

AGRICULTURAL SCIENCES

ASD No. 1

**DEVELOPMENT OF BACTERIAL BLIGHT RESISTANT
HYBRID RICE PARENTAL LINES THROUGH
BI-DIRECTIONAL MARKER-AIDED SELECTION**

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Although hybrid rice (*Oryza sativa* L.) technology has been widely adopted in China, it is relatively new in tropical Asia. Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama 1922) Swings et al. 1990 (*Xoo*), has posed serious threats in the success of hybrid rice seed production and cultivation in the Philippines. To improve the level of resistance, BB resistance from donor lines carrying *Xa4*, *Xa7*, and *Xa21* genes were successfully introgressed into IR58025B, IR62829B, LianB, 913B and BoB maintainer lines. To further refine the selection process, bi-directional marker-aided selection (MAS) was utilized in selecting for the advanced generation (BC_3F_1) of IR58025 progenies. Morpho-agronomic evaluation facilitated by molecular markers enabled the selection to be more efficient. To determine the level of parental genotype recovery among the improved progeny lines, DNA fingerprints were generated. Results indicate a high level of similarity with the original parental genotype. While the rest of the improved maintainer lines will serve as potentially useful hybrid rice germplasm with enhanced level of bacterial blight resistance for future breeding purposes, the selected IR58025B and IR58025A with improved BB resistance can now be used to produce a BB-resistant *Mestizo*, the most widely used hybrid in the Philippine government's hybrid rice commercialization program. Moreover, IR58025A is the female parent of most hybrids released in Asia. With the AxB and AxR seed production technology

well worked out and found to be economically feasible, these BB-resistance enriched lines thereby promise to create both national and international impact.

Keywords: hybrid rice, bacterial blight, marker-aided selection, *Oryza sativa* L., *Xanthomonas oryzae* pv. *oryzae*

ASD No. 2

INULIN AVAILABILITY IN TRANSGENIC RICE

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Inulin is a linear fructan abundant in 15% of flowering plants. Inulin lowers blood lipids, reduce serum triglycerides and most importantly enhance bifidobacteria and lactobacilli for increased Fe, Mg, Ca, and Cu absorption. The staple rice is a good target to contain inulin since it lacks many micronutrients needed in the human diet, most specially, vitamins, the essential amino acids and iron. The two inulin synthesizing enzymes saccharose-saccharose-1 fructosyltransferase (SST) and fructan-fructan-1-fructosyltransferase (FFT) were isolated from artichoke and obtained from Max-Planck Institute. The two genes were cloned in a binary vector containing the phosphomannose isomerase selectable marker gene and placed under two different endosperm-specific promoters. This binary vector pInulin 2 was introduced to *Agrobacterium tumefaciens* LBA4404 and used to transform immature embryos of Taipei 309. A total of 128 putatively transformed plants were obtained and 7 transformation events were confirmed through Southern blot analysis. Reverse transcriptase PCR of the T1 seedlings proved the presence of the 3 transgenes. Fructose analysis of the T2 seeds that is being continuously conducted in the University of Stuttgart showed that line 4-1 has 0.5 mmol/L and line 5-1 has 1.5 mmol/L inulin. Highly expressing lines that will be obtained will be seed-increased for possible feeding test in animals.

Keywords: inulin, rice, saccharose-saccharose-1 fructosyltransferase, fructan-fructan-1- fructosyltransferase, *Agrobacterium tumefaciens*-mediated transformation

ASD No 3

**DEVELOPMENT OF LOCALLY-ADAPTED
RICE VARIETIES WITH HIGH BETA-CAROTENE CONTENT
IN THE GRAINS (GOLDEN RICE)**

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A two-pronged strategy is being implemented to develop locally-adapted Golden Rice, a genetically modified rice capable of producing high amounts of pro-Vitamin A (beta-carotene) within the endosperm. In the first strategy, Golden Rice versions of popular varieties PSB Rc82 and Mabango1 are being developed through molecular marker-aided backcrossing with emphasis on foreground and background selection. In the second strategy, new elite Golden Rice lines that are resistant to tungro and bacterial blight diseases are being bred through multiple crosses, anther culture and marker-aided selection. A total of 26 cross combinations were performed using three Syngenta Golden Rice 1 events (with up to 8 mg/g beta-carotene content) as donor of the beta-carotene biosynthetic genes. All three events are in the background of US rice variety Cocodrie and have a single copy of the transgene cassette. Morphological evaluation and PCR assay using primers specific to the transgenes *phytoene synthase* and *phytoene desaturase* confirmed the hybrid nature of the F1 plants. BC1F1 progenies were produced by crossing the F1 plants from selected cross combinations to their respective recurrent parents.

Variations in grain color (yellow) intensity, an indicator of beta-carotene content, were observed both in the BC1F1 and F2 grains suggesting gene dosage effect. Individual BC1F1 plants that will be selected based on genetic similarity to their recurrent parent and/or resistance to tungro and bacterial blight will be used for further backcrossing and selection. Locally-adapted Golden Rice varieties are envisaged as an additional strategy to combat the persistent problem of vitamin A deficiency (VAD) in the country.

Keywords: Golden Rice, vitamin A deficiency, betacarotene, marker-aided backcrossing, biofortification

ASD No. 4

**EFFICIENT *IN VITRO* SEED CULTURE, GERMINATION, SHOOT
REGENERATION AND MUTANT INDUCTION TECHNIQUES IN LANSONES
(*LANSIUM DOMESTICUM* CORREA)**

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Langsat or lansones (*Lansium domesticum* Correa) is a very popular fruit in South-East Asia. In this species parthenocarpy is the rule and apomixis as evidenced by multiple seedlings due to polyembryony is quite common. No breeding has been attempted, and propagation is limited to seeds and through grafting to achieve fruiting in half-time (5-7 years). Mutation breeding by ionizing radiation is a potent strategy to induce and select for improved traits particularly early fruiting, dwarfing, improved fruit quality and disease resistance in lansones. This study involved the establishment of *in vitro* culture requirements for aseptic seed culture, germination and shoot regeneration in lansones 'Paete' followed by gamma-irradiation of cultured seeds.

The first two replicated experiments involved the culture of 81 seeds in MS basal medium with 30 g L⁻¹ refined sugar and 2 mg L⁻¹ BAP (L1 medium). On the average, 86.6% germination was achieved in 30 d of culture with 1.5 and 2.1 shoots produced per seed at 30 and 60 d, respectively. The third experiment involved the comparison of germination and shoot regeneration as influenced by four culture media namely L0 (MS basal medium with no growth regulator), L2 (B5 basal medium with 1 mg L⁻¹ each of BAP and GA₃), and L3 (WPM medium with 1 mg L⁻¹ each of BAP and GA₃) with L1 medium as check. Higher % germination was achieved using L0 (92.9%) and L2 (92.6%) as compared with L3 (81.3%) and L1 (80.4%). However, earlier shoot germination was recorded in L2 (24.4 d) and L3 (25.2 d) as compared with L1 (34.6 d) and L0 (33.0 d). Highest number of shoots produced after 60 d was obtained in L1 (2.5 shoots) followed by L2, L3 (1.9 shoots) and lowest in L0 (1.5 shoots). Addition of BAP alone or BAP and GA₃ had significant effects on days to germination, number of shoots and shoot lengths. The fourth experiment involved exposure of cultured lansones seeds in L1 medium to gamma rays (0, 10, 20 and 40 Gy) followed by shoot regeneration. The number of shoots regenerated was significantly reduced from 2.6 (control) to 2.0 (10 Gy), 2.1 (20 Gy) and 1.6 (40 Gy). Shoot tip and nodal explants from irradiated seedlings were successfully advanced through micropropagation.

Keywords: langsat, *Lansium domesticum* Correa, micropropagation, mutation breeding, tissue culture, tropical fruit tree

ASD No. 5

**MICROGRAFTING TECHNIQUE FOR SOMATIC EMBRYO
RESCUE AND MUTANT RECOVERY IN AVOCADO
(PERSEA AMERICANA MILLER)****Renato A. Avenido*, Julita G. Dimaculangan and Julieta N. Welgas**

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The successful plant regeneration via somatic embryogenesis of the two Philippine avocado varieties 'RCF Purple' and 'Semil' at the Institute of Plant Breeding (IPB-CA, UPLB) has promoted the use of plant biotechnology through somaclonal variation and *in vitro* mutation as potential strategies for avocado crop improvement. In the last three years, we have produced over 250 shoot regenerants as putative variants/mutants following tissue culture and gamma-irradiation treatments. These materials are being micropropagated, and screening for genetic variations using molecular markers has started with very promising results. Among the challenges met were the limited recovery of rooted (bipolar) plantlets from somatic embryos, slow growth of shoot regenerants and the losses due to contamination and callusing problems among long-term avocado tissue cultures. Micrografting and *ex vitro* grafting are viable tools available to promote shoot growth of putative mutant lines and fast-track the screening process for mutant selection. For the purpose of developing avocado micrografting technique, we have used two zygotic embryo-derived shoot cultures namely 'Semil' as scions and the *Phytophthora* root rot-resistant and faster growing line 'Mainit' as rootstocks. Scion shoots with matching V-shaped base were inserted into slit made on the rootstock shoots, with grafts secured using sterile filter paper strip and thread. The micrografts were re-cultured in agar-solidified B5 basal medium with 1 mg L⁻¹ each of BAP and GA₃. Depending on scion type, micrografting was 72-100% successful. A modified procedure of micrografting either zygotic and somatic embryo-derived shoots of 'Semil' onto rooted *in vitro* germinated seedlings as rootstocks resulted in 90 and 61% success, respectively. Growth of successful shoot micrografts in shoot cultures and in the rooted rootstocks are being evaluated for subsequent *ex vitro* grafting and potting out, respectively.

Keywords: Avocado, *ex vitro* grafting, mutation breeding, micropropagation, *Persea Americana*, shoot cultures, somatic embryogenesis, tissue culture

ASD No. 6

SEARCH FOR TUBER-ASSOCIATED PROTEIN GENES IN CASSAVA

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Cassava ranks second among the five major tuber crops in terms of worldwide production as an important source of starch or carbohydrates for human food, animal feed and industry. But the protein content of the cassava tuber is relatively low (1 to 2%) compared to other cereal crops such as rice and corn (8 to 12%). Conventional breeding efforts to increase protein content in cassava from 2 to 7% were successful but with major drawbacks such as poor agronomic traits and increased cyanide content.

A molecular approach to increase the protein content without such drawbacks is through genetic engineering. The primary requirement is to isolate a gene encoding a tuber-associated protein from cassava and regulate the expression of this gene using a strong constitutive plant promoter. Primers were designed and constructed based on the N-terminal sequence of a non-glycosylated 40 kDa globulin protein (pI of 6.5) isolated and purified from the cassava tubers. PCR and RT-PCR of genomic DNA and total RNA from cassava leaves/tubers generated a 600 bp PCR fragment. Based on DNA sequence analyses, two genes (CSV1 and CSV2) encoding putative tuber-associated proteins were identified. CSV1 is approximately 187 amino acids of 20.6–21.5 kDa molecular mass while CSV2 consisted of 183 amino acids with a molecular mass of 19.5 kDa. Phylogenetic analyses suggest that CSV1 and CSV2 are more related to patatin than dioscorin, sporamin and tarin. Unexpectedly, the deduced amino acid composition showed that both are highly charged basic (cationic) proteins (pIs of 9.8 and 11.6). Two highly conserved domains (LSGRQ and WISAEFAL) were identified in both CSV1 and CSV2.

Keywords: cassava, *Manihot esculenta*, tuber proteins

ASD No. 7 ✓

TOWARDS MAP-BASED ISOLATION OF DOWNY MILDEW RESISTANCE GENE IN MAIZE**Hayde F. Galvez*, Alma O. Canama and Desiree M. Hautea**

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Downy mildew caused by *Peronosclerospora philippinensis* Weston (Shaw) is one of the most important diseases limiting maize production in the country. The use of resistant varieties remains the most effective control measure against downy mildew.

Bulk segregant analysis (BSA) coupled with simple sequence repeat (SSR) and resistance gene analog (RGA) marker analyses was employed to saturate the quantitative trait loci (QTL) for downy mildew resistance (DMR) and to identify tightly linked SSR/RGA markers. Nine RGA markers were mapped in DMR QTL regions especially in chromosomes 2 and 3, and in the major QTL region in chromosome 8. RGA marker *srga3* mapped exactly within the interval of the major QTL, which is flanked by RFLP markers *umc150* and *asg52*. Four EST-derived SSRs were also mapped in this region and confirmed 100% linked to DMR by BSA.

The *srga3* fragment in maize is currently being cloned for sequencing. This is to characterize this putative plant resistance gene ortholog, develop DMR-specific DNA markers for marker-assisted selection, and to isolate the underlying R genes via a QTL map-based approach. RGA *srga3* marker is derived from a plant disease resistance gene sequence of soybean.

Keywords: maize, downy mildew resistance, QTL, disease resistance gene sequence

ASD No. 8

**NEW TECHNIQUES IN HYBRID RICE CULTIVATION:
PARACHUTE OR SCATTERED TRANSPLANTING METHOD**

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Parachute or scattered transplanting is a new method used widely in China specifically in hybrid rice production. It is a technique where rice seedlings are grown in plastic trays, pulled or uprooted from the tray, with a soil ball and tossed like a parachute toy on a puddle field. In 2003, because of the interest shown by PhilSCAT researchers on the method, 30,000 trays were sent to the Philippines through the center. Thus formal research for the adoption of parachute transplanting has now started in the country.

This research aimed to compare three transplanting methods commonly used for hybrid rice production. It specifically aimed to seek the advantages of the parachute transplanting method over mechanical transplanting and conventional (manual) method in terms of yield and economics of production.

Parachute transplanting showed significantly better results than manual or conventional transplanting and mechanical transplanting in terms of number of productive tillers, number of tillers per hill, yield and net income. With the parachute transplanting method, the seedlings were able to reach maximum tillering faster and thus they had more productive tillers.

In the two-season trials, parachute transplanting gave the highest net income of P 23,657.34 for wet season (WS) and P 32,465.55 for the dry season (DS). Mechanical transplanting gave an income of P 22,827.74 (WS) and P 28,872.13 (DS) while conventional transplanting was the lowest as it registered P 19,710.32 (WS) and P 23,766.21 (DS).

Based on the two season trials, parachute transplanting is worth considering due to the following reasons: (a) seedlings can be established easier and (b) grow faster, (c) it promotes tillering (more number of tillers per hill and productive tillers), (d) it provides higher yield, and (e) higher net income.

Keywords: parachute, hybrid, tillers, panicles, rice cultivation

ASD No. 9

DESICCATION AND FREEZING TOLERANCE OF MANDARIN (*Citrus reticulata* Blanco) SEEDS**Olivia P. Damasco and Luzminda C. Refuerzo**

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Citrus seeds are classified as intermediate, thus, seed conservation under conventional storage conditions defined for orthodox seeds (3 to 7% moisture content (MC) at -20°C storage) cannot be applied. Cryopreservation is the only viable option for long-term storage of *Citrus* germplasm. One of the critical factors ensuring success in cryopreservation is the amount of water present in the cell prior to freezing. This study aimed to determine the moisture content that would allow seeds of mandarin species to survive cryopreservation without deleterious effects on germination and seedling recovery. The tolerance of seeds of mandarin (*Citrus reticulata* Blanco) varieties 'Calamandarin', 'Ladu', 'Szinkom', and 'Tai Cat' to desiccation and liquid nitrogen freezing was determined.

Surface sterilized seeds excised from mature fruits were dried over silica gel (100 g) in an airtight container for varying time intervals. Desiccated seeds were subjected to rapid freezing in liquid nitrogen (-196°), rapid thawing at 50 °C, and cultured onto MS basal medium for seedling recovery and germination.

Mandarin seeds desiccated to about 9% MC showed germination rates similar to fresh seeds (80 to 100% germination). Lowering the MC to <5% resulted to significant reduction in germination rates (<50% germination). The highest survival after cryopreservation, ranging from 21.5 to 27.8%, was observed only for seeds with 9.1% MC for 'Calamandarin', 6.1% MC for 'Szinkom', and 3.8% MC for 'Tai Cat', respectively. Among the different mandarin varieties tested, 'Ladu' showed the least survival (13%) after cryopreservation. Results show that mandarin seeds were moderately tolerant to desiccation (could be desiccated to ~9% MC without significant reduction in germination), but sensitive to liquid nitrogen freezing. Hence, cryopreservation using seed desiccation technique is not a viable option for long-term conservation of mandarin seeds.

Keywords: mandarins, cryopreservation, seed desiccation, citrus

ASD No. 10

**FIELD PERFORMANCE OF SELECTED BBTV
RESISTANT MUTANT LINES OF BANANA CV LAKATAN**

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Banana Bunchy Top Virus (BBTV) is a major problem of the Philippine banana industry. It has gravely reduced yields for the small-scale farmers and even wiped out the Lakatan industries in some regions of the country.

Bananas are not amenable to sexual breeding because they are male and female sterile. Thus, bunchy top virus resistance cannot be introgressed into banana by conventional breeding methods. Gamma irradiation and *in vitro* technologies were used to develop BBTV resistance in banana cv Lakatan. Thirty two (32) selected BBTV resistant mutant lines obtained from the earlier study were micropropagated and planted in BBTV infected field under high disease pressure for confirmation of stability of BBTV resistance and evaluation of agronomic characters of Generation 1 and 2 (G1 and G2) mutant lines.

Of the 32 mutant lines evaluated, ten (10) promising lines consistently showed lower BBTV disease incidence compared with the tissue culture (TC) control plants 3 to 19 months after planting. The %BBTV-free G1 plants at harvest was significantly higher for the ten selected mutant lines (58.3% to 95.8%) compared with the TC control plants (32.4%). Yield parameters such as bunch weight, number of hands per bunch, weight per hand and number of fingers per hand were comparable with the TC control plants. Some selected mutant lines have shorter number of days from planting to harvest; harvest period of 15 to 32 days earlier than the TC control plants.

Keywords: gamma irradiation, *in vitro* techniques, BBTV resistance, mutant lines

ASD No. 11

IN VITRO TECHNIQUES FOR MICROPROPAGATION AND LONG-TERM CONSERVATION OF INDIGENOUS CITRUS SPECIES**Olivia P. Damasco* and Luzminda C. Refuerzo**

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Indigenous citrus species are important part of the culture and livelihood of the native and rural communities in the Philippines. These species are becoming endangered, and at present found only in the wilds and remote areas of the country. Collection, propagation and conservation are urgently needed for further utilization and possible commercialization of these species. This study aimed at developing *in vitro* techniques for conservation and use of indigenous citrus species.

Indigenous citrus species such as 'Gapas-gapas' (*Citrus hystrix* var *boholensis*), 'Kubot'/'Kulubot'/'Kalpi' (*Citrus* spp), 'Dalayap'/'Dayap' (native lime, *C. aurantifolia*) and 'Suwa' (*C. limon*) were collected in different areas in Luzon (Quezon, Isabela, Nueva Viscaya, Quirino) and Mindanao (Cagayan de Oro). *In vitro* techniques for micropropagation and long-term conservation through cryopreservation are being developed for the different species.

Somatic/nucellar embryogenesis in 'Gapas-gapas' and 'Kalpi' was obtained using immature and mature seeds cultured onto MS basal medium or MS basal medium supplemented with 2,4-D and BAP. Techniques for long-term conservation through cryopreservation of desiccated seeds were developed. The % germination after cryopreservation ranged from 68 to 75% for native lime 'Dalayap'/'Dayap', 74 to 93% for 'Suwa' and 34 to 80% for a range of 'Kubot'/'Kulubot' genotypes. Results of the study show that seeds of indigenous citrus species, though heterogeneous, could be cryopreserved and this technique would allow long-term conservation of these species.

Keywords: indigenous citrus species, cryopreservation, micropropagation, *in vitro* techniques

ASD No. 12

A CANDIDATE GENE SEQUENCE FOR BACTERIAL WILT RESISTANCE IN TOMATO

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Tomato is one of the most important vegetable crops grown in the Philippines. Bacterial wilt is a serious production constraint in tomato production especially during the off-season planting. Genetic sources for bacterial wilt resistance (BWR) have been identified and quantitative trait loci (QTL) have been mapped. However, the BWR-QTL identified need to be fine-mapped and validated for use in marker-assisted breeding and to isolate the BWR genes.

Integrating mapped SSR as anchor markers, six (6) of the 12 linkage groups previously constructed for BWR were assigned to their respective chromosome numbers in the tomato genome. The major QTL for BWR (AFLP *af02*) was assigned in tomato chromosome 6. The QTL is being fine-mapped to possibly characterize and/or clone the underlying resistance gene. Through bulk segregant analysis (BSA), 20 RGA and one (1) SSR markers were identified linked to BWR.

Gene-specific markers are being developed based on *af02* marker. Based on sequence homology, *af02* was found to be highly identical with a gene sequence of the signal recognition particle (SRP54) specific protein of tomato, EST and other tomato exon sequences, and with a putative plant disease resistance protein of *Solanum demissum*. For further applications, SCAR markers were designed based on the sequence information of *af02*. These markers are being used to screen and validate the susceptible mutants induced by physical and chemical mutagenesis. The markers are also used as starting regions in the map-based isolation of BWR genes.

Keywords: tomato, bacterial wilt resistance, QTL, fine mapping, bulk segregant analysis, map-based gene isolation

ASD No. 13

**GERMPLASM COLLECTION, MULTIPLICATION, MAINTENANCE AND
EVALUATION OF NATIVE AND EXOTIC VARIETIES OF
UBI AND TUGUI UNDER ILOCOS CONDITION**

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The collection of different accessions started in 2001. To date, a total of 19 tugui and 26 ubi accessions have been collected and maintained at MMSU for conservation. Characterization using the IPGRI Descriptor for Yam was undertaken to establish authenticity. For tugui, the most prominent differentiating characteristics were vine color and tuber characteristics such as hairiness, shape, size and presence/denseness of thorns. On the other hand, variability of the ubi collections were apparent both on the foliage and on the tubers. These include leaf shape, color, density and the presence of aerial tubers. For the tubers, flesh color were either purple, white, yellowish, off-white, a combination of white and purple with either color as the primary flesh color, purple with a shade of white or white with a shade of purple. Among the collections, four tugui accessions coded as MMSU Tugui # 3, 6, 7 and 9 and two ubi accessions coded as MMSU Ubi # 2 and 4 were identified promising. These could yield an average of 4.59-5.54t/ha for tugui and more than 11 t/ha for ubi. In addition, these accessions are very acceptable to consumers. Moreover, the farmers' cultural management practices which only include planting and harvesting were documented and fine-tuned to develop a technology for increased productivity. With such, farmers could engage to a more profitable yam production, making the marginal and idle lands productive and thereby be used as a means to support the governments program on food security.

Keywords: yam, ubi, tugui, accession

ASD No. 14

**INDIGENOUS SEMI-TEMPERATE VEGETABLES OF THE HIGHLAND
CORDILLERAS**

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Indigenous vegetables are hardy, nutritious and a storehouse of desirable traits. A thorough survey and inventory of the indigenous vegetables in the various municipalities in Benguet and Mountain Province and including Baguio City was undertaken through a participatory approach with the local folks. Each plant was closely studied and aptly described botanically.

This step hopes to come up with strategies for the genetic conservation, propagation and eventual commercialization of the most promising (in terms of nutrient content, hardiness, acceptability, etc) indigenous semi-temperate vegetables in the Cordillera. Results of the chemical analysis revealed that carbohydrates, fat, fiber, crude protein; vitamins, minerals, etc of the indigenous semi-temperate vegetables were comparable with those of the cultivated crops.

Forty-nine (49) plants belonging to 23 families were eaten as vegetables utilizing the tops or young shoots. Few make use of the fruits and flowers for food. Of the 23 families, a greater number (18.4%) belong to Asteraceae, followed by Solanaceae (10.2%), then Amaranthaceae (8.2%).

A very important output of this project is a handbook with the title "Indigenous Semi-Temperate Vegetables of Cordillera" which will surely boost the awareness and on the utilization of indigenous vegetables.

Keywords: indigenous, vegetables, semi-temperate, highland, cordillera, sustainable, conservation, propagation.

ASD No. 15

GERMINATION AND GROWTH OF COCONUT EMBRYOS

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Gibberellic acid (GA_3) concentrations ranging from 30–40 μM significantly promoted germination and fresh weight of 'Laguna Tall' embryos in comparison with the control. Shoot emergence of germinated embryos was significantly enhanced with the addition of 10–50 μM GA_3 but did not significantly promote root and shoot growth *in-vitro*. In addition, various *in vitro* soil support systems including coconut coir dust, vermiculite and garden soil were tested for suitability during *in vitro* acclimatization to improve growth of germinated seedlings in comparison with the hybrid protocol. These support systems had the same effect as the control (hybrid protocol) on the promotion of leaf production, leaf width expansion, leaf elongation, girth expansion and enhancement of leaf quality (greening of leaves). Seedlings acclimatized *in vitro* in coconut coir dust and vermiculite, and in the control had similar leaf quality four months after culture, but those in garden soil had pale green or yellowish leaves and had lower leaf quality rating. This quality of seedlings was carried up to the *ex vitro* acclimatization phase where seedlings previously acclimatized *in vitro* in coconut coir dust and vermiculite had greener leaves than those in garden soil. Percent survival of the *ex vitro* acclimatized seedlings previously grown in coir dust (*in vitro* acclimatization) and the control is higher (83.3%) than those acclimatized *in vitro* in vermiculite and garden soil (66.7%). Furthermore, the collective acclimatization method applied to newly *ex vitro* acclimatized seedlings, using a wooden box covered with a transparent plastic lid, produced better quality seedlings with greener and shinier leaves than was possible using the plastic bag method used in the hybrid protocol.

Keywords: coconut, gibberellic acid, *in vitro* soil

ASD No. 16

**ORGANIC, VIRUS-FREE AND TRUE-TO-TYPE
TISSUE-CULTURED GARLIC (*ALLIUM SATIVUM* L.)**

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Garlic is one of the most economically important crops in the Philippines. Average yield is low compared to other countries due to low quality planting materials which can be traced to its conventional method of propagation, i.e. by cloves. Traditionally, farmers use previous crop's harvest as planting materials on a continuous basis. This poses a high risk of transmitting diseases from one generation to the next and of losing varieties. To solve this problem, we developed the technology of producing virus-free and true-to-type garlic planting materials and adapted it for mass production of bulbs for distribution to farmers. In addition, we incorporated organic farming in increasing bulb production to add value to the crop.

The technology consisted of (1) improved tissue culture protocol (with cold pre-treatment and thermotherapy) to produce small-tissue-cultured bulbs (in vitro bulblets we referred to as G_0 bulblets), (2) Enzyme-Linked ImmunoSorbent Assay or ELISA to index the materials for presence/absence of virus, (3) isozyme (protein) markers to check if the tissue-cultured materials were true-to-type, i.e. without genetic variations from the initial materials and (4) field planting (using organic farming) of tissue cultured bulblets to produce more bulbs ($G_0 > G_1 > G_2 > G_3$) and increase bulb size. Small bulbs were obtained from in vitro bulblets in the first generation (G_1) while normal size of bulbs was obtained in the 2nd generation (G_2). Increase of planting materials from G_2 to G_3 was 6x for Ilocos White, 5x for Mindoro and 7x for Tan Bolter. While low cost, imported garlic is available in the market, what we have produced are bulbs which are disease-free, true-to-type and with added value of having been produced organically.

Keywords: *Allium sativum* L., garlic, thermotherapy, tissue culture, virus-free

ASD No. 17

SOMATIC EMBRYOGENESIS IN COCONUT (*COCOS NUCIFERA* L.) USING PLUMULE EXPLANTS OF 'MAKAPUNO', 'LAGUNA TALL', AND 'BAYBAY TALL'**Lilian F. Patena, Claudette N. Dacumos, and Ramon C. Barba**

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Coconut is among the woody species which is very difficult to tissue culture. It is propagated only by seed. Through biotechnology, we are developing other systems for its propagation.

Sixteen batches of plumule explants from fresh and pre-germinated embryos of coconut var. 'Makapuno', 'Laguna Tall' and 'Baybay Tall' were inoculated onto four media for calloid formation and somatic embryogenesis. The media consisted of Y3 (Euwen's, 1976), BP (Barba and Patena, 2002), modified BP and MS (Murashige and Skoog, 1962) basal salts supplemented with different levels of 2,4-D, BAP and 2-iP. Fruit weight, embryo size, plumule and calloid weight, calloid color and degree of calloid formation were noted. 'Laguna Tall' had the highest increase in embryo length, followed by 'Baybay Tall' while 'Makapuno' remained very slow growing except in the first 14 days of pre-germination. Calloid formation was observed one month after inoculation. Calloid weight increase was greatest with 42-day pre-germinated embryos of 'Laguna Tall' and 'Baybay Tall' while the effect of media was variable on these varieties. Calloid weight increase in 'Makapuno' embryos was greatest using Y3 medium, then BP medium and last, MS medium. White calloids were observed on the three varieties using BP medium. Few to profused (rating of 1 to 3) calloid formation was observed using BP medium while cultures turned brown using Y3 and modified BP media.

Keywords: calloid formation, coconut, *Cocos nucifera* L., somatic embryogenesis,

ASD No. 18

**TISSUE CULTURE OF DIFFERENT STRAINS OF 'CARABAO' MANGO
(*MANGIFERA INDICA* L.) AND THEIR CHARACTERIZATION USING SSR
MARKERS**

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'Carabao' mango is the variety of commerce in the Philippines, both for local consumption and for export. There are several strains of the 'Carabao' mango and SSR markers were used to characterize them.

Somatic embryos (1^o, 2^o, 3^o, ... n^o) were produced on a continuous basis using the protocol of Pateña, et al. (2002). Depending on the strain, primary (1^o) somatic embryo induction ranged from 33 to 100% while succeeding somatic embryo proliferation after 23 months (subculture cycle 8, S8) ranged from 25-100%. Cotyledonary leaf and root formation was best in modified Gamborg's B5 and Barba and Patena's BP basal media supplemented with either Ki or BAP and maintained in lighted condition. An increase of 40-50% in frequency of shoot formation was observed in BAP-containing media. True leaf formation was obtained 4-8 weeks after subculture onto the regeneration medium compared to 1-2 years the previous years. 2,4-D inhibited root formation. Initial transplant of plantlets to soil was done and more plantlets are being regenerated for subsequent transplant. Approximately 6-gram leaf samples were used for DNA extraction. DNA extraction was better using yellowish green, young leaves and adding PVP and mercaptoethanol to the extraction medium. The modified CetylTrimethylAmmonium-Bromide (CTAB) Method used in genomic DNA extraction from rice leaves was successfully used for genomic DNA extraction of mango. Different primers were tested for DNA amplification using PCR and characterization of the different 'Carabao' strains using SSR markers is in progress.

Keywords: mango, *Mangifera indica* L., tissue culture, SSR markers

ASD No. 19

MICROSATELLITE POLYMORPHISM AND DISEASE RESPONSE TO BACTERIAL BLIGHT (*XANTHOMONAS ORYZAE* PV. *ORYZAE*) OF RICE AMONG RESTORERS, MAINTAINERS AND THERMOSENSITIVE GENIC MALE STERILE LINES IN PHILIPPINES HYBRID RICE BREEDING

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Many of the problems associated with different breeding programs based on phenotypic characterization of an agronomic trait can be eliminated by the use of DNA-based diagnostics. The analysis of genetic relationships among lines is necessary in determining widely diverse genotypes for developing heterotic hybrid combinations and ensuring wide genetic base of future hybrids that will be developed. In this study, 61 hybrid rice materials including 28 restorer (R) lines, 15 maintainer (B) lines, 6 thermo-sensitive genetic male sterile (TGMS) lines and 12 advanced breeding lines were analyzed for extent of genetic relationships in 64 microsatellite loci spanning the 12 rice chromosomes. UPGMA cluster analysis showed that both IRRI-bred and PhilRice-bred and developed B and TGMS were distinct in terms of genetic background. B lines of IRRI clustered at 59.2% genetic similarity and are separated from the B lines of PhilRice hybrid genepool except for IR73328B and IR78367B. The TGMS materials including IR68301s, IR73827-23s and IR73834s, grouped at 76.7% genetic similarity. IR73827-23s and IR73834s showed 96.4% genetic similarity in 64 loci and 129 alleles. TGMS1 exhibited 63.5% similarity with the TGMS lines from IRRI while TGMS4 and TGMS6 clustered at 90.1% similarity. All 61 parental cultivars were susceptible to *X. oryzae* pv. *oryzae* race 6, PXO99. IR62161-184-3-1-3-2R, pollen parent of NSIC114H demonstrated high level of resistance to *Xoo* race 10, PXO341 including IR68897B, IR79123B, IR78378B, PR3B, IR68301s (TGMS) and three advanced breeding lines.

Keywords: restorer, maintainer, TGMS, hybrid rice, *Oryza sativa*, bacterial blight, microsatellites, genetic diversity

ASD No. 20

**STORAGE ROOT DEVELOPMENT OF
FIVE VARIETIES OF SWEETPOTATO (*Ipomoea batatas*)
UNDER LA TRINIDAD CONDITION**

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The storage root development of five varieties of sweetpotato was evaluated at the BSU-Institute of Plant Breeding (UPLB-CA) Highland Crops Research Station in Benguet State University, La Trinidad, Benguet from September 2004 to March 2005 to identify early and high yielding variety.

Sweetpotato storage root development increased with time of harvest from two to five months after planting exhibiting a sigmoid curve which varied among the five varieties of sweetpotatoes studied such as: SG 98-01-03, PSBSp 17, NSICSp-27 (Bengueta), Kalbo-oy and Haponita. Sweetpotato varieties showed significant differences on the number and weight of storage root per plot, diameter and length of storage root, plant weight and harvest index. SG 98-01-03 and PSBSp 17 significantly produced the highest number and weight of storage root, storage root diameter and plant weight. These varieties were identified early maturing and high yielding under La Trinidad, Benguet condition during September to March production season. Haponita was medium maturing variety while NSICSp-27 and Kalbo-oy were late maturing varieties based on the storage root diameter, weight of storage root per plant and computed total yield per hectare.

(SG-sweetpotato genotype; PSBSp-Philippine Seed Board Sweetpotato; NSICSp-National Seed Industry Council Sweetpotato)

Keywords: sweetpotato, storage root, development, early, medium and late maturing varieties

ASD No. 21

**BIOEFFICACY OF TOBACCO (*Nicotiana tabacum* L.) SEED POWDER
AGAINST CORN WEEVIL (*Sitophilus zeamayz*)**

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The use of botanical pesticides for agricultural stored food stuffs is the most environment-friendly way to safeguard the consuming public from the risk of possible pesticidal residue poisoning.

The laboratory and *in situ* bioefficacy tests of tobacco seed powder (TSP) against corn weevil were evaluated. Dry tobacco seeds were powderized and different TSP quantities at 1, 3, 5, 7, 9, 11, and 15 g were prepared and mixed separately with 7 g of uninfected corn kernels previously placed in a petri dish. Percent (%) mortality of the test insects was evaluated 6 h after application and every 6 h thereafter for 72 h.

The 15 g TSP outperformed the other treatments including the positive control. The commercial insecticide and the 15 g TSP showed 100% mortality of the test insects after 66 and 72 h, respectively.

The *in situ* test used the best treatment from the laboratory experiment. The 15 g TSP was then tested and was evaluated in two preparations: the TSP in powder form and TSP in tea bag. Each of the TSP preparations was used to treat 100g uninfected corn kernels placed in a rectangular box of 6.5 x 4.5 inches dimension and which were previously infested with 30 healthy corn weevils. After one month observation period, TSP in pure form showed the highest and fastest insecticidal action at par with the TSP in tea bag including the commercial pesticide.

Furthermore, percent (%) damage of the corn kernels was least pronounced in pure TSP. Corn kernels treated with pure TSP gave 100% germination while those seeds treated with the commercial insecticide, negative control, and TSP in tea bag gave 98%, 45% and 82%, respectively.

Keywords: Tobacco seed powder (TSP), pesticidal, mortality, bioefficacy

ASD No. 22

**ECOTYPIC VARIATION OF THE ASIAN CORN BORER,
OSTRINIA FURNACALIS (GUENEE) POPULATIONS IN THE PHILIPPINES**

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Barrion *et al.* (1981) and Mendoza *et al.* (1994) reported differences in terms of morphometric, DNA and isozyme analysis in the Asian corn borer populations from Laguna, Leyte, Bukidnon and South Cotabato. There is the possibility also that other differing populations may be occurring in other corn growing areas. What is the significance or what are the implications of these possible different populations particularly on the management of the corn borer during production. Likewise, what is its implication in corn breeding program for ACB resistance and release of commercial varieties?

Each geographical region(s) has its own set of environmental conditions including climatic and other factors. Hence, a crop variety's responses may vary for each site. In corn, the expression of resistance may differ not only because of the abiotic factors in the agroecosystem but more importantly it is because of the varying levels of diversity among local populations of its primary pest, the Asian corn borer. Therefore, the differential varietal responses against the ACB can be a reliable and direct indicator in confirming the presence of significantly different populations of ACB in the major corn growing areas of the country. This study is aimed to establish the occurrence of these populations and assess their infective ability through the field responses of selected corn varieties in six major corn-growing areas of the Philippines.

This study was conducted in six (6) selected sites representing different corn growing areas as follows: Laguna, Isabela, Bicol (Camarines Sur), Leyte, Bukidnon, South Cotabato using the following corn varieties: Yellow corn hybrid (Opaque) – IPB 911, Yellow corn hybrid (Flint) – NK 8840, Sweet Corn – IPB Philippine Supersweet, White corn hybrid – C 818, Tiniguib, Lagkitan.

Wet season trial results showed that Philippine Supersweet was the most susceptible variety against ACB across locations followed by Lagkitan and Tiniguib. The most ACB resistant varieties were the two hybrids NK 8840 and C818. Intermediate response (tolerance to ACB feeding) was exhibited by IPB 911. NK 8840 and C818 had the highest yield followed by IPB 911. Philippine Supersweet followed by Lagkitan had the lowest yield.

Keywords: ACB resistance, IPB 911, Yellow corn hybrid (Flint) – NK 8840, Sweet Corn – IPB Philippine Supersweet, White corn hybrid – C 818

ASD No. 23

***QUADRSTICHUS ERYTHRINAE* KIM (HYMENOPTERA: EULOPHIDAE),
A NEW INVASIVE PEST INFESTING, GALLING AND KILLING DAPDAP
TREES (*ERYTHRINA* SPP.) IN THE PHILIPPINES**

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Dapdap trees (*Erythrina* spp.) are commonly planted as ornamental trees and their usually bright red flowers and attractive form make them important landscape material. In the last two years at least, however, many dapdap trees have died and only a few trees are left and struggling to recover. The culprit is an insect that causes masses of galls on leaves, stems and shoots, eventually defoliating trees and drying up new and old growth. Damage assessments place the percentage of mortality among trees from around 20 to as high as 60%. Affected plants range from seedlings to pole stage young trees to very mature ones. Recovery, if at all, is very slow and usually death of trees follows, often signaled by the final attack of scavenging maggots. This paper reports the identity of the species as *Quadrastichus erythrinae* Kim, a wasp belonging to the order Hymenoptera, family Eulophidae and recently described in 2004 from Singapore, Reunion Island and Mauritius. The species is believed to be Afrotropical in origin but its manner of introduction into the Philippines and the penultimate source is still unknown. The adult insect, its larvae and the nature, development and extent of damage are described and photodocumented. There are large- and small-type galls which seem to correspond with the sizes of the haploid males and diploid females, the species apparently exhibiting haplodiploidy like most Hymenoptera. This is the

third invasive insect species that entered the country in a span of less than 5 years, following the buff coconut mealybug, *Nipaecoccus nipae* (Maskell) and long palm leaf beetle, *Brontispa longissima* Gestro. Poor implementation and/or widespread non-compliance to quarantine rules and regulations are always suspect underlying causes of these new pest problems.

Keywords: dapdap, dapdap gall wasp, Eulophidae, forest insect pests, Hymenoptera, invasive species, *Quadrastichus erythrinae* Kim

ASD No. 24

IDENTIFICATION OF MUTATION SITES IN THE ACETOLACTATE SYNTHASE (ALS) GENE OF THE RESISTANT *Rotala indica*

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Rotala indica, a lowland weed, developed resistance to an ALS-inhibiting herbicide, bensulfuron methyl(BSM). Most studies on the resistance mechanism to ALS inhibitors report that resistance is due to mutations in the ALS gene. This study aimed to identify amino acid substitutions in conserved regions of the resistant *R. indica* ALS gene that could explain its resistance to ALS inhibitors.

First strand cDNA was synthesized from the total RNA extracted from leaves of the resistant and susceptible *R. indica*. These served as templates for the polymerase chain reaction (PCR) amplifications. The target was a 230-bp sequence that contains two conserved regions of the ALS Domain A. Amplification was verified by agarose gel electrophoresis, and the excised product was cloned using the TA-cloning technique. Double-stranded plasmid DNA was purified and DNA sequencing was performed using an ALF autosequencer.

Comparing the partial DNA and amino acid sequences of the resistant and susceptible ALS, seven nucleotide substitutions were detected but these did not result in mutations within the conserved regions. There was, however, an obvious difference in a single amino acid right after the AFQETP region (Isoleucine₂₁₁ in the

R-biotype; Threonine₂₁₁ in the S-biotype). There was no amino acid substitution at Proline₁₉₇ of the AITGQVPTKVIGT and at Alanine₂₀₅ of the AFQETP sequences. Instead, a three-amino acid sequence after Proline₁₉₇ of the AITGQVPTKVIGT was found. This sequence is common for both biotypes but not with other plants. This is the first report of a unique sequence within this conserved region. It is not conclusive yet to state that the resistance mechanism of *R. indica* is attributed to that single amino acid substitution after the AFQETP region, but it can be hypothesized that it may have an influence on the responses of the weed to ALS inhibitors.

Keywords : *Rotala indica*, herbicide resistance, acetolactate synthase inhibitor, mutation, bensulfuron methyl

ASD No. 25

INSECT RESISTANCE MANAGEMENT (IRM) OF BT CORN IN THE PHILIPPINES

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Bt corn was commercially sold in the Philippines market in 2002. The Bt corn has the ability to withstand the Asian corn borer infestation as compared to traditional varieties and hybrids that are susceptible to it. In order to prolong the use of this new technology, insect resistance management (IRM) is needed as a proactive method on risk mitigation.

In 2005, the Bureau of Plant Industry, the technology providers namely: Monsanto, Pioneer and Syngenta, non-government organization represented by the Biotech Coalition of the Philippines, the academe through the U.P. Los Baños, selected local governments and corn farmers planned and joined efforts to address IRM issues. This group recently launched concerted actions related to insect resistance management on Bt corn (IRM) in the Philippines thru an awareness briefings to farmers in five provinces of Mindanao and four provinces of Luzon from July 18-27. A total of 926 Bt corn adoptors participated. They were informed on modern biotechnology and Bt corn. The 80:20 bag in a bag method to prolong

the technology of Bt corn usage was discussed. This insect resistance management strategy involves the planting of 80% Bt corn and 20% non Bt corn. Different planting designs were presented to the farmers. Later they were asked if they were willing to practice this IRM strategy or not. About 56.40% were not willing to implement such scheme while 43.60%. The figures are quite close to each other as far as the acceptance and non acceptance of 80:20 IRM strategy is concerned. The results of this briefing cum survey were used in the review of DA Memorandum Circular # 17 (S 2003) which provides for the requirements for the IRM of Bt corn in the

Keywords: Bt corn, insect resistance management, risk mitigation, alliance

ASD No. 26

**PRODUCTION AND UTILIZATION OF *TRICHOGRAMMA EVANESCENS*
TRICHOGRAMMA PARASITOIDS IN TARLAC**

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This study is a joint undertaking among corn farmers, the Provincial and Municipal governments of Tarlac, the Tarlac College of Agriculture and the National Crop Protection Center - UP Los Baños on the production and use of *Trichogramma* in Tarlac. It is a continuation of the project on *Community Based Mass Production and Utilization of Trichogramma Parasitoids Against Lepidopterous Pest of Corn*.

The National Crop Protection Center which is now a part of the Crop Protection Cluster, College of Agriculture, UPLB, extended a technology transfer scheme to the Tarlac College of Agriculture in 2004 through a memorandum of agreement. Four staff and five students of the college were trained on the production and utilization of *Trichogramma*. The Tarlac College of Agriculture on the otherhand forged a partnership with the governor's office of Tarlac to provide farmers *Trichogramma* parasitoids as a part of an IPM package for corn borer.

Trichogramma evanescens Westwood releases were done three times during the 2004-2005 growing season. These were at 30, 45, and 60 days after corn emergence. Twenty five farmers tilling sixty four hectares of Dekalb hybrid corn used this technology. A total of 4,445 *trichogramma* cards were released.

As of April 2006 the information drive for farmers was continued at the Tarlac College of Agriculture. A total of 50 farmer leaders from ten towns of Tarlac participated. More farmers are targeted for this year. Meanwhile the production of *Trichogramma* using *Sitotroga* as host commenced last December 2005 in addition to the initial *Cocycra* host that was utilized. Likewise *Trichogramma chilonis* will be reared for use against corn earworm, tomato fruit worm and eggplant fruit and shoot borer.

Keywords: Central Luzon, corn borer, partnership, *Trichogramma*, Tarlac, corn

ASD No. 27 X

IDENTIFICATION OF MUTATION SITES IN THE ACETOLACTATE SYNTHASE(ALS) GENE OF THE RESISTANT *Rotala indica*

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Comparing the partial DNA and amino acid sequences of the resistant and susceptible ALS, seven nucleotide substitutions were detected but these did not

result in mutations within the conserved regions. There was, however, an obvious difference in a single amino acid right after the AFQETP region (Isoleucine₂₁₁ in the R-biotype; Threonine₂₁₁ in the S-biotype). There was no amino acid substitution at Proline₁₉₇ of the AITGQVPTKVIGT and at Alanine₂₀₅ of the AFQETP sequences. Instead, a three-amino acid sequence after Proline₁₉₇ of the AITGQVPTKVIGT was found. This sequence is common for both biotypes but not with other plants. This is the first report of a unique sequence within this conserved region. It is not conclusive yet to state that the resistance mechanism of *R. indica* is attributed to that single amino acid substitution after the AFQETP region, but it can be hypothesized that it may have an influence on the responses of the weed to ALS inhibitors.

Keywords : *Rotala indica*, herbicide resistance, acetolactate synthase inhibitor, mutation, bensulfuron methyl

ASD No. 28

EFFICIENCY OF HIGH FREQUENCY RESONANT PEST-KILLING LAMP IN CONTROLLING COTTON BOLLWORM, *Helicoverpa armigera* (Hubn.)

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The efficiency of the imported light trap from china, known as the high frequency resonant pest killing lamp for pest control was evaluated on cotton. Ten insect orders with various number of families were noted, namely, coleoptera (23 families), hemiptera (11 families), homoptera (2 families), hymenoptera (4 families), lepidoptera (6 families), odonata (1 family), orthoptera (4 families), dermaptera (1 family), diptera (4 families) and neuroptera (1 family), with all described for their trophic roles and some insects identified to the species level. An increasing trend in *helicoverpa armigera* population was noted from 45 dap – 78 dap while *amrasca biguttula* population was minimal.

About 20% increase in seedcotton yield was obtained from cotton with the light trap over the comparative farm. Further, there was only one chemical spraying for cotton with the trap while the comparative farm received 3x sprayings, thus, savings of two sprayings and six labor man-days for the former. Partial budget analysis showed an advantage of ca. Php 2000 for the new technology vis-à-vis chemical insecticides.

It is highly recommended that the technology be marketed soon in the country through government intervention so that its merits as pest control tactic are harnessed for other economic crops.

Keywords: high frequency resonance lamp, cotton bollworm, seedcotton yield, chemical insecticides

ASD No. 29

**PLANT RESOURCES USED AS BUILDING MATERIALS IN TINOC, IFUGAO
CORDILLERA ADMINISTRATIVE REGION, LUZON, PHILIPPINES**

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The towering mountains and dense forests surrounding Tinoc, Ifugao, one of the provinces of the Cordillera Administrative Region, has made it one of the remotest areas in the country. As such, the Kalanguya inhabiting the area has preserved their culture. The tribe members have managed to survive the harsh life in the mountains. This study highlights their ingenuity on plant use especially those used as building materials. Information regarding traditional or indigenous use of plant resources among the Kalanguya was gathered using interview schedules, focused group discussions and direct observation. The information gathered from interviews was enhanced by field observations. Results showed that there are 38 plant species distributed in 36 genera and 29 families identified for building purposes. These plants are further classified into their specific uses, i.e. house construction, fencing, kitchen paraphernalia, baskets, chicken cages and coffin.

Key words: traditional or indigenous uses, Kalanguya, plant resources, Tinoc, Ifugao

ASD No. 30

**PRELIMINARY ASSESSMENT OF LAND USE
AND WATER QUALITY OF SOME LAKES
IN SAN PABLO, LAGUNA, PHILIPPINES**

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Land conversions brought about by increasing population and the apparent increase of agricultural, residential and commercial activities in San Pablo have affected the quality of the water resources in the area. Agriculture and built-up areas comprised the majority of land use. Their assessment generated new interpretative maps after integrating remotely sensed data such as Landsat image and aerial photographs to various land use studies and surveys.

The degradation of surface water quality was manifested by the physico-chemical analyses of sediments deposited in selected rivers and lakes. Sediments are important repositories of information regarding water quality and chemical composition of water bodies. Sediments from Palacpaquen Lake and adjoining rivers were assessed. The sulfate values of samples taken from different sites range from 47.79 to 292.29 ppm, phosphates from 0.79 to 5.53 ppm, nitrates from 146.98 to 370.50 ppm, and organic carbon from 0.98 to 4.56 ppm. The observed variability of nutrient concentrations were attributed mainly to anthropogenic activities around the lake and rivers which include agriculture, domestic and aquaculture.

Land use has also affected groundwater resources being one of the principal sources of drinking water. Untreated spring and well water are vulnerable to industrial, domestic and agricultural pollution. Spring and well water from selected lakes such as Sampaloc, Mohicap and Palacpaquen were monitored for total and fecal coliform contamination levels. There were varied levels of contamination. In most cases the microbiological water quality in the sampled sites did not pass the drinking water standards of the Department of Health and the Department of Environment and Natural Resources.

Proper land and water resource planning and management should be implemented to address the problem of water quality degradation in San Pablo.

Keywords: land use, water quality, San Pablo City, remote sensing, nutrient analysis, coliform analysis

ASD No. 31

**EFFECTS OF FILAMENTOUS GREEN ALGAE (*ENTEROMORPHA*
INTESTINALES LINN.) AND COMMERCIAL FEEDS TO MILKFISH (*CHANOS-CHANOS* FORSSKAL) FINGERLINGS REARED IN CAGES AT
THE MARINE WATERS OF TIGUIS, PORO, CEBU**

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Bangus or milkfish being our national fish is the focus of some aquaculturists to be reared in any system of culture in order to meet the demands of the consuming public. Camotes Islands as one of the fishing grounds in the Philippines have areas where fishpen and cage culture can be done That is why this study was conducted in order to find out the effects of the filamentous green algae and commercial feed to bangus fingerlings in cages in terms of body weight, total length and weight to supply acclimated fingerlings to the prospective fishpen and fishcage owners.. This study was also conducted in order to find out the survival and mortality rate of the fish.

Bangus fingerlings were reared in cages and there were four treatments in the study with four replicates using Randomized Complete Block Design (RCBD). Treatment 0 (control) no feed given; Treatment 1 (fed with filamentous green algae) ; Treatment 2 (Commercial feeds) and Treatment 3 (Combination of filamentous green algae and commercial feeds). Feeding was done twice a day based on 25% of its biomass Sampling was done every 15 days for 1 1/2 months.

Results show that in terms of body weight, Treatment 2 has gained 7.1 grams followed by Treatment 3 which has a gain of 5.5 grams. Third is Treatment 1 which has 2.7 grams and the last is Treatment 0 which is 2 grams. For the total length, Treatment 2 got first which has an increase of 4.5 cm, Treatment 3 has 3.4 cm; Treatments 1 has 2.7 cm and Treatment 0 has 2 cm respectively. For the body depth, Treatment 2 got first with an increase of 0.8 cm followed by Treatment 3, Treatment 1, and Treatment 0 which is 0.4 cm For the survival and mortality rate, it shows that Treatment 2 and Treatment 3 has 100% survival rate compared to Treatment 0 and Treatment 1 which is 97.73%.

Results further show that there was no significant mean difference on the rate of growth of milkfish fingerlings in terms of body weight, body depth, total length and mortality rate.

Keywords: *Chanos chanos*, Poro, Cebu and *Enteromorpha intestinales*

ASD No. 32 31

TOLERANCE OF GIANT FRESHWATER PRAWN (*MACROBRACHIUM ROESNBERGII* DE MAN) POSTLARVAE TO HIGHER SALINITIES REARED IN AQUARIA AT THE LABORATORY OF CEBU STATE COLLEGE OF SCIENCE AND TECHNOLOGY, SAN FRANCISCO, CEBU CAMPUS

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The tolerance of the postlarva of giant freshwater prawn to higher salinity was studied using behaviour, movements, and feeding characteristics as indicators. There were five treatments in the study where Treatment 0 (control 0- 0.5 ppt); Treatment 1 (1-5ppt); Treatment 2 (6-10ppt); Treatment 3 (11-15 ppt) and Treatment 4 (16-20ppt). The postlarvae having a weight of 0.9 to 1.2 grams were stocked in the aquaria using 8 grams of prawn per liter of water as the ratio. Increase in salinity in each treatment was done gradually every two days by adding saltwater in each treatment measured by using refractometer until the desired salinity level in each treatment is reached. Movements, behaviour, feeding characteristics and mortality were observed throughout the experimental process.

Results show that the scattered swimming behaviour of the freshwater prawn has increased from 2.5 cm per minute in the Treatment 0 (0-5 ppt) to 2.9 cm per minute in the Treatment 4 (16-20 ppt). Rapid up and down movements has reduced from 17 times per second in T0 to 9 times per second in T4. Motionless behaviour has increased from 25 times per second in T0 to 27 per second in T4. As to the bodily movements, it showed that there was an increase of the movements of the walking legs from 21 times per minute in the T0 (control) to 29 times per minute in T4. Antennae movements have also increased from 29 times per minute in T0 to 41

times per minute in T4. And on the swimmerets movements it decreased from 501 times per minute in T0 to 204 times per minute in T4. On the effects of salinity to the feeding characteristics of the shrimp it was found out that there was an increase on the time the fish reacted to the feed. That is from 15 seconds in T0; 48 seconds in T1; 120 seconds in T2; 58 seconds in T3 and 65 seconds in T4. For the survival rate it showed that the highest survival mean was in T0 which is 94.12 % followed by T4 which is 84.98 %; T3 is 80.45 %; T1 is 80.44 % and the last is T2 61.26 %.

Results further show that increase in salinity has affected the physiological characteristics of the freshwater prawn that the higher the salinity, the faster are the movements of its antennae and walking legs. On the contrary slower movements of the swimmerets were observed at increasing salinities. Food consumption appears to be lower at higher salinity.

Keywords: Tolerance, Giant Freshwater prawn and Salinity

ASD No. 33

EFFECTS OF FIRST FOOD ON THE HEART OF 30-DAY OLD NILE TILAPIA *Oreochromis niloticus* L.

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First food for fish culture is the initial feed material introduced to the fry stage of the fish upon yolk resorption. This study was conducted to determine if the quality of first food, based on five different diets has any effect on the histology of the heart of month-old tilapia. The diets used were: I – zooplankton Moina; II – 40% fish meal and 60% rice bran; III – commercial brand fry mash; IV – combination of I and II; and V – combination of I and III. After the treatment period of one month, the heart of the fish specimens were dissected out and processed for histological analysis. Thickness of heart layers and spaces between muscle fibers were compared to those of the control, the fish given diet I. Body weight, length and muscle fiber diameter were subjected to statistical analysis. Diet I produced the greatest length and weight while diets IV and V induced the formation of the largest muscle fiber diameters. Differences were statistically significant using SPSS ver 10.0.1 program.

Keywords: heart, tilapia, first food quality, fish meal, rice bran, commercial brand fry mash

ASD No. 34

**LEAF-LITTER PATTERN OF MAJOR COMPONENT SPECIES IN
SECONDARY TROPICAL RAINFOREST IN PHILIPPINES**

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Continuous litterfall measurements were made from 1993-1997 at the secondary tropical rainforest of Mt. Makiling Forest Reserve, Philippines. Mean annual litterfall was 15.3 t ha⁻¹ yr⁻¹, composed of 64.79% leaves, 18.68% woody materials, 3.46% reproductive organs and 12.99% trash. Total litterfall and total leaf fall exhibited a bimodal pattern while wood and trash fall have a unimodal litterfall pattern. The bulk of the litterfall consisted of the leaves and maximum leaf shedding was during dry season. The dominant tree species had a unimodal leaf pattern characterized by a semi-deciduous trees while evergreen trees showed a bimodal pattern of leaf fall. The major leaf producing species were *Celtis luzonica*, *Diplodiscus paniculatus* and *Parashorea malaanonan*. Based on dissimilarity grouping, phenological observations on the species and climatic characteristics, the study indicated that the tropical rainforest of Mt. Makiling Forest Reserve comprised primarily of evergreen trees and to a lesser degree, semi deciduous tree species. Compared with similar studies on other old growth and secondary forest in SouthEast Asia, the litterfall value of this study is unusually high. The pattern of leaf shedding was species-specific and varied from markedly seasonal to nearly continuous shedding with small seasonal fluctuations.

Keywords: bimodal, leaf-shedding pattern, litterfall, unimodal

ASD No. 35**THE COMMUNITY STRUCTURE OF LOGGED-OVER TROPICAL RAIN FOREST IN MT. MAKILING FOREST RESERVE, PHILIPPINES****Amelita C. Luna¹ and Katsuhiko Osumi²**¹College of Forestry and Natural Resources,
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Community structure and species diversity were studied at a 4-ha tropical rain forest in Luzon Island, Philippines, 50 years after selective logging. The once selectively logged forest had high species diversity and complex guild structure. The 4-ha plot recorded 3,648 trees and palms of 179 species with larger than 5cm in diameter breast height (dbh). The dbh size of all trees and palms showed an inverse-J distribution with a maximum of about 150 cm. Species diversity indices for 4-ha plot fell within the range of the indices previously reported for old-growth tropical rainforest in Southeast Asia. The dbh size distribution in the population of each component indicated that the community was composed primarily of shade-tolerant species. Most species with more than 1% density or basal area of the plot total had an aggregate distribution, and nearly half of them showed a significant topographic preference. The smaller presence of dipterocarp species, which were the dominant in the original flora, indicated that the species have suffered heavy utilisation in the past, with the result that numerous non-dipterocarp tree species now formed a species-rich secondary tropical rain forest.

Keywords: community structure, guild structure, shade-tolerant species, species diversity

ASD No. 36**OPTIMIZING CONDITIONS FOR KABIR POULTRY RAISING****Renecilia B. Paz de Leon**College of Science, University of Rizal System- Morong, Rizal
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Today, poultry raisers are facing crisis due to rising cost of antibiotics, feeds, labor, and infrastructure requirements. Birds if not fully supplied with medicines become vulnerable to disease and stress, thus, requiring expensive raising. An

attractive alternative for today's mass-produced industrial broiler is "Kabir", strong, antibiotic free chicken breed with firm and savory meat.

The study sought to verify Kabir's optimum responses when subjected to varying temperature and space conditions. Kabir's feeding, water, sanitation requirements and reproduction behaviors were also taken into account. Experimental research design and descriptive methods were used to establish the optimum temperature and space requirements for chicks ranging from 0-4 weeks.

Kabir is a dynamic breed of chicken whose ancestral basis can be traced from Israel. The breed is superior for meat conversion because of its rapid growth, good body conformation and efficient feed conversion. Study revealed that the optimum temperature depends on the age of the chicks. Temperature ranging from 33-35 °C gave best survival rate for week old chicks, 31-33 °C for two weeks, 29-31 °C for three weeks and 27-29 °C for four weeks. In terms of space, 1 m x 1 m is the most ideal space measurement for twenty chicks whose age ranges from 0-3 weeks.

The type of feeds depends on the age of the chicks. Beyond four weeks, Kabir can be exposed to a free range environment. One male Kabir is capable of breeding with 15 to 20 females. The breed begins laying after 26 weeks and can lay 200 eggs/cycle.

Kabir raising is a potential livelihood opportunity to families that desire to have an additional income, and a source of "native style" meat with low cholesterol table eggs.

Keywords: poultry, Kabir, feeding

ASD No. 37

**DETECTION OF FOREIGN GENES INTRODUCED INTO *Zea mays* L. BY
DUPLEX PCR ANALYSIS**

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Polymerase Chain Reaction (PCR) - based detection of genetically modified organisms (GMO) requires the use of oligonucleotide primers targeting transgenes introduced into GM varieties. In this study, new primers were designed and evaluated by PCR that can be used as basis for developing a GM corn seed detection kit. Duplex PCR procedures were developed for detecting structural gene and terminator DNA sequences in three transgenic maize lines: insect-resistant Yieldgard*

MON810, glyphosate-tolerant Roundup Ready[®] GA21 and Roundup Ready[®] NK603.

Three sets of primers CrF/CrR, AbcF/AbcR and NosMf/Nosr targeting the *cry1Ab* delta-endotoxin gene from *Bacillus thuringiensis* subs. *kurstaki*, optimized transit peptide and modified maize enolpyruvylshikimate-3-phosphate synthase (*OTP-mepsps*) gene and Nos termination sequence from *Agrobacterium tumefaciens* respectively were used for the specific detection of recombinant DNA sequences in GM corn samples. One set of primers Zmf/Zmr was used to amplify the endogenous corn gene *zein* which served as an internal control target in non-GM IPBVar1 and GM corn. Duplex PCR combining: Zmf/Zmr and CrF/CrR primers resulted in the amplification of *zein* (589bp) and *cry1Ab* (400bp) fragments in Yieldgard[®] MON810. Zmf/Zmr and AbcF/AbcR produced the expected *zein* and *OTP-mepsps* (213bp) PCR products in Roundup Ready[®] GA21 and Roundup Ready[®] NK603. Zmf/Zmr and NosMF/Nosr primers generated the expected *zein* and *nos* (162bp) amplicons in all transgenic corn samples. The combination of primers targeting native and recombinant gene sequences amplified the target DNA segments distinguishing non-GM from GM corn varieties.

Keywords: genetically modified corn, transgenic maize, GM corn detection

ASD No. 38

MULTIPLEX PCR DETECTION OF FOREIGN GENES INTRODUCED INTO SOYBEAN (*GLYCINE MAX* L.)

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Polymerase chain reaction (PCR) primers targeting foreign genes introduced into genetically modified (GM) soybean (*Glycine max*) were designed and used in this study for multiplex PCR detection of transgenic soybean Roundup Ready[®] GTS 40-3-2. Three primer sets that resulted in distinguishable profile for transgenic soybean Roundup Ready[®] soybean line GTS 40-3-2 were simultaneously used to amplify a 300-bp fragment traversing the cauliflower mosaic virus (CaMV) 35S promoter and a modified form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (*m-epsps*), a 173-bp fragment of the nopaline synthase (*nos*) 3' terminator, and a 430-bp native lectin fragment that is present in both GM and non-GM seed samples. As expected, only the lectin gene fragment was amplified in the non-GM control soybean IPB PSBSY2. The three primers were also tested on

non-GM soybean samples containing 5%, 2%, 1%, 0.5%, 0.1%, and less than 0.03% transgenic Roundup Ready® soybean. Detection limits for the 300-bp CamV 35S/*m-epsps* and the 173-bp *nos* terminator gene fragments by the multiplex PCR procedure used in this study were 0.5% and 1.0%, respectively. The three new primers designed and tested in this study can now be used for multiplex PCR detection of GM soybean containing the transgenes *CamV/EPSPS* and *nos* terminator and can be utilized in developing a commercial detection kit for such transgenes in GM soybean.

Keywords: genetically modified (GM) soybean, *Glycine max*, multiplex PCR, PCR detection limit

BIOLOGICAL SCIENCES

BSD No. 1

CHALLENGES IN THE STUDY AND CONSERVATION OF PHILIPPINE *RAFFLESIAS*

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Rafflesia (Rafflesiaceae) is a genus of plants obligately parasitic to *Tetrastigma* (Vitaceae), lianas of the lowland rainforests in tropical and subtropical Asia. To date, 21 species of *Rafflesia* have been described: one species in Thailand, eight in Indonesia (five in Sumatra and three in Java), eight in Malaysia, and four in the Philippines including the most recently described, *R. mira*, from Compostela Valley in Mindanao. Our recent explorations in Luzon have added two more new species. Three potentially new species, yet to be described, are currently known from photographic evidence only. Once formally described, they would make the Philippines the global center of *Rafflesia* with a total of at least nine species, the