8:00 AM to 2:00 PM
Friday, August 4, 2006
SEARCA Auditorium
College, Laguna

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PROGRAMME

7:30 Registration

8:00 Invocation
National Anthem

8:10 Welcome Remarks & Introduction to the Molecular Biology and Biotechnology Program
Dr. Evelyn Mae Tecson-Mendoza
Research Professor & Acting Chair, Molecular Biology and Biotechnology Program Management Committee

8:30 Dissection of drought responsiveness in rice using microscopy and molecular biology
Dr. John Bennett,
Senior Scientist, International Rice Research Institute &
Adjunct Professor, University of the Philippines Los Baños

9:15 Open Forum

9:25 Snacks, Poster Viewing and ISAAA Video Presentation

PRESENTATIONS I

9:40 Molecular characterization of beef cattle from selected regions of the Philippines
Genevieve Mae B. Aquino

9:55 Isolation and molecular cloning of the cocosin promoter from coconut (*Cocos nucifera* L.)
Jorge Gil C. Angeles

10:10 Molecular analysis of resveratrol synthase genes in peanut (*Arachis hypogaea* L.)
Aileen N. Bayot

10:25 Expression analysis of WOX (WUSCHEL-related homeobox) genes in rice (*Oryza sativa* L.).
Rico L. Gamuyao

10:40 Deregulation of lysine synthesis improves the protein quality of rice endosperm
Russel Julian

10:55 Open Forum

PRESENTATIONS II

11:05 Molecular studies in coconut
Dr. Rita P. Laude (Genetics)

11:20 The Biochemistry Laboratory of the Institute of Plant Breeding: An active partner of the MBB program
Dr. Antonio C. Laurena (Biochemistry)

11:35 *Dr. Asuncion K. Raymundo* (Microbiology)

11:50 Open Forum

PRESENTATIONS III

12:00 Design and assembly of a novel fused double gene construct of 1-Aminocyclopropane-1-Carboxylic Acid (ACS) Synthase and Papaya Ringspot Virus (PRSV) coat protein genes for RNA interference (RNAi)
Neil H. Tan Gana

12:15 Biological and molecular characterization of Sweet Potato Feathery Mottle Virus (SPFMV) from Luzon, Philippines.
Medino Gedeun N. Yebron Jr.

12:30 Biological and Molecular characterization of sweet potato chlorotic stunt virus (SPCSV) in some sweet potato [Ipomoea batatas (L.) Lam.] growing areas in the Philippines
Jenny A. Panopio

12:45 Biochemical and molecular characterization of Philippine Solo papaya with delayed ripening trait
Cerrone S. Cabanos

1:00 Open Forum

1:10 Closing Remarks

Lunch

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College, Los Baños, Laguna 4031, Philippines
Telephone: (63-49) 5362361 / 5362363 / 5362365 to 67 | Fax: (63-49) 5367097

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ORAL PRESENTATIONS

Molecular Characterization of Beef Cattle from Selected Regions of the Philippines

Genevieve Mae B. Aquino

The success of the smallholder beef industry in the Philippines has been limited by a lack of knowledge on the true nature of the genetic composition of present-day Philippine Native cattle. To identify and characterize the genetic variation of the generally-accepted types of the Philippine Native breed of cattle, 16 autosomal microsatellite markers were surveyed in blood samples from selected regions of the country. Genotypic data was analyzed through various software programs for population genetics. Allelic frequencies of microsatellites revealed the presence of possible diagnostic population-specific alleles for all four groups of native cattle. Intrapopulation and interpopulation genetic diversity were estimated alongside principal components analysis of allelic frequencies. Microsatellite data was supplemented by sequence analysis of a 300-bp fragment in the hypervariable region of the mitochondrial displacement loop (D-loop). Analysis of population admixture and genetic introgression suggests that the Philippine Native breed descended from three parental populations, presumably the zebu (*Bos indicus*), taurine (*Bos taurus*), and banteng (*Bos banteng*) cattle. This research provides basic information for the development and implementation of rational and effective breeding programs in the Philippines.

Keywords: cattle, microsatellite markers, genetic variation

Isolation and Molecular Cloning of the Cocosin Promoter from Coconut (*Cocos nucifera* L.)

Jorge Gil C. Angeles

Cocosin is a major storage protein in the coconut endosperm. Expression of the cocosin gene is tissue-specific and developmentally regulated.

Multiple sequence alignment analysis generated from the amino acid sequences of 11S globulin and their homologues showed consensus regions of RCAGVS and ILRALP olipeptides that are located near the N- and C- terminal domains, respectively. Based on these conserved regions, gene-specific primers were designed via backtranslation and weighing against the published coconut codon preference table. *In-silico* verification of these consensus oligopeptides and the DNA sequences of the designed primers showed significant homology towards 11S and other related proteins.

Isolation and cloning of the endosperm-specific promoter from coconut was done using extracted genomic DNA from young coconut leaves as templates which generated PCR products whose DNA sequences represent the partial 5' end of the cocosin gene and some of its upstream regulatory regions. The primers used were based from the highly conserved RCAGVS domain near the N-terminus of the acidic chain of the cocosin protein and the RY repeat conserved region in various 11S globulin promoters. The PCR products were cloned in TOPO TA cloning vectors (Invitrogen) and partially characterized by sequence analysis.

Sequence analysis of the plasmid DNA of two bacterial clones reveal putative promoter sequences containing the reported RY repeat conserved reion, putative TATA boxes and the AGVS consensus oligopeptide found following the initial ATG transcription

codon. Moreover, the plasmid DNA Sequence of one clone show the conserved SVR(G)S oligopeptide corresponding to the N-terminal sequence of the acidic chain of the cocosin promoter.
Post-thesis research show *in-vivo* expression of one of the promoters when subjected to transient *GUS* assays. *In-silico* analysis reveal other elements in the promoter.

Keywords: 11S globulin, coconut, cocosin, promoter

Molecular Analysis of Resveratrol Synthase Genes in Peanut (*Arachis hypogaea* L.)

Aileen N. Bayot

The transformation of resveratrol synthase (RS) into crops has been an attractive option because it is the key enzyme in the synthesis of resveratrol (3, 4', 5-trihydroxystilbene), a stilbene phytoalexin that has anti-leukemic, antioxidant and chemopreventive properties. In this study, the isolation and cloning of the full length RS gene was done using genomic DNA from germinating seeds of peanut (*Arachis hypogaea* L.) by PCR using RS-specific primers. This generated a 1.5 kb product. Sequence analysis of the isolated genes showed that they have high similarity with known RS genes. Further analysis revealed the presence of two exons (exon 1: 180 bp and partial exon 2: 197 and 670 bp) and one intron (331 bp). The conserved MVSVSG and RSMAI that flanked the RS gene were also found. However, due to sequencing limitations about 150 bp of the isolated gene had no sequence data. Unfortunately, this could be the region that has the highly conserved active site containing cys_{169}. Further sequencing should be done to obtain the full sequence of the isolated RS genes. Differences in the partial restriction sites in the exons and introns suggested that at least two RS genes have been isolated. In conclusion, the isolated resveratrol synthase gene could be expressed in important crops thereby providing them with protection from microbial infections and increasing their nutraceutical value by resveratrol synthesis.

Keywords: resveratrol synthase, stilbene phytoalexin, *Arachis hypogaea*, genomic DNA, PCR

Expression Analysis of WOX (WUSCHEL-related homeobox) Genes in Rice (*Oryza sativa* L.).

Rico L. Gamuyao

Induction of adventitious embryogenesis is one of the approaches for the development of synthetic apomixis to maximize the high yield potential of hybrid rice. In *Arabidopsis*, WUSCHEL (WUS) gene encoding a homeodomain transcription factor functions in the transition from vegetative growth to somatic embryogenesis which suggests WUS gene as one of the candidate genes to induce an apomictic embryo. It is also involved in specifying shoot and floral meristem integrity. However, WUS orthologue in rice is not yet known. *In silico* search of rice genome database revealed the presence of 14 rice WOX genes which encode putative homeodomain-containing proteins possessing 41-77% identity to WUS homeodomain. Expression analysis was performed for all rice WOX genes using RT-PCR and RNA *in situ* hybridization. Two criteria based on expression patterns were considered to identify probable rice WUS orthologue: cell-specific expression (1) in the shoot meristem's organizing center and (2) in the ovule's nucellus. Unexpectedly, the putative rice WUS (CAE04846) was expressed in almost all tissues examined including the vegetative tissues (i.e. shoot, leaf, root). Spatio-temporal expression analysis of rice WOX genes demonstrated that no WOX gene satisfies such requirements to be a plausible WUS. The data presented here suggest that the shoot meristem maintenance in rice may involve different regulatory pathways (i.e. absence of the organizing center) which could be a consequence of the loss of an acidic motif at the C-terminal of WOX proteins.

Keywords: apomixis, embryogenesis, homeodomain, shoot meristem, acidic motif

Deregulation of Lysine Synthesis Improves the Protein Quality of Rice Endosperm

Russel Julian

The improvement of the quality of protein in *Oryza sativa* L. is a cost-effective way to combat the protein-calorie malnutrition in Asia. We have increased the lysine content of rice endosperm by introducing lysine-feedback insensitive aspartate kinase (AK, EC 2.7.2.4) and dihydrodipicolinate synthase (DHPS, EC 4.2.1.52), two enzymes that regulate the lysine biosynthetic pathway, through biolistic transformation. The *Corynebacterium dapA* gene for AK and *E. coli lysC M4* for DHPS were each linked to a chloroplast transit peptide sequence and expressed through a seed-specific promoter. Expression of both lysine-insensitive AK and DHPS resulted in a two-fold increase of total lysine and total protein in transgenic seeds. Histochemical localization of protein in transgenic seeds showed a strong yellow fluorescence of protein bound to 8-anilino-1-naphthaline-sulfonic acid (ANS) in the endosperm. The level of lysine (the first limiting amino acid in the human diet) obtained may provide approximately half the lysine requirement of a 60-kg adult man in a rice-based diet.

Keywords: lysine, rice, aspartate kinase, dihydrodipicolinate synthase, biolistic transformation

Design and Assembly of a Novel Fused Double Gene Construct of 1-Aminocyclopropane-1-Carboxylic Acid (ACS) Synthase and Papaya Ringspot Virus (PRSV) Coat Protein Genes for RNA Interference (RNAi)

Neil H. Tan Gana

Two cDNAs (*ACS2* and *cp*PRSV) were utilized to develop a plant transformation vector cassette to initiate delayed ripening trait and PRSV resistance in papaya by RNA interference (RNAi). An endogenous ripening-related cDNA, *ACS2* was previously isolated, cloned and characterized from *C. papaya* cv. Philippine Davao Solo. To clone the coat protein (*cp*) of the papaya ringspot virus (PRSV), the total RNA was isolated from PRSV-infected papaya leaves by RT-PCR. The E-RNAi software identified two 100 bp DNA fragments (cp001 and cp004) from the 870 bp long *cp*PRSV cDNA with the highest probability to initiate RNAi at 68.49% and 64.38% respectively. Similarly, the 100mer cDNA fragments (acc001/acc003 and acc002/acc004) from the 1,192 bp-long *ACS2* cDNA gave the highest probability of initiating RNAi (35.62-38.36%). These 100mer fragments from *ACS2* and *cp*PRSV were fused into a single entity using the VectorNTI program and the highest probability of RNAi initiation calculated by the E-RNAi software. The best combination of the fused 200mer *ACS2* and *cp*PRSV was acc004 and cp002 with 38.5% RNAi efficiency. Primer pairs (ASacs04cp02fpPstI/ASacs04cp02rpHindIII and acs04cp02fpXbaI/acs004cp002rpClaI) generated the synthetic sense and antisense versions of the acc004|cp002 fused gene fragments and assembled with the 7mer intron (TCAAGAG) after restriction digestion and ligation. The recombinant DNA construct, antisense cp002|acc004-intron-sense acc004|cp002 was finally inserted in between the cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase (NosT) terminator of the plant expression vector pGTVa. Correct orientation and composition of the various genetic elements in the final gene construct was validated by restriction digestion analyses, PCR and DNA sequencing. The final gene construct is expected to generate a 230 bp double stranded RNA (dsRNA) with a hairpin structure that would down-regulate climacteric ripening and at the same time provide viral resistance against PRSV in papaya through RNAi.

Keywords: RNA interference (RNAi), 1-Aminocyclopropane-1-Carboxylic Acid Synthase (ACS2), coat protein (*cp*), papaya ringspot virus (PRSV), cauliflower mosaic virus (CaMV) 35S promoter, nopaline synthase (NosT) terminator, double stranded RNA (dsRNA),

Biological and Molecular Characterization of
Sweet Potato Feathery Mottle Virus (SPFMV) from Luzon, Philippines

Medino Gedeun N. Yebron Jr.

Sweet Potato Feathery Mottle Virus (SPFMV) is considered as the most important viral disease in sweet potato. For studies aimed at distinguishing differences between virus isolates at the molecular level, cloning and sequencing of SPFMV CP is necessary. Virus isolates from Bataan, Zambales, Tarlac and Laguna were collected and indexed. The selected isolates were biologically characterized using different plant hosts. The isolates from Bataan, Saysain and Binukawan, did not react with the *Chenopodium* local lesion hosts. The isolates Nagbunga and Sapang, from Zambales and Tarlac respectively, resulted in chlorotic local lesions in *Chenopodium murale* while isolate Tranca, from Laguna, showed chlorotic local lesions in *C. murale* and *C. amaranticolor*. Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) and TA cloning procedures were used to produce SPFMV CP clones that were sent for sequencing using T7 and SP6 primers. Sequence comparisons of the two isolates Saysain and Binukawan gave nucleotide and amino acid sequences with 96.7% similarity with two Common (C) isolates Sor and Ken25/4a (Soroti, Uganda and Kenya, respectively). The Nagbunga isolate had nucleotide and amino acid sequence similarity at 98.2% and 99.6%, respectively, with Russet Crack isolate RC (North Carolina, USA) while the Sapang isolate had nucleotide and amino acid sequence homologies with Russet Crack isolate RC at 98% and 99.6%, respectively. The Tranca isolate had 95.3% and 99.6% nucleotide and amino acid sequence similarity with East African (EA) isolate Nam1 (Namulonge, Uganda). These confirm the presence of three SPFMV strains in the Philippines, namely: Common (C), Russet Crack (RC) and East African (EA). Two sets of specific primers were designed from these sequences: RCeg-185F and RCeg-881R for the RC supergroup strains, and Say-172F and Say-835R for the C group strains.

Keywords: sweet potato feather mottle virus, sweet potato, Luzon, Philippines, RT-PCR

Selection and Characterization of Transgenic Papaya with Delayed Ripening Trait

Cerrone S. Cabanos

The Philippines ranked 14th in world production and 7th as an exporter of papaya (FAOStat data, 2005). Papaya is a climacteric fruit and like other climacteric fruits, one of its main problems in its production is the significant postharvest loss due to uncontrolled ripening. Our strategy was to control ripening is to downregulate ethylene biosynthesis through antisense expression of the ripening-related ACC synthase gene.
Fifty-four putative transgenic lines were PCR-screened and confirmed for the presence of transgenes, the kanamycin resistance marker and antisense ACC synthase2 gene using specific primers. The transgenes were also detected in the leaves, fruits and peduncles of the transgenic papaya trees, indicating the non-chimeric character of the transformed plants. On the basis of molecular analyses and desirable phenotypic traits, including delayed ripening trait, seven confirmed transgenic papaya plants from six unique lines were selected for subsequent relevant biochemical and proximate analyses. Southern blot analysis revealed a single insertion site and single copy of the transgene in the genome transgenic lines. In general, the selected transgenic papayas exhibited 1 to 2 days longer time to attain full yellow from color break to attain full yellow from color break and 6 to 7 days delayed tissue softening relative to conventional papayas. Other than these, the proximate composition, TSS values, beta-carotene, ascorbic acid and benzyl isothiocyanate content of the transgenic lines were comparable and substantiall equivalent to conventional solo papaya lines and proximate values reported for papaya cultivars. At 100% yellow stage, the proximate analyses of the fruits of the different lines and control papayas showed: 88.15–89.23% moisture, 0.602–0.709% protein, 0.797–0.880% crude fiber, 0.119–0.131% fat, 0.512–0.566% ash, and 9.653–10.57% carbohydrate. Beta-carotene ranged from 500–769 µg/100 g while ascorbic acid ranged from 41.6–80.35 mg/100g. Free BITC ranged from 0.7–1.7 ppm. TSS or total sugars ranged from 10.1–14.2^{o}B.

Keywords: transgenic papaya, delayed ripening, substantial equivalence, antisense technology

POSTER PRESENTATIONS

Integrated Location/Expression Candidate Approach for the Analysis of RTSV resistance

T. De Leon[1], K. Satoh[2], M. R. Madamba[1], D. Y. Kwak[3], R. Mauleon[1], B. C. Lee[3], P. Q. Cabauatan[1], R. C. Cabunagan[1], M. Muhsin[1], S. Kikuchi[2], H. Leung[1] and I.-R. Choi[1]
[1]International Rice Research Institute, Los Banos, Laguna, Philippines, [2]National Institute of Agrobiological Sciences, Kan'nondai, Tsukuba, Japan, and [3]Yeongnam Agricultural Research Institute – NICS, RDA, Milyang, Korea

Rice tungro disease (RTD) is one of the most serious threats to rice production in tropical Asia. RTD is caused by the interaction between rice tungro bacilliform virus and rice tungro spherical virus (RTSV). An Indonesian cultivar, Utri Merah was identified to be resistant to RTSV. Genetic analysis showed that RTSV resistance in Utri Merah is inherited through a single recessive locus. DNA marker analysis using near isogenic lines (NIL) derived from Utri Merah revealed that a region near 22 Mb of Chromosome 7 is tightly associated with RTSV resistance, although the involvement of other loci may not be excluded. Comparative genome-wide expression analysis performed between RTSV-free and -infected plants of NIL identified arrays of common and line-specific RTSV-responsive genes. Multiple comparisons of gene expression between a susceptible and a resistant NIL at different conditions detected conditional and constitutive genes which are differentially expressed between the two lines. Some of the differentially expressed genes were found to cluster in several genome regions including the 22 Mb region of Chromosome 7. DNA marker scanning indicated that the genome regions containing the clusters of differentially expressed genes are also genotypically polymorphic between the two lines, implying the association of DNA introgression with differential gene expression. Collectively these results suggest that cross-referencing between genotypic polymorphism and expression clustering facilitates the extraction of candidate genes for certain traits such as RTSV resistance in Utri Merah.

Keywords: rice, tungro, resistance, candidate genes

Molecular Characterization of Beef (Zebu) Cattle (*Bos indicus* L.) in Agroecological Zones of the Philippines

Aquino GMB[1], Laude RP[2], Sevilla CC[3], Han J[4], and Hanotte O[4]
[1] Animal Breeding Division, Animal and Dairy Sciences Cluster, University of the Philippines, Los Baños, Laguna, Philippines; [2] Genetics and Molecular Biology Division, Institute of Biological Sciences, University of the Philippines, Los Baños, Laguna, Philippines; [3] Animal Production Division, Animal and Dairy Sciences Cluster, University of the Philippines, Los Baños, Laguna, Philippines; and, [4]International Livestock Research Institute, Nairobi, Kenya

The success of the smallholder beef industry in the Philippines has been limited by a lack of knowledge on the true nature of the genetic composition of present-day Philippine Native cattle. To identify and characterize the genetic variation of the generally accepted types of the Philippine Native breed of cattle, namely the Ilocos,

Batangas, Ilo-ilo, and Philippine Bali types, 16 autosomal microsatellite markers were surveyed in blood samples of 127 individuals from selected regions of the country. Genotypic data was analyzed through various software programs for population genetics. A total of 175 alleles were detected in all four populations over the 16 microsatellite loci. Allelic frequencies revealed the presence of possible diagnostic population-specific alleles for all four types of native cattle, including alleles that differed by one base pair in length from the other alleles. Intrapopulation genetic diversity based on the mean number of alleles, allelic richness and heterozygosity was highest in the Batangas population, closely followed by the Ilocos, Ilo-ilo and Philippine Bali populations. Estimation of genetic subdivision using F-statistics indicated 15.7% to 16.0% genetic differentiation among populations, and 5.4% to 6.7% genetic differentiation within populations. Interpopulation genetic diversity was evident in the phylogenetic trees based on the standard genetic distance (D_s), modified Cavalli-Sforza distance (D_A) and shared-allele distance (D_{AS}) matrices. Principal components analysis of allelic frequencies indicate that 86% of the variation reveals the split between Philippine Bali Cattle and the other types, while 10% of the variation is between Ilo-ilo cattle from the Luzon populations and 4% separates the Batangas and Ilocos types. Analysis of population admixture suggests that the Philippine Native breed descended from three parental populations, namely the banteng (*Bos banteng*), zebu (*Bos indicus*) and taurine (*Bos aurus*) cattle. The Philippine Bali type, shown to be a banteng-zebu hybrid, could be described as a separate breed from the Philippine Native breed, composed of the indigenous zebu-taurine hybrids from the other populations included in this study. This provides basic information for the development and implementation of rational and effective breeding programs in the Philippines.

Keywords: cattle, microsatellites, genetic diversity, Philippine Native breed

Associating Candidate Defense Genes with Quantitative Resistance to Rice Blast

Carrillo G.[1], J. Wu[1], B. Liu[1], N. Sugiyama[1], I. Oña[1], M. Variar[2], J.C. Bhatt[3], E. Javier[1], P. Goodwin[4], B. Courtois[5], J. Leach[6], H. Leung[1], C. Vera Cruz[1]

[1]IRRI, DAPO Box 7777, Metro Manila Philippines; [2]CRURRS, Hazaribag, India; [3]VPKAS, ICAR, Almora, India; [4]University of Guelph, Guelph, N1G 2W1, Canada; [5]CIRAD-CA, Montpellier Cedex 5, France, [6]Colorado State University, Fort Collins, 80523-1177, USA

We used the candidate gene (CG) approach to accumulate different mechanisms involved in quantitative resistance to rice blast in 84 selected lines derived from advanced backcross populations of Vandana/Moroberekan. Using sequence information and molecular markers from the rice genome, we designed gene-based markers and identified SSRs within CGs. These markers were used to conduct a genome scan to determine the proportion of CG alleles from Moroberekan in 108 introgression lines (84 selected for blast resistance and 24 for drought tolerance). Single-marker analysis identified three CGs significantly correlated with blast resistance. Oxalate oxidase and peroxidase were correlated with yield under blast as well as with panicle blast in Almora, a blast hotspot in India. Thaumatin is correlated with neck blast incidence in Cavinti, Philippines. Significant two-gene interactions (P<0.001)were observed in seedling blast

(HSP90 x thaumatin and thaumatin x oxalate oxidase) and neck blast (chitinase x thaumatin and thaumatin x oxalate oxidase-like protein). Of the lines selected for blast resistance, 23 yielded more than Vandana under natural drought stress. These include two lines (IR78221-19-6-7-B-B and IR78221-19-6-99-B-B) that are also high-yielding under high blast pressure in Almora. IR78221-19-6-7-B-B has alleles of oxalate oxidase, peroxidase, and HSP90 from Moroberekan. IR78221-19-6-7-B-B has only oxalate oxidase allele from Moroberekan. We are testing if additional candidate defense genes are responsible for the observed resistance in these lines. By combining multi-location tests and molecular analysis, we have identified gene-based markers strongly associated with partial blast resistance. We are now in a position to combine blast resistance with drought tolerance in advanced breeding populations for a target environment in India.

Keywords: rice, candidate genes, blast

In Silico Analysis and Molecular Characterization of Rice Oxalate Oxidase, a Candidate Gene Associated with Quantitative Resistance to Rice Blast

G. Carrillo[1], M. Reveche[1], P. Goodwin[2], J. Leach[3], H. Leung[1], C.M. Vera Cruz[1]
[1]IRRI, DAPO Box 7777, Metro Manila Philippines; [2]University of Guelph, Guelph, N1G 2W1, Canada; and, [3]Colorado State University, Fort Collins, 80523-1177, USA

Converging evidence points to a role for oxalate oxidases in the defense response of plants to fungal pathogens. Oxalate oxidase is an H2O2-generating enzyme catalyzing the reaction: oxalate + O2 + 2H+ -> 2 CO2 + H2O2. Active oxygen species, such as H2O2, have been suggested to be involved in plant defense responses against several fungal pathogens. Oxalate oxidase is a member of the cupin superfamily of functionally diverse proteins with the conserved domains G(x)5HxH(x)3,4E(x)6G and G(x)5PxG(x)2H(x)3N. Scanning the rice genome resulted in the identification of 74 proteins with cupin domains that are dispersed across nine chromosomes. Phylogenetic analyses using distance matrix revealed two major groups of cupins in the rice genome: the germin-like proteins which include oxalate oxidase and the seed storage proteins which include legumins. *In-silico* analysis of these protein sequences as well as *cis*-elements related to defense response in the 1 kb upstream regions of each gene is in progress. Four putative oxalate oxidase sequences were located on chromosome 3. These oxalate oxidases are associated with variation in resistance (i.e., lesion numbers) in a rice blast-resistant mapping population derived from Vandana/Moroberekan and are highly similar to a barley oxalate oxidase (gi|2266667), previously reported to encode a cell wall-localized oxalate oxidase involved in the response to powdery mildew infection. To identify the functional variants of oxalate oxidase important for conditioning resistance in rice, we are sequencing these genes from 48 rice accessions and evaluating the reaction of these accessions to several rice blast isolates.

Keywords: rice, oxalate oxidase, blast

Development of Non Gel-Based, Low Cost Technologies for Marker–Assisted Selection in Rice and Maize

B. Collard[1], M.Y. Reveche[1], E. Mercado[1], G. Carrillo[1], M. Bernardo[1], J. Wu1[2], J. Chen[1,2], R. Babu[3], E. Redoña[4], P. Kadirvel[5], M. Bustamam[6], V. Valerie[7], M. William[8], and C.M. Vera Cruz1

[1]International Rice Research Institute, Metro Manila, Philippines; [2]China National Rice Research Institute, Hangzhou, China; [3]VPKAS, ICAR, Almora, India; [4]Philippine Rice Research Institute, Nueva Ecija, Philippines; [5]Mahyco Research Foundation, India; [6]ICABRRD, Bogor, Indonesia; [7]IRD-Agropolis, France, 8CIMMYT, Mexico

DNA markers are invaluable tools for increasing the efficiency and precision of plant breeding. Currently, the main obstacle to the implementation of DNA markers in plant breeding programs using marker-assisted selection (MAS) is the high cost for marker genotyping. Furthermore, the majority of DNA markers are detected by gel electrophoresis, which is time-consuming and often limits the large number of samples that require testing. Thus, three new genotyping methods are currently being developed Two of them – a dot blot method and a PCR-ELISA-based method - were specifically designed for low cost genotyping approaches that do not require gel electrophoresis or expensive and specialized laboratory equipment. These methods are being developed for adoption in plant breeding laboratories with limited resources. The third method - a microarray-based SNP genotyping method – is being developed as a cost-effective, high-throughput genotyping procedure that could be utilized in core genotyping plant breeding institutes that also assist NARES partners. In order to validate and optimize these methods, we have focused on two important traits: bacterial blight resistance (controlled by *xa5* and *Xa21*) in rice, and protein quality in maize (controlled by the *opaque2* allele, *o2*). Using the donor R allele of target genes and several S alleles from recipient varieties, we have designed allele-specific oligonucleotide probes for dot blot and PCR-ELISA methods based on divergent regions, indels and SNPs. Using dot-blot hybridization, a pair of SNP-based probes hybridized with the expected target alleles. Optimization of allele-specific hybridization conditions for PCR-ELISA and microarray-based methods is also in progress. DNA of popular recipient varieties and parental hybrid lines from NARES partners in China, India, Indonesia and the Philippines have been obtained and are also being used for sequence analysis of susceptible alleles of *Xa21* and *Xa5* to design specific primers for genotyping the progenies. The DNA sequences of these alleles are providing invaluable information for accurate marker genotyping of a collection of varieties for improving resistance to bacterial blight. Using this set of recipient and donor DNA for these target genes, we are exploring non gel-based allele detection technologies for low cost application of gene-based MAS.

Keywords: marker-assisted selection, rice, bacterial blight, microarray

Characterization of an IR64-Derived Mutant Line for the Reaction to Rice Tungro Disease

Negussie S. Zenna; Pepito Q Cabauatan; Hei Leung; Il-Ryong Choi

Approximately 23,000 mutant lines derived from IR64 were evaluated for the reactions to rice tungro disease (RTD). One of the diepoxybutane-mutaginized lines, M3D6 93–1 was identified to have enhanced resistance to RTD by the insect-transmission test of rice tungro virus complex. Insect transmission of rice tungro spherical virus (RTSV) revealed that the rate of RTSV infection in the mutant line (~ 20 %) is significantly lower than in wild type (~ 80 %), although reduction in the virus titer in the infected mutant plants was not observed. Inoculation of rice tungro bacilliform virus (RTBV) to the mutant through the *Agrobacterium*-mediated method indicated that the resistance is not effective to RTBV. The mutant line was crossed with susceptible varieties IR64 (wt) and Taichung Native 1 to produce progeny and backcross populations. Based on the rate of RTSV infection by insect transmission, the F1 plants were found to be susceptible, whereas the resistant and susceptible phenotypes of the F2 progenies segregated in a 1:3 ratio, suggesting that the resistance trait is controlled by a single recessive locus. The vector resistance test demonstrated that the mutant exhibited a high level of antixenosis against the vector as well. Comparative gene expression analysis performed with non-inoculated plants of the mutant and wt showed that, although genes encoding such as RNA-binding protein, zinc metalloprotease and senescence-associated protein are differentially expressed between the mutant and wt, no significant differences in the expression of genes encoding NBS-LRR proteins were observed.

Keywords: IR64, tungro disease, rice

Molecular Analysis of Resveratrol Synthase Genes in Peanut (*Arachis hypogaea* L.)

Aileen N. Bayot[1] and Antonio C. Laurena[2]

[1]National Institute of Molecular Biology and Biotechnology
[2]Institute of Plant Breeding, University of the Philippines Los Baños, College, Laguna 4031

The transformation of resveratrol synthase (RS) into crops has been an attractive option because it is the key enzyme in the synthesis of resveratrol (3, 4', 5-trihydroxystilbene), a stilbene phytoalexin that has anti-leukemic, antioxidant and chemopreventive properties. In this study, the isolation and cloning of the full length RS gene was done using genomic DNA from germinating seeds of peanut (*Arachis hypogaea* L.) by PCR using RS-specific primers. This generated a 1.5 kb product. Sequence analysis of the isolated genes showed that they have high similarity with known RS genes. Further analysis revealed the presence of two exons (exon 1: 180 bp and partial exon 2: 197 and 670 bp) and one intron (331 bp). The conserved MVSVSG and RSMAI that flanked the RS gene were also found. However, due to sequencing limitations about 150 bp of the isolated gene had no sequence data. Unfortunately, this could be the region that has the highly conserved active site containing cys_{169}. Further

sequencing should be done to obtain the full sequence of the isolated RS genes. Differences in the partial restriction sites in the exons and introns suggested that at least two RS genes have been isolated. In conclusion, the isolated resveratrol synthase gene could be expressed in important crops thereby providing them with protection from microbial infections and increasing their nutraceutical value by resveratrol synthesis.

Keywords: resveratrol synthase, stilbene phytoalexin, *Arachis hypogaea*, genomic DNA, PCR

Mitochondrial DNA Analysis of Genetic Introgression in Selected Philippine Cattle Populations

Genevieve Mae B. Aquino[1]*, Rita P. Laude[2], Jianlin Han[3], and Olivier Hanotte[3]
[1] *Animal & Dairy Sciences Cluster, University of the Philippines, Los Baños, Laguna, Philippines*
[2] *Institute of Biological Sciences, University of the Philippines, Los Baños, Laguna, Philippines*
[3] *International Livestock Research Institute, Nairobi, Kenya*
**for correspondence: 49-536-4116 (tel); gm.aquino@gmail.com*

For the future improvement of beef cattle breeding programs in smallholder farming systems, mitochondrial DNA (mtDNA) displacement loop (D-loop) sequence variation was examined to supplement existing basic information on the genetic composition of Philippine Native cattle. To evaluate the degree of genetic introgression in native cattle populations representing the Ilocos, Batangas, Ilo-ilo and Philippine Native types, a fragment of the hypervariable region in the mitochondrial D-loop was amplified and sequenced from a total of 100 individuals used in a previous study on autosomal microsatellite variation. Multiple sequence alignment with published D-loop sequences of foreign pure breds and subsequent phylogenetic analysis revealed three major clades of zebu (*Bos indicus*), taurine (*B. taurus*) and banteng (*B. banteng*) maternal ancestry. Individual sequences did not cluster significantly into geographic groups, providing evidence of migration between populations though trade relationships between these regions. However, haplotypes of Native cattle were distinct from those of the foreign breeds, implying the evolution of the Philippine populations from their foreign ancestral populations. Patterns of mitochondrial DNA variation indicate that the Ilo-ilo type was of taurine ancestry with zebu genetic introgression, and the Philippine-Bali type was of zebu ancestry with banteng introgression. The hybrid zebu-taurine composition of mtDNA from the Ilocos and Batangas populations confirmed the results of previous autosomal microsatellite analysis. Molecular characterization based on combined mitochondrial and microsatellite analysis provides a clearer perspective on the genetic composition of Philippine cattle populations and showed the potential of existing populations in the geographically-isolated islands of the Visayas for future genetic conservation and breed development programs.

Keywords: cattle, mitochondrial DNA, D-loop, genetic diversity, Philippine Native breed

Substantial Equivalence between Transgenic Papaya with Long Shelf Life and the Conventional Solo Papaya Confirmed by Biochemical Analyses

Cerrone S. Cabanos, Evelyn Mae Tecson-Mendoza,
Roberta N. Garcia, and Antonio C. Laurena
Institute of Plant Breeding, College of Agriculture
University of the Philippines Los Baños
Email: aclaurena@yahoo.com

Several transgenic lines of papaya containing the ACC synthase 2 or ACS 2 gene in the antisense orientation grown in the IPB BL2 greenhouse were observed to exhibit the delayed ripening trait. Unintended effects may result from the random and stable integration of the transgene and therefore, compositional analyses of the macro- and micronutrients, anti-nutritional and toxic factors were conducted to determine substantial equivalence between the genetically modified (GM), transgenic or biotech papaya and the conventional solo papaya.

The proximate composition, total soluble sugars (TSS) values, beta-carotene, ascorbic acid and benzyl isothiocyanate (BITC) content of the transgenic lines were found comparable and substantially equivalent to the nontransgenic solo papaya lines and proximate values reported for papaya cultivars. At 100% yellow stage, the proximate analyses of the fruit of the different lines and control papayas showed: 88.15 – 89.23 89.23% moisture, 0.602-0.709% protein, 0.797- 0.880% crude fiber, 0.119-0.131% fat, 0.512 – 0.566% ash, and 9.65 – 10.57% carbohydrate. β-carotene ranged from 500 – 769 ug 100 g^{-1} while ascorbic acid ranged from 41.6 – 80.35 mg 100 g^{-1}. Free BITC ranged from 0.7 – 1.7 ppm, while TSS ranged from10.1 – 14.2 °B.

Keywords: Philippine Solo, papaya, substantial equivalence, biochemical analyses

Genetic Variability in Nematode Resistance and Molecular Characterization of Genetic Introgression from *Oryza glaberrima* into *O. sativa*

I.K. Bimpong, A.L. Carpena, G. Reversat, L. Fernandez, D. D. Tambalo, D.S. Brar

EcoTILLING Candidate Genes for Drought Tolerance in Rice

HeHe Wang[1], Ma. Elizabeth Naredo[1], JianLi Wu[1], Bradley J. Till[2], Elizabeth A. Greene[2], Steven Henikoff[2], Luca Comai[3], Hei Leung[1], and Kenneth L. McNally[1]
[1] Genetic Resources Center and [2]Entomology and Plant Pathology Division, IRRI, DAPO Box 7777, Metro Manila 1301, the Philippines; [2]Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle WA 98109, USA; [3]Department of Biology, Box 355325University of Washington, Seattle WA 98195, USA

EcoTILLING of diverse germplasm allows the discovery of SNPs and the delineation of haplotypes at loci of interest (Comai *et al* 2004). This technique is based on TILLING

(Targeted Induced Local Lesions IN Genomes, Colbert *et al* 2001) and relies on the enzymatic cleavage of heteroduplex molecules formed between reference and query lines by an S1 type single-strand endonuclease from celery, CEL1. Dual-labeling of the PCR amplicons by different fluorescent tags allows the detection of the cleavage products on denaturing PAGE with automated genotyping. The appearance of new bands against the background products allows the detection of single nucleotide polymorphisms. Furthermore, the banding patterns across a range of diverse germplasm can be grouped into haplotypes. This combination of haplotyping and identifying SNP loci reduces the cost associated with SNP discovery so that only those germplasm carrying SNP differences need be sequenced in order to establish the identity of the nucleotide difference. As such, EcoTILLING is a powerful, inexpensive tool for the detection of natural variation.

Keywords: rice, candidate genes, EcoTILLING, TILLING

Biochemical Analyses of Cry1Ab Content in YieldGuard Maize Samples Through Indirect ELISA Assays

Jorge Gil C. Angeles[1], Marie-Sol Hidalgo[1], Maribel M. Zaporteza[1], Cleotilde Caldo[1], Antonio C. Laurena[1], and Arnold Estrada[2]

[1]Biochemistry Laboratory, Institute of Plant Breeding, College of Agriculture, University of the Philippines, College, Laguna and [2]Monsanto Philippines, Alabang, Muntinlupa, Metro Manila

Optimal conditions on the extraction and analyses through indirect ELISA of the Cry1Ab from YieldGuard maize samples were conducted. The use of phosphate-buffered saline (pH 7.4 to 8.0) buffer on leaves and carbonate buffer (pH 9.6) on roots and stem tissues extracted the greatest quantity of the Cry1Ab protein. Optimal antibody titer showed that a 1:4000 antibody against the Cry1Ab and a 1:2000 anti-rabbit IgG conjugated with alkaline phosphatase (Sigma) can detect a minimum concentration of 30 pg/mL of a standardized Cry1Ab solution. A 20-minute incubation with the *p*-nitrophenyl phosphate for alkaline phosphatase is sufficient to detect a 30 pg/mL solution of the standardized Cry1Ab. The average Cry1Ab protein content is 1.140 µg Cry1Ab per g fresh weight tissue (fwt) in leaves, 2.933 µg Cry1Ab per g fwt in stems, and 1.291 µg Cry1Ab per g fwt in roots. These data are lower than those published in literature.

Keywords: Cry1Ab, corn, ELISA, fresh weight tissue

Bioinformatics Analyses for Gene Discovery and Characterization Using Informax's VectorNTI Suite

Jorge Gil C. Angeles[1], Roberta N. Garcia[1], Amy Emiliana N. Bernardo[1], Tri Rini Nuringtyas[1,2], Ellen S. Regalado[1,3], Antonio C. Laurena[1], and Evelyn Mae Tecson-Mendoza[1]

[1]Biochemistry Laboratory, Institute of Plant Breeding, College of Agriculture, University of the Philippines, College, Laguna 4031 and [2]Current address: Laboratorium Biokimika, Universitas Gadjah Mada, Bulaksumur, Jogjakarta, 55281 Indonesia, and [3]Current

Address: Asian Maize Biotechnology Network, International Maize and Wheat Improvement Center, c/o International Rice Research Institute, Los Baños, Laguna 4031

InforMax's VectorNTI is an indispensable tool in the multifaceted bioinformatics analyses for gene discovery and characterization that are relevant to agricultural productivity and enhanced crop quality. It illustrates lucid graphical representations as well as descriptive molecular biological and biochemical information of database-mined and annotated nucleic acid and protein sequences. The software is also used to investigate phylogenetic relationships and homologies between sequences that are used for other molecular biology applications. Moreover, hydrophobicity plots of proteins may also be developed using the software suite.

Relevant works of past and current researches who use the application will be discussed. Its future utilizations will include the annotation and contig construction of cloned sequences of many economically-important crops such as coconut and abaca.

Keywords: bioinformatics, *in-silico* analyses, software, VectorNTI

Isolation and Partial Characterization of the Oleosin Gene from the Coconut (*Cocos nucifera* L.) Endosperm

Ellen S. Regalado, Jorge Gil C. Angeles, Antonio C. Laurena and Evelyn Mae Tecson-Mendoza*
Biochemistry Laboratory, Institute of Plant Breeding, College of Agriculture, University of the Philippines Los Baños, College, Laguna 4031
*Corresponding author: emtmendoza@nast.ph

Oleosins are a unique class of hydrophobic proteins found in the oil bodies of diverse organisms. In plants, they are most abundant in the lipid-storing bodies of seeds comprising up to 8% of the total seed protein. In this study, we report the isolation of coconut oleosin cDNAs and the partial characterization of their nucleotide sequences.

A gene-specific primer was designed based on the conserved sequence of known oleosin genes. Using this, two gene isoforms coding for oleosin were isolated from the total mRNA of a six month after pollination-old coconut using reverse transcription polymerase chain reaction (RT-PCR) with molecular sizes of approximately 500 (ole500+) and 300 (ole300) base pairs. These isoforms were ligated into the pGEMT® Easy Vector and maintained in *E. coli* DH5α cells.

Sequences of the six clones analyzed reveal that these are distinct and homologous to the published oleosin gene sequences. The homology of the five ole500+ sequences range between 60%-99.4% among each other and averages at 91%. These sequences show an average homology of 91% with the oil palm (*Elaeis guineensis*) oleosin OPZE1A gene (Accession No. AF273023.1). The sequence of the ole300 clone is 96% homologous to the rice (*Oryza sativa* L.) 16kDa isoform R16 gene (Accession No. AF022148.1). The deduced amino acid sequences of these cDNAs were found to contain the conserved oleosin domain when searched against known oleosin proteins.

By Southern Blot analyses, the ole500+ cDNA was found to have two copies in the coconut genome. These results indicate the isolation of oleosin cDNA sequences which could be present as multiple copies in the coconut genome.

The analysis of the sequences of the oleosin gene and its putative isoforms will provide the molecular basis for construction vectors that will carry important hydrophobic proteins and designer oils in future genetic engineering studies and in studies to isolate sequences regulating the oleosin gene expression.

The study was funded by the Department of Science and Technology – Philippine Council for Agriculture and Natural Resources and Development and the University of the Philippines Los Baños.

Keywords: coconut, oleosin, isoforms, RT-PCR

Establishing a New Cocon Preference Table for the Coconut (*Cocos nucifera* L.)

Jorge Gil C. Angeles[1], Marni E. Cueno[2], Ma. Genaleen Q. Diaz[2], Hazel L. Gaza[2], Antonio C. Laurena[1], Rita P. Laude[2] and Evelyn Mae Tecson-Mendoza[1]

[1]Institute of Plant Breeding, College of Agriculture; and, [2]Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, 4031 College, Laguna

e-mail: jgcangeles@gmail.com

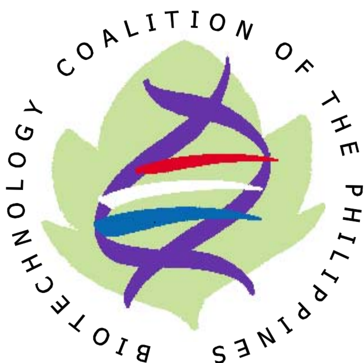
The triplet codes of 18 sequences derived from ten genes isolated from coconut (*Cocos nucifera* L.) were tallied. Relative frequence percentages of the individual codons encoding each amino acid were calculated. The highest percentage of the codon for each amino acid was chosen as that amino acid's most preferred codon. Comparative analysis to determine whether a change of codon preference and degeneracy was done between the published codon preference table (Nakamura *et al*, 2000) and from a new one generated from the sequence data of the coconut genes. A similar analysis was done for the sequence data of the genes derived from the normal and the makapuno phenotypes.

Analysis between the published codon preference table and the tabled derived from this study showed that 30% of the amino acids retained the existing published preference codon, 35% of the amino acids prefer a non-degenerate codon while 35% had a change of codon preference especially at the third nucleotide position of the codon. Moreover, arginine prefers a degenerate codon with an additional change at the first nucleotide position over the reported codon preference.

Analysis between the genes derived from the normal and makapuno phenotypes show that 70% of the amino acids utilize the same codon across the two phenotypes; 5% has a distinction with the normal coconut phenotype preferring a degenerate codon while its makapuno counterpart utilizes a non-degenerate codon; and, 25% of the amino acids had a change in codon preference between the two phenotypes.

This is a pioneering study in establishing the coconut codon preference table, a molecular biology tool much-needed by researchers in gene discovery and other applications in instances where molecular biology data is limiting

Keywords: codon preference, coconut, *Cocos nucifera* L.



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