

## 2007



Generation Challenge Programme

CULTIVATING PLANT DIVERSITY FOR THE RESOURCE-POOR



### **Generation Challenge Programme**

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### **Poster Abstracts**

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## **Theme 1: Exploiting allelic diversity**

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#### 1.1 Introgression of New Alleles

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Tolerance to drought is a complex phenotype that continues to challenge plant breeders. The quantitative nature and complexity of response to water-limited conditions have hindered breeding efforts in the past. Although several mechanisms have been described that can lead to improvements in drought tolerance, most of these tend to reduce productivity. As a result there have been considerable interest and energy expended on attempting to understand and improve the way in which plants sequester and use water efficiently. Discovery of the genes controlling these traits would greatly improve our understanding of drought tolerance and aid in the development of more tolerant varieties.

Fortunately in barley, there exists an enormous amount of natural diversity within the landrace and wild genepool for tolerance to both biotic and abiotic stress; many of these barleys being able to sustain an acceptable yield level under water-limited conditions. By tapping into this natural diversity and combining these with a range of analytical approaches, that dissect the complex drought tolerance phenotype into simple and measurable traits, we aim to identify chromosomal regions (and ultimately genes) underlying the major component trait loci in this material.

#### **1.2 Controlling and Monitoring Water Stress in** Contrasting Environment for Drought Tolerance Phenotyping of Cereals and Legumes

Reinaldo Lúcio Gomide\*, Paulo Emílio Pereira de Albuquerque, Camilo de Lelis Teixeira de Andrade, Cleber Morais Guimarães, Edson Alves Bastos, Walter Quadros Ribeiro Júnior, Luiz Balbino Morgado, João Herbert Moreira Viana

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Drought is considered the main source of grain yield instability for cereals and legumes in tropical regions among other environmental stresses. The drought process studies require knowledge of some target environment factors and how these factors interact with plants genotypes performance under water constraint condition. The objectives of this work are to describe the procedures and practices of controlling and monitoring water stress in contrasting environment for drought tolerance phenotyping of cereals and legumes to better understand the effects of plants genetic and environmental (GxE) interactions for grain yield, identifying and characterizing the causes which will result in genotypes yield reduction due to water shortage. Irrigation water needs are being determined through computation of reference (ETo) and crop (ETc) evapotranspiration, using modified Penman-Monteith equation and crop coefficient (kc). Each genotype ETc is being determined through multiplying ETo by Kc. Irrigation management strategy and timing criteria are being performed based on a spreadsheet water balance program, including ETo, ETc, and soil water content at different depths. The water stress treatments are being obtained with different ETc replacement, generating different water depth applications in the plots at pre-defined crop growth phases, defined for each genotype, in order to establish the water stress level.

#### 1.3 Diversity analysis of 3365 sorghums in the GCP composite germplasm set based on allelic variation at 41 SSR loci

C. Tom Hash\*, Claire Billot, Ramu Punna, Jean-Francois Rami, Laetitia Gardes, Rolf T. Folkertsma, Ronan Rivallan, Hari D. Upadhyaya, Monique Deu, Yu Li, Tianyu Wang, and Ping Lu \*ICRISAT

The GCP Global Composite Germplasm Collection of 3372 wild and cultivated sorghums includes 280 elite breeding lines and improved cultivars, 250 Chinese germplasm lines to be provided by CAAS, 64 wild accessions, and >3000 landrace accessions selected from previously defined core collections, for resistance/tolerance to production constraints, and/or for variation in other traits. A set of 48 sorghum SSR markers distributed across all ten linkage groups was chosen following preliminary analysis of 48 diverse genotypes with 104 available SSRs complemented by additional SSRs from CIRAD and ICRISAT. Diversity analysis was performed on 3367 accessions genotyped with 41 SSRs by CIRAD and ICRISAT.. Breeding lines and wild accessions clustered separately from landraces, which exhibited structure explainable by geographic origin. Landrace population substructure was further characterized within racial groups (five basic races and ten hybrid races). Race bicolor showed little evidence of population structure, congruent with it being the original domesticate. Race kafir (largely from Southern Africa) was distinct. Accessions of the durra, caudatum and guinea races each formed four distinct geographic subgroups. The guinea race margaritiferum group formed its own cluster, suggesting independent domestication. Intermediate races behaved similarly. A reference subset of 384 accessions was then defined for allele mining.

#### Notes:

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#### 1.4 Translating short-term environmental effects on leaf growth rate into differences in whole-plant profiles of maize leaf area

Karine Chenu\*, Scott C. Chapman, Graeme L. Hammer, Greg McLean, François Tardieu \*INRA, France

Leaf growth is one of the first processes affected by environmental changes. Physiological studies often concentrate on short-term mechanisms, which are a long way from whole-plant models designed to predict biomass accumulation, transpiration and yield in field conditions. We propose here a model to bridge this gap for maize leaf growth in response to temperature, evaporative demand (VPD) and water deficit (estimated with the predawn leaf water potential,  $\Psi$ ). The model was calibrated with data from field, growth chamber and greenhouse experiments. It combines (i) the estimation of key developmental stages for leaf growth, and (ii) environmental responses of leaf elongation rate that are applied to every leaf. Each leaf thus expands during a fixed period at a rate determined by the environmental factors. This model of leaf growth and development was interfaced with the crop model APSIM for simulation at canopy level, extending existing physiological knowledge of leaf elongation responses to environmental conditions. Most of the parameters are stable characteristics of a genotype and can potentially be related to QTL. This model could thus be applied to determine how the genetic controls of leaf responses translate into yield differences in various set of environmental conditions.



1.5 Characterization and Transferring of resistance genes to Rice yellow mottle virus (RYMV) from African	Notes:
cultivated rice (O. glaberrima) to O. sativa by	
molecular markers	
Deless THIEMELE <sup>*</sup> , Laurence ALBAR, Sophie PEREZ.	
Séverin AKE, Marie-Noëlle NDJIONDJOP, Yacouba SERE,	
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Cocody, Côte-d'Ivoire.	
The African cultivated rice species, Orvza glaberrima harbors useful	
traits for resistance/adaptation to several biotic and abiotic stresses	
which gives the opportunity to introgress those genes into O sativa	
genetic background and to develop marker-assisted selection. The	
project is focused on the resistance against Rice yellow mottle virus	
(RYMV), one of the main pathogen of rice in Africa and on the	
Rymv1 resistance gene conferring complete resistance to the different	
strains of the virus. Analysis of allelic diversity of this gene and	
marker-assisted selection for RYMV resistance represent a case	
study anticipating the same scenario when gene/QTLs for drought	
resistance will be available. In order to identify new sources of high	
resistance to RYMV, a collection of 300 representative accessions of	
O. glaberrima is presently screened at WARDA (Benin) by visual	
symptom scoring and by assessment of virus content (Elisa).	
Preliminary results reveal a higher frequency of resistance in this	
species. In parallel, sequencing of <i>Rymv</i> 1 gene in twelve confirmed	
resistant accessions showed at least 3 different alleles compared to O.	
sativa. Corresponding accessions are involved in on-going	
competitive and commissioned research GCP projects. Specific	
primers nave been designed to facilitate the selection of the different	
aneres in interspecific progenies.	

## *1.6 iBridges*: Interspecific bridges that give full access to the African rice allele pool for enhancing drought tolerance of Asian rice

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*iBridges* is a new GCP project with the objective to overcome the sterility barrier separating the two cultivated rice species and to enlarge the genetic diversity of Asian rice germplasm. The project is based on a partnership between LGDP (France), two CGIAR Centers (CIAT, WARDA), and five NARS (IER-Mali, INERA-Burkina Faso, Fedearroz-Colombia, PhilRice-Philippines, University of Punjab -India), to jointly develop *interspecific bridges* between selected O. glaberrima accessions and reference O. sativa recipient cultivars. A marker-assisted selection will be carried out on backcross progenies and will be focused on the  $S^1$  locus, which is the key-factor of the interspecific sterility. The fertility restoration will be monitored along three generations to derive fertile Backcross Inbred Lines (BILs) with improved crossability towards O. sativa. Second, the genetic material will be scored for its O. glaberrima genome content in using a novel marker technology revealing Single feature Polymorphisms (SFPs). Last, fine genetic and physical maps of the  $S^{1}$ locus will be done to bring it to positional cloning using the OMAP molecular resources (O. glaberrima BAC libraries). The resulting material will be suitable for new evaluations and genetic studies to identify original genes/QTL with an accent on drought resistance and better water use efficiency.

## 1.7 Mining allelic diversity associated with drought and salinity tolerance in the reference subset of chickpea germplasm collections

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Drought, salinity, and extreme variations in temperature are the major abiotic constraints to chickpea production worldwide. With support from GCP, we developed a global composite collection (3,000 accessions), profiled its structure and diversity using SSRs (50), and a reference set of 300 accessions (211 chickpea mini core + 89 additional diverse accessions) identified using DARwin-5.0. This reference set captured 78% (1403 alleles) of the 1791 alleles of the composite collection. Currently, we are saturating this reference set with additional SSRs and have plans to survey this reference set using DArT markers once the DArT technology is established at ICRISAT. Techniques for screening for drought (root traits) and salinity tolerance have been standardized. Published studies revealed genetic variability for traits associated with drought and salinity tolerance among chickpea mini core germplasm accessions. We plan to extensively evaluate this reference set (300 accessions) for drought and salinity tolerance besides agronomic and quality traits and associate this variation with allelic diversity present in the reference subset. The genetically diverse accessions with contrasting response to drought and salinity will be identified for diverse uses in chickpea genomics and breeding.



### **1.8 Large variation in gravimetric TE exists in wild and cultivated groundnut germplasm**

#### Notes:

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Transpiration efficiency is considered as one trait that can contribute to achieving higher yields under conditions of limited water, and variations for TE exist in groundnut. We have measured TE gravimetrically in 440 genotypes, encompassing the mini-core collection of groundnut, and have found a 4-fold range of variation for TE (0.62-2.75 g biomass  $kg^{-1}$  water transpired). The relation between the TE values measured under well-watered and water stress conditions was significant, but the correlation coefficient was weak (r=0.23). The surrogate traits for TE, SLA and SCMR, did not show any significant relation with TE, regardless of water regime. By contrast, we found that the rate of water loss per unit of leaf area was inversely related to TE. We also measured TE under well-watered conditions in 21 accessions of peanut wild relative and 10 accessions of cultivated groundnut. TE varied 1.99-4.93 g.kg<sup>-1</sup> in the wild and 2.27-3.50 g.kg<sup>-1</sup> in the cultivated. On average, TE was higher in the wild peanut than the cultivated. We found that the rate of water loss per unit of leaf area was lower in the wild peanut, and was well and negatively related to TE (r = 0.56).

## 1.9 Completing genotyping of composite germplasm set of barley

Wafaa Choumane, Jan Valkoun, Stefania Grando and Michael Baum

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ICARDA was assigned the responsibility for developing the composite set of 3000 barley accessions, representing the range of diversity of the crop and its wild progenitor, Hordeum vulgare ssp. spontaneum. The objectives of this study were to assembe a composite collection in barley, characterising the collection using EST-derived and genomic SSR marker, then extract a core collection harbouring about 70% of the genetic diversity of the composite collection. The barley composite set consists of 2692 accessions collected from 85 countries and composed of 445 accessions of H. vulgare ssp. spontaneum, 1935 accessions of landraces and 612 genotypes from improved germplasm. The analysis with 15 SSR markers allowed the detection of a total of 310 alleles with the number of alleles varying between loci and between accessions. The analysis of results with structure 2.1 allowed the detection of structured genetic variability. A reference sample set was developed as a core collection holding more than 70% of the genetic diversity existing in the whole collection. The set will be used to establish a GCP resource to quickly capture the value of results obtained in the most advanced genetic studies on candidate genes for drought tolerance, for crop improvement of our target species.

#### 1.10 Allele Mining Based On Non-Coding Regulatory SNPs in barley germplasm

von Korff M., RadovicS, Choumane W, Stamati K, Udupa SM, Grando S, Ceccarelli S, Langridge P, Morgante M, Powell W, BaumM International Center for Agricultural Research in the Dry Areas, ICARDA, SYRIA

Mutations in cis-regulatory DNA sequences, which affect gene expression levels, have been proposed to play a central role in gene expression cascades under abiotic stress. We tested allele specific expression of 30 genes putatively involved in drought tolerance in F1-hybrids of barley (5 crosses, F1, RF1) subjected to drought stress and measured at two different developmental stages. The analysis demonstrated that 57% of the genes showed allelic differences in expression of up to 9-fold due to cis-regulatory variation in at least one cross by treatment/stage combination. Drought stress induced in seven gene/cross combinations significant imbalance in expression ratios indicating differences in the linked drought responsive ciselements. In addition, seven gene/cross combinations showed differences in allelic ratios between the two developmental stages. Our findings suggest that the *cis*-regulatory variation is a common phenomenon in barley and may provide a molecular basis of transgression. We could also demonstrate that the occurrence of allelic imbalance and hence differences in cis-regulatory elements is closely correlated with the genetic divergence between the parents of the crosses. In addition, cis-regulatory variation plays an important role in governing expression under drought stress and in different developmental stages. Identification and targeted manipulation of cis-regulatory elements will help breeding for improved crops with a better adaptation to changing environments.



## 1.11 Progress in orthologous candidate gene diversity assessment

Dominique This\*,Brigitte Courtois, Claire Billot, Romain Philippe, Jean François Rami, Merideth Bonierbale, Reinhart Simon, Roland Schaftleitner, Dave Hoisington, Rajeev Varshney, Spurthi Nayak, Jayashree B, Dominique Brunel, Pierre Mournet, Ken McNally, Michael Baum, Wafaa Choumane, Tom Hash, Matthew Blair, Hari Upadhyaya, Martin Fregene \* Montpellier SupAgro, Agropolis, France

The ADOC project aims to characterize allelic diversity at orthologous loci of candidate genes for drought tolerance in seven GCP crops (rice, sorghum, barley, potato, cassava, bean and chickpea), working on reference collections of around 300 accessions for each crop. Six gene families (ERECTA, DREB, SS, SPS, ASR and INV) were selected, during an initial scientific exchange with gene specialists and advisers, as the initial subset of target genes. Consensus and specific primers amplifying those sequences were defined, in order to complete our information on those gene families and facilitate the allelic sequence project. Crop partners have fulfilled the selection of the reference collection, extracted DNAs and made them available for allelic sequencing. Those collections have been screened for anonymous markers and field performance in other programs and should be made available for collaborative phenotyping projects and additional genotyping. Ortho-labs have improved their knowledge of the gene families and design specific primers for some candidate genes. The comparison of the first sequences obtained to date provides some interesting features that will be presented in this poster.

#### 1.12 Molecular characterization of maize landraces and various subspecies of teosinte from Mexico and Central America

Dreisigacker, S\*., Taba, S., Bedoya, C., Franco, J., Charcosset, A., Mir, C., Zhang, S., Xie, C., Prasanna, B.M., Hearne, S., Muthamia, Z., Yunus, M., Jampatong, C., Cuong, B.M., and Warburton, M.L. \*CIMMYT, Mexico

Teosinte (Zea sp.) are the closest wild related species to maize, (Zea mays mays) and contains the progenitor of domesticated maize, (Zea mays ssp. parviglumus). This study was undertaken to compare the diversity of SSR markers in 20 teosinte subspecies with the diversity of 10 maize landraces from the same countries and 10 maize landraces from geographical regions who have been separated from the center of origin of maize for several centuries. The teosinte relationships shown in this study agreed with previously published results based on markers, morphology, and geography, with the addition of some subspecies not previously studied. The diversity within the cultivated maize landraces is extensive, even compared to the diversity in the wild species. Of a total of 386 alleles identified in the data set, 138 (36%) were unique to the teosinte species, and 38 (1%) were unique to the maize. Of these, 33 alleles were present only in the Asian maize, and only 5 were present in both Asian and Latin American maize landraces. The search for unique alleles in maize populations that are sympatric to teosinte accessions indicate the probability of diversity in maize being influenced by gene flow directly from wild maize subspecies.



#### 1.13 Exploiting genetic diversity among unadapted wheat landraces from different geographical origins revealed by SSRs

Dreisigacker, S\*., Zaharieva, M., Balfourier, F., Miloudi, N., Zhang, X., Warburton, M. \*CIMMYT, Mexico

Over several thousand years, landraces of hexaploid wheat have developed under a variety of different edaphic and climatic environments. This has led to the evolution of a large number of ecotypes adapted to specific local environments, an important source of genetic variation in wheat. Landraces have been characterized phenotypically and found to contain good levels of resistance to pests such as stem rust, leaf rust, or Russian wheat aphid and for tolerance to abiotic stresses like heat, and these have been used in the CIMMYT wheat improvement program. At the molecular level, considerable diversity indicates new variation that may be tapped for continued wheat improvement in the future. Molecular diversity can be used to determine relationships between accessions, and to select diverse core subsets for further phenotypic characterization. In this study, five hundred hexaploid wheat landraces from over 30 countries were analyzed with 35 SSRs. Substantial levels of genetic variation was found in the selected materials. It was clearly demonstrated that genetic differentiation was closely correlated with geographical origin. The accessions were combined into groups of European, Asian or American origin. Differences between clusters of accessions were mainly defined by differences in the frequency of the SSR defined alleles.

#### 1.14 Improvement of Documentation of Wheat Relatives for More Efficient Breeding

Zaharieva M\*., Dreisigacker S., Hernandez E., Rodriguez F., Payne T., Warburton M. \*Cimmyt, Mexico

In order to breed the crop plants required for the future, the efficient use of genetic resources is essential. During the last decades thousands of Triticum and Aegilops accessions have been collected and stored in various genetic resources centers. An accurate documentation of this conserved germplasm is critical for its effective management and utilization. In the present study, morphological and genomic descriptive information was collected from 150 accessions of different wheat relatives held in the CIMMYT Gene Bank, and part of the wheat composite collection of Generation Challenge Programme. These accessions were assembled in an herbarium of dried plant specimens and in a virtual (photographic) herbarium, providing support for taxonomic documentation. All this information, along with passport data, guarantees a quality control from the genebank accession to the genotyping data. It can also be used to develop a well-documented set of wheat relatives to be further phenotyped, enabling plant breeders to make decisions regarding the material to be used in wheat improvement programmes.

## 1.15 Preparing IITA-cassava reference germplasm for distribution and association mapping

*Odu B.O\*., Ferguson M. and Dumet D.J. \*International Institute of Tropical Agriculture (IITA)* 

This project aims at producing virus-free seedlings of 198 selected cassava accessions in order to facilitate their distribution to GCP partners located outside Nigeria (IITA, CIAT and EMBRAPA). Standard approach for virus-free seedling production of cassava involves thermo-treatment of cuttings and meristem tip culture. 10 meristems (i.e. 10 lines) of each accession were excised from thermotreated cuttings and introduced/multiplied in vitro. They were then indexed for African cassava mosaic virus genus Bigeminivirus, East African cassava mosaic virus, and other possible geminiviruses infecting cassava. Indexing was performed on both, in vitro seedlings and fully developed plants after acclimatization in screen house. Out of the 198 accessions selected, 166 were successfully introduced in vitro i.e. produced well established culture, 18 are still at various stages of in vitro development and 14 did not respond to standard meristem culture. For the latter, *in vitro* culture conditions need to be adjusted. Out of the 166 accessions producing fully developed seedlings, 153 were virus free at in vitro seedling stage. This result was confirmed for 134 after indexing on full plant. These are awaiting certification by the Nigeria Plant Quarantine Service while new lines are presently tested for the non-virus free accessions.

## 1.16 Exploring genetic diversity in yam (*Dioscorea* spp) core collection.

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The core sub-set of yam germplasm from IITA GeneBank collection, which contains 3200 accessions, was made up of a total of 342 accessions representing six economically important yam species (D. alata, D. bulbifera, D. cavenensis, D. dumentorum, D. esculenta and D. rotundata) with diverse geographical origin in West and Central Africa and was evaluated for inter- and intra- specific variability using microsatellite markers. The accessions were analyzed using set of 23 SSR markers that showed polymorphism across species. Pairwise distance matrices using Jaccard coefficients resulted to similarities varying from 58% to 93%. Principal component analysis showed distinct separation between D. rotundata and D.alata with some overlapping genetic divergence between other species. A close genetic association was observed between D. rotundata and D. cavenesis. Genetic diversity as revealed by tree construction of the molecular marker data using unweighted neighbour-joining method showed large number of inter- and intra- specific polymorphisms that enabled us to reliably discriminate between the investigated core set. These genetically diverse accessions with various potentials will be valuable source for breeders worldwide to exploit diverse traits towards the development of hybrid yams.



## 1.17 Yield response to pre-flowering drought stress in a population of Chromosome Segment Substitution Lines (CSSLs) of rice

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Bocco R, Cissoko M,

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Water stress has been identified as one of the most important rice production constraints in West African environments. It severely reduces rice vields in rice ecologies. Introducing new allelic combinations in the genetic backgrounds of high vielding varieties will broaden their genetic base and improve stress tolerance. A population of CSSLs comprising 93 lines developed from the cross of Caiapo and O. glaberrima (IRGC103544) was screened for drought tolerance under upland conditions in Cotonou, Benin during the dry season of 2006/2007. The drought stress treatments lasted 21 days starting at 45 days after sowing (DAS) and comprised of a moderate stress treatment imposed by irrigating every third day during the 21 day stress period and the second stress treatment included continuous drought stress throughout the 21 day stress period. The third treatment was a control which was irrigated from sowing till maturity. Mean yields under continuous irrigation (8.56 g/plant) were significantly different from that under moderate stress (7.55 g/plant) as well as under severe stress (7.70 g/plant). Transgressive segregants that out-yielded Caiapo, the high-yielding parent, were detected under both continuous irrigation and drought stress. This shows the positive contribution of O. glaberrima alleles in enhancing grain yield of rice under both stress and non-stress conditions.

1.18 Supporting distribution of reference germplasm

(Musa)

Roux N.; Hippolyte I.; Jenny C.; Dolezel J. and Rouard M. \*Bioversity International

A DNA collection currently comprising 52 Musa accessions was assembled using the GCP criteria: genetic diversity representation; duplication at Bioversity's International Transit Centre (ITC): use in breeding programmes; and part of an 'in-trust' collection. For each of the accessions, ten extractions, each of three grams of frozen leaves, were made with the Matab method. About 1 miligram of DNA was obtained per accession. The DNA samples are stored and distributed in PCR grade water. The DNA samples are distributed upon request to the Musa research community via the Musa Genome Resource Centre (MGRC) based at the Institute of Experimental Botany (IEB), Olomouc, Czech Republic. The MGRC operates at a cost-recovery basis and the recipients are expected to cover packing and shipment costs. In order to maximize the number of researchers benefiting from this service, a maximum of 1 µg of DNA per accession is provided and only after signing an agreement. Information on each accession of the reference list, the 22 SSR markers enabling distinction between the 52 reference accessions, and the protocol for DNA amplification and detection can be found online in the Global Musa Genomics Consortium website: www.musagenomics.org

Poster Abstracts, GCP Annual Research Meeting 2007

#### 1.19 Genotyping of Composite Germplasm Set, Tier 1,

Musa

*Roux N.; Hippolyte I.; Kolesnikova M.; Perrier X. and Rouard M.* 

\*Bioversity International

The aim of this project was to genotype the global diversity of Musa held in three important in vivo Musa collections (CIRAD, Guadeloupe; IITA Nigeria and CARBAP, Cameroun). DNA samples were extracted from 549 accessions representing wild Musa acuminata colla (A genome 2n=2x=22) and Musa balbisiana colla (B genome 2n=2x=22) accessions coming from different geographic areas, AB interspecific cultivars, triploid mono or interspecific cultivars (AAA, AAB, ABB) and a few tetraploids. In order to have a broad view of the Musa complex, some species such as Musa basjoo and Musa schizocarpa were added to this study. Two phylogenic trees were built, one by CIRAD scientists using 22 SSR markers and the other by IITA scientists using 20 SSR markers. The analyses took into account the difference in ploidy levels of the genotypes. These trees not only confirm previous results obtained with molecular markers and fit with the known botanical classification, but they also have uncovered genetic relationships between triploids and their putative diploid ancestors. Another important output from this analysis is its possible use by the Musa research community, more specifically by Musa breeders.

#### 1.20 HaplOryza – SNP Analysis of the Genetic Diversity along the Rice Genome

Claire Billot\*, Ken McNally, Brigitte Courtois, Gaetan Droc, Amr Abdelkalik Farouk, Guy Clément, Nour Ahmadi, Dominique Brunel, Aurélie Bérard, Mark Lathrop, Gérard Second \*CIRAD

Asian cultivated rice occurs as two major types, indica and japonica that appear to have arisen from two independent domestication events from wild relatives that have diverged for more than two million years. Even though rice is a predominantly self-pollinated crop, both types can frequently be found within the same region allowing the prospect for genetic exchange between them. Particular situations with profuse gene exchange between the two types enable genetic analysis of those traits that differentiate the two types. Based on the analysis of both complete sequences of the rice genome, a set of 1536 SNPs used for an Illumina cheap has been derived. 357 Mb of the genome is covered by 1 SNP per 320 kb and 10 specific regions are covered by 1 SNP per 50 kb. They include 8 regions carrying candidate genes for drought tolerance (about 700 kb each, 5.9 Mb), the short arm of Chr. 6 (15.4 Mb), and two regions of low SNP density on Chr. 7 and 2 regions of normal density on Chr. 12 (~ 4 Mb). 550 rice accessions (out of 900) have just been genotyped. They include a core collection (MiniGB) previously analysed with SSR markers, highland rices from Madagascar and some wild samples. Preliminary data concerning linkage disequilibrium are presented



### 1.21 Race structure and relationships among ecotypes in cultivated common bean (Phaseolus vulgaris L.)

Matthew Blair\*, Hector Fabio Buendia, Lucy Diaz, Juan Manuel Diaz, Myriam Cristina Duque, Steve Kresovich, Sharon Mitchell, Maria Jose Peloso, Rosana Brondani, Xiaoyan Zhang, Shumin Wang, Teresa Avila, Ximena Rojas, Andrea Davila, Sandra Lorigado

\* CIAT, Colombia

Cultivated common bean germplasm is especially diverse due to the existence of two genepools in the Mesoamerican and Andean centers The two genepools can be morphologically of diversity. distinguished into various races, however the association of these phenotypic divisions with genetic structure has not been clear. In the GCP genotyping project for common bean we addressed this through a thorough analysis of international and national germplasm collections representing wide genetic variability from both primary and secondary centers of diversity using genomic and genic microsatellites. We found the division between the Mesoamerica race and the Durango-Jalisco group to be very evident in the Mesoamerican genepool while the Andean genepool showed somewhat less diversity overall and a continuum between the Nueva Granada and Peru races. The Chile race could not be distinguished within the Andean genepool but there was some support for a Guatemala race within the Mesoamerican genepool. Introgression between the genepools was evident as was probable introgression between cultivated and wild common beans. In conclusion, this study has shown that common bean has very significant populations structure that could help guide the construction of genetic crosses that maximize diversity as well as serving as a basis for future association studies.



## 1.22 Targeted Genotyping of *Oryza* Germplasm by Agarose-based EcoTILLING

Ma. Elizabeth B. Naredo\*, Michael B. Gamalinda, Genelou A. Atienza, Reneeliza Jean A. Melgar, Millicent D. Sanciangco, Chitra Raghavan, Hehe Wang and Kenneth L. McNally

\*T.T. Chang Genetic Resources Center, IRRI, Philippines

To discover novel alleles and establish genetic variation in rice germplasm, targeted genotyping was accomplished by Ecotilling. At IRRI, agarose-based ecotilling was developed to simplify the TILLING procedure, alleviating the need for labeled markers. Candidate genes were selected through convergent evidence, and primers were designed for both upstream regulatory and coding regions using the Nipponbare genomic sequence. Primers for the following genes, DREB2 (dehydration-responsive element binding protein), ERF3 (ethylene-responsive element binding protein), 14-3-3 (membrane associated signal cascade), MAPK (protein kinase domain containing protein), Ext (Extensin), SucSase (sucrose synthase), BZIP (ABA responsive element binding factor), TPP (trehalose-6-phosphatase) and ADF (actin depolymerzing factor) were tested on 1536 accessions of O sativa, 145 accessions of O. glaberrima, and 95 accessions AA genome wild Oryza species. Nipponbare (japonica) and IR 64 (indica) were used as contrasts against O. sativa and AA genome species; O. glaberrima was contrasted against NB and the O. glaberrima accession, IRGC 96717. Mismatches in the form of SNPs or indels were detected for all genes. Sequenced samples representing mismatch patterns largely agreed with patterns detected on agarose. For the wild Oryza accessions, EcoTILLING also proved to be a useful tool to differentiate among the non-Asian AA genome species.

#### 1.23 OryzaSNP – Genome-wide SNP discovery in Diverse Rice

K.L. McNally\*, K.L. Childs, V. Ulat, R. Clark, R. Bohnert, G. Zeller, G. Rätsch, D. Weigel, D. Hoen, T. Bureau, R. Stokowski, D. Ballinger, K. Frazer, D. Cox,, R. Bruskiewich, D. Mackill), C.R. Buell, R. Davidson,

J. Leach, and H. Leung \*IRRI, the Philippines

The OryzaSNP project (http://www.oryzasnp.org) has undertaken genome-wide SNP discovery across the unique fraction of the Nipponbare rice genome for 20 diverse varieties. We chose 100 Mb of the rice genome (IRGSP release 4) corresponding to the fraction with little or no repetitiveness for SNP discovery. The diverse varieties included representatives from all variety groups with Nipponbare as a control. SNPs were identified by array-based resequencing technology using very high-density oligomer arrays at Perlegen. Perlegen's model-based algorithms predicted 259,721 non-redundant SNPs - sites in Nipponbare where one or more of the other 19 varieties differed. This translates into approximately 2.6 SNPs per kb consistent with values from pairwise comparisons of indica and japonica rice varieties. If linkage disequilibrium extends to 50 kb, the SNP set is sufficient to cover 73-88% of the genome. Efforts are in progress to verify data quality and assess the recall and false discovery rates. We also attempt to complement Perlegen's model-based calls with additional predictions of SNPs and highly polymorphic regions using the machine learning algorithms developed for the Arabidopsis SNP discovery project. Annotation relative to the Rice Annotation Project release 2 and TIGR release 5 gene models has been accomplished, and the OryzaSNP annotation database (release 1) will be opened to the public on October 16, 2007.



#### **1.24 Population Structure of the Rice Composite Set** Notes: K.L. McNallv\*, R. Manzano, M. Macatangav, M. Redondo, V. Lacorte, M. Zaidem, J. Detras, M. Barile, R. Angeles, S. Quilloy, M. Gamalinda, M. Sancinagco, D. Pabale, N.R. Sackville Hamilton, L. Benoit, R. Rivallan, B. Courtois, C. Billot, A. Garavito, M. Lorieux, C.P. Martinez, T. Borba, R.V. Brondani, C. Brondani, M. Cissoko, M.-N. Ndjiondjop, A. Famoso. S.R. McCouch \*T.T. Chang Genetic Resources Center, IRRI, Philippines The population structure of the composite rice collection of 2757 accessions was investigated using genotypic data for 48 out of a panel of 50 SSR markers. The current dataset (version 2) has undergone revision and quality assessment through re-scoring. Statistics have been computed using PowerMarker version 3.25 with average major allele frequency of 0.34, average gene diversity of 0.78, and average PIC value of 0.75. Clustering was accomplished using DARwin5 with the simple matching coefficient for distances followed by unweighted Neighbor Joining. The groupings observed by cluster analysis are analogous to those obtained for sets of sativa and glaberrima at Cornell. Model-based inference of population structure has been accomplished using Structure v2.1 allowing for admixture and correlated allele frequencies. To account for the inbred nature of rice, InStruct (version 1) analyses have been accomplished. Whereas the Evanno approach on Structure models indicated that the best K value for the O. sativa population was K =11, the Deviance Information Criteria from InStruct indicated that the best K-value is K = 5. InStruct models result in groupings that are in better agreement to the known variety structure of O. sativa. Additionally, representative samples have been selected using the structural disequilibrium feature of DARwin5 and PowerCore software.

## 1.25 Development of allele size standards for potato genotyping

Jorge Núñez\*, José Pignataro, Ma. del Rosario Herrera, and Marc Ghislain \*CIP, Peru

A new Potato Genetic Identity (PGI) kit of 24 markers was developed from the large genotyping data deposited in the GCP SSR marker data repository. In order to identify precisely each SSR allele across gels, labs and possibly platforms, we developed allele size standards for each allele of the 24 SSR markers. We used SSR genotyping data of the database and accessions of the DNA bank from CIP to select genotypes displaying complementary alleles to develop molecular weight ladders. We selected these genotypes based on the following criteria: good separation among the alleles (>3bp), inclusion of high frequency alleles, and, the presence of the minimum and maximum size of the range of alleles when possible. The number of alleles of the ladder ranged from 3 (STM5121) to 9 (STI0012). Genotype combination made for each SSR marker ranged from one genotype (such as for STM5121) to four genotypes (such as for STM0019). A list of genotypes used to develop each ladder and a list of the 24 allele size standards (ladders) with their respective annealing temperature are showed. These SSR allele size standards and the new PGI kit are been used to genotype the whole CIP germplasm.

# Theme 2: Genomic resources and gene/pathway discovery

#### 2.1 GCP Informatics Platform Support for Crop Research

Richard M. Bruskiewich\*, Mylah Anacleto, Supat Thongjuea, Ramil Mauleon, Trushar Shah, Samart Wanchana, Mathieu Rouard, Marcos Costa, Victor Jun Ulat, Arllet Portugal, Ryan Carlo Alamban, Roque Almodiel, Lord Hendrix Barboza, Kevin Manansala, Michael Jonathan Mendoza, Jeffrey Morales, Barry Peralta, Rowena Valerio, Michael Echavez, Sergio Gregorio, Joseph Hermocilla, Jan Michael Yap, Yi Zhang, Matthieu Conte, Alexis Dereeper, Kouji Satoh, Koji Doi, Shoshi Kikuchi, Manuel Ruiz, Reinhard Simon, Edwin Rojas, Masaru Takeya, Guy Davenport, Martin Senger, Thomas Metz, Graham McLaren and Theo van Hintum.

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- Third-party tools like the MAXD microarray system, the TIGR Multiple Experiment Viewer, Cytoscape network visualization, MAPMAN pathway visualization, the ATV phylogenomic tree visualization, the JalView multiple sequence alignment, and the Apollo genome browser, customized to match GCP needs and integrated into the GCP platform through GCP scientific domain model and data source integration standards.
- GCP-partner specific tools, like the EMBRAPA "Genoma" workbench, converted to communicate with GCP platform data sources.
- GCP-specific standalone and web query tools that directly manage GCP domain model structured genomic data.
- Access to High Performance Computing (HPC) resources, via the above tools.

## 2.2 The development of a novel data mining tool to find *cis*-elements in rice gene promoter regions

Koji Do\*i, Aeni Hosaka, Toshifumi Nagata, Kouji Satoh, Kohji Suzuki, Ramil Mauleon, Michael Jonathan Mendoza, Richard Bruskiewich, and Shoshi Kikuchi \*NIAS, Japan

We have developed a novel tool to search cis-element candidates from the upstream regions of differentially regulated genes, and opened it publicly through http://hpc.irri.cgiar.org/tool/nias/ces. The tool first lists up conserved motifs as *cis*-element candidates from upstream sequences of the given gene set. Then the likelihood of these motifs are evaluated by association rule analysis, a popular data mining method to discover significant relationships. Candidates showing the highest likelihood (specificity) are retained in the final cis-element candidate list. We have recently improved the developed tool mainly in two points. First, down stream and coding regions have become covered, as well as upstream region. As quite limited information has been accumulated in these regions, this improvement should be valuable for comprehensive understanding of gene expression regulatory mechanism. Secondly, multiple alternative reference gene sets have been prepared, and users can now select the most appropriate one for their focus. Reference data sets are essential to evaluate likeliness of cis-element candidates. Focused references should enable precise evaluation of cis-element with particular roles. Refinement of the reference data sets and new findings using the tool are issues in next step.
# 2.3 Analysis of the repetitive sequences in the component genomes of peanut using BAC end sequences and *insitu* hybridization.

PM Guimarães\*, K Proite, O Garsmeur, G Seijo, W Martins, S Nielen, C Foo, CD Town, A D'Hont, SCM Leal-Bertioli, D Bertioli \*Embrapa Genetic Resources and Biotechnology, Brazil

Cultivated peanut, Arachis hypogaea is an allotetraploid with an AABB genome. It is of recent, and monophyletic origin. Analysis of data from molecular markers, cytogenetics, morphology and geographical distributions support that A. duranensis and A. ipaënsis are the direct ancestors of cultivated peanut. Recently we constructed two BAC libraries, one for each of the diploid species. In order to begin to understand the component genomes of allotetraploid cultivated peanut genome we have used a number of approaches. Here we report the study the component genomes at the sequence level using in-situ hybridization and large scale endsequencing of randomly selected BAC clones from the diploid libraries. The 7,581BAC end-sequences representing 5,545,270 bp were searched against several databases and significant homology was found to mitochondria and chloroplast (2.0%), transposons and repetitive sequences (81,5%) and proteins (1,2%). A comparison of the datasets also shows that the component genomes are substantially different in respect to their repeated sequences/transposable elements content. In contrast, the existing available evidence indicates that the gene order in the AA and BB genomes is substantially conserved. We conclude that the component AA and BB genomes of peanut harbour largely syntenic gene frameworks embedded within guickly evolving repetitive DNA.

#### 2.4 Task 31 (SP4) -Comparative Stress Gene Catalog and Ortholog Function Display Tool Development

Samart Wanchana, Supat Thongjuea, Victor Jun Ulat, Mylah Anacleto, Ramil Mauleon, Barry Peralta, Lord Hendrix Barboza, Mathieu Conte, Manuel Ruiz, Mathieu Rouard, Kimmen Sjölander, Nandini Krishnamurthy, Balaji Jayashree, Masaru Takeya, Marcos Costa, Shoshi Kikuchi, Hei Leung, Theo van Hintum, and Richard Bruskiewich

\*Crop Research Informatics Laboratory, IRRI

Comparative biology provides valuable insights into the function and evolution of organisms, highlighting the divergence and conservation of gene families and biological processes. To facilitate cross-species gene functional analysis, the Generation Challenge Programme commissioned a project to assemble tools for the compilation and visualization of comparative information about stress-responsive genes. An online resource documenting stressresponsive genes comparatively across plant species has been developed. This public resource is a compendium of protein families, phylogenetic trees, multiple sequence alignments, and associated experimental evidence. The central objective of this resource is to elucidate orthologous and paralogous relationships between plant genes that may be involved in response to environmental stress, mainly abiotic stresses such as water deficit ("drought"). The web-based graphical user interface (GUI) of the resource includes query and visualization tools that allow diverse searches and browsing of the underlying project database. The web interface can be accessed at http://dayhoff.generationcp.org.



#### 2.5 Deciphering the genetic and environmental interaction of leaf expansion under drought in rice (Oryza sativa L.) J.E. Cairns\*, Impa S., D.C. Liu R. Mabesa G. Dimaguya

and R. Serraj

\*IRRI, the Philippines

Leaf expansion is one of the most sensitive processes to water deficits, and an important parameter in determining plant ability to intercept light and convert it into biomass. We are using a combined approach to dissect the underlying physiological and genetic mechanisms controlling expansion. The overall aim is to identify QTLs/candidate genes controlling tissue regulation, develop models to predict how allelic combinations impact growth under different drought scenarios, and, ultimately, develop new selection criteria for breeding. Leaf expansion and morphogenesis under vegetative drought stress was investigated in field experiments using two backcross populations (Vandana/Moroberekan). OTL for leaf elongation, area, biomass partitioning and accumulation under water-limited and control conditions were identified. Detailed analysis of leaf elongation and emergence under progressive soil drying (using the Fraction of Transpirable Soil Water, FTSW, as a covariable) was investigated in contrasting genotypes. Leaf elongation rate per unit thermal time was also determined under VPD and temperature regimes at different levels of soil moisture. Expression profiles of expansins and genes related to drought response were measured, at three FTSW levels and simultaneously in the well-watered controls. These data will be used for analysing the genotype x environment interactions on tissue growth regulation under water-limited conditions.

#### 2.6 The salicylic acid signalling pathway confers tolerance to a biotrophic rust pathogen in pearl millet

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Studies were undertaken to assess induction of defence response pathways in the pearl millet, in response to infection with the leaf rust fungus Puccinia substriata. Pathology studies indicated that pretreatment of pearl millet with salicylic acid (SA) conferred resistance to a virulent isolate of the rust fungus, whereas methyl jasmonate (MeJA) did not significantly reduce infection levels. These results suggest that the salicylic acid defence pathway is induced in response to rust infection. Microarray gene expression profiling was performed in order to contrast MeJA and SA responses in pearl millet, and identify transcripts that are uniquely expressed in response to SA treatment. Gene expression analysis revealed substantial overlap in gene expression responses between treatments, with MeJA and SA treatments exhibiting 75 co-induced transcripts. However there were 108 transcripts that were differentially expressed in response to SA treatment, but not in response to MeJA treatment. Sequence analysis indicated that these SA responsive transcripts included genes involved in SA biosynthesis, defence response, signal transduction, cellular detoxification in response to pathogens/oxidative burst, protein synthesis and photosynthesis, as well as transcripts with no significant homology to sequences in the Studies are currently underway to knockout the GenBank. expression of a selection of SA induced genes in pearl millet in order to characterise their role in conferring tolerance to rust.

## 2.7 DREB1A promotes root growth and enhances water uptake under water stress in groundnut

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Previously, we have shown that several transgenics groundnut events carrying single inserts of the At rd29::DREB1A genes had an enhanced transpiration efficiency (TE) compared to wild type (WT) parent JL24. Here we tested whether DREB1A could improve water capture under water deficit, and enhance drought tolerance during reproduction. We measured water uptake and roots using a lysimetric system where plants were exposed to well-watered and waterstressed conditions. The transgenics developed more profuse rooting (circa 30%) in all soil layers than the WT JL24 under water stress, whereas rooting was similar in all genotypes under well-watered conditions. Similarly, water uptake in the transgenics was 16-30% higher than in WT JL24, whereas it was similar under well-watered conditions. We measured the pod number in plants exposed to a drought spell at various stages during reproduction, and then rewatered until maturity, in comparison to plants kept well-watered throughout. Stress imposition during mid-pod filling had little consequence on the final pod number. By contrast, stress exposure at flowering had a severe effect on the relative pod number, which was higher in the transgenic event RD2 (82%) than in WT JL24 (63%), and similar to drought tolerant genotype ICGV91114 (93%).

#### 2.8 Is there a common genetic determinism between source and sink strengths in maize plants subjected to water deficit ?

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Leaf growth and Anthesis-Silking Interval (ASI) are the main determinants of source and sink strengths of maize via their relations with light interception and yield, respectively. They depend on the abilities of leaves and silks to expand under fluctuating environmental conditions, so we raise the possibility that they may have a partly common genetic determinism. Maximum leaf elongation rate and the slopes of the responses to evaporative demand and soil water status were measured on the Phenodyn platform, in a mapping population which segregates for ASI. ASI was measured in 3 and 5 fields under well-watered and water deficit. respectively. For each RIL, the maximum elongation rate per unit thermal time was reproducible over several experiments in the absence of water deficit. It was accounted for by 5 QTLs, among which three co-localised with OTLs of ASI in well-watered conditions. The alleles conferring high leaf elongation rate conferred a high silk elongation rate. The responses of leaf elongation rate to evaporative demand and to predawn leaf water potential had 3 QTLs in common with ASI of plants under water deficit. The alleles for leaf growth maintenance were those for maintained silk elongation rate. These results may have profound consequences for modelling the genotype x environment interaction and for designing drought tolerant ideotypes.



#### 2.9 Near-isogenic lines for disease QTL in maize

J. Zwonitzer\*, C.-L. Chung, J. Longfellow, R.J. Nelson, and P. Balint-Kurti \*North Carolina State University

To allow detailed characterization of OTL for disease resistance, we have used a range of methods to develop near-isogenic lines (NILs) of maize differing for loci affecting quantitative disease resistance (QDR). In this poster, we present the approaches used to extract QDR-NILs, the set of available lines resulting from this project, available data on the lines and plans for their further characterization. The available ODR-NILs have been extracted from the following sources: NC250, CML 52, Mo17, DK888, B73, and Tx303. QDR-NILs are being systematically tested for resistance to multiple diseases. In at least one case, an introgression was shown to provide resistance to two distinct diseases. ODR-NILs showing interesting phenotypes at the macroscopic level are being analyzed by histopathological techniques to identify those that interfere with pathogen development in potentially complementary ways. Further genetic dissection of disease QTL, using the QDR-NILs, provides a pathway for gene isolation.

#### 2.10 Integration of genomic tools with conventional screening for developing NERICA rice cultivars for West Africa

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The Asian rice (Oryza sativa subspecies indica and japonica) and African rice (O. glaberrima) are the two cultivated rice species. It is notable that it was at WARDA that high yielding rice varieties were bred between O. glaberrima and O. sativa for the first time. The new rice varieties are called NERICA and they are interspecifics that bridge the genetic gap between the two rice species. The development of new NERICAs with tolerance to drought and other constraints in West and Central Africa is still a top priority. A field trial was thus conducted in Cotonou, Benin to characterize O. glaberrima accessions and new interspecific lines for drought tolerance traits. In the trial, 372 genotypes of rice comprising 225 O. glaberrimas, 22 O. sativas and 115 interspecifics were subjected to drought stress treatments lasting 21 days starting either at 35 or 45 days after sowing (DAS) and the third treatment was a control which was continuously irrigated throughout the trial. Significant genetic variation was exhibited for vegetative stage vigor, plant height during and after drought stress and tiller number after stress in both stress treatments. The most discriminating parameter under both stress treatments was tiller number at 73 DAS

#### 2.11 Targeted Musa Genome Sequencing and Frame Map Construction

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Developing basic genomic tools is important for Musa (banana) germplasm exploitation, especially in the context of adding value to Musa diversity. Moreover, it is also a top priority for sequencing the whole Musa genome. The initiation of genomic sequencing to identify genes will allow the use of breeding-relevant genetic variation within Musa. The GCP has promoted the development of publicly available, well characterized genomic resources in order to integrate them with ongoing trait-based research in Musa. This project has allowed consolidating and characterizing existing publicly available Musa genomic resources and making new resources available. 41 BACs bearing genes mainly related to abiotic and biotic stresses have been sequenced, representing a total of 3.303Mbp. ESTs have been used as probes to identify the BACs to be sequenced. Two cDNA libraries from Pisang Klutuk Wulung (BB) expected to be tolerant to drought, were produced. International mapping populations and a consensus genetic map were established. Several annotation studies are ongoing, especially on biotic and abiotic stress. BACs showing micro-synteny (based on BAC-ends) with rice are being analysed. The most advanced study is related to RGAs. Further information can be found online on the Global Musa Genomics Consortium website: http://www.musagenomics.org

#### 2.12 The Molecular, Genetic, and Physiological Basis for Aluminum Tolerance in Sorghum and Maize

Leon V. Kochian, Jurandir Magalhaes, Jiping Liu, Owen Hoekenga, Claudia Guimaraes, Lyza Maron, Vera Alves, Robert Schaffert, Ubiracy Lana, Miguel Pineros, Jon Shaff, and Michael Lyi

Aluminum (Al) toxicity on acid soils represents a major worldwide constraint for crop production. Therefore, understanding the genetic and molecular mechanisms underlying Al tolerance has been a major focus for many researchers. Here, we report on progress for GCP grant IC69 on the identification and characterization of Al tolerance genes and associated physiological mechanisms in sorghum and maize. In sorghum, we have shown that our recently identified Al tolerance gene, AltSB, which is a member of MATE family of membrane transporters, is an Al-activated root citrate efflux transporter. Differential sorghum Al tolerance is conferred, in part, via differences in Al-inducible root tip  $Alt_{sp}$  expression. This differential expression may involve a MITE-type transposable element in the  $Alt_{sp}$  promoter that is highly polymorphic across members of the diversity panel. In maize, we have employed an interdisciplinary approach based on expression profiling, analysis of maize homologs of the two identified tolerance genes, ALMT1 and Alt<sub>se</sub>, and knowledge of physiological mechanisms of maize Al tolerance to identify candidate genes. Association and linkage analysis of these candidate genes has identified a number of putative tolerance genes. Some of these genes are homologs of ALMT1 and AltSB, while others may be involved in novel tolerance mechanisms.

#### 2.13 MetaQTL: a package of new computational methods for the meta-analysis of QTL mapping experiments

Jean-Baptiste Veyrieras<sup>\*</sup>,Bruno Goffinet and Alain Charcosset <sup>\*</sup>INRA. France

Integration of multiple results from Quantitative Trait Loci (OTL) studies is a key point to understand the genetic determinism of complex traits. Many efforts have been made by public database developers to facilitate the storage, compilation and visualization of multiple OTL mapping experiment results. We present a new computational and statistical package, called MetaOTL, for carrying out whole-genome meta-analysis of QTL mapping experiments. First, MetaOTL implements a new statistical approach to merge multiple distinct genetic maps into a single consensus map which is optimal in terms of weighted least squares and can be used to investigate recombination rate heterogeneity between studies. Secondly, assuming that QTL can be projected on the consensus map, MetaQTL offers a new clustering approach based on a Gaussian mixture model to decide how many OTL underly the distribution of the observed QTL. As expected, simulations also show that this new clustering algorithm leads to a reduction in the length of the confidence interval of QTL location provided that across studies there are enough observed OTL for each underlying true OTL location. The usefulness of our approach is illustrated on published QTL detection results of flowering time in maize. MetaQTL is freely available at http://bioinformatics.org/mgtl.

# 2.14 Comparative mapping of peanut (Arachis hypogaea) with the model legumes Lotus japonicus and Medicago truncatula.

David Bertioli<sup>\*</sup>, Niels Sandal, Marcio Moretzsohn, Lene Madsen, Soraya Leal Bertioli, Patricia Guimarães, Jakob Fredslund, Birgit Hougaard, Leif Schauser, Anna Marie Nielsen, Satoshi Tabata and Jens Stougaard.

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Most agriculturally important legumes fall within the warm-season or cool-season legume clades. However peanut falls in the more basal, mostly tropical, Dalbergioid clade. It is an allotetraploid (genome type AABB), with very low genetic diversity and this has hampered its genetics. Under the framework of the Generation Challenge Program we have been working to provide an improved genetic framework for peanut, making diploid and tetraploid maps using a common set of markers. Within this framework, the diploid AA genome map has been chosen as the central reference map because of its very high polymorphism. Clearly, the utility of this map would be greatly enhanced if it could be used to help to access the near-complete genome sequences of Lotus japonicus and Medicago truncatula. With this aim, we placed more than 100 single copy gene markers on this map and analyzed the synteny between Arachis and the model legumes. We were also able to identify the main affinities of the Arachis and model legume linkage groups. In addition, some substantial regions of marker co-linearity are evident. The inclusion of peanut in a unified genetic system for legumes is now feasible, and likely to be very informative.

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#### 2.15 Comparative genomic analysis and assessment of candidate genes in the *Pup1* locus, a major QTL for phosphorus deficiency tolerance in rice.

Sigrid Heuer\*, Xiaochun Lu, J.H. Chin<sup>1</sup>, T. De Leon, M. Yano, Abdelbagi M. Ismail, Matthias Wissuwa \*IRRI, Philippines

The *Pup1* locus, a major QTL for tolerance of phosphorus (P) deficiency was mapped on Chr.12 in Nipponbare (intolerant) x Kasalath (tolerant) mapping population, and the fine-mapped Pup1 region was recently sequenced in Kasalath. Kasalath Pup1 locus is 272 kb, but the corresponding region in Nipponbare is about 150 kb and almost 800 kb in 93-11. The observed differences can largely be attributed to transposable elements, in addition to introgression from other chromosomes. Many inversions, as well as truncations and mutations render it difficult to reliably predict Pup1 gene models in Kasalath. Many genes show limited sequence similarity to known genes or are not found in databases, and at least one gene seems indica specific. After in silico assessment of the predicted genes and RT-PCR expression analyses, we short listed few genes for further analyses by overexpression and RNAi, and rice transformation is currently ongoing. None of these candidate genes has formerly been associated with P-response and it is currently unclear how Pup1 confers tolerance. Interestingly, the Pup1 locus seems to overlap with published QTLs associated with tolerance of other biotic and abiotic stresses such as drought, opening new perspectives on how to assess the function of Pup1 genes.

#### 2.16 Identifying Genes Responsible for Failure of Grain Formation in Rice and Wheat under Drought (SP2-1)

J. Bennett\*, R. Dolferus, K. McNally, R. Serraj, Z.-Q. Ma, S. Kikuchi, K. Satoh, and R. Chandra Babu <sup>\*</sup>IRRI, Philippines.

Although wheat is generally more drought-tolerant than rice, both crops are most sensitive to drought stress at the reproductive stage when grain is formed. This progress report describes new screening methods that have been developed to identify germplasm with enhanced resistance during the most drought-sensitive events. such as peduncle elongation, pollen meiosis and anther dehiscence. Particular attention has been given to the mobilization, transport and utilization of stored carbohydrate in response to drought stress, with anther, peduncle and grain being key sink tissues. Unlike rice, wheat stores fructans, which are believed to aid drought tolerance. We show that fructan biosynthesis genes that evolved in the Pooideae such as wheat are absent from rice. We are currently using RNAi to downregulate these genes in wheat to establish their relevance to grain formation under stress. The impact of drought stress on peduncle elongation and anther development has been studied using microarray analysis and microscopic tools such as RNA in situ hybridization. Key emergent issues are the regulation of ABA breakdown during re-watering after drought stress, ABA-GA antagonism during peduncle elongation, and the primary site of drought action on the cell cycle and cellular elongation.

## 2.17 Characterization of maize and bean diversity in Central Europe

Peter P. Papp\*, László Holly, Marilyn Warburton, Matthew Blair, György Botond Kiss, Jean-Marcel Ribaut

\*Agricultural Biotechnology Center, Gödöllő, Hungary

Two projects have been initiated to characterize the Hungarian germplasm in the context of global germplasm diversity. Collaborations between the Institutes of Hungarian Ministry of Agriculture (Institute for Agrobotany and Agricultural Biotechnology Center) and GCP affiliated Institutes (CIMMYT and CIAT) have been planned to compare the allelic diversity of Hungarian maize and bean collections with the diversity present in GCP's reference sets.

Two collections (Active Collection with 73.968 samples and Base Collection with 11.042 samples), from a total of 1.538 collection sites all over Hungary in the last 50 years, have been set for medium and long-term conservation by Institute for Agrobotany. These collections can supply initial material for breeding programs as well as for botanical, biochemical, cytological and genetic research. Seed samples are distributed together with relevant information to users in Hungary and world-wide. Using the SSR microsatellite kits developed by GCP, 100-100 maize and bean accessions will be characterized, representing most of the variations in the Hungarian collections. The allelic diversity obtained for both the Hungarian material and the GCP reference set will be compared. The projects also support the training of Hungarian scientist on molecular characterization, high-throughput molecular screening techniques, developed to rapidly genotype the collections, and different strategies for marker-assisted selection.

2.18 Identification of quantitative trait loci for field resistance to rice blast in an advanced backcross population derived from two indica upland varieties, Way Rarem and Oryzica Llanos-5	Notes:
M. Bustamam <sup>*</sup> K.R. Trijatmiko, P. Lestari, A.K. Dewi, A.	
Warsun, K. Basyirin, D. Agisimanio, Iasiian, E. Lubis Suwarno I Ona MYV Reveche R I Nelson – H	
Leung, C.M. Vera-Cruz	
*Indonesian Center for Agricultural Biotechnology and	
Genetic Resources Research and Development, Indonesia	
Blast caused by <i>Magnaporthe grisea</i> is a devastating disease in rice worldwide. The Advanced Backcross OTL (AB-OTL) strategy was	
used to identify QTLs for field resistance to rice blast in a population	
from Indonesia, Way Rarem, and a durable and highly blast resistant	
variety from CIAT, Oryzica Llanos-5. A total of 123 BC2F2 plants were genotyped by means of 113 DNA markers (111 SSR and 2 SNP	
markers). The BC2F3 and BC2F4 families were evaluated for field	
Lampung, and in a blast nursery of International Rice Research	
Institute, Philippines. Greenhouse blast evaluation was also conducted using 8 different Indonesia isolates. OTLs were identified	
using single-point analysis and interval mapping performed using OGene. A total of 16 OTLs located at chromosome 1, 3, 5, 6, 7, 8, 9	
11 were detected using LOD > 3 threshold. The range of R <sup>2</sup> value of	
these Q1Ls was 11.31 – 45.11 %. The Oryzica Llanos 5 allele was	

favorable for 19% of the QTLs.

# Theme 3: Marker development and breeding applications

#### 3.1 Optimum Breeding Strategies for Selecting Drought Tolerant Wheat by Simultaneously Pyramiding Nine Favorable Genes

Jiankang Wang\* and Scott Chapman. \*Institute of Crop Science and CIMMYT China Office, Chinese Academy of Agricultural Sciences, Beijing

Wheat drought-suitable lines should be semi-dwarf with long coleoptiles, resistant to multiple diseases, have good dough properties, and have productive tillers. To achieve this, nine target alleles need to be combined into one genotype. Three parent lines were used: Sunstate, a commercial Australian line; HM14BS, a germplasm line combining an allele for height reduction and long coleoptiles; and Silverstar+tin, a derivative of Silverstar with a restricted tillering allele. The largest target genotype frequency was found in the Silverstar+tin/HM14BS//Sunstate topcross. The optimum MAS strategy to combine the nine target alleles from this topcross could be divided into three steps: (i) selection for Rht-B1a and *Glu-B1i* homozygotes, and enrichment selection of *Rht8c*, *Cre1*, and *tin* in TCF1, (ii) selection of homozygotes for one target allele, e.g. *Rht8c*, and enrich the remaining target alleles in TCF2, and (iii) selection of the target genotype with doubled haploid lines or recombination inbred lines. Enrichment of allelic frequencies in TCF2 reduced the total number of lines screened from >3500 to <600. While eight target alleles were present at frequencies after selection, the tin reduced-tillering allele frequency was lower due to its strong repulsion-phase linkage to Glu-A3 and the incomplete linkage of the tin marker. Therefore, the presence of the *tin* gene needs to be further confirmed by other methods.

#### 3.2 Enhancing Tolerance to P- deficiency in Upland Rice Varieties by integration of Pup-1 locus using Molecular Markers

M. Bustamam<sup>\*</sup>, J. Prasetiyono, I. H. Somantri, T. Suhartini, S. Abdulrahman, S. Moeljopawiro and Abdelbagi M. Ismail \*ICABIOGRAD, Indonesia

To accomplish self sufficiency in rice production, the possible extension of rice growing area was toward marginal area which mostly acid soils where P-deficiency is the most common constraint and poor for food crop production. Improved upland rice varieties released were mostly aimed at fertile soil with sufficient rainfalls. Some were tolerant to drought and Al toxicity but none of them tolerant to P-deficiency. This study were to identify suitable recipient cultivars of upland rice, to develop phenotyping procedures for Pdeficiency tolerant rice, to validate candidate genes (Set of NILs for the *Pup1* locus) in the developed backcross populations, and to evaluate material developed in hotspots in farmers' fields. In greenhouse evaluation similar to the hotspot areas, three Indonesian upland rice varieties susceptible to P-deficient soil were selected. These varieties were crossed to three Pup-1 donor parents from IRRI. Out of nine BC1F1 populations, 180 plants were selected and nine of which were used to develop BC2F1 populations. The effectiveness of candidate gene present in Pup-1 donor parents was verified in the field and in the greenhouse using P-deficient soils. Ten BC3F5 lines of Wayrarem//Oryzica llanos-5 were included in the field experiments. The responses of them to P-deficiency will also be presented.

#### 3.3 Evidence for genetic origin of aroma gene in Nerica1

Maxwell Darko Asante\*, Lingxia Huang, Sandra Harrington, Paul Kofi Dartey, Richard Akromah, Marian Quain, Mande Semon, Marie-Noelle Ndjiondjop and Susan McCouch

\*CSIR-Crops Research Institute, Ghana.

Aromatic rice is popular in many parts of the world including Africa. Nerica 1 is the only aromatic rice accession amongst the 18 upland Nericas developed by the African Rice Center. However. the supposed parents of Nerica 1, WAB56-104 (Oryza sativa) and CG14 (O. glaberimma) are both non-aromatic. A mutation in the BAD2 gene is reported to be the likely cause of aroma in Jasmine and Basmati-styled rices and an allele-specific amplification (ASA) marker for aroma has been developed. Our study indicated that the 8 bp deletion in BAD2 is associated with aroma in Nerica 1. Haplotype analysis around the BAD2 gene showed the fingerprint of Nerica1 to be similar to that of Basmati 217; aroma in Nerica 1 is, therefore, likely to have been introgressed from an aromatic genotype. Association studies conducted with 66 rice accessions from Ghana showed perfect co-segregation between the aromatic phenotype and the functional ASA marker. This suggests that screening for aroma using molecular markers will be possible in the rice breeding program in Ghana.



#### 3.4 The Many Faces of GenoMedium

Kyle Braak\*, Sebastian Ritter, Andrew Farmer, Kevin Manansala, Ryan Carlo Alamban, Richard M. Bruskiewich, Graham McLaren, Luís Ávila, Reinhard Simon, Guy Davenport \*CIMMYT. Mexico

Critical to the success of the GCP's genomics-driven plant breeding is the development of the 'GCP Pantheon Informatics Platform' (http://pantheon.generationcp.org) which aims to integrate the distributed information resources of the consortium members and provide an improved set of bioinformatics tools. One such tool under development is GenoMedium - a plug-in based standalone user interface developed using the Eclipse Rich Client Platform (RCP) platform (http://www.eclipse.org/rcp). In brief, it allows the user to query, analyze, and visualize data using the array of tools built in accordance with the Pantheon Informatics Platform. As a result, GenoMedium is fully customizable and extendible in terms of a list of services and data sources available. GenoMedium can also construct conceptual linkages between data which assist the user in making sensible decisions. Furthermore, data can be exchanged seamlessly between tools as the data outputted from one tool can be shuttled as input to another. Ultimately, the end-user enjoys a very intuitive and user-friendly experience.

GenoMedium is entirely open sourced and licensed with the software and technical documentation available at: <u>http://www.genomedium.org</u>

#### 3.5 Marker-assisted Selection of Genes Associated with Drought Tolerance in Wheat (Triticum aestivum L.) Rui-Lian JING\*, Xiao-Ping CHANG, Xin-Guo MAO, Xing

XU, Hui-Min XIE

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Drought is a major limiting factor in wheat production in semi-arid and arid areas in China. Discovering and utilizing genes to improve wheat drought tolerance is our approach for generating superior lines. We have identified genes/OTLs involved in drought tolerance for integrating marker-assisted selection (MAS) into conventional breeding for the improvement of wheat in Northern China. We used doubled haploid lines constructed from a cross between the Chinese common wheat cultivars Hanxuan-10 and Lumai-14 and recombinant inbred lines derived from the cross Opata85 × W7984 to establish QTLs controlling important traits associated with drought tolerance. Traits scored included physiological traits, such as staygreen, chlorophyll fluorescence, leaf water status, canopy temperature, accumulation and remobilization of stem water-soluble carbohydrates, and agronomic traits, such as flowering date, plant height, spike number per plant, spike length, ratio of spikelet setting, thousand-grain weight and grain yield, among others. Phenotypic characters were recorded in different locations and over several years under two water regimes, drought stress and well-watered conditions, and QTLs  $\times$  environment interactions were analyzed. Some target gene-derived 'Functional Markers' have been developed and mapped on genetic linkage maps with selection ongoing for stable lines carrying markers from the introgression lines by MAS. Theme: SP3

### 3.6 Genetic studies on early vigour in low phytic acid, QPM and tropical inbred lines of maize

R. Naidoo\*, P. Tongoona, J. Derera and M.D. Laing \*African Centre for Crop Improvement, University of KwaZulu-Natal, South Africa

Maize has high levels of phytic acid inhibiting absorption of essential minerals such as iron and zinc: and also lacks in essential amino acids (tryptophan and lysine). The low phytic acid (lpa1-1) gene has been sequenced and molecular markers can be designed and used to track the trait in leaf material. However lpa mutants have reduced vigour. therefore knowledge of linkage between lpa and vigour can aid in developing a selection strategy. The objective of the study was to investigate gene action and linkage between lpa and seedling vigour, and use of MAS. F, hybrids generated from a diallel mating design and selected F, families will be evaluated for seed germination, vigour, grain yield and lpa and tryptophane levels. Linkage between early vigour and low phytate will be determined in F<sub>2</sub> materials from lpa X QPM crosses. Heritability, GCA, and SCA will be estimated. Markers linked to lpa traits will aid in breeding for lower lpa content in normal tropical maize in BC selection. Parental lines and BC progeny will be fingerprinted using SSRs and AFLPs. This study will result in increased efficiency and progress in breeding maize cultivars that combine lpa and quality protein traits.

# 3.7 Accelerating varietal development using high throughput, low cost, gene-based MAS technologies in cereals

C.M. Vera Cruz\*, M.Y.V. Reveche, E.F.L. Mercado, J. Chen, B. Collard, J.H. Chin, D. Skinner, M.G.C. Carrillo, J. Wu, Y. Xu, M. Bernardo, M. Bustamam, J. Agarcio, V. Verdier, D. Sanchez, D. Joshi, J. Ulat, J. Crouch, and D.J. Mackill

\*International Rice Research Institute (IRRI), Philippines

High throughput, low cost technologies for marker-assisted selection (MAS) that utilize SNP-based probes were developed using the genes Xa21 for bacterial blight resistance (rice) and opaque2 (maize) for quality protein maize as models, in a collaboration between IRRI, CIMMYT and NARS/SME partners in China, India, Indonesia and the Philippines. The dot blot and FRET methods have been refined and validated using elite lines of Basmati-derived lines in a replicated yield trial and Hui 593-derived population with partners from China, India, Indonesia and the Philippines. Cost reduction experiments, including use of home-made reagents versus commercially available kits, have been investigated and have shown that sensitivity of the assays had been maintained. The dot blot method is now being adapted to detect the allelic state of the submergence tolerance gene (sub1) in breeding population. The microarray- and microplate/PCR-ELISA® -based genotyping technologies is being further optimized and refined using the Xa21 resistance gene. Management of the data generated and accessibility of the same data is being done in collaboration with the IRRI Crop Research Informatics Laboratory. To transfer and validate these technology platforms to national program partners of the project, two separate workshops were held in Jalna and Hyderabad, India in collaboration with Barwale Foundation.



# **3.8** Phenodyn : a phenotyping platform and an information system to dissect the genetic variability for growth and transpiration rates in response to water deficit.

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W Sadok, B Parent, O Turc, N Hilgert, F Tardieu F \*INRA-SupAgro Montpellier, France

Tolerance to water deficit is identified at early stages of the vegetative cycle of monocot species, especially maize. This is based on a genetic analysis of the responses to water deficit of organ growth and gas exchanges. The rationale is that (i) genotypes which do not maintain gas exchanges and leaf growth during water deficit cannot perform well in dry fields, (ii) the expansive growth of several organs (e.g. leaves and silks) have common genetic determinisms. Phenodyn imposes common fluctuating climatic and soil moisture conditions to 420 plants in greenhouse and growth chamber. Temperature, evaporative demand and soil water content are measured every 15 min, together with leaf (or silk) elongation rate and plant transpiration. Outputs are managed in a database for real time monitoring of experiments and for post-analyses of thousands of diurnal time courses of growth rate or transpiration (Sadok et al. (2007) PCE, 30: 135-146). It was used for analyzing mapping populations, a panel of lines for association genetics or insertion lines. It has also been tested for rice. The identification of early QTLs of response can be combined with models which predict leaf area, light interception and, potentially, yield. In addition to genetic analyses of physiological processes associated with plant adaptation, this platform might be a potent tool in the breeding for drought tolerance.

#### 3.9 Marker-aided Cassava Germplasm Improvement in Nigeria: Partnerships that Strengthen Capacity for Food Security

C.N. Egesi<sup>\*</sup>, E. Okogbenin, K. Shuaibu, O.N. Eke-Okoro, E.N.A. Mbanaso, O. Ogundapo, S. Baiyeri and M. Fregene.

\*National Root Crops Research Institute, Nigeria.

Cassava's increasing importance in Nigeria for food, animal feed, and industry has spurred rapid expansion of cassava production. However, an array of constraints in combination with the crop's long reproductive cycle limit efficient introduction of genes for key useful traits. Among the greatest needs in cassava development in Nigeria is to diversify its use as basic raw material for industrial purposes in order to enhance its marketability. Breeding for traits that incorporate genes for pest and disease resistance and economic traits will greatly improve the livelihoods of small-scale cassava producers. African cassava varieties could be improved through germplasm from Latin America, cassava's center of origin, but most Latin American varieties are highly susceptible to the devastating cassava mosaic disease. Under the Generation Challenge Programme's project, "Development of Low Cost Technologies for pyramiding useful genes from wild relatives of cassava into elite progenitors", molecular markers associated with a CMD resistance gene, was used to breed for resistance in Neo-tropical cassava gene pools that were subsequently introduced and tested for release in Nigeria. In the process, both formal and hands-on training on molecular breeding of Nigeria's cassava breeders was achieved. The achievements, potential impacts and future prospects of the partnership are discussed.



#### 3.10 Developing salt tolerant rice (Oryza sativa L.) cultivars through conventional breeding and doubled haploid approach S. Kashenge-Killenga\*, P. Tongoona., J. Derera and Z.

Kanveka.

\*African Centre for Crop Improvement (ACCI), South Africa

Salty soils are among the most important abiotic stresses limiting lowland irrigated rice production. This silent but life threatening constraint is gaining importance in most African countries including the northern and eastern rice belts of Tanzania. The study focuses on developing salt tolerant varieties as a key intervention to enhance rice productivity in salt prone environments. Three main phases are planned: Phase 1 will involve searching for new genetic sources of salt tolerance. A wide gene pool (elite lines, varieties, landraces) will be screened under hydroponic salt condition (12 dS/m) to identify tolerant genotypes. In Phase 2, the genes for salt tolerance will be transferred to popular and high yielding cultivars using 8 x 8 parent full diallel mating scheme. The F1 progenies will be advanced to F2 by selfing and used to explore the nature of gene action and inheritance of salt tolerant traits. Selected F2 progenies will be further advanced through the doubled haploid approach to shorten the breeding cycles. Phase 3 will involve testing of derived products using incomplete block designs under stress and non-stress environments. The output will include conventional-derived and doubled haploid homozygous lines combining salt tolerance, high grain yield potential and farmer preferred traits.

#### 3.11 Tailoring Superior Alleles for Abiotic Stress Genes for Deployment into Breeding Programs: A Case Study Based on Association Analysis of Alt<sub>sb</sub>, a Major Aluminum Tolerance Gene in Sorghum

Jurandir Magalhaes\*, Leon Kochian, Stephen Kresovich, Robert Schaffert, Alexandra Casa, Sharon Mitchell, Theresa Fulton, Jiping Liu, Owen Hoekenga, Antônio Marcos Coelho, Claudia Guimaraes, Vera Alves, Issoufou Kapran, Soumana Souley, Maman Nouri, Magagi Abdou, Adam Kiari, Fatouma Beidari \*Embrapa Maize and Sorghum, Brazil

One of the most important factors limiting agriculture in developing countries involves the large areas of acid soils found therein. On acid soils, toxic levels of aluminum (Al) ions damage roots and impair their growth and function, resulting in reduced nutrient and water uptake, with concomitant reductions in crop yield. Building upon our recent success in isolating Alt<sub>se</sub>, a novel Al tolerance gene in sorghum, we will now apply association genetics to undertake a comprehensive scan for agronomically better versions of this gene for deployment into sorghum breeding programs. Through the use of cutting edge genomics and statistical genetics approaches, this research will bridge the gap between basic research on Al tolerance and applied breeding programs, to develop the tools that plant breeders can use to efficiently and effectively breed for improved acid soil tolerance. The long-term goals of this research are to generate sorghum genotypes expressing improved Al tolerance that ultimately can be distributed to farmers who till acid soils in Africa and other developing regions, thus exploiting a wide range of still hidden genetic variation for Al tolerance. Increasing the Al tolerance of staple crops, such as sorghum, will help increase yields and thus food security worldwide.

tolerance to gray leaf spot (GLS), northern corn leaf blight (NCLB) and common rust. However, these efforts have been hampered by the quantitative nature for which these traits are controlled. This paper, reports the results of a study carried out to employ marker assisted selection to map the QTLs in 41 parent/inbred lines to augment conventional breeding. We selected 28 SSR markers from linkage groups 2 and 10. Linkage was determined by Log-likelyhood (LOD) threshold of 3.0 and linkage maps constructed using pairwise recombination estimates between 3 and 80%. And a LOD score larger that 3.0 from Kosambi's mapping function were taken as QTLs of larger effect. Linkage groups were scanned for QTLs at 5 cM intervals with LOD thresholds corresponding to a genome wide error rate of 5% calculated by 2000 permutations of the data. Thirteen lines had positive marker trait association for both GLS and common rust. Four of these were parental lines while nine were inbred lines

#### 3.12 Quantitative trait loci (QTL) for resistance to GLS and common rust

Fungal diseases have continued to constraint maize production in Kenya by reducing the average yield by 18-40%. Conventional breeding methods have produced several maize lines with acceptable

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#### 3.13 Marker Development and Marker Assisted Selection for Striga Resistance in Cowpea

S. Muranaka<sup>\*</sup>, M. P. Timko, N. Cisse, M. Wade, C. Fatokun, A. Raji, D. J. Kim and B. Ousmane \*IITA, Nigeria

Striga gesnerioides (Willd.) is a parasite of cowpea and a major constraint of cowpea production in West and Central Africa. To tackle this Striga problem, under GCP project "Marker development and marker assisted selection for *Striga* resistance in cowpea". IITA. UVA, CERAAS and CRNA have been seeking to develop molecular markers and establishing Marker Assisted Selection (MAS) method in cowpea for effective and efficient breeding of Striga resistance. The Striga hotspot trial clearly displayed that the ability of cowpea genotypes to resist Striga parasitism depends on geographic origin of the parasite. Several genotypes were selected from this trial with favorable agronomic traits and adaptability to each region of West and Central Africa. These lines will serve as potential parent combinations in further breeding program thorough MAS. So far, two SCAR markers, 61R and MahSE2, were identified as reliable markers for Striga race 3 with high efficiencies, 79% and 77% respectively. New markers have also been developed for several Striga races in West and Central Africa. Four populations have been newly developed and are under MAS efficiency test with the markers to establish MAS proto

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### 3.14 On-going Molecular Breeding Activities in the Faculty of Agriculture at Makerere University

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We have ardently pursued integration of molecular markers through fidelity testing and/or development of potential new markers linked to important traits for effective use in the National breeding programs. To do this a modest laboratory with capacity for genomics (PCR, RNA and DNA blots, autoradiography, proteomics (2-D systems) has been established. We continue tested strategies for effective implementation of molecular markers. Almost all our research projects are run as student theses research projects which aimed at ensuring that a continuation supply of need human capacity is developed. A summary of past and on-going research work is provided.

### 3.15 The African Molecular Marker Applications Network (AMMAnet)

#### R. Edema\*, Interim Coordinator

\**Faculty of Agriculture, Makerere University, Uganda* The African Molecular Marker Applications Network (AMMAnet) was established to help build and support the capacity of National Agricultural Research Systems in Africa to adopt biotechnology tools in the improvement of African Crops. In Asia, regional networking involving a centre of excellence and a group of highly motivated NARS from developing countries has demonstrated the cost-effectiveness in transferring technology in a short period. The main role AMMAnet is to identify and consolidate a body of local plant biotechnologists and breeders for the improvement of key crops in Africa. The costs of creating such local research networks are likely to be small and the benefits yielded are important and lasting, namely:

- Reduced transaction costs and enhancing economies of scale, scope and size.
- Provide a suitable framework to build synergies and support efficiency of the research process through capacity building in the region.
- An opportunity to build a functional network of excellence that allows Africa utilizes the available resource more effectively.
- Create a pool of scientists and enhance collaboration through sharing of resources and peer support.
- Create a dynamic platform for developing capacity and support innovations in biotechnology and other emergent sciences.

In this poster paper, AMMAnet's key achievements, challenges it faces and opportunities available are discussed.

#### 3.16 Characterisation of transpiration profile in response to water deficit in Arachis species and synthetic amphydiploids

Soraya C. M. Leal-Bertioli\*, Vincent Vadez, Patricia M. Guimarães, Ana Claudia G. Araújo, Pedro Í. T. Silva, Luciano F. M. V. Moraes & David J. Bertioli.

\**Embrapa Genetic Resources and Biotechnology, Brazil.* Peanut, *Arachis hypogaea* is widely cultivated in the tropics, where unpredictable drought stress is limiting for production. However, wild species are found in diverse environments. Here we describe the response of leaf gas exchange to progressive soil drying of wild, cultivated and synthetic accessions of *Arachis*.

Large variations of response to progressive water deficit were observed. In general, in wild accessions, transpiration decreased when the fraction of transpirable soil water was as high as 0.8 - 0.6 (conservative behavior). Conversely, transpiration of cultivated peanut varieties declined at lower soil water content (FTSW *c*.0.2, opportunistic behavior).

Transpiration response of synthetic amphidiploids (tetraploids, oportunistic) was dramatically different from the wild parentals (diploids, conservative), and similar to cultivated peanut. This could be attributed to effects of polyploidy. To evaluate that, stomatic type and index were determined. Amphidiploid (KG30076 x V14167)<sup>4x</sup> had stomatic type and index more similar to paternal parent (V14167) and to cultivated peanut (tetraploid) than to maternal parent (KG30076). This suggests that these characteristics do not, alone, explain the variation of transpiration behavior observed. These are important results, because they show that some drought-tolerance assays on diploid wilds may have limited predictive power as to the behavior of synthetic amphidiploids.

#### 3.17 Identification of resistance sources and mapping of resistance QTLs to African strains of Xanthomonas oryzae pv. oryzae causing Bacterial Leaf Blight in rice

Gustave Djedatin\*, Mathias Lorieux, Thierry Mathieu, Alain Ghesquière, Marie-Noëlle Ndjiondjop and Valérie Verdier.

\*WARDA and Université d'Abomev-Calavi. Bénin Rice bacterial blight caused by Xanthomonas orvzae pv. orvzae (Xoo) was reported in Africa in the 80s, since then it is increasing in importance. Breeding strategies for durable BB resistance need to be urgently developed in Africa. The objectives are to identify sources of resistance to Xoo in African germplasm and to characterize genes for durable resistance. 28 accessions of African cultivated rice Orvza glaberrima (selected for the Generation iBridges project) were screened using two African Xoo strains MAI1 and BAI3. A reference IR64 x Azucena RI population made of 172 lines was mapped with the same strains. The accessions were grown under controlled conditions in glasshouse. Leaf clipping inoculation was performed on 5 week-old plants. Phenotypic evaluation was performed 3 weeks after inoculation by measuring leaf lesion length. All O. glaberrima accessions were susceptible to BAI3 while CG14, TOG6308 and TOG6356 showed a high resistance to MAI1. QTL mapping based on ANOVA evidenced five putative QTLs common to the two Xoo strains and are located on chromosomes 1, 4, 7, 10 and 11 respectively with a major QTL on chromosome 7 explaining 29.8 % of the total variance. Two additional OTLs were strain-specific and were located on chromosome 3 and 8 for strain BAI3 and MAI1 respectively. Most of the OTLs detected differ from those previously characterized.

#### 3.18 Using Molecular Breeding to fast-track release of Delayed PPD and Disease Resistant Cassava in Ghana

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\* Crops Research Institute, Ghana

Cassava is a major staple in Ghana, contributing 22% of Agricultural GDP. It is a high and reliable cheap source of carbohydrates for over 200 million people and has currently assumed industrial and cash crop status in Sub-Saharan Africa. Cassava is very susceptible to ACMV and other devastating diseases and pests, as well as post harvest physiological deterioration. Crosses started at CRI under the GCP project on pyramiding useful genes in cassava. Marker Assisted selected (MAS) disease and pest resistant lines and inter-specific hybrids with delayed PPD properties from CIAT were crossed with preferred land races. Under this project a functional molecular laboratory started and 25 participants were trained in cassava crosses and field plot techniques. Preliminary results showed four of the inter-specific hybrids of *M.walkerae* with disease resistance in all ecological zones. Clone CR 52A-25 additionally showed no signs of post harvest physiological deterioration after the seventh day and AR 14-10 unique industrial starch properties. A family, TAI 8 + C-243showed exceptional resistance to disease and high fresh tuber yields between 36 to 58 t / ha in all locations. Useful clones obtained indicate that MAS could accelerate the improvement of cassava clones for release in the near future.

#### 3.19 Creating a Unified Genetic Map Resource for Peanut.

Marcio Moretzsohn\*, Soraya Leal-Bertioli, Patricia Guimarães, Marcos Gimenes, Rajeev Varshney, Aruna Rupakula, David Hoisington, Vincent Vadez, José Valls, David Bertioli.

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Cultivated peanut is an allotetraploid (AABB genome) with very limited genetic diversity. This, coupled with the complexity of tetraploid genetics has constrained the advances in genetics necessary for modern breeding. Under the framework of the Generation Challenge Program we have worked to overcome these limitations. Firstly, we dissected the tetraploid genetics of peanut by constructing two diploid maps: one for the AA, and one for the BB genome. For this we used populations derived from the most probable wild ancestors of peanut crossed with closely related wild species. Secondly we are constructing tetraploid maps: one from a population derived from a cross of a cultivated peanut and a synthetic amphidiploid, and one from a cross of two cultivated. For markers we used microsatellites, because they are co-dominant and highly polymorphic, have good transferability between populations, and are ideal for breeders. We have used the same marker sets for all maps, thus generating a framework for map comparison and the mapping of QTLs in different genetic backgrounds. Finally to enrich the information content of the maps, we have placed candidate genes, and anchor markers on the AA genome map.
# 3.20 Evaluation of drought tolerance contrasting cassava varieties under semi-arid environment

Alfredo A. C. Alves \*, Miguel A. Dita, Alineaurea F. Silva, Luis Duque, Tim L. Setter \*Embrapa Cassava and Tropical Fruits, Cruz das Almas,

Brasil

Cassava varieties are being field evaluated under irrigation and rainfed conditions in the semi-arid environment of Petrolina, Brazil. where the annual average rainfall and potential evapotranspiration are around 560 mm and 1500 mm, respectively. Plants has been submitted to two water treatment: irrigation and water deficit (irrigated only in the first 3 months after planting). Trials were evaluated in different periods using growth and physiological parameters, aiming to identify most contrasting traits that can be useful to select genotypes for drought tolerant attributes. From the preliminar analysis of the trials'data (not completed yet), the results have showed significant effect of water deficit treatment on production's parameters such as tuber roots fresh weight, number of tuber roots, dry matter and harvest index, with different responses among varieties. The most contrasting traits that have showed correlation to the varieties performance are leaf retention, leaf conductance, and early bulking. Additional analyses, such as abscisic acid, sugars and starch accumulation are currently undergoing. These results will be useful, not only for characterizing the varieties as tolerant or susceptible to drought, but also for defining the better traits for genotype selection in the breeding program.

# 3.21 Evaluation of cassava interspecific hybrids for resistance to pests and diseases in different environments of Brazil

Alfredo A. C. Alves\*, Alba R. N. Farias, Miguel A. Dita, Alineaurea F. Silva; Anthony C. Bellotti; Martin A. Fregene

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Cassava interspecific hybrids were evaluated for pest and disease resistance in diferent environments of Brazil. Experiments were established in São Miguel das Matas (SMM), Tancredo Neves (TN), Cruz das Almas (CA) in the Bahia State and in Petrolina (PT), in the Pernambuco State. SMM, TN and CA are caracterized as subhumid environments with different annual average rainfall varing from 1000 to 1800 mm and PT is representative of the semi-arid (around 560 mm). Evaluations were performed every month starting 6 months after plantation (MAP) to 12 MAP. In all the places, the most relevant pest were cassava green mite (Mononychellus tanajoa) and cassava mealy bug (Phenacoccus manihoti), respectively, with the highest incidence in PT. Diseases were increasingly severe in TN, SMM and CA. Anthracnose (Colletotrichum gloesporioides f sp. manihotis), brown spot (Cercosporidium henningsii) and rust (Uromvces manihotis), were the most severe diseases, in this order, Hybrids with high levels of resistance to both pests and diseases were identified in all the places. These hybrids are promising genotypes, not only for the introgression of resistance traits into elite cassava varieties, but also to understanding the genetic basis of resistance to pests and diseases.

# **3.22** QTL mapping and marker-assisted backcrossing for improved salinity tolerance in rice.

Abdelbagi M. Ismail\*, Michael J. Thomson, Marjorie de Ocampo, James Egdane, Meggy Katimbang, M. Akhlasur Rahman, Eduardo Blumwald, Clyde Wilson, Zeba Seraj, Timothy J. Close, Rakesh K. Singh, Glenn Gregorio \* International Rice Research Institute, Philippines

Salt stress is a major constraint across many rice producing areas because of the high sensitivity of modern rice varieties. We aim to develop an improved marker-assisted backcrossing (MAB) system for salinity tolerance in rice by dissecting tolerance mechanisms through fine-mapping of quantitative trait loci (QTLs) and developing gene-based markers at the target loci. Previously, a mapping population between IR29 and the salt tolerant landrace Pokkali was used to map several tolerance OTLs, including Saltol, a major OTL on chromosome 1. Fine-mapping of the Saltol locus is in progress and graphical genotypes using microsatellite and candidate gene-based markers have defined the Pokkali introgressions in a population of near-isogenic lines (NILs). Additional Pokkali OTLs are being targeted for NIL development to allow testing of individual QTL effects and combinations of QTLs. We are employing a precision MAB system to efficiently transfer these salt tolerance OTLs into popular varieties. We have also identified novel sources of tolerance using a diverse set of germplasm from Bangladesh, and have begun developing new mapping populations with selected landraces. The long term goal is to identify and combine QTLs controlling different physiological mechanisms to achieve a higher level of salt tolerance in high yielding rice varieties.

Notes:

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# 3.23 Evaluation and Deployment of Transgenic Drought-Tolerant Varieties (SP3-19)

J. Bennett\*, R. Serraj, I. Rao, M. Reynolds, V. Vadez, F. Ezeta and K. Yamaguchi-Shinozaki \*International Rice Research Institute, Philippines.

Drought-response elements (DREs) are found in the promoters of many genes induced by drought, cold and salt stresses. DREBs are transcription factors that bind to DREs and help to mediate abiotic stress responsiveness. Enhancement of stress tolerance by overexpression of DREB genes under the control of stress-responsive promoters (such as rd29A or OsLip9) is an emerging paradigm that needs validation across crops and under physiologically relevant conditions. This progress report describes the effectiveness of DREB constructs in groundnut (Arachis hypogaea), wheat (Triticum aestivum) and rice (Oryza sativa). In groundnut (JL24 background), more than thirty independent T<sub>1</sub> transformants containing the rd29A::AtDREB1A construct were screened in PVC cylinders; higher transpiration efficiency was detected in three lines, and higher root dry-weight and higher root/shoot ratio were detected in six lines in response to drought. In wheat (BobWhite background),  $170 \text{ T}_1 - \text{T}_4$ transformants containing the above construct were screened in pots; fifteen showed enhanced biomass and/or water use efficiency. In rice (Palmar and CICA8 backgrounds), 49 T<sub>1</sub> transformants carrying the OsLip9::AtDREB1A or OsLip9::OsDREB1B were screened under drought stress in large trays; seven OsLip9::AtDREB1A lines showed enhanced resistance to leaf rolling. Future work will focus on the impact of transgenes on yield under field stress.

# 3.24 Marker Assisted Selection: A Paradigm for Germplasm Transfer and Genetic Improvement of Cassava in Africa

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Marker assisted selection (MAS) activities were initiated to develop improved cassava varieties by exploring the useful plant genetic resources from Latin America, where cassava originates. Latin American (LA) germplasm are susceptible to the cassava mosaic disease (CMD), and molecular markers associated with CMD resistance (CMD2 gene) were used to introgress CMD resistance into LA germplasm introduced to Nigeria and Ghana and then selected based on marker data for CMD. F, seeds generated from parents selected for beta-carotene (pre-cursor of Vitamin A) were also introduced to Africa to develop cassava varieties with improved nutritional qualities. Results obtained indicate that 146 high performing CMD resistant genotypes were identified in Nigeria and 23 in Ghana. In Nigeria, three genotypes (CR 14A-1, CR 41-10 and AR 38-3) are undergoing multi-locational testing nationwide which is a key step towards their release. In collaboration with the Genotyping Support Service (GSS), two new sources of CMD genes were identified using NRCRI germplasm. These genes will facilitate the pyramiding of CMD resistance genes in elite germplasm towards building durable and stable CMD resistance in Africa. Through the newly built MAS laboratories initiated under the GCP project in Nigeria and Ghana, molecular breeding activities will play a key role in developing elite cassava varieties for improved productivity in Africa.

# 3.25 Stacking disease QTLs with drought tolerance in

# rice

C.M. Vera Cruz\*, G. Carrillo, I. Oña, M. Variar, M. Bustamam, R. Trijatmiko, J. Cairns, R. Mauleon, B. Courtois, E. Javier, J.E. Leach, R. Lafitte, R. Serraj, R.J. Nelson, and H. Leung. \*IRRI, Philippines

An objective of our project is to demonstrate the feasibility of combining traits essential for drought-prone environments. For upland rice in India and Indonesia, germplasm with improved drought tolerance must have blast resistance to have any chance of adoption. Thus, selection for drought tolerance should be done in conjunction with selection for disease resistance. We accumulated different disease OTL from Moroberekan into Vandana, a droughttolerant variety and evaluated the advanced backcross progenies in blast hotspots in India and the Philippines. Selected F<sub>6</sub> lines showing resistance to blast and agronomically similar to Vandana were evaluated for yield performance under managed drought. Six candidate genes (CGs) co-localizing with known dQTLs - aldose reductase, chitinase, oxalate oxidase, oxalate oxidase-like proteins (OXLP), peroxidase and thaumatin, including several SSR markers were significantly associated with resistance to blast. We identified IR78221-19-6-33 and IR78222-20-8-7-148-2B as the two best lines containing the CG alleles from Moroberekan. These two lines showed agronomic similarity to Vandana but with added blast resistance. In Indonesia, two lines (BC<sub>2</sub>F<sub>5</sub>-168 and BC<sub>2</sub>F<sub>5</sub>-49) derived from Way Rarem/Oryzica Llanos 5 were found to show resistance to blast and perform well under drought. Our data, though preliminary, did not show obvious antagonistic effects in combining blast resistance with drought tolerance.



### **3.26 A European Grain Legume Integrated Project** (GLIP) for comparative mapping of legume species *A. Seres\*, G. Deák, C. Iliescu, P. Kaló, T. Pálfv, G. Tóth.*

A. Seres\*, G. Deak, C. Illescu, P. Kalo, I. Palfy, G. Toth T. H. N. Ellis, G. B. Kiss

\*Agricultural Biotechnology Center, Hungary

To carry out comparative genetic mapping between legume species, Medicago truncatula and legume specific gene based (intronspanning) primer pairs were designed and synthesized followed by the determination of the map position of the genes in cicer, lens, lupin, vicia, clover and pea. Intron-spanning (targeting) primers amplify in most cases ortholog genes therefore the genetic position of these genes can be compared for cross-species comparative mapping purposes. 710 primers were tested on parental DNAs by PCR amplification using touch-down amplification programs. PCR products were run on agarose gel to detect amplification and length polymorphism. Uniform fragments were denatured, re-annealed and digested with Cell enzyme followed by running on polyacrilamyde gel to detect cut products of heteroduplexes ( Cell cuts at mismatches). This strategy has a considerable advantage over other methods: it is a simple polymorphism detection technique, there is no need to sequence the amplification products or screen for restriction endonuclease cutting sites to find polymorphism! The results of this mapping activity will be presented in the poster and demonstrate that this is a simple, cost-effective way to perform comparative gentic mapping. This study was supported by the European grant "Grain Legumes for Food and Feed" (grant no. FOOD-CD-2004-506223), and by the Hungarian Ministry of Agriculture and Rural Development.

### 3.27 Inheritance of Seed-Transmitted Cowpea Aphid Borne Mosaic virus (serotype D) Resistance in Cowpea (Vigna unguiculata (L.) Walp.) Jean Baptiste Tignegre, racinetignegre@yahoo.com

During 1999 season, an experiment was conducted at Kamboinse research station. The objective of this research work was to identify sources of resistance to Cowpea aphidborne mosaic virus (CABMV) transmitted through seed, and to determine the inheritance of this character in cowpea. Four hundred and forty four (444) genotypes were inoculated using strain "D" of Cowpea aphid-borne mosaic virus (CABMV) under an artificial inoculation in field conditions. An Enzyme Linked Immunosorbent Assay (ELISA) method was used to detect the virus in seeds collected from infected plants. ELISA tests indicated that the transmission of CABMV through seed did not occur with 129 over a total of 444 genotypes screened to identify resistant lines. Crosses and reciprocal crosses realized between Moussa local, a landrace with a high rate of CABMV transmitted through seed and KVX 396-4-4, a variety with a low rate of CABMV seed transmission. Successfully infected plants from these different parents were crossed to get the seed transmission rate of the virus in  $F_1$  seeds. Then, the  $F_1$  seeds were planted and the subsequent infected seedlings from each of the above F<sub>1</sub> seeds were counted to determine the rate of virus transmission from F<sub>1</sub> seed to F<sub>1</sub> seedlings...



# Theme 4: Support Services and enabling delivery

# 4.1 Development of Online Bioinformatics Course

Nelzo C. Ereful\*, Jeffrey Detras, Victor Jun Ulat, and Richard Bruskiewich \*IRRI, Philippines

Various databases, methodologies, protocols and technology in molecular biology and plant breeding are currently updated or developed each day. To fill in the gap between hands-on research and the rapidly growing electronic biological information, the Crop Bioinformatics online tutorial course is presently developed at the Crop Research Informatics Laboratory, IRRI. The course is designed as an online self-tutorial and will allow scientists and researchers to learn at their own pace, how to cope up with the increasing number of biological information resources. The course covers practical Bioinformatics meant as a guide to plant genome informatics and bioinformatics useful for modern plant breeding, genomics and comparative genomics, data analysis and interpretation. In consultation with community experts, the materials uploaded were designed as an educational resource or reference tool for a two-week course incorporating protocols, key references, photographs, concept illustrations, examples, and suggested applications. Currently, 75% of the modules have been completed, covering topics such as an introduction to biological databases, sequence analysis, Quantitative Trait Loci mapping and microarray analysis. Modules on proteomics, biochemical pathway analysis, metabolomics and systems biology are currently being developed. The online course will be formally launched on December 2007. The evolving course accessible curriculum i s a t http://cropwiki.irri.org/gcp/index.php/SP5/Bioinformatics101/inde x.html.

# 4.2 Management of the GenerationCP Central Registry

Tom Hazekamp\*, Guy Davenport, Samy Gaiji, Raj Sood, Milko Skofic \* Bioversity International

The Central Registry provides the GCP with a central facility where datasets can be registered, stored and shared with the entire GCP community and beyond. Data are presented on the number of datasets registered and files that have been uploaded in the Central Registry from November 2005 to September 2007. Additional statistics on the contribution of projects are also on display.

# 4.3 Generation Challenge Programme: Making data accessible

# Tom Hazekamp and Guy Davenport \*Bioversity International and CIMMYT

The research projects within the Generation Challenge Programme (GCP) generate a large amount of data. The GCP is committed to making these data fully accessible to any authorized user in such a way that they can be fully interpreted and easily converted to any other format. The Bioinformatics and Crop Information Systems subprogramme has developed DATA TEMPLATES and a CENTRAL REGISTRY to help GCP scientists make their data accessible to others. The poster describes how GCP scientists can use these tools to publish their data.

# 4.4 The Interactive Resource Center & Helpdesk: Resources for Scientists

### Theresa M. Fulton Institute for Genomic Diversity, Cornell University

The lack of access to research-related information, trained personnel, key literature, and a support system can be limiting factors in the progress of scientists' research programs, particularly in developing countries. The Interactive Resource Center & Helpdesk (http://irc.igd.cornell.edu) was initiated to address these problems. Currently available on the IRC are protocols, lists of funding opportunities, free literature, tutorials, learning modules, and more. Last year a section was added specifically for sorghum and millet researchers, and now includes literature, links to maps and other resources, and SSR data. Most recently added was a much-requested list of suppliers of laboratory reagents and equipment, and a News section including 2 highlighted journal articles each month as well as other news. The IRC also includes a helpdesk (IGDResourceCenter@cornell.edu), where users can ask questions relating to their research and get personal, interactive support. The Resource Center has had more than 2300 unique visitors in 2007 alone. User emails have been mainly from the continent of Africa, but have also included Iran, Mauritius, India and even within the US. A survey is now being conducted to help select next priorities. Please see http://irc.igd.cornell.edu to take part.

# 4.5 Characterization of Maize germplasm found in Ghana, using the bulking technique

A.Oppong\*, M..Warburton, M. Ewool, R. Thompson, K. Poku-Sekyere, J.N.L. Lamptey, M.D. Quain. \*CSIR-Crops Research Institute, Ghana

Maize steak virus disease and drought are major constraints to maize production in Ghana, as well as in other parts of Africa. They can lead to yield losses of between 15%-80%. The CSIR-Crops Research Institute (CRI) of Ghana and CIMMYT, with the support of the GCP. are making efforts to address these constraints. Maize germplasm collected in Ghana will be characterized both morphologically and genotypically using molecular markers and the bulked fingerprinting technique. A core subset will be created that has been extensively characterized, and will be accompanied by data on origin, pedigree, phenotypes, and markers. The core can be used to develop varieties with desirable traits, the variation for which has been reported in Ghanaian maize populations that will go into the core. The human resource capacity of CRI will also be developed through training in the application of biotechnology tools such as marker assisted selection for desirable traits for variety development.

# 4.6 Task 22 (SP4) – Development and Application of the GCP Domain Model and Associated Ontology

Richard Bruskiewich\*, Jeffrey Detras, Kevin Manansala, Tom Hazekamp, Adriana Alercia, Elizabeth Arnaud, Arllet Portugal, Rowena Valerio, Yunlong Xia, Isaiah Mukema, Thomas Metz, Martin Senger, Guy Davenport, and Graham McLaren

\* *IRRI-CIMMYT Crop Research Informatics Laboratory* The GCP domain model design with associated documentation (<u>http://pantheon.generationcp.org/demeter</u>) was released for production use late last year. Incremental refinements of the model are being made in 2007 based on feedback from various users of the model.

Primary attention in 2007 moved to the formalization of controlled vocabulary and ontology (CVO) used to parameterize the GCP domain model to more precisely capture full research data semantics. Existing third party tools such as OBO-Edit (www.oboedit.org), and controlled vocabulary component of the GMOD Chado relational database (www.gmod.org), as well as, GCP funded software tools are being used to establish and populate an online database of GCP CVO (http://ontology.generationcp.org) for GCP platform software access.

This project relying heavily on importing CVO from existing international initiatives such as the FAO-IPGRI list of multi-crop passport descriptors (MCPD), Plant Ontology, Gene Ontology, MIAME and other sources. For example, this year, the core task team focused on germplasm ontology development derived from MCPD. For microarray analysis, MIAME-Plant (see www.mged.org) ontology is incorporated in the GCP platform version of maxdLoad2 (http://koios.generationcp.org/maxdLoad2/). Members of the task team also participated in a two-day ontology workshop hosted by the Plant Ontology Consortium, with participants from USDA-GRIN and GCP.



# 4.7 Building a Community of Rice Biotechnology in the Mekong Region

Theerayut Toojinda\*, Jonaliza L. Siangliw, Sureeporn Katengam, Watcharapong Wattanakul, Men Sarom, Monthathip Chanpengsay and Toe Aung \*Rice Gene Discovery Unit, National Center for Genetic Engineering and Biotecnology, Thailand

Countries in Mekong Region share similar rice planting ecosystems and constraints in rice production. Application of biotechnology like marker-assisted selection (MAS) in rice breeding had been proven effective in Thailand. Rice Gene Discovery Unit (RGDU), BIOTEC had the opportunity to convey knowledge to its neighboring countries through trainings thus molecular breeding of rice in Mekong Region begun when long term training on MAS was initiated in 2004 as sponsored by Rockefeller Foundation. This training aims to develop popular rice varieties like CAR3, TDK1 and IR53936 from Cambodia, Laos and Myanmar, respectively which lack traits that may improve quality and adaptation in the local area. This made them realize the potential of biotechnology, thus under the GCP project, the lines initially developed can be continued until target location testing. Molecular techniques on OTL/gene identification and MAS were introduced to participants from CARDI (Cambodia), NAFRI (Laos) and DAR (Myanmar) inside and outside RGDU. On site workshops further intensify the enthusiasm of each institute to learn and apply biotechnology not only in breeding programs but also in protecting rich germplasm collections in each country. Moreover, they are seeking for more capacity-building and human resource development programs that will strengthen research and development.



# 4.8 Implementation of Web Services technology in the GCP Consortium

Milko A. Skofic\*, Samy Gaiji, Rajesh Sood, Tom Hazekamp, Mathieu Rouard, Martin Senger, Markus Döring, Javier de la Torre \*Bioversity International

The availability and sharing of data is crucial to the success of the GCP, web services technology has been selected as the main vector to achieve this goal. The poster illustrates the main steps involved in data flow, explains the various operations needed to harmonise information and the technologies used.


### 4.9 Detecting and fine-mapping QTLs with major effects on rice yield under drought stress for deployment via marker-aided breeding

A. Kumar<sup>\*</sup>, R. Venuprasad J. Bernier, R. Serraj, P. Perraju, H.E. Shashidhar, , N.P. Mandal, P.K. Sinha, J.L. Dwivedi, P. Swain, O.N. Singh, L. Bose, S. Robin, R. Chandrababu, D. Tao, D. Spaner and G. Atlin \*IRRI, Philippines

Drought causes substantial yield losses in the rainfed rice ecosystems. Success in backcross breeding to enhance vield in dry environments depends on the ability of one or a few donor genes to generate large effects on tolerance when introgressed into a megavariety. To date, few QTLs with large effects on grain yield under drought have been mapped but none have yet been fine-mapped in rice. IRRI initiated a program in 2002 to systematically identify QTLs with large effects on the grain yield under drought stress. Four major QTLs with drought-specific effects on yield have been identified. Small introgressions from Aday selection in BC<sub>2</sub>-derived sister lines IR77298-14-1-2 and IR77298-5-6, developed with IR64 as a recurrent parent were also shown to differ substantially in vield under severe lowland stress, despite sharing a coefficient of parentage of more than 0.9. The present study will fine-map these OTLs, clarify their physiological effects, confirm performance in the target environment with the IRRI-India Drought Breeding Network, assess their utility in MAB by evaluating them in BC<sub>1</sub> or BC<sub>2</sub> populations with mega varieties as the recurrent parents, and develop efficient protocols allowing initial detection of major loci with effects on yield under stress using bulked segregant analysis (BSA).



4.10 Developing and disseminating resilient and productive rice varieties for drought-prone environments in India: a network approach

A. Kumar\*, J. Bernier, R. Serraj, R. Anitha, S.B. Verulkar, H.E. Shashidhar, S. Hittalmani, N.P. Mandal, P.K. Sinha, J.L. Dwivedi, P. Swain, O.N. Singh, L. Bose, S. Robin, R. Chandrababu, and G. Atlin

\*IRRI, Philippines

Drought regularly affects 23 million ha of rainfed rice in South and Southeast Asia. Cultivars that combine drought tolerance with high vield potential under favorable conditions are therefore an important breeding objective. However, limited progress has been made so far, despite considerable research effort. The Generation Challenge Program supports the IRRI-India Drought Breeding Network that links IRRI and eight Indian breeding programs from the droughtprone regions with the objective of developing drought tolerant cultivars. The network in the first two years of collaborative research has identified breeding lines which yield 1.0 tha<sup>-1</sup> under severe drought stress in uplands, 1.5-2.0 tha<sup>-1</sup> under severe drought stress in shallow lowland, and 2.4 tha<sup>-1</sup> under severe drought stress in medium lowland respectively. These lines also maintain the high yield potential under the non-stress situations in their respective ecosystems. Under the same level of stress, popular varieties now grown in these regions either failed or yielded less than 1.0 tha<sup>-1</sup>. The lines with drought-related QTL RM 511 on chromosome 12 yielded at least three times more than random lines without OTL under severe stress with no difference in yield under nonstress situation.



### 4.11 Ex Ante Impact Assessment of Marker-Assisted Selection Technologies

George W. Norton, \* Vida Alpuerto, Ndrem Rudi \* Virginia Polytechnic Institute and State University

Ex ante impact analysis is being used to provide early estimates of economic benefits of GCP supported research to (a) discover genes for tolerance to saline and phosphorous-deficient soils to enhance rice productivity and (b) develop low cost technologies for pyramiding genes from wild relatives into elite progenitors of cassava. Rice and cassava projects were selected for the impact assessment because they address significant problems on major crops and have advanced sufficiently to identify economically useful products. The assessment approach should be useful to the GCP in documenting progress, and assisting with research prioritization. Economic benefits are projected based on the situation with and without the new traits. Benefits consider (a) area planted to crops affected by target stresses, projected changes in that area, and production of the crops in specific countries, (b) nature of markets for the crops, (c) projected yield and cost changes due to the technologies, (d) estimated time to develop and deploy the DNA marker technologies, (e) estimated time to develop and disseminate superior new cultivars, and (f) the discount rate for future benefits and costs. Significant benefits are due to savings in research time and costs due to molecular-assisted breeding.

# 4.12 Helping breeders to determine the potential geographical utility of adaptation of a variety

Reinhard Simon\*, Anthony Collins, Edson Plasencia, Magna Schmitt, Luis Avila, Henry Juarez, Anne Forbes, Merideth Bonierbale, Roberto Quiroz \*CIP, Peru

Breeders need to plan for the potential geographical distribution of a variety. Amongst the most relevant factors determing the distribution are climate variables. While weather station data exist they are usually not available in a uniform format nor spatially evenly distributed. Thus, geographical information systems (GIS) use interpolated climate surfaces. While some climate surface datasets are globally available they may not be suitable for breeders for having low resolution or reflecting long term averages. A 'weather' database would allow breeders to produce high resolution surfaces for specific regions and growing seasons. We are developing a toolset that will help to fill this gap. The toolset will allow breeders to incorporate their own weather station data and combine it with freely available data sets to achieve a resolution of up to 90 m. This surface can then be used in a GIS in combination with other datasets like soil and road maps. The toolset makes use of a freely available climate interpolator and the GCP bioinformatics platform. A first betaversion and user interface is available via the DIVA-GIS application. The climate interpolator is suitable for subtropical and tropical latitudes. The value added by this activity consists in convenience of access.

### 4.13 The GCP Microarray Data Integration and Analysis System

Ramil Mauleon\*, Guy Davenport, Andreas Magusin, Kouji Satoh, Koji Doi, Violeta Bartolome, Emily Deomano, Yi Zhang, James Wagner, Michael Jonathan Mendoza, Trushar Shah, Richard Bruskiewich, Shoshi Kikuchi, and Hei Leung \*IRRI, Philippines

The Generation Challenge Program Subprogramme 2 (Comparative Genomics for Gene Discovery) leverages the availability of genome sequence across GCP crops and microarray technology to enable rapid discovery of genes potentially useful for many crops. The need for integrative analysis of SP2-generated data led to the commission of SP4 project 2006 Task 08, with the objective of developing a database/data analysis system and compilation of microarray analysis best practices. Publicly available resources (such as published analysis algorithms/methods, open source or free analysis and database software originally developed for human and model organisms studies) are implemented/adapted to fit data from the GCP crops of interest. The types of analysis available are <1> Significance analysis (for multifactor experiments), <2> Enrichment/Association analysis (determining regulatory elements, gene function categories, genome regions, e.g. OTLs, or relevant pathways associated with gene subsets, and <3> Genome-structure dependent expression profile analysis (Regions of Correlated Expression, Aggregation of Differentially Expressed Genes). Result outputs can be imported by visualization tools such as MAPMAN, Generic Genome browser and Apollo. Component software can be for stand-alone use, and development is underway for <1> ability of the component software to use GCP data sources for analysis and <2> integration of these software to the CGIAR High Performance Computing platform. The software suite and accompanying documentation of the system is available at http://cropforge.org/projects/gcpmicroarray/.

# 4.14 A sustainable future for the GCP cluster/grid HPC facilities

Anthony Collins\*, Jayashree Balaji, Denis Diaz, Reinhard Simon, Richard Bruskiewich et al \*CIP, Peru

This project aims to consolidate the 3 global High Performance Computer (HPC) sites created by the GCP into a sustainable cluster/grid facility serving the GCP and it's partners with substantial cost benefit, and with specific support for associated GCP goals. Systems management of the overall global facility is focusing upon client support and usage monitoring to develop a persuasive cost/benefit case leading towards eventual sustainability of the facility beyond the GCP funding umbrella.

A load and user reporting system is being developed to monitor and provide usage statistics of the global facility, as the basis of a capacity needs study.

Further use cases are underway, complementing and extending the achievements to date, as part of the overall suite of facilities available for the GCP community. The 3 sites target:

# <u>CIP</u>

GIS-based site characterization

### <u>ICRISAT</u>

Extending the software resources available in the comparative genomics and population genetics toolbox on the ICRISAT HPC

<u>IRRI</u>

Regions of Correlated Expression analysis of microarray data using the HPC as computation engine (I have a cgi job submission form to show) R/MAANOVA (Microarray Analysis of Variance) significance analysis of microarray data

# 4.15 Strategic approaches to targeting technology generation: Assessing the coincidence of poverty and drought-prone crop production

Glenn Hyman\*, Sam Fujisaka, Peter Jones, Stanley Wood, Carmen de Vicente and John Dixon \*CIAT, Colombia

The world's poorest and most vulnerable farmers on the whole have not benefited from international agricultural research and development. Past efforts have tried to increase the production of countries in more favourable environments; farmers with relatively higher potential for improvement benefited most from these advances. This study prioritizes areas of high poverty, the key problem of high drought risk and the crops grown and consumed in these areas. We used global spatial data on crop production, climate and poverty (as proxied by child stunting) to identify geographic areas of high priority for crop improvement. Using spatial overlay, drought modeling and descriptive statistics, we identified where best to target technology generation to achieve its intended human welfare goals. Analysis showed that drought coincides with high levels of poverty in 15 major farming systems, especially in South Asia, the Sahel and eastern and southern Africa, where high diversity in drought frequency characterizes the environments. Twelve crops make up the bulk of food production in these areas. We developed a database for use in agricultural research and development targeting and priority setting to raise the productivity of crops on which the poor in marginal environments depend.

# 4.16 Video and radio bridging worlds

# M. Malick Soumah\*, Dr Paul Van Mele \*APEK Agriculture, Guinea

The partnership aims to build on complementary skills, knowledge and networks to test the convergence between participatory approaches and media in order to build capacity of R&D and farmers in community-based seed production (CBSS) and participatory learning and action research (PLAR), scale up farmer experimentation and learning and stimulate South-South exchange of local innovations. Rice seed health videos produced with rural women in Bangladesh were translated by WARDA into French and various African languages, and distributed to more than 15 countries. In Guinea, WARDA and the national agricultural research institute (IRAG) established community-based seed production systems with multiple NGOs, including Association for Economic Development of Kindia (APEK). The seed health videos re-inforced capacity among farmers and partner organizations. More than 5,200 farmers were reached in 6 months. The videos contributed to better seed quality in local and improved varieties. Furthermore, they enabled linkages of APEK with all local actors. The methodological backstopping (PLAR and CBSS) of West African Rice Development (WARDA), provision of first generation foundation seed, strengthening international exchange of (local) innovations and learning tools, an Agricultural Research Institute of Guinea (IRAG) with the national coordination assure the production of second generation foundation seed, training of trainers in CBSS and PLAR and the Association for Economic Development of Kindia (APEK) is doing the local networking with producer groups, projects and partners; training seed producers and organising video shows. Train partners in developing high quality farm radio and video programs jointly produce radio and video programs with farmers on CBSS and PLAR.



For more information on the Generation Challenge Programme, please visit our Web site at www.generationcp.org

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#### **Consortium members**

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