

UTILIZATION OF POLYMERASE CHAIN REACTION IN CLONING & SEQUENCING OF GENES: *XANTHOMONAS ORYZAE* PV. *ORYZICOLA*

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Polymerase chain reaction (PCR) allows the amplification of a discrete piece of DNA a million fold. With the use of the right primers, any gene of interest can be amplified, cloned and sequenced. In *Xanthomonas oryzae* pv. *oryzicola*, the causal organism of the bacterial streak of rice, primers of the upstream region of the hypersensitivity (*hrp*) gene were synthesized based on the published sequence of the *hrp* gene of a closely related species, *Xanthomonas oryzae* pv. *oryzae*, the causal organism of the bacterial blight of rice. PCR was done using these primers and the following parameters: 95°C for 10 minutes, 30 cycles of 95°C for 2 minutes, 56°C for 2 minutes, followed by 27°C for 10 minutes to complete the extension. A 189 basepair PCR product resulted. This was cloned in plasmid pBluescript digested with *Pst*I and *Bgl*III restriction enzymes. The resulting clone was sequenced by dideoxy sequencing procedure. Direct sequencing of the PCR products from other strains was done to confirm sequence of that region of the *hrp* gene through a modified procedure. Other genes can be cloned and sequenced in the same manner.

HEALTH SCIENCES

PHYTOCHEMICAL STUDIES ON THE PHARMACOLOGICALLY-ACTIVE COMPOUNDS OF *VITEX NEGUNDO*, L.

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Vitex negundo, L. ("lagundi"), is a shrub which is widely distributed in the tropics from the Philippines to India. Both the leaves and seeds are utilized for their medicinal properties. Aqueous decoctions of the leaves are used traditionally as an antipyretic, anti-asthma, and antitussive (Quisumbing 1978).

This study is part of the results of the efforts of the National Integrated Program on Medicinal Plants (NIRPROMP) and the PCHRD to investigate the scientific basis for the pharmacological action of traditional medicinal plants such as *V. negundo*. The pharmacological efficacy of the use of *V. negundo* for cough treatment has been demonstrated in clinical trials and no adverse effects have been noted (Maramba *et al.* 1989). Detailed bioassay studies showed that the ethyl acetate and methanol extracts relaxed cat's trachea (Estrada, research reports 1986). From these active fractions we isolated and identified 11 compounds: five flavonoids (casticin, chrysosplenol D, lutcolin, lutcolin -7-0-

glucoside, and isoorientin), two common aromatic acids (p-hydroxybenzoic acid and protocatechuic acid), and four iridoids (agnuside, 2'-p-hydroxybenzoyl mussaenosidic acid, 6'-p-hydroxybenzoyl mussaenosidic acid, and lagundinin) (Dayrit *et al.* 1987; 1989; 1993a; 1993 b).

A large number of flavonoids are known to be active as anti-inflammatory compounds by virtue of their antioxidant properties. Three of the flavonoids from the active fraction of lagundi – chrysoeriol D, luteolin, and isoorientin - - were known to inhibit the release of histamine from rat mast cells. Isoorientin inhibited the enzyme 3-lipoxygenase (Sankawa 1987).

In this paper, we describe the isolation and identification of these compounds and discuss their possible roles as pharmacologically-active constituents of lagundi.

CORNEAL ENDOTHELIAL CELL CULTURE IN WOUND HEALING, GROWTH AND VIABILITY STUDIES USING LOCALLY AVAILABLE CHEMICALS

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The corneal endothelium, which has the following characteristics: (1) acts as a barrier to ions; (2) site for active transport; and (3) has a limited ability for cell division or regeneration, is an important factor in the maintenance of corneal transparency. The Institute has therefore been interested in characterizing human normal and pathologic endothelial cells by clinical specular microscopy *in vitro* to observe, quantify and correlate morphological changes in these corneas. The Institute, however, realizes that tissue culture of mammalian (rabbit or human) endothelial cells is a necessary step toward studying and understanding the factors that may increase its cell growth and viability and eventually its capacity to regenerate and increase its functional property of active transport via increasing its active pump sites in these cells. The Institute has demonstrated using locally available chemicals of pharmaceutical grade that endothelial cell culture is possible as verified by contrast-phase microscopy contrary to popular belief that only pure and tissue grade chemicals are useful in its culture. The ability to culture endothelial cells *in vitro* has enormous applications in drug hormone, chemical, and natural substance promoters and enhancers, which may totally change present concepts of endothelial wound healing, viability and growth.

PERFORMANCE AND CONSTRAINTS TO ADOPTION OF COTTON PRODUCTION TECHNOLOGIES

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The adoption of the recommended cotton production technology, garlic-cotton intercropping and minimum tillage technologies were evaluated in selected cotton growing municipalities in Ilocos Norte, Ilocos Sur, Pangasinan, Tarlac, Isabela and Cagayan from July 1991-July 1992.

Farmers activity monitoring forms and interview schedules were used to gather production-related, economic and demographic data. Analyses used were Cobb-Douglas production function, multiple regression, financial and some descriptive statistics.

The recommended cotton production technologies were generally modified as a result of the interplay of climate, general attitudes of farmers to the technology components, and the services attached to the financing and technical services for cotton production provided by private financiers.

Adoption of a package of technology is a function of farmer's experiences in cotton growing, frequency of extension visit, farmer's organizational affiliation and age.

Acceptability of garlic-cotton intercropping were affected by the lack of financing for garlic. The selectivity of areas suited for minimum tillage was a factor that slowed rate of adoption. Pest control concentrated on chemical use with higher dosage application for low dosage recommendation and vice versa. Economic viability of garlic-cotton and minimum tillage technologies was noted. Farmers' cotton production practices were productive and high but lower in economic returns.

SWEETPOTATO HOUSEHOLD GARDENING DEVELOPMENT

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Household gardening is increasingly becoming an important supplementary source of food and income as more and more families are faced with rapidly increasing food prices, poverty, pollution and other health hazards. Hence, gardening development is encouraged. Sweetpotato is among the versatile homegarden crops in the Philippines especially in the Cordilleras, and as such, this report summarizes the experiences learned from the first year of a participatory research where seminar-workshops were conducted in a series to: (1) promote sweetpotato-based homegardening among households and school

children; and (2) encourage partnership in developing a user-friendly sweetpotato-based gardening technology.

Among the promising technologies introduced for home gardeners were sweetpotato varieties for evaluation by the households. After one cropping, most of the cooperators plan to replant again all their stocks including their chosen varieties. This means that the selection process for a desired sweetpotato variety will take more than one cropping. So far, good taste described as sweet or neither sweet nor bland, and with a dry or mealy texture surpassed other criteria in the choice of a variety. Further, organic fertilization and double digging which are components of the bio-intensive gardening techniques were not completely adopted. To some extent, the traditional way of organic fertilization and sweetpotato management prevailed. These only show that research and extension efforts must always incorporate the traditional practices taking into account the existing situation as springboard for any development activity.

HORMONAL EFFECT OF THE *JATROPHA CURCAS* L. OIL ON *HELICOVERPA ARMIGERA* (HUBN.)

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The insecticidal principle from the physic nut, *Jatropha curcas* seed kernel or chloroform was studied. The crude chloroform-extracted leaf oil incorporated in the diet of second instar *Helicoverpa armigera* larvae did not inhibit feeding and assimilation. However, the conversion of the treated diet to biomass was negatively affected, thus, a reduced growth rate. Larval development was inhibited resulting in a delay in molting.

The crude oils caused production of larval-pupal intermediates and abnormal adults, indicating an Insect Growth Regulatory (IGR) effect. Based on ID 50, the crude petroleum ether-extracted seed kernel and leaf oils were the most effective. Also, the oils had a partial chemosterilizing effect on the moths, indicating action as a juvenile hormone (JH) mimic.

Histopathological changes were noted on the tissues of the treated insects. The larva reared on oil-treated diet or the pupa from this larva had few hemocytes and the epidermal cells were cuboidal and disarranged instead of being columnar and intact as in the normal insects. Oocytes from ovarioles of females that emerged from the treated larvae had disintegrated follicular epithelium or were atrophied. Males from the treated larvae had few spermatozoa.

COMPREHENSIVE RUBBER TECHNOLOGY TRANSFER PROGRAM (CRTTP)

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To dramatize the massive cultivation of rubber especially for the smallholders, a program on rubber techno-transfer was implemented in the province of Cotabato. This was done by conducting a series of pilot demonstration farms in potential expansion areas for rubber showcasing the following updated and recommended technologies: rubber nursery techniques and management, tapping system and yield stimulation and rubber processing. Effective and efficient technology transfer activities and/or series of trainings on the above technologies were conducted. Likewise, pamphlets were distributed to the training participants.

Rubber nursery techniques and management were piloted in Linao, Matalam; Malapag, Carmen; Avas, Pres. Roxas and Alamada. One polybag and ground rubber nursery was established in each location. Likewise, budwood nurseries were established which served as sources of budstick and budwood during the budding operation. A total of 7,276 two-leaf storey polybag budded rubber seedlings ready for market were produced.

Tapping system and yield stimulation were piloted in Magpet, Antipas and Pres. Roxas. Prior to project implementation, the tapping panel had numerous bumpy scars due to wounding of the cambium. The angle of slope was undulating and bark consumption was too fast due to thick bark shavings. All these were corrected such that the tapping panel was laid 150 cm from the ground. The angle of slope of tapping was adjusted to 30° and bark consumption was controlled to 2.5 cm per month. Moreover, monthly application of yield stimulant (2.5% ethrel) was done except during the wintering and defoliation period of the trees (March-April). An average yield increase of 11.28% was obtained following the improved tapping techniques.

Four units each of rubber sheeters and agro-waste dryers were fabricated. One sheeter and dryer was installed in Magpet, Antipas and Pres. Roxas. One unit was installed at USMARC which served as demo to the academe, students, visiting farmers and other interested groups.

A total of seven (7) trainings on the following technologies: intercropping and covercropping, nursery establishment, tapping management and yield stimulation and rubber processing were conducted for 202 small rubber farmers of Avas, Pres. Roxas; Antipas; Magpet; Linao; Matalam; Greenfield, Arakan; Malapag, Carmen and Alamada.

Standardized training materials (pamphlets) on the above technologies were distributed to the training participants.

GROWTH PERFORMANCE OF GOATS FED WITH NAPIER SUPPLEMENTED WITH *DESMANTHUS* AT TWO RATES AND FEEDING INTERVALS

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Using a 2 x 2 factorial arrangement in a Randomized Complete Block Design, the growth performance of 16 growing female goats were evaluated, involving two rates (0.3 and 0.6% bodyweight-BW) of *Desmanthus* supplementation levels and two intervals (daily and every 2 days) of supplement feeding. The animals were fed grass *ad libitum* daily. The feeding trial lasted for a period of 100 days.

The total, final and daily gain in weight, and feed efficiency of the experimental animals were not significantly affected by the treatment diets. Numerically, however, slightly higher daily gain was observed with daily supplement feeding and with higher levels of supplementation. Likewise, goats fed *Desmanthus* at 0.3% BW were more efficient feed converters. Total voluntary feed intake (VFI), while not significantly affected by rate of supplementation and the interaction of rate and intervals of feeding, significantly increased from daily to every-2-days supplement feeding. Grass intake remain unchanged as affected by the two factors while *Desmanthus* intake significantly varied showing greater intake with every two days feeding and higher supplementation rate. Increasing levels of supplement feeding every two days slightly increased grass intake by 0.1 unit.

Lower cost per gain was observed with every two days *Desmanthus* feeding with 0.3% BW supplementation level. The results of the study suggest that *Desmanthus* as a supplement to goat rations need not be fed daily to the animals to obtain comparable growth performance. In large-scale goat farming, this could significantly reduce labor cost due to a less-frequent harvesting and feeding of *Desmanthus* as a supplement.

EFFECTS OF CALCIUM AND PHOSPHORUS SUPPLEMENTATION ON SOME PRODUCTION AND REPRODUCTION PARAMETERS IN GROWING PHILIPPINE INDIGENOUS SHEEP

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A 16-week feeding trial in a 3x2 factorial experiment involving three levels of Ca and P in the ration (A-2.6g Ca and 1.17 g P/kg DM, B-3.85 g Ca and 1.76 g P/

kg DM and C-5.06 g Ca and 2.3 g P/kg DM) and two sexes (male and female) in a Randomized Complete Block Design was conducted to determine the effect of Ca and P supplementation on the growth, wool production, slaughter characteristics and on some reproductive parameters.

Lambs fed ration B had significantly higher DMI and liveweight gain. Wool growth was significantly faster in lambs fed ration B. Mean measurements of some slaughter, male and female reproductive parameters did not differ among rations but animals fed ration B tended to have higher values.

Histological examination of the seminiferous tubules showed the presence of more layers of cells in the germinal epithelium of animals fed ration B.

Based on these results, it appears that growing Philippine indigenous sheep performed better in all parameters studied with a ration (B) containing 3.85 g Ca (0.36% of ration) and 1.76g P (0.16% of ration) per kg DM.

CALCIUM AND PHOSPHORUS REQUIREMENTS OF GROWING PHILIPPINE INDIGENOUS SHEEP

ROGELIO R. CALUYA and C.C. SEVILLA. Mariano Marcos State University, Batac, Ilocos Norte

A 16-week feeding trial in a 3 x 2 factorial experiment involving three levels of Ca and P in the ration (A-2.6 g Ca and 1.17 g P/kg DM, B-3.85 g Ca and 1.76 g P/kg DM and C-5.06 g Ca and 2.3 g P/kg DM) and two sexes (male and female) in a Randomized Complete Block Design was conducted to determine the requirement for Ca and P, the effect of supplementation on the Ca and P status and metabolism.

Lambs fed ration B significantly consumed more Ca and had significantly higher DMI and liveweight gain. Serum Ca in the males significantly decreased while it increased in the females with time of collection. The mean serum P concentration was increased by the increased level of P in the diet and intake but decreased with time in the lambs fed ration A. Calcium and phosphorus were mainly excreted through the feces and only minimal in the urine. Fecal Ca and P, apparent absorption and retention of Ca and P were highly positively correlated to P intake. Calcium and P balance were negative in the lambs fed ration A while those fed rations B and C were in positive balance except for a slightly negative Ca balance among ewe lambs fed ration B.

The pH of the digesta of each compartment of the gastrointestinal tract were not significantly different and were within normal levels. Calcium concentration in the rumen-reticulum, omasum and cecum, and P concentration in the omasum were significantly higher in animals fed rations B and C.

Based on these results, it appears that a 15-kg growing male Philippine indigenous sheep should consume 131 mg Ca and 57 mg P/kg LW/day of ration B while the females should consume 167.6 mg Ca and 82.8 mg P/kg LW/day of ration C.

MICROBIAL INOCULATION FOR QUALITY ORGANIC FERTILIZERS FROM FARM WASTES

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Experiments were conducted to evaluate the effects of microbial inoculation on the composting of rice straw-chicken manure and mushroom wastes, and the effects of the resulting composts on test crops. Composting of rice straw-chicken manure with combined inoculation of *Trichoderma* sp. SS 33 and *Azotobacter* sp. IBF 4b yielded composts with high values for total sugars (0.72%), total N (1.61%), and counts of nitrogen-fixing bacteria (13.9×10^8 cells/g). Uninoculated controls and those treated with 10% urea gave significantly lower values. In tests in wetland rice for two growing seasons, inoculated composts gave significant average yield increase of 21% over the control comparable to the yield levels of those treated with chemical fertilizer alone (60-30-30). Combined inoculation of mushroom wastes with *Trichoderma* sp. BCF 36 and *Azotobacter* sp. BCB 42 gave composts with high levels of substrate decomposition (23%), population of nitrogen-fixing bacteria (3.9×10^8 cells/g), acetylene-reduction activity (20.32 nmol C_2H_4 /hr/g) and total nitrogen (0.63%). These values were significantly higher than those of the uninoculated controls. When tested in pechay, composts with combined inoculation produced significantly higher dry matter yield (8.13 g/pot) than the uninoculated control (2.22 g/pot) or those treated with 2% urea (4.58 g/pot). The results demonstrated the beneficial effects of microbial inoculation in composts increasing crop yields.

PARTICIPATORY ON-FARM SWEETPOTATO VARIETAL EVALUATION SELECTION IN THE PHILIPPINE HIGHLANDS

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In the Philippine highlands, sweetpotato farmers and consumers have varied varietal preferences depending on location, cultural management practices and utilization. During Phase I of the project, preliminary and general yield trials were conducted in the experimental station confining evaluation and selection to the breeders' and researchers' points of view. Varietal evaluation

under this scheme limits performance of a given clone and the chance that it will be selected by farmers. Involvement of farmers during initial selection saves time and resources and ensures wider adoption of the cultivar.

The paper presents results of the "participatory approach" followed as an alternative strategy in varietal evaluation and selection under farmer's field condition during Phase II of the project.

PERFORMANCE OF *Gmelina arborea* WITH VAM INOCULATION IN ACID SOIL

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A field experiment to determine the effects of VAM inoculation on the growth and survival of *Gmelina* in acid soil was conducted in Villa, Baybay, Leyte from November 1992 to June 1993. A Randomized Complete Block Design with six replications per treatment was used. The treatments were *Gmelina* seedlings with and without VAM inoculation.

Survival rates of both the control and VAM treatments were 100 percent. VAM inoculated seedlings were significantly taller than the uninoculated trees. *Gmelina* inoculated with VAM was significantly bigger and produced higher biomass compared to the control.

BODY TEMPERATURE, RESPIRATORY FREQUENCY AND HEART RATE PATTERNS UNDER THERMONEUTRAL AND SHORT-TERM THERMAL STRESS IN GOATS

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In order to gain further insights into the mechanisms of thermal polypnea in ruminants during conditions of high ambient temperature, the changes in the patterns of thermoregulatory functions that were affected by exposing goats to short-term severe heat stress were investigated.

In a thermoneutral condition (ambient temperature = 20°C; relative humidity = 50 - 60%), mean body temperature (T_{bo}), respiratory frequency (f) and heart rate (HR) were found to be higher in light than dark period. This largely reflects activity and feeding during the day and rest during nighttime. Fluctuations were observed within a 24-hour period, with T_{bo} showing a mean daily variation of 0.964°C. A biphasic pattern was observed for T_{bo} and HR patterns with two distinct peaks after the feeding periods.

When ambient temperature was raised to 40°C, mean T_{bo} and f were significantly higher than before heating, with the increase in f of about 7.3 times.

This suggests that peripheral simulation of up to 40°C was enough to induce polynea in goats. T_{bo} pattern showed a transient decrease at the onset of heat before increasing as response to the continuing heat load. There was no apparent effect in the mean HR before and during the heat period.

CHALLENGES AND PROSPECTS OF SUSTAINING SUGARCANE PRODUCTIVITY

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While sugarcane production costs are increasing due to the increase in the cost of imported fertilizers, machineries/spare parts, oil, high labor costs and interest charges, sugarcane yields are declining (Mendoza 1991). In recent years, the sucrose content (PS/TC) of the sugarcane crop has been decreasing. This decrease in PS/TC occurs despite the use of modern agricultural practices consisting of high yielding variety (HYV) fertilizers, cultivation and irrigation (Alva *et al.* 1990).

Historical yield data from 1933 to 1993 were obtained (source: sugar Regulatory Administration), and yield trends were analyzed. The yield trend analysis indicated the following:

- a) the improvement in yields in the 60s were mainly achieved by increasing tonnage;
- b) the 70s onward was characterized by declining PS/TC from a high of 1.77 in 1933-63, an average of 1.58 in 1977-83, to as low as 1.36 in 1987-93.

MAXIMUM LIKELIHOOD AND BAYESIAN ESTIMATORS FOR INSECT DAMAGE ON RICE

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From a previous study, it has been found that under natural field conditions and low level of damage on rice caused by insects, the distribution of the number of damaged tillers per hill fitted the Poisson distribution model very well. Given the situation that the data follows a Poisson distribution with parameter (mean number of damaged tillers per hill), there is a need to find a more efficient and precise method for estimating. Thus this paper derived a procedure for estimating using the maximum likelihood method and the Bayesian procedure using a gamma distribution prior for ϕ . Two measures of relative efficiency (RE) were derived, one as the ratio of the variance of estimates and the other as the ratio of mean squared errors. By applying the results to actual data on infestation on rice caused by insects, the relative efficiency of the Bayesian

procedure over that of the maximum likelihood method were computed, graphically presented, and interpreted.

POSTHARVEST LOSS ASSESSMENT IN YAMBEAN AND GINGER AT VARIOUS HANDLING POINTS

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Loss assessment at various handling points was conducted to quantify and identify causes of losses in yambean and ginger. This study was done in Mangaldan, Pangasinan and Dupax del Sur, Nueva Vizcaya. These were transported to La Trinidad, Benguet for further assessment.

In yambean, harvest losses were mainly due to mechanical injuries, very small-sized tubers and slight to moderate skinning observed during bulk transport. In retailing, weight loss ranged from 3% to 6% for 7 to 14 days holding in the local market.

The loss assessment in ginger indicated immediate need of integrating appropriate production, crop protection, and postproduction management systems. Postharvest losses were high ranging from 5% to 100%. At harvest, the highest losses were primarily due to disease infection and differed from one farm to another. Mechanical injuries and bruises were moderate and attributed to poor harvesting, transporting techniques and poor roads. Washing slightly contributed to mechanical breakage of rhizomes but improved appearance. Losses during retailing was not recognized much for the reason that retailers have ways of including the undesirable root during packing in plastic containers. Percentage decay in storage was high (100%) in the selected roots obtained from infected farms; the lowest was 2%. Other causes of strong losses included weight loss, shriveling, and sprouting.

AGROTECHNOLOGY SUITABILITY EVALUATION SYSTEM (ASES): DEVELOPMENT AND IMPROVEMENTS

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The Farming Systems and Soil Resources Institute (FSSRI) developed expert systems which could easily provide accessible and understandable agricultural information. One of these is the Agrotechnology Suitability Evaluation System (ASES) wherein queries on crop suitability and required farming systems technology for a given area could be answered immediately. The required inputs and management needed to overcome the limiting land characteristics are easily identified.

ASES consists of location, soil, climate, crop requirements and fertilizer recommendation files. Prolog version 2.2 is used to develop a storage and retrieval system of agro-environmental information and crop requirements. Fertility capability interpretation for upland crops and lowland rice are adopted to complement the crop suitability. Recommendations could be established based on suitability ratings after considering the predicted performance, constraints and input requirements of the agrotechnology to be transferred to the farmers' fields.

ASES used provinces of Laguna and Batangas as initial pilot areas. The characteristics of each soil mapping unit or the soil of each land management unit are obtained from the recent soil survey or the land resource evaluation report of the province, respectively. It is user-friendly specifically to provide extension workers with a well-functioning, accessible and understandable information system. It can now be used in farming system resource management in different agro-ecosystems and agricultural planning projects of the towns of the provinces.

DEVELOPMENT OF OTHER USES OF TOBACCO

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With the inevitability of a "smoke-free" world, new and beneficial uses of tobacco must be developed to save the tobacco industry. The poster will showcase the new products from tobacco and the technologies to produce them developed by the Products Development and Waste Utilization Division of NTA. These products include food, feeds and oil from the seeds; paper, particleboard and handicraft from the stalks and midribs; and antimicrobial and pesticidal extracts from the leaves. Comparisons of these products to commercially available ones will also be presented.

STEM BORERS ATTACKING *GMELINA ARBOREA*

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This paper presents three new destructive stem-boring insect pests of yemane, *Gmelina arborea* Roxb. The stem borers, detected during a series of surveys conducted from January to March 1994 in 12 yemane stands in an industrial plantation in Surigao del Sur, include two moth species – *Xyleutes* sp. (Lepidoptera: Cossidae) and *Cossus* sp. (Lepidoptera: Cossidae) – and a species of long-horn beetle, *Acalolepta rusticator* Fabr. (Coleoptera: Cerambycidae). The distinguishing characteristics, nature of damage, and other hosts of the pests are given. The information will aid reforestation workers,

agroforestry technicians and farmers, as well as researchers in prompt detection and identification of the borers. *G. arborea* constitutes one of the principal species in the National Forestation Program, a major tree crop in agroforestry farm development, and a commonly used cover crop for soil and water conservation projects in the country.

DEVELOPMENT OF DOUBLE ENDOSPERM MUTANT VARIETIES IN MAIZE

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Endosperm mutant genes affecting sugar, starch and amino acid concentrations were combined in a common genotypic background in order to improve green corn kernel eating and nutritional quality. A double mutant involving shrunken and waxy genes was formed by crossing ASUKAR and LB Lagkitan followed by a series of selfing, testcrossing, and recombinations. Another combination involving brittle and opaque genes was formed by crossing HS #9 and HICli. The two new varieties were named DL Umali Pearl Sweet (DLU PS) and Nutrisweet, respectively.

Yield trials revealed that the marketable ear yields of DLU PS and Nutrisweet were comparable to the check varieties. Sensory and biochemical evaluations showed a significant improvement in eating and nutritional quality of the two varieties. Both of them had elevated total free sugar content giving them the supersweet taste. DLU PS exhibited an increased amylopectin content resulting in the glutinous consistency of the endosperm. Nutrisweet gave a dramatic improvement in the concentrations of two essential amino acids, tryptophan and lysin, showing an increase of two times more than the normal endosperm. Kernel tenderness was rated excellent for both varieties.

IN VITRO PROPAGATION OF BUHO BAMBOO (*SCHIZOSTACHYUM LUMAPAO*)

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Buho (*Schizostachyum lumampao*) was propagated by *in vitro* culture techniques. Excised embryos which were expanded on Murashige and Skoog medium + 1 ppm BAP + 1 ppm 2, 4 - D formed loose-type calli which when transferred to various culture media, yielded yellowish compact calli on MS + 2 ppm 2, 4-D + 2% sucrose. Rates of multiplication of the embryogenic calli differed for the four (4) compact callus lines. The compact-type embryogenic calli regenerated plantlets after subculture to MS medium devoid of 2, 4-D. Plantlets were potted out, and transplanted to the field.

ISOLATION OF PLANTLETS FREED OF BANANA BRACT MOSAIC VIRUS FROM INFECTED BANANA CV SEÑORITA

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The recovery of virus-free plants from banana bract mosaic-infected banana cv Señorita, was studied. Several protocols for recovering virus-free plants were tried and plantlets were indexed during the *in vitro* rooting stage, potting out, and during nursery care. Virus-free plants were obtained in meristem culture, heat treatment and combinations of heat treatment-meristem culture. The results for bract mosaic virus elimination are discussed in light of elimination procedures for banana mosaic and bunchy top virus and rapid multiplication techniques used for bananas at present.

DEVELOPMENT OF NEAR-ISOGENIC LINES WITH RESISTANCE OR TOLERANCE GENES TO TUNGRO DISEASE IN RICE

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Isogenic lines are useful for identifying races or strains of tungro virus, for studying the mechanism for resistance and for tagging resistance genes with restriction fragment length polymorphism (RFLP) markers. The development of near-isogenic lines was initiated to establish a basis for research on resistance or tolerance to tungro. The genes for resistance from different donors are being transferred on the genetic background of highly susceptible varieties such as Taichung Native 1 (TNI) and IR22. The procedure mainly involves an alternate backcross and selection phases. To date, the most advanced generation is BC₈F₁. The homogeneity for tungro resistance or tolerance of these lines will be tested subsequently in BC₈F₁, BC₈F₃, and BC₈F₄ generations. Parallel to this, selected resistant or tolerant plants from the other backcross generations were used for progeny test of homogeneity for tungro resistance or tolerance. Thus, the developed near-isogenic can be used in monitoring the distribution of races and biotypes, as testers for genetic analysis and as donors in breeding programs for resistance to pests.

ANATOMICAL DIFFERENCES BETWEEN A NEW CMS SOURCE AND ITS MAINTAINER LINE IN RICE (*ORYZA SATIVA* L.)

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To understand the mechanism causing pollen abortion in rice, a new CMS source derived from a wild relative of rice (*Oryza perennis*) and its maintainer line were comparatively investigated in terms of vasculature of the stamen and development of the characters related to anther dehiscence. Comprehensive studies were conducted using advanced microtechniques such as semi-thin sectioning, Safranin-Fast Green staining combination and light microscopy.

The CMS lines have undifferential vascular tissues, degenerated tapetum, disorganizing vascular cylinder, flattened pollen sacs, underdeveloped endothecium and absence of the anther dehiscent cavity (ADC).

The observed anatomical abnormalities in the CMS line can be used as an index in the screening and continuous development of CMS lines of rice with variable genetic and cytoplasmic background.

CYTOGENETIC STUDIES OF IRRADIATED BITTERGOURD (*Momordica charanta* L.) AND THE F₁, BC₁ and F₂ PROGENIES

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F₁, BC₁ and F₂ populations from mutants (with suppressed shoot growth) generated from irradiation of seeds and pollen of *M. charanta* together with their parentals were studied cytogenetically.

Meiotic analysis showed 11 bivalents to diakinesis in all populations studied. Abnormalities in the meiotic behaviour such as laggards and differential disjunction in early stages did not affect telophase II. Consequently, there was a large percent of pollen fertility. The results observed suggest that the suppressed shoot growth was caused by genic mutation rather than gross chromosomal changes.

PRELIMINARY EVALUATION OF A PROMISING PAPAYA (*Carica papaya* L.) HYBRID

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Inbred lines, developed through our breeding project that were rated as moderately tolerant to the papaya ringspot virus (PRSV), were intercrossed. Preliminary evaluation of the F₁ hybrids revealed that cross 5648 x 4172 is a

promising combination in terms of fruit quality and tolerance to PRSV. Fruits of this hybrid are medium sized (1.5 kg), very sweet (11-12^oB), have thick and deep yellow orange flesh, and have excellent fruit flavor. The hybrid is semi-dwarf and moderately tolerant to PRSV. It is intermediate between the two parents (a yellow solo variety and a big-fruited local collection) in most attributes but is superior in terms of fruit flavor and sweetness.

MOLECULAR MAPPING OF 11 ISOZYMES AND 3 CLONED ALPHA-AMYLASE GENES IN RICE (*O. SATIVA* L.)

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A total of 11 isozyme markers and 3 cloned alpha-amylase genes were localized via linkage with RFLP markers in order to map and integrate these genes in the existing molecular map. The mapping populations consist of 135 double-haploid lines from a cross between IR64 and Azucena, and backcross population from an interspecific cross between *O. sativa* cv. BS 125 and *O. longistaminata* (WLO2) wherein an RFLP map covering the 12 chromosomes was available. The three cloned alpha-amylase genes were mapped using non-radioactive labelling and detection procedures. The technique includes the use of polymerase chain reaction (PCR) for incorporation of digoxigenin-11dUTP in DNA molecule and the use of a chemiluminescent compound (AMPPD) for signal detection. The use of non-radioactive procedure provides sensitive and convenient alternative to ³²p-based systems due to the cost, health hazard and instability of radio-labelled probes.

GENE TRANSFER FOR RESISTANCE TO BROWN PLANTHOPPER FROM *ORYZA MINUTA* AND BACTERIAL BLIGHT FROM *O. BRACHYANTHA* INTO RICE (*O. SATIVA* L.)

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Among various biotic stresses, reducing rice productivity, bacterial blight (BB) and brown planthopper (BPH) are the most important. Wild species of rice are an important source of resistance to BPH and BB. Following embryo rescue, we have produced hybrids between elite breeding lines of rice and several wild species representing BBCC, CC, CCDD, EE, FF and species with unknown genomes. Backcrossing with the recurrent parents and embryo rescue have been used to derive fertile disomic progenies ($2n = 24$) from various cross-combinations. From a cross of IR31917-45-3-2, an elite breeding line of rice (susceptible to BPH biotype 1) and *O. minuta* (BBCC $2n = 48$) Acc 101141 (resistant to BPH), several progenies have been derived. Of the 168 BC4F3 progenies, 2 segregated for resistance to biotype 1. In addition, 784 BC3F3 progenies derived from the cross of IR56 x *O. brachyantha* (FF $2n = 24$) Acc 101232 were screened for resistance to different BB races. Progenies resistant to BB have been identified. Monosomic alien addition line and RFLP analyses are being carried out to characterize introgression of BPH and BB resistance into rice from *O. minuta* and *O. brachyantha* respectively.

CALLUS INDUCTION AND MAINTENANCE IN CHINESE CABBAGE (*BRASSICA PEKINENSIS* L.) CV. CORAZON

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Two base media, MS (Murashige and Skoog 1962) and SH (Schenk and Hildebrandt 1972) and combinations of auxin (2, 4-dichlorophenoxyacetic acid) and cytokinins (6-benzylaminopurine and kinetin) were tested for callus induction maintenance in Chinese cabbage (*Brassica pekinensis* L. cv. Corazon) using different explants (embryo, leaf, root and hypocotyl).

Best callus induction was observed using leaf explant inoculated onto SH medium containing 0.1 mg/L BAP and 1.0 mg/L 2,4-D. Calli induced were profuse and friable.

Callus cultures were established and maintained by consistent monthly transfer onto fresh medium. Rapid proliferation of calli was observed when the initial materials came from root explant.

LILAK, A BANANA (*MUSA* SPP) SELECTION FROM TISSUE CULTURE

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Lilak, is a new banana introduction from tissue culture. This selection may be a potential export banana. It has excellent eating quality with flavor and aroma compared to that of "Lakatan". The fruit is oblong, cylindrical with blunt tips and weighs from 102-207 g. The skin is smooth, dark yellow, thick and firm similar to "Lakatan". The flesh is light yellow orange with total soluble solids ranging from 19-27°Brix. The fruit stalk (pedicel) attachment is remarkably strong which is a desirable characteristic when fruits are to be transported locally or overseas. Under greenhouse conditions, the yield is high. The number of hands per bunch ranges from 6-8 while the number of fingers per hand ranges from 14-16. In terms of overall quality, size and appearance, it may be superior to "Lakatan".

Suckers from greenhouse-grown plants have now been planted in the field for further evaluation. The origin of the initial material and explants used in the establishment of *in vitro* cultures is also being traced. The plant has been *in vitro* propagated using the method developed at IPB.

GROWTH OF TISSUE-CULTURED SHALLOT (*ALLIUM CEPA* VAR GROUP AGGREGATUM) AND GARLIC (*ALLIUM SATIVUM* L.) UNDER GREENHOUSE CONDITION

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Tissue-cultured bulblets of 6 and 13 accessions of shallot and garlic, respectively, were transplanted to soil and grown under greenhouse condition at the Bureau of Plant Industry, Baguio City. Growth was good two weeks after

transplant. However, marked difference on survival and growth of plants of shallot and garlic was observed 4 and 8 weeks after transplanting. Shallot survived better than garlic with survival percentage of 96.67% and 78.61%, respectively, on the 4th week and 81.32% and 33.27%, respectively, on the 8th week. These initial results indicate that the requirements for transplant of garlic differ from those of shallot and that modification of the protocol is needed to suit the requirements of garlic.

CYTOLOGICAL INVESTIGATION OF ENDOSPERM EXPLANT AND LONG-TERM MAINTENANCE OF ENDOSPERM-DERIVED CALLI OF CALAMANSI (*X CITRO FORTUNELLA MITIS* J. INGRAM AND H. MOORE)

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Endosperm explants excised from calamansi fruits harvested 58-65 days after pollination were studied cytologically and their triploidy ($3n = 27$) was confirmed. Calli were successfully induced from these triploid endosperm explants. Cytological investigation of these endosperm-derived calli is in progress.

Endosperm-derived calli which were initiated in November 1991 were maintained *in vitro* for two years and two months. This long-term maintenance of calli (which we refer to as ageing) was necessary for shoot regeneration. Shoot regeneration was observed earlier from calli induced from mixed nucelli-endosperm explants. However, when the calli were derived solely from the endosperm, the expected response was not observed. The study on shoot regeneration is in progress. Ultimately, the project aims to produce triploid plants bearing seedless fruits.

OVERCOMING INCOMPABILITY IN A CROSS OF *ORYZA SATIVA* (L.) AND *PORTERESIA COARCTATA* (Tateoka. Roxb.)

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Reciprocal crosses were made between rice (*Oryza sativa* $2n = 24$) and a salt tolerant grass species (*Porteresia coarctata* $92n = 48$). As many as 9,100 florets of *O. sativa* and 2,658 florets of *P. coarctata* were pollinated with *P. coarctata* and *O. sativa*, respectively. In order to overcome incompatibility barrier, hormones such as GA_3 (75 ppm), 2,4-D (75 ppm), ACA (250 ppm), and IAA (2 ppm) were sprayed singly and in combination on the pollinated florets. In order to produce hybrids, ovary culture and embryo rescue were employed. After 1, 6, 12 and 24 hours of pollination, pollen grain germination and pollen tube growth were studied. Majority of the pollen tubes either burst or were

swollen and failed to reach the micropylar region. However, some florets sprayed with combinations of hormones produced pollen tubes up to the micropylar region. Most of the seeds produced from these pollinations were devoid of embryos but with water fluid. From *O. sativa* x *P. coarctata* cross, 572 embryos were cultured and based on plant morphology and isozyme analysis, none of the 48 regenerated plants showed any evidence of hybridity. In a cross where *P. coarctata* was the female parent, none of the 32 cultured embryos grew into seedlings.

PRODUCTION OF SEED TUBERS OF ASN 69.1 FROM ROOTED STEM CUTTINGS, MICROTUBERS AND FROM THEIR FIRST GENERATION TUBERS

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Rooted stem cuttings (RSC) and microtubers of three size grades (based on diameter) and their first generation tubers were compared as planting materials for ASN 69.1. Percent survival was highest for microtubers of 10 mm size grade (83%) and lowest for rooted stem cuttings (57%). Tuber yield was also significantly highest in terms of number (157/m²) and weight (4.2 kg/m²) for 10 mm microtubers. Yields of smaller microtubers (<10 mm) and RSC were similar. The mean number of seed tubers per hill was not significantly different among planting materials.

For the first generation tubers, tuber yield was significantly affected by the size of seed tubers used rather than seed source. The traditionally acceptable seed tuber size (i.e. 28-35 mm) yielded more seed-sized tubers than smaller tubers (7-8 vs. 5 pcs per hill).

RICE POTENTIAL PRODUCTION IN THE PHILIPPINES USING CROP GROWTH MODEL

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Simulation Model for Rice-Weather Relationships (SIMRIW) is a crop model that integrates ontogenic development, biomass accumulation and yield formation. This model predicts crop growth and yield from daily weather data. Using crop parameters derived from field experiments at IRRI, the model has accurately simulated the dry season yield of 9.3 t/ha. We incorporated in the model the effect of CO₂ on photosynthetic rate and the effect of temperature on spikelet sterility to simulate the impact of climate change on rice production. Model runs were performed using 30 years of weather data from Los Baños. The model predicted a yield reduction of 12% as a result of 4°C mean daily

temperature increase. Additional 50 ppm CO₂ to the ambient CO₂ concentration slightly increased yields.

SIMRIW was used to simulate potential production under current and future climate scenarios from other weather stations in the Philippines with at least 10 years of historic weather data. Generally, increased temperature hastened crop development by at least 7 days, producing lower yields; while CO₂ enhancement generally increased yields. By using a geographic information system, it was demonstrated that the areas with high potential production which are currently in the central parts of the country will move away from the equator in response to climate change.

HISPATHOLOGICAL EVENTS DURING *RHIZOCTONIA SOLANI* INFECTION IN RESISTANT AND SUSCEPTIBLE CORN PLANTS

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Rhizoctonia banded leaf and sheath blight resistant and susceptible corn plants were sampled for hispathological study. Results from scanning electron microscopy showed that in both resistant and susceptible samples, *R. solani* exhibited stomatal penetration. However, only one hypha was observed to penetrate stomata in resistant samples while two hyphae were frequently seen in susceptible material. There was lobate appressorium formation and penetration pegs produced from this structure penetrated the plant surface directly. The susceptible sample showed more severe damaged cells than the resistant tissue.

INCREASED NITROGEN MITIGATES GROWTH AND YIELD REDUCTION OF UPLRi-5 RICE CULTIVAR DUE TO *MELOIDOGYNE GRAMINICOLA*

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Effects of *M. graminicola* on growth and yield of upland rice cultivars UPLRi-5 were studied under glasshouse experiment. *M. graminicola* juveniles were inoculated near the base of the plant in five splits at an interval of three days at the rate of 0, 10, 50, 100, 500, and 1000/kg of soil. The levels of nitrogen used were 0, 40, 80 and 160 kg/ha applied in three equal splits 10 days after seeding, at maximum tillering and during panicle initiation.

Plant height, leaf and shoot weights, leaf area, root weight, depth of rooting and, the number of panicles and grains were reduced by the nematodes. Grain yield was reduced by 30-70% when the number of infective juveniles present around young seedlings at transplanting (Pi) was ≥ 375 . Increased nitrogen (N) application, increased growth and yield of plants compensated for yield loss caused by nematodes. However, since yield loss remained approximately con-

stant for a given P_i across the range of N used, N application did not reduce the relative nematode effect.

CONTROL OF *HIRSCHMANNIELLA ORYZAE* USING *SESBANIA ROSTRATA* AND ITS RESIDUAL EFFECTS IN A CONTINUOUS RICE CROPPING

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A four continuous cropping experiment was conducted to determine if the yield increase in rice could be attributed to the decrease in *Hirschmanniella oryzae* population when grown in a sequential cropping with *Sesbania rostrata* and to evaluate if there is a residual effect of the treatment on subsequent rice crops. The treatments included the growing of *S. rostrata* and rice during the first cropping and incorporation of the legume before the second rice crop. Carbofuran was added to the same set of treatments for comparison. Increase by 30-45% in the yield of the first rice crop after treatment application resulted when *S. rostrata* was grown regardless of incorporation as green manure and in all treatments with carbofuran. A significant correlation of yield and nematode population densities was also observed. A 16-25% increase in yield was observed in treatments with carbofuran after the second rice crop and a significant correlation with nematode population was noted. There was no significant differences on the yield and nematode population after the third rice crop. The increase in rice yield, therefore, could be attributed not only to the fertilizer effect brought about by growing of *S. rostrata* or green manure application but to the control of the rice root nematode. The treatments were effective in two rice croppings only after growing of *S. rostrata* and/or incorporation as green manure.

DETECTION OF LEAF CURL VIRUS IN PEPPER BY USING NON-RADIOACTIVE DNA PROBE

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Experiment was conducted on the use of non-radioactive DNA probe produced from the gene of tomato yellow leaf curl virus (TYLCV), Thai isolate, to detect gemini virus in leaf curl pepper.

Results on the detection by NBT, x-phosphate and chemiluminescence AMPDD methods showed that 25 out of 63 samples collected could be hybridized with the TYLCV-DNA probe. Weed plants collected from pepper planting field such as *Physalis flordina* and *Eclipta alba* also reacted with the probe as well.

SHOOT SOLUBLE CARBOHYDRATE CONTENT AS A MEASURE OF DAILY C-ASSIMILATION IN RICE

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Current methods for measuring carbon assimilation require a large number of photosynthesis measurements at different times of the day in order to give an accurate estimation on a daily basis. This is impractical and expensive in terms of labor and equipment. Large scale applications such as cultivar screening require a simple method needing only one or two samples. Measuring the soluble sugar levels in the plant may give a better estimate of carbon assimilated over the day. This relies on a direct relationship between shoot carbohydrate content and daily radiation. To test this, main stems of IR72 plants growing in the field were harvested at four growth stages: newly transplanted seedlings, mid-tillering, panicle initiation and flowering. Soluble sugar levels in the shoot gave high correlation with daily radiation ($R^2 = 0.94$, $n = 16$) at panicle initiation but not in the other three stages. Low correlations at these stages may be due to translocation of carbohydrates to rapidly growing tissues or for grain filling. The excellent correlation with daily radiation shows that shoot soluble carbohydrate content at panicle initiation gives an accurate measure of carbon assimilation over the whole day.

NEW PLANT TYPES FOR IRRIGATED RICE: MANIPULATING PANICLE HEIGHT FOR INCREASED LIGHT INTERCEPTION

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A simulation model that identifies plant attributes for greater yield potential predicts that a 10 cm decrease in panicle height will increase light interception by leaves and hence yield by 15%. Panicles usually shade the flagleaf and lower incident radiation reaching the leaf canopy, decreasing photosynthetic rate and carbohydrate supply to panicles. Another advantage of lower panicles is reduced lodging tendency due to a lower center of gravity. This study focused on the use of gibberellic acid to increase and paclobutrazol to decrease panicle height. Treatments as to what part of the plant, when and how much of the growth regulators to apply were evaluated in the greenhouse so that only panicle height and not plant height was affected. Based on the greenhouse results, light interception and yield of IR50 grown in the field were evaluated to test the hypothesis.

It is possible to manipulate panicle height of IR50 from 78 cm to 65 cm by applying growth regulators on the base of plants during booting. Field data

showed that panicles intercepted 10% of the light available to the whole canopy and 40% of the light available to leaves below them. Flag leaves of low panicle plants had less shading and were able to intercept 10% more light than high panicle plants. Low panicle plants had heavier panicles compared to high panicle plants.

PROCESS OF SEEDLING ESTABLISHMENT AS INFLUENCED BY ORGANIC MATTER APPLICATION TO FLOODED SOIL

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Organic matter application to flooded soil may affect seedling establishment by lowering the redox potential and producing toxic substances. This study was conducted in greenhouse to clarify how seedling establishment was impaired by organic matter application. Cultivars tolerant of soil hypoxia ASD1 and IR41996-50-2-1-3 and a control IR72 were sown in flooded Maahas clay soil into which 0, 0.25, 0.50, and 1.00% (w/w) rice straw was incorporated. The soil was either incubated for 14 days by flooding or nonincubated. Pregerminated seeds were sown at 25 mm soil depth and 25 mm water depth. Rice straw incorporation did not affect coleoptile length but reduced leaf and root growth. Leaf and root development was inhibited in nonincubated soil. The redox potential in nonincubated soil seedling establishment might be controlled by redox potential while in incubated soil, the accumulation of organic acids from anaerobic decomposition might inhibit leaf and root development. The tolerant cultivar ASD1 consistently performed better than the control even in nonincubated soil with high straw incorporation.

CHEMICAL CHARACTERIZATION OF TWO ORGANIC MATTER FRACTIONS IN IRRIGATED LOWLAND RICE SOILS

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Much of the N fertilizer is applied to irrigated rice paddies cycles through soil organic matter (SOM). Chemical characterization of SOM may help predict soil N supply and N balance. Characterization is often performed on SOM extracted with NaOH. Soil Ca can bind to SOM, protecting it from complete microbial degradation-thus facilitating its maturation into more recalcitrant forms. NaOH extraction of SOM with or without initial soil decalcification allows fractionation of younger, more labile SOM called mobile humic acids (MHA) from more recalcitrant SOM bound to Ca (Ca-humates, Ca-HA). Both SOM fractions were extracted from a field experiment in which rice was double-cropped

for 10 years without N inputs and N-source treatments with equivalent N inputs as prilled urea, azolla, *Sesbania rostrata*, or rice straw (116 kg N ha⁻¹ and 58 kg N ha⁻¹ in the dry and wet seasons, respectively). The MHA had 22% higher total N and 50% more acid-hydrolyzable amino acids than the Ca-HA. The MHA may have a smaller molecular weight and be less aromatic and condensed than the Ca-HA. Although the quantity of total organic carbon and HA was greater in soil with organic N-sources, chemical characteristics varied only slightly by N source. These results indicate that using organic N sources can increase SOM content without changing the chemical composition of young and older HA fractions in soils cropped under submerged conditions.

RELATIONSHIP BETWEEN N, P AND K UPTAKE AND YIELD IN RAINFED LOWLAND RICE

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Rainfed lowland rice response to applied fertilizer is often poor. The advantage of applying fertilizer nutrients to a pre-rice manure crop (*Sesbania rostrata*) to enhance nutrient availability was evaluated in experiments at six sites in Northeast Thailand from 1987 to 1991. At all sites, treatments were arranged in split-plot design with three replicates. Main plots were nutrient sources for the pre-rice crop (*Sesbania*, *Sesbania* + 13 kg P/ha, *Sesbania* + 3 t/ha farmyard manure (FYM), fallow) and subplots were nutrient levels for wet season rice crop (0, 50 kg N/ha + 13 kg P + 25 kg K/ha, 3 t/ha FYM). This paper examined the relationship between rice yields and N, P and K uptake.

Generally, rice grain yield was linearly related to N-uptake over sites and years ($R^2 = 0.75^{**}$), except at Khon Kaen in 1987 and at Ubon in 1990. There were no exceptions, however, for the linear relationship between DM and N uptake ($R^2 = 0.67^{**}$) indicating that drought stress during reproductive growth limited grain yield. The relationship between grain yield and P-uptake was not consistent over years. Uptake of K was not closely related to grain yield or total rice dry matter. These relationships will be used to estimate the N & P balance and N & P uptake efficiency.

FUNCTIONAL EQUILIBRIUM OF THE RICE ROOT SYSTEM

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The effect of nutrient supply on root length and distribution of rice was examined in field studies at PRR1 (Nueva Ecija) and IRRI (Laguna). Roots from 0-5, 5-10, 10-20, and 20-30 cm, and above ground biomass were sampled at

flowering in two cultivars (IR72 and IR29723-143-3-2-1) with and without NPK inputs (+/- NPK) in 1991 WS and 1992 DS. In the 1993 DS, a pot experiment was conducted in the IRRRI phytotron under controlled day/night temperature (28.5/21/5°C). Treatments and measurements were the same as in the field experiments. Grain yield was 2.7 t ha⁻¹ in -NPK and 4.5 t ha⁻¹ in +NPK in the WS, and 3.4 and 7.8 t ha⁻¹ in the DS, respectively. At flowering, shoot weight in +NPK treatments was 70% (WS) and 190% (DS) greater than in -NPK. By contrast, root length increased by only 15% (WS) and 24% (DS) in +NPK. In the pot experiment, shoot dry matter increased by 180% and root mass by 33% with +NPK, while total root length was equivalent for + and -NPK. Stability of root relative to shoot growth resulted in a decrease in the root/shoot dry matter ratio from 17% in -NPK to 8% in +NPK. Tiller number per pot increased from 16 in -NPK to 43 in +NPK, but root length per tiller decreased from 48 m in -NPK to 14 m in +NPK. These results indicate a dynamic functional equilibrium in root-shoot partitioning of rice in response to the external nutrient supply such that when nutrients are deficient, rice allocates relatively more dry matter to root development than when nutrient supply is abundant.

COMPONENTS OF PERCOLATION LOSSES IN IRRIGATED PUDDLED RICE FIELDS

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The very low water use efficiency of irrigated lowland rice system is partly due to water losses by percolation. The components of percolation losses in a puddled rice field with highly permeable subsoil were quantified using detailed field experiments conducted at IRRRI last 1993 dry season. Water balance components were measured independently in each of the five 11.5-m x 6.2-m experimental plots for Scenarios 1 to 3. In Scenario 1, water losses in the well puddled field were measured. Scenario 2 included the water losses through the non-puddled areas. Water losses by underbund percolation, which results from lateral movement of water from the flooded fields into the bunds and then vertically down to the water table, were accounted in Scenario 3.

Inclusion of small nonpuddled area (1.5 m² per 100 m² of puddled soil) within the field of 5-cm ponding water depth (PWD) increased water losses from 2.7 mm d⁻¹ to 14 mm d⁻¹. Underbund percolation increased percolation rate 10 mm d⁻¹ further in a typical farmer's field of 25 x 100 m and even more in smaller fields. Maintaining shallow PWD does not significantly affect percolation loss through a homogeneously puddled soil but greatly reduces losses in nonpuddled spots and underbund percolation. The latter can be further reduced if horizontal conductivity of the bunds is reduced, e.g. via sealing of macropores with mud.

The importance of the processes and its implications in water-savings in irrigated puddled rice fields are discussed.

INCREASING RAINFED FARMERS' INCOMES THROUGH AN APPROPRIATE POSTHARVEST TECHNIQUE

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An evaluation of a postharvest technique of farmers in a rainfed lowland area in Guimba, Nueva Ecija, revealed that lack of storage facilities can be offset by stacking harvested palay in the upper part of the field near the farmers' houses. In 1991 and 1992, harvesting commenced the last week of October and continued through the second week of November. No rainfall was expected within the period of stacking. The amount of rainfall expected at 80% probability was less than 1 mm per day. Farmers dried the harvested palay in the field for 1-2 weeks and then stacked them up for another 1-4 weeks to wait for a better price for palay. Average labor and power costs for stacking was P386/ha. Farmers reported no insect, bird or rat damages and were not aware of any possible postharvest losses in stacking.

Even with an assumed 4% loss due to handling and stacking, farmers got a gross margin 30% higher than if had they immediately threshed and sold their products. The high income gained from stacking encouraged farmers to continue practicing the technique for more than 30 years. This technique offers an opportunity of overcoming the income loss due to low price of palay at harvest time and augmenting the farmers' meager resources.

TOWARDS AN INTEGRATED LINKAGE MAP OF MUNGBEAN (*VIGNA RADIATA* L. WILCZEK) - PROGRESS AND PROSPECTS

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Mungbean (*Vigna radiata* L.) is a leguminous species or pulse crop grown primarily in Asia. It is an important source of inexpensive protein most Asian diets and a significant component of various cropping systems. We have been developing an integrated linkage map of mungbean consisting of morphological, physiological, isozyme and DNA markers in order to facilitate the development of improved cultivars, to clone plant genes and to understand genome evolution in this crop. This report summarizes some of the significant accomplishments of our collaborative efforts during the past three years. These were: (a) construction of RFLP map with 171 RFLP markers, (b) mapping of agriculturally important genes such as resistance to bruchid and powdery mildew and for seed weight,

(c) comparative mapping of mungbean, cowpea and soybean genomes, (d) identification, inheritance and linkage studies of isozyme and morphological markers, and (e) development of additional mapping populations from interspecific crosses, recombinant inbred lines (RILs) and near-isogenic lines (NILs). We also initiated the development of pulsed-field gel techniques for physical mapping and gene cloning in mungbean. Extensive breeding programs for mungbean are continuously being undertaken in international and national agricultural research centers. With a well-developed map and appropriate technologies for developing countries, it will now be possible to integrate marker-based technology into conventional breeding programs for mungbean and improve production of local cultivars.

CYTOLOGY, MORPHOLOGY AND POLLEN FERTILITY OF INTERSPECIFIC HYBRIDS BETWEEN *ORYZA SATIVA* AND *O. OFFICINALIS*

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UPL Ri3 was crossed with *Oryza officinalis*, a source of resistance against brown planthopper, green leafhopper and white-backed planthopper. Eleven to fourteen-day old embryos were cultured *in vitro* resulting in identical hybrid plants. Very few F₂ seeds obtained from F₁ plants were germinated *in vitro* and grown in the greenhouse for evaluation. This study aimed to analyze the morphology, pollen fertility and cytology of both the F₁ and F₂ plants.

The F₁ hybrids had semi-erect, grassy and vigorous growth. Like the wild parent, they had perennial growth cycle, rigid awns, well exerted panicles and purple stigma, auricles and basal leaf sheath. F₂ plants had soft-textured awns, moderately exerted panicles and longer anthers, flagleaf, panicles, grain and culm. Variation in tillering ability, panicle type, grain size and color of the leaves, basal leaf sheath, awn, apiculus and internode were noted among the F₂ plants.

Pollen fertility of the F₂'s ranged from 0-0.45%, similar to the value obtained from the F₁'s. Cytological examination of the microsporocytes of both the F₁'s and the F₂'s showed a wide array of chromosomal aberrations like the congression, extra chromosomes and chromosomes elimination. The abnormal meiotic behavior may explain pollen sterility.

AGRONOMIC EVALUATION OF DOUBLED HAPLOIDS DERIVED FROM RICE ANTHER CULTURE

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Agronomic performance of doubled haploid (DH) rice plants derived from anther culture of two purelines (IR 43 and Momay) was evaluated in terms of 11

characters. Three sources of variation were examined: variation among anthers, among regenerants within an anther and among tillers within regenerants. Variations were noted among regenerants within an anther and regenerants coming from different anthers. DH lines of IR 43 differed significantly from the check (seed-derived plants) by having higher yield, longer days to heading, more productive tillers and higher panicle sterility. DH lines of Momay had shorter, wider and smaller grains and more tillers. Surprisingly, there was also variation among tillers of an IR 43 regenerant in terms of days to heading, panicle length, number of filled grains and 100 seed gram weight.

Theoretically, doubled haploids derived from anther culture of purelines should be similar to the check. Present results deviated from the expected, indicating that there may be some genetic changes occurring during *in vitro* culture. These changes could take place during any stage of callus formation, proliferation, differentiation. Regenerants could also arise from groups of cells with mutant sectors and tillers could have originated from these sectors. Stability of these changes needs a follow-up study.

ANTHER CULTURABILITY OF SOME PHILIPPINE TRADITIONAL RICE VARIETIES

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The response to anther culture of 17 Philippine traditional rice varieties and elite lines, currently being used as parents in the crossing work, was initially evaluated using N₆ and SK₃ as basal media, supplemented with 2 mg/l 2,4-D, 0.5 mg/l kinetin and 60 g/l sucrose. Callus induction was observed in 14 genotypes with callus induction frequency ranging from 0-0.17%. Regeneration efficiency (based on the total number of anthers plated) was 0-0.71%. Among the responsive genotypes were UPLRi5, C2982-5-3-1, IR 43, Momay, Basura, and Tuhaw. Albinism among the regenerants was a major concern.

Although anther culture of rice appears to be a routine procedure, problems associated with the technique need to be investigated to improve its efficiency and practical application in rice breeding.

ISOZYMES FOR GENETIC MAPPING IN MUNGBEAN: OPTIMIZATION OF CONDITIONS FOR GEL ELECTROPHORESIS AND IDENTIFICATION OF POLYMORPHISMS

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Electrophoresis using starch gel and polyacrylamide systems were used to resolve 24 isozymes from the dry seeds, cotyledon and young leaflets of

three *Vigna* sp. (*V. radiata*, *V. radiata* ssp. *sublobata* and *V. mungo*). Nine extraction buffers and ten gel/electrode buffer systems were tested.

Five extraction buffers in specific combination with four starch gel electrophoresis buffer systems effectively resolved 15-20 different isozymes from the dry seeds, cotyledon and young leaf samples. The 20 isozymes resolved were: acid phosphatase (ACP), alcohol dehydrogenase (ADH), alanine aminopeptidase (AlAP), arginine aminopeptidase (ArAP), catalase (CAT), diaphorase (DIA), endopeptidase (ENP), esterase (EST), B-glucosidase (B-GLU), glucose-6-phosphate dehydrogenase (G6PDH), glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), peroxidase (PER), phosphogluconate dehydrogenase (PGD), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM) and shikimate dehydrogenase (SDH). However, the best resolution for LAP, PER, PGD and SDH from young leaves were obtained using a 12% polyacrylamide non-denaturing gel system.

All 20 enzymes were subsequently assayed for polymorphisms in mungbean (*V. radiata* and its two closely related species, *V. mungo* and *V. radiata* ssp. *sublobata*). More isozyme polymorphisms were observed between mungbean and *V. mungo* than between mungbean and *V. radiata* sp. *sublobata*.

KINETIC PROPERTIES AND ACTIVITY LEVELS OF AMMONIA AND CARBON ASSIMILATION ENZYMES IN TWO *VIGNA* SPECIES.

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Induction of high levels of activity of ammonia and carbon assimilation enzymes of various legume nodules has been observed to parallel the development of high N₂ fixation. These enzymes are: glutamine syntase (GS), glutamate syntase (COGAT), glutamate dehydrogenase (GDH) and phosphoenolpyruvate carboxylase (PEPC). We have been developing the potential use of these enzymes as biochemical indices of N₂-fixation in mungbean (*Vigna radiata*) and cowpea (*V. unguiculata*). This report describes the kinetic properties and the variation observed in the activity level of each enzyme in developing and mature nodules of the two *Vigna* species.

Mungbean nodule GS showed an apparent Km value of 12.0 uM for NH₄ and 4.95 uM for hydroxylamine. The Km values for NADH and a-ketoglutarate of nodule GOGAT were 33.3 and 50.0 uM, respectively; for GDH, 76.3 uM for NADH and 20uM for a-ketoglutarate. The Km value for PEP is 86.0 uM. The pH optima for nodule GS < GOGAT, GDH and PEPC were estimated to be 7.0, 5.0, 7.0 and 8.0, respectively.

In yardlong bean nodules, the apparent Km values observed for: (a) GS, 13.4 uM for NH₄ and 4.95 uM for hydroxylamine, (b) GOGAT, 91.2 uM for NADH and 2.85 uM for a-ketoglutarate, (c) GDH, 14.0 uM for NADH and 8.76

uM for α -ketoglutarate, and PEPC, 82.0 uM for PEP. GS and GDH showed similar pH optimum at 7.0. GOGAT and PEPC were observed to have a pH optimum of 7.6 and 8.0, respectively.

All four enzymes were stable at 26-30°C.

Small developing nodules showed significantly higher GOGAT, GDH, and PEPC activities than mature large nodules. Percent increase ranged from 1.1 for PEPC to 26.8 for GDH. GS activity was lower in small nodules. Similarly, all enzyme activities were higher in nodules found in the root crown than in nodules located elsewhere.

INHERITANCE OF RESISTANCE TO RICE TUNGRO SPHERICAL VIRUS IN RICE

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The genetics of resistance to infections of rice tungro spherical virus (RTSV) in resistant cultivars Utri Merah, Utri Rajapan and Pankhari 203 was studied. The F_1 and F_3 progenies of crosses between resistant cultivars and Taichung Native 1 (TNI) or IR22, cultivars susceptible to RTSV, were tested to determine the mode of inheritance for resistance to RTSV. The allelic relationships of resistance genes among the resistant cultivars were also determined.

Utri Merah is found to possess two independent recessive genes for TSV resistance. Resistance in Pankhari 203 and Utri Rajapan is supposed to be under monogenic control. Allelic tests among three resistant cultivars segregated no susceptible F_3 lines indicating that all the cultivars possessed the same or closely linked genes. Therefore, we would propose gene symbols for RTSV resistance gene, *tsv-1* in Utri Merah, Utri Rajapan and Pankhari 203, and *tsv-2* for another RTSV resistance gene in Utri Merah.

ASSOCIATION OF p12 PROTEIN OF RICE TUNGRO BACILLIFORM VIRUS TO VIRIONS

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Rice tungro bacilliform virus, a member of badnavirus, encodes proteins with molecular weights of 24k, 194k, and 46k. The 194k polyprotein encodes the coat proteins, viral proteinase, reverse transcriptase, and ribonuclease H. The functions of the other proteins are still unknown. To examine the function of 12k protein (p12), the anti p12 antiserum was raised by injecting rabbits with synthetic peptide covering the 16 amino acids of the N-terminus. Immunoblotting

using the antiserum revealed the presence of p12 in partially purified and purified virus preparations but not in the crude extracts of infected plants and healthy controls. The p12 was not detected when polyclonal antibodies against the virus (purified using driselase) were used. This means that p12 was lost during the driselase treatment p12 in addition to the major coat protein, which is probably a constituent of the viral capsid. The results suggest that p12 may play a role similar to that of the gene III product of caulimoviruses.

DIRECT TISSUE BLOTTING FOR DETECTION OF RICE VIRUSES

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Most serological diagnostic methods for plant viruses require extraction of plant sap. Such a process limits its use in a field. Direct tissue blotting which does not need a sap extraction step, was applied for field testing of rice viruses such as rice tungro bacilliform virus (RTBV), rice tungro spherical virus (RTSV), rice grassy stunt virus (RGSV), and rice ragged stunt virus (RRSV). Rice plant parts, culm, sheath, and leaf, were cut by a razor blade. The cut surface was directly blotted by pressing it gently onto a 0.45 μ m pore size nitrocellulose membrane. Signal for virus infection was detected using direct and indirect immunoblotting. Of the three tissues, the sheath samples gave the best signal. The indirect method is more sensitive than the direct one in detecting all viruses. Optimum dilutions of immunoglobulin (IgG) for the indirect method were 1/4000 for RGSV and 1/1000 for RTBV, RTSV, and RRSV. After adding the substrates, initial reaction started within 15-30 min for RGSV and 30-45 min for RTBV, RTSV, and RRSV. The whole procedure took 4-5 h. The results showed that tissue blotting is specific, sensitive, rapid and is a convenient method to assay a large number of samples.

"RED STRIP"; A NEW RICE DISEASE IN THE PHILIPPINES

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A new "disorder" has been observed in various rice fields in the Philippines. It occurs in IR72 (IRRI farm), in IRBB7 (Calauan, Laguna), in IR74, and PSBc4 (Zamboanga), and in Bordagul cultivar (Davao del Sur and Davao del Norte, North Cotabato, Zamboanga). This "disorder" is commonly observed in areas of intense rice cultivation, with high nitrogen fertilization. At the early flowering stage, the lesions occur more frequently on the lower leaves. At the ripening stage, the lesions are usually at the flag leaves, and on the other young leaves. The disorder starts as pin-point yellow specks on the leaves. The lesions are circular to oval in shape. As the lesion grows older, it darkens in color and becomes yellow-orange, sometimes with the center of the lesion

becoming more distinct. A yellow streak then extends from the lesion to the tip of the leaf, covering the entire length of the leaf. The lesion then turns reddish brown and the streak becomes orange or rusty in color. The whole leaf eventually becomes necrotic and dries up. Old lesions often become gray at the center. So far, the etiology of the disorder has not been established. There are some indications that this may be a complex disease, resulting from an interaction between soil fertility and a weak pathogen. Work in IRRI is focused on establishing the etiology of the disease.

CONCENTRATING GENES FOR DOWNY MILDEW RESISTANCE AND DESIRABLE AGRONOMIC TRAITS IN CORN

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Following a simple phenotypic recurrent selection technique, two corn populations which are highly resistant to *Peronosclerospora philippinensis* (Weston) Shaw, the causal pathogen of Philippine downy mildew, were developed. These populations, designated as CPRP1 and CPRP3, were from base populations earlier formed out of the resistant plants for the F₂ generation of commercially-available hybrids. After several cycles of selection under high inoculum pressure, a significant increase in the level of resistance was observed in both populations when compared to the reaction of the original F₂ populations. As each population is highly diverse genetically, visual selection for agronomic characters, such as plant height, ear height, general vigor, ear size, and tassel size, was likewise done. Selection for resistance to other pathogens that attack the crop at subsequent stages of growth is possible in these populations.

MAJOR CHARACTERISTICS OF PHILIPPINE TRADITIONAL UPLAND RICE VARIETIES

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Data from documented experiments with traditional upland rice varieties in the Philippines were collected and stored in a database. The information gathered was used in obtaining both quantitative and qualitative insights about traditional rice varieties.

Traditional upland rice is usually grown under long growing season in degraded areas with serious problems such as poor, unfertile soils, mainly acidic with blast, drought and weeds. The increasing population considered as the

"poorest of the poor" continues to grow rice in these areas. Under such environment, the idea that traditional cultivars are poor yielders is wrong, especially when considering that the rice growers can hardly identify achievable input levels.

ORYZA GLABERRIMA AS AN INDICATOR PLANT FOR RICE TUNGRO VIRUSES

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Oryza glaberrima (IRGC Acc. No. 100139) was identified and evaluated as an indicator plant for rice tungro viruses (RTVs). This accession was evaluated for its sensitivity to infection with RTVs, susceptibility to both virus and vector, and suitability as virus source.

IRGC 100139 was not only susceptible but also very sensitive to infection with RTVs. When inoculated at seedling stage with rice tungro spherical virus (RTSV), a latent virus, infected plants showed stunting, reduced tillering and pale green leaves 3-4 weeks after inoculation. Visual score based on these symptoms was highly accurate when compared with serological score. This accession could also be used to distinguish plants infected either with rice tungro bacilliform virus (RTBV) alone or with both RTBV and RTSV. Generally, plants infected with both viruses died within three weeks of inoculation while those infected with RTBV alone survived beyond three weeks but died before maturity. Similar symptoms were observed when this accession was inoculated by leafhoppers collected from the field for monitoring viruliferous insects. Visual score based on symptom severity and life span of infected plants correlated well with serological score. IRGC 100139 was also susceptible to the leafhopper vector *Nephotettix virescens* comparable to rice cv Taichung Native 1. It was also a good virus source. Hence, this accession has the attributes of an ideal indicator host and it would be useful in studying the epidemiology of rice tungro disease without the need for expensive serological assays.

OPTIMIZATION OF A SWEETPOTATO-PEANUT COOKIE FORMULATION

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The formulation of cookies containing sweetpotato flakes and peanuts was optimized. A 3 x 3 full factorial experiment using three levels each of sweet potato flakes (0-120g) and egg white (30-90g) in formulation containing 120 g of peanuts and 80 g sugar was conducted. Consumer acceptance tests for color, shape, appearance, flavor, mouthfeel and overall acceptability were done using 9-point Hedonic scales and 60 panelists. Response surface methodology (RSM) was used to optimized the formulation. Objective measurements of color and texture were also determined. All formulations containing all levels of egg white and up to 15 g of sweetpotato flakes resulted in formulations with overall acceptance of color and appearance but had deleterious effects on mouthfeel. Verification trials revealed the predictive ability of all models developed, except for flavor. However, all optimum formulations would likewise have acceptability scores for the sensory properties and physical measurements.

IMPROVEMENT OF YIELD AND FIBER QUALITY IN LUFFA SPP. THROUGH INTERSPECIFIC HYBRIDIZATION

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Interspecific hybridization in *Luffa* was done to obtain a cultivar with a combination of traits from *L. cylindrica* such as good yielding ability, tolerance to pests and environment stresses as well as ease of processing and traits from *L. acutangula* such as sponge fiber compactness, fineness, and thickness. Two trials were conducted involving 5 hybrids of *Luffa* in 1991 and 8 hybrids in 1992, together with their respective parental lines and Talisay (OP) as check variety. The interspecific hybrids excelled in terms of yield which was largely attributed to heterosis. Furthermore, the hybrids showed field resistance to melon fly (*Dacus cucurbitae*) and improved sponge thickness and fiber compactness. Hybrid SmP-1 x RdP-1 (or LTH-9101) showed consistent performance with yield advantage ranging from 240% to 279% over the check 'Talisay.' Initial results of on-farm trials showed farmers' preferences for the interspecific hybrid LIH-8101 over the farmers' variety.

