.

RupR corn NK 603/MON 818 was highly tolerant to glyphosate at either dosages applied at early or middle vegetative stage. It was highly resistant to corn borer infestation. With inherent glyphosate-tolerance and insect resistance to corn borer properties, this hybrid should be evaluated under different local corn growing conditions for adaptation assessment, in addition to consideration of possible effects on the environment.

<sup>1</sup>Project funded by Monsanto Phils., Inc.

**Keywords:** glyphosate-tolerance, RupR, *Bacillus thuringiensis*, Asiatic corn borer



**ASD No. 32**

**SCREENING OF  $R_1$  PAPAYA LINES DERIVED FROM  
MICROPROJECTILE BOMBARDMENT FOR RESISTANCE  
TO PAPAYA RINGSPOT VIRUS**

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Transformation of Davao 'Solo' papayas was done to develop resistance to ringspot virus using microprojectile bombardment. Twenty-seven  $R_1$  papaya lines derived from  $R_0$  lines were utilized in the screening for resistance to papaya ringspot virus (PRSV). Resistance to the disease was based on symptomatology and serology whereby virus was detected by enzyme-linked immunosorbent assay (ELISA) after 4-8 weeks of inoculation with IPB-PRSV isolate. One hundred and seven individual plants coming from 17  $R_1$  lines including PB 4.3, PB 4.6, PB 4.8, PB 4.10, PB 4.11, PB 5.1, PB 5.5, PB 5.8, PB 5.12, PB 6.8, PB 8.2, PB 8.5, PB 12.9, PB 13.6, PB 13.8, PB 13.10, and PB 15.6 were considered putative resistant  $R_1$  lines. The putative resistant plant lines were free of PRSV infection and negative to ELISA after four to eight weeks of inoculation. In contrast, the susceptible plant lines had typical symptoms of PRSV that developed 14 days after inoculation. The symptoms include: leaf mottling, mosaic, chlorosis, vein-clearing, severe leaf malformation, stunting and elongated oily streaks on the stem and petioles.

**Keywords:** microprojectile bombardment, papaya ringspot virus,  $R_1$  lines, symptomatology, enzyme linked immunosorbent assay, resistance, transformation

**ASD No. 33**

**DEVELOPMENT OF TRANSGENIC PAPAYA WITH DELAYED  
RIPENING CHARACTERISTICS CONTAINING THE ACC OXIDASE  
GENE VIA THE *Agrobacterium*-MEDIATED TRANSFORMATION**

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ACC oxidase is one of the regulatory enzymes that control ethylene biosynthesis. By controlling this enzyme, the effect of ethylene during ripening

of climacteric fruits such as papaya can also be regulated. The partial cDNA of ACC oxidase (isolated from ripening papaya fruits) was inserted in a binary vector pGA643 in the antisense orientation and the resulting gene construct was transfected into *Agrobacterium tumefaciens* strain LBA4404 via electrophoretic techniques. Transformation of papaya somatic embryos and multiple shoots was done using an *Agrobacterium*-mediated co-cultivation method. After a brief exposure of plant tissues to the bacteria, putatively transformed somatic embryos and multiple shoots were transferred and maintained in Solid Embryo Induction Medium (SEIM) supplemented with carbenicillin and kanamycin. Approximately 22% (178 out of 802) of the somatic embryos and 6% of the multiple shoots (48 out of 780) are currently grown in SEIM with carbenicillin to get rid of the bacterium. Nineteen per cent (19%) of the somatic embryos and 77% of the multiple shoots are already grown in the kanamycin selection medium in preparation for plant regeneration.

**Keywords:** *Agrobacterium*, ACC oxidase, Transformation, Somatic Embryo Induction Medium (SEIM)

#### ASD No. 34

#### **BANANA BUNCHY TOP VIRUS RESISTANCE IN BANANA (*Musa* sp.) Cv. LAKATAN DEVELOPED BY IRRADIATION OF SHOOT CULTURES**

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Banana bunchy top virus (BBTV) is the most destructive virus disease of banana in the Philippines. Incorporation of resistance to this virus disease by conventional hybridization is not possible due to sterility of most commercial banana cultivars. Gamma irradiation coupled with *in vitro* technology was explored as a means to develop BBTV resistance.

The sensitivity of banana shoot cultures to varying doses (5 to 100 GY) of Cobalt 60 gamma radiation was determined. Irradiated shoot cultures were micropropagated for three to five cycles and plants regenerated were potted out and evaluated for BBTV resistance. Plants were indexed for BBTV using symptomatology, ELISA and PCR techniques.

A total of 6,012 plants regenerated from irradiated shoot cultures were subjected to artificial BBTv inoculation using the aphid vector *Pentalonia nigronervosa*. From these plants, 64 putative BBTv resistant lines were selected in the field after 36 months of evaluation. The selected putative BBTv resistant plants exhibited varying degree of resistance reaction to the virus. Twenty six (26) lines showed no BBTv symptom expression in both irradiated and first generation sucker plants. The other 38 lines exhibited limited symptom expression. Yield and agronomic characteristics of some putative resistant lines were comparable to non irradiated micropropagated plants. Suckers from these putative BBTv resistant lines were collected; micropropagated and plants are now being evaluated for the second cycle stability of BBTv resistance trait.

**Keywords:** banana, mutation, BBTv, gamma radiation, *in vitro* technology

**Funded by:** IAEA, PCARRD-DOST, IPB-UPLB

#### **ASD No. 35**

### **EFFECT OF DRYING OILS ON PROPERTIES OF PHENOLIC VARNISH FROM CASHEW NUT SHELL LIQUID**

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The total production of cashew nuts during the last ten years averaged around 100,000 MT per year. The average amount of cashew nut shell liquid (CNSL) generated by the cashew industry for the last ten years is estimated to be around 25,000 MT. Utilization of CNSL into varnish can bring benefit to the furniture industry.

The effect of different drying oils on properties of phenolic varnish from cashew nut shell liquid was evaluated. Drying oils tested include linseed oil, tung oil, soya bean oil and rubber seed oil with levels varied at 20, 30 and 40 parts by weight (pbw). Results showed that drying time of CNSL varnishes proceeded from the shortest to longest time in this order: tung oil<linseed oil<soya bean oil<rubber seed oil. CNSL varnishes with tung oil followed a decrease in drying time with an increased in amount of oil from 20-40 pbw. CNSL varnishes with linseed oil, soya bean oil and rubber seed oil have longer drying time with

increased amount of oil from 20-40 pbw. All other properties such as adhesion, gloss, resistance to hot coffee, soft drinks and 45% alcohol, hot-and-cold check resistance were comparable with commercial varnish.

**Keywords:** cashew nut shell liquid, tung oil, linseed oil, soya bean oil, rubber seed oil, adhesion, hot-and cold check resistance

**ASD No. 36**

**UTILIZATION OF FRESH GOLDEN SNAIL MEAT  
AS FEEDS FOR SWINE**

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Feeding trials using fresh golden snail meat as replacement for commercial mash and soybean oil meal were conducted to compare the growth performance of pigs fed with and without fresh golden snail meat and to assess the profitability of using fresh golden snail meat on growing-finishing pigs. The study was conducted in seven municipalities of Ilocos Norte, namely Bacarra, Batac, Dingras, Pagudpud, Piddig, Sarrat and Solsona. One cooperator was selected in each municipality. Two dietary treatments in each trial were randomly assigned to piglets distributed to each cooperator using Randomized Complete Block Design (RCBD). Dietary treatments were pure commercial mash (CM) and 90% CM + 10% equivalent amount of fresh golden snail meat (FGSM) in the first feeding trial and formulated rations (FR) with and without FGSM in the second feeding trial.

Result showed that pigs fed with CM + 10% equivalent amount of FGSM had comparable growth performance, feed consumption and backfat thickness to those fed with CM. However, required less amount of feeds to produce a kg gain in weight. Thus, the gain obtained per pig is PhP815.00 higher than those fed with pure CM.

Similarly, pigs fed FR with and without FGSM had comparable total and daily gain in weight, feed consumption, feed conversion efficiency and backfat