



# Poster abstracts

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CULTIVATING PLANT DIVERSITY FOR THE RESOURCE-POOR

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## **CGIAR Generation Challenge Programme**

**General Research Meeting  
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### **Poster abstracts**

#### **Generation Challenge Programme (GCP)**

**Hosted by CIMMYT**

(Centro Internacional de Mejoramiento de Maíz y Trigo;  
the International Maize and Wheat Improvement Center)

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# TABLE OF CONTENTS

|  |           |
|--|-----------|
| <b>Theme 1: Comparative and applied genomics.....</b>  | <b>1</b>  |
| <b>Cassava .....</b>   | <b>2</b>  |
| 1.1: Development of a genetic resource base for drought and biotic stress improvement in cassava .....   | 2         |
| <b>Maize .....</b>   | <b>3</b>  |
| 1.2: QTLs and genes for aluminium tolerance in maize.....  | 3         |
| <b>Pigeonpea .....</b>   | <b>4</b>  |
| 1.3: Single-nucleotide polymorphisms (SNPs) for diversity analysis and linkage mapping in pigeonpea (Cajanus cajan L.) .....   | 4         |
| <b>Rice .....</b>  | <b>5</b>  |
| 1.4: Genetic architecture of aluminium tolerance in rice (O. sativa) determined through genome-wide association analysis and QTL mapping .....                             | 5         |
| <b>Sorghum.....</b>  | <b>6</b>  |
| 1.5: Improving phosphorus efficiency in sorghum by the identification and validation of sorghum homologues for Pup1, a major QTL underlying phosphorus uptake in rice..... | 6         |
| <b>Theme 2: Integrated crop breeding .....</b>   | <b>7</b>  |
| <b>Beans .....</b>   | <b>8</b>  |
| 2.1: Improving drought adaptation in Pinto bean for the semiarid highlands of Mexico .....   | 8         |
| 2.2: Improving common bean productivity for marginal environments in sub-Saharan Africa .....  | 9         |
| <b>Cassava .....</b>   | <b>10</b> |
| 2.3: Participatory clonal evaluation of introduced cassava genotypes in agro-ecological zones of Ghana .....   | 10        |
| 2.4: Integrated breeding strategies for disease resistant and nutritious cassava varieties in Africa .....   | 11        |
| 2.5: Drought tolerance performance of cassava genotypes in the savannah ecological zone of Ghana .....   | 12        |
| <b>Chickpeas.....</b>  | <b>13</b> |
| 2.6: Deployment of molecular markers for developing high yielding wilt resistance chickpea genotypes.....  | 13        |
| 2.7: Improving drought tolerance of Kenyan chickpea variety by introgressing root QTL traits using Marker Assisted Backcross Crossing (MABC) .....                         | 14        |
| 2.8: Integrated chickpea breeding approach to enhance crop responses to climate change in Ethiopia .....   | 15        |
| 2.9: Development and implementation of genomic and genetic resources for improving chickpea productivity for marginal environments in Sub-Saharan Africa and Asia.....     | 16        |

|  |           |
|--|-----------|
| <b>Cowpeas</b> .....   | <b>18</b> |
| 2.10: Application of Marker-Assisted Selection for Striga Resistance in Cowpea ( <i>Vigna unguiculata</i> L. WALP) .....   | 18        |
| <b>Groundnuts</b> .....  | <b>19</b> |
| 2.11: Pattern of flowering and yield components under water stress imposed during reproductive phase in groundnut ( <i>Arachis hypogaea</i> L.) .....  | 19        |
| 2.12: Consensus genetic maps for drought tolerance related traits and foliar diseases resistance traits in cultivated groundnut ( <i>Arachis hypogaea</i> L.) .....                              | 20        |
| <b>Maize</b> .....   | <b>21</b> |
| 2.13: Maize genetic analysis of gray leaf spot resistance .....  | 21        |
| 2.14: Development of Maize Drought Tolerance in Indonesia: Status and Progress .....   | 22        |
| 2.15: Screening for tolerance to Al toxicity and P-efficiency in Kenyan maize germplasm .....  | 23        |
| 2.16: Exploiting the genetic diversity of available maize germplasm using markers to cluster inbred lines collection at KARI Katumani .....  | 24        |
| 2.17: AMDROUT: Perspective and Progress .....  | 25        |
| <b>Rice</b> .....  | <b>26</b> |
| 2.18: The use of crop growth performance data for characterization and mapping of variability in soil water holding capacity.....  | 26        |
| 2.19: Pyramiding of resistance QTL/gene into Mekong mega varieties using marker-assisted backcrossing .....  | 27        |
| 2.20: Root phenotyping for drought tolerance: evaluation of the OryzaSNP panel using a range of methods .....  | 28        |
| 2.21: Is drought screening in dry season reliable for genotype selection for rainfed lowland rice- a case study in Laos?.....  | 29        |
| 2.22: MAGIC Rice: production, characterization, and its use in breeding networks .....   | 30        |
| 2.23: Development of drought-tolerant rice varieties adapted to West Africa lowland agrosystem .....   | 31        |
| <b>Sorghum</b> .....   | <b>32</b> |
| 2.24: Variability for post-flowering drought tolerance in sorghum reference set.....   | 32        |
| 2.25: Development of sorghum isogenic male sterile and restorer lines and isogenic hybrids for use as a tool in field validation of the effect of the gene for aluminium tolerance - AltSB ..... | 33        |
| 2.26: Phenotyping of panicle characteristics in a sorghum mapping population in Mali.....  | 34        |
| 2.27: Development and evaluation of drought-adapted sorghum germplasm for Africa and Australia.....  | 35        |
| <b>Wheat</b> .....   | <b>36</b> |
| 2.28: Exploiting AB genome diversity for creating new wheat germplasm for enhanced drought/heat tolerance.....   | 36        |
| 2.29: Association mapping of dynamic developmental plant height in common wheat .....  | 37        |
| 2.30: Physiological trait based phenotyping of International core set for drought tolerance in wheat.....  | 38        |

|   |               |
|---|---------------|
| <b>Theme 3: Crop information systems .....</b>  | <b>39</b>     |
| 3.1: Construction of Genetic Linkage Maps and Mapping of Quantitative Trait Loci in the Integrated Software Package QTL IciMapping v3.1.....  | 40            |
| 3.2: Molecular characterisation of Burkina Faso rice landraces using 22 microsatellite markers and establishment of a core collection .....   | 41            |
| 3.3: Coverage based consensus calling (CbCC) of short sequence reads and comparison of CbCC-results for the identification of SNPs in chickpea, a crop species without a reference genome ..... | 42            |
| 3.4: ISMU: An easy-to-use pipeline for identification of SNPs based on next generation sequencing (NGS) data.....   | 43            |
| 3.5 & 3.6: Expansion of the crop ontology by adding cassava (3.5) and Musa (3.6) trait ontologies .....   | 44            |
| 3.7: To contribute crop traits lists using the new online Crop Ontology Curation and Annotation tool.....   | 45            |
| 3.8: QuMARS: A QU-GENE application module to simulate marker assisted recurrent selection and genomic selection .....   | 46            |
| 3.9: A new web interface for rice germplasm and breeding data: integrated data browser and StudyMart.....   | 47            |
| 3.10: Molecular Breeding Services at IBP/GCP .....  | 48            |
| 3.11: Generation Atlas .....  | 49            |
| 3.12: Information management and data curation support services of IBP/GCP .....  | 50            |
| <br><b>Theme 4: Capacity building and Product delivery.....</b>   | <br><b>51</b> |
| 4.1: Exploiting the benefits of sharing knowledge and technology among research partners towards a cassava MAB-based program: experiences from Uganda.....                                      | 52            |
| 4.2: TLI students for analysis of drought tolerance in common bean .....  | 53            |
| 4.3: Introgression of Saltol and fine-mapping of reproductive stage QTLs to develop rice varieties highly tolerant of salt stress.....  | 54            |
| 4.4: Communities of Practice Generation Challenge Initiative for Rice in West Africa .....  | 55            |



# **Theme 1:** Comparative and applied genomics



## Cassava

### 1.1: Development of a genetic resource base for drought and biotic stress improvement in cassava

*Morag Ferguson\*, Luis Augusto Becerra Lopez-Lavalle, Jorge Franco, Dominique Dumet, Daniel Debouck, Peter Kulakow, Hernan Ceballos, Geoffrey Mkamilo, Elizabeth Parkes, Chiedozie Egesie, Melaku Gedil, Ismail Rabbi, Edward Kanju and Clair Hershey*

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It can be argued that for a germplasm reference set to be broadly useful to plant breeders it should not only represent diversity but also encompass germplasm with a range of responses to traits of interest. The objectives of this project are to (1) define a reference set in cassava that incorporates diversity as well as germplasm with specific traits of interest to breeders (2) to characterise this germplasm with 1,536 SNPs using an Illumina GoldenGate assay (Illumina Inc., San Diego, CA), and (3) make the reference set available to collaborating NARS breeding programs. Initially approximately 550 cassava varieties will be genotyped using SNPs. This will consist of 100 varieties from southern, eastern and central Africa, 200 IITA and CIAT breeder-preferred germplasm lines and 250 IITA and CIAT diverse germplasm lines based on 30 SSR markers. From the SNP results a set of about 200 genotypes will be selected that reflect both diversity and breeder preferred traits to form the GCP reference set. This reference set will be dynamic and should be reviewed and updated on a regular basis as new gene combinations become available. Cassava varieties in the GCP reference set will be placed *in vitro* and, as far as phytosanitary regulations allow, will be available for distribution under SMTA, but initially through this...

*Related GCP project– G7010.01.01: Improvement and evaluation of the existing cassava reference set for Africa (PI: Morag Ferguson, IITA)*

**Notes:**



## Pigeonpea

### 1.3: Single-nucleotide polymorphisms (SNPs) for diversity analysis and linkage mapping in pigeonpea (*Cajanus cajan* L.)

Rachit K Saxena,\* Serah Songok, Anuja Dubey, R Varma Penmetsa, Hari Upadhyaya, KB Saxena, Paul Kimurto, Doug Cook, Greg May, Rajeev K Varshney

\*International crops research institute for the semi-arid tropics (ICRISAT), Patancheru, Hyderabad, India; email: r.k.varshney@cgiar.org; r.saxena@cgiar.org

Pigeonpea is one of the most important legume crops for human and livestock feed. However, the paucity of the genomic resources coupled with low genetic diversity in cultivars has hindered the application of molecular-assisted breeding programmes. A crucial step in the successful application of molecular assisted breeding programmes is the development of high-throughput and cost-effective molecular markers, such as single-nucleotide polymorphisms (SNPs). Here, we describe the development of KASPar assays for 1,834 SNPs. A set of 184 pigeonpea genotypes comprising of parental lines of mapping populations, breeding lines and wild relatives representing 10 different countries around the globe, were chosen for genotyping with the developed SNP markers. As a result 1,226 SNPs showed polymorphisms in the germplasms analyzed. Less genetic variation was observed within cultivated species; however, phenetic analysis clearly distinguished all wild species genotypes from each other and from the cultivated genotypes. The close relationships of *C. cajanifolius* with cultivated pigeonpea (*C. cajan*) has been verified in this study. By using 188 F2 lines from ICP 28 × ICPW 94 cross, a linkage map comprising 875 SNP loci has been developed that should be useful for accelerating molecular genetics and breeding applications in pigeonpea.

*Related GCP project–G4008.47: Genomic resources (ESTs, markers and maps) developed for pigeonpea to enable trait/gene identification (PI: Gregory D May, NCGR)*

## Notes:

5

## Sorghum

1.5: Improving phosphorus efficiency in sorghum by the identification and validation of sorghum homologues for *Pup1*, a major QTL underlying phosphorus uptake in rice

*Jurandir Magalhães,\* Alvaro Vilela Resende, Barbara Hufnagel, Claudia Guimaraes, Eva Weltzien, Fred Rattunde, Jiping Liu, João Herbert Moreira Viana, Leon Kochian, Martha Hamblin, Matthias Wissuwa, Randy Clark, Robert Schaffert, Sam Gudu, Sharon Mitchell, Sigrid Hewer, Soumana Souley, Steve Kresovich, Sylvia Morais de Souza, Theresa Fulton, Vera Alves, Willmar Leiser and Zhangjun Fei*

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Low productivity due to soil constraints is a serious problem in many parts of Africa, where sorghum is a staple food supporting millions of the rural poor. *Pup1* is a major QTL located on rice chromosome 12 that underlies phosphorus uptake. The aluminium tolerance gene, *Alt<sub>SB</sub>*, is an Al-induced root citrate efflux transporter and citrate can mobilize P that is fixed in the soil clay. Association analysis is being used to identify significant associations between allelic variation at *Pup1/Alt<sub>SB</sub>* and P efficiency. A sorghum panel was phenotyped for P-efficiency traits in the field in 2010 and a second experiment is now underway both at EMBRAPA and JIRCAS. Root traits were assessed in controlled conditions. Sequence similarity analysis identified 7 *Pup1* candidate genes in sorghum and Sb07g00284, located on chromosome 7, was the top hit. Using Primer-blast, we designed primers specific to each gene family member. We are now sequencing amplicons for SNP discovery and association analysis will follow with both field and root morphology data. Primers for quantitative analysis of gene expression were also designed and optimized to assess *Pup1* candidate gene expression in 6 sorghum accessions. This approach is expected to identify functional homologues of *Pup1* in sorghum.

*Related to GCP project—G7010.03.06: Improving phosphorus efficiency in sorghum by the identification and validation of sorghum homologues for Pup1, a major QTL underlying phosphorus uptake in rice, and identification of other P efficiency QTLs (PI: Jurandir Magalhães, EMBRAPA)*

**Notes:**

## **Theme 2:** Integrated crop breeding

Jorge A. Acosta-Gallegos\* and Edgar Espinoza-Trujillo

This project aims to improve drought adaptation and productivity by crossing two drought resistant cultivars, Pinto Villa and Pinto Saltillo. Trials were conducted on 289 F<sub>5</sub> families under irrigation and terminal drought during 2010 and 2011 at two locations in Guanajuato, Mexico. Data from the 2010 trials were used to conduct a stepwise multiple regression analysis. Traits that showed to be significant in the model were, biomass, HI, length of the reproductive stage, days to flowering and 100-seed weight. From these, biomass and HI contributed largely to the observed variation in yield (53 and 33%). The yield of both parents under stress was similar to the highest yielding families. In 2011 data were taken on leaf temperature (LT) and leaf water content (LWC) at the pod elongation stage among other traits. Under both moisture conditions families displayed large variation for all recorded traits (data to be presented). Under stress, plants displayed higher average LT, 23.6 vs 22.4; lower temperature differential, 9.8 vs 11.0 and lower LWC, 3.6 vs 4.2 A 't' test at p≤0.01 indicated highly significant differences between moisture conditions. A low relationship between LT and LWC was observed. The genotyping of this population is being conducted.

*Related GCP project—G4008.11: Dry bean improvement and marker-assisted breeding for diseases and abiotic stresses in Central America and the Caribbean (PI: Jorge A Acosta-Gallegos, INIFAP)*

**Notes:**

[illegible]

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*Related GCP projects—G6007.03/G6010.03: Improve common bean (*Phaseolus vulgaris* L) productivity for marginal environments in sub-Saharan Africa (PI: Steve Beebe, effective October 2010; previous PI: Matthew Blair (CIAT))*

[illegible]



## Cassava

### 2.3: Participatory clonal evaluation of introduced cassava genotypes in agro-ecological zones of Ghana

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A major staple in Ghana and contributing 22% of Agricultural GDP, cassava has assumed industrial and cash crop status in the country. However, this good and reliable cheap source of carbohydrates is susceptible to cassava mosaic virus (CMD) and other devastating diseases and pests, as well as post harvest physiological deterioration. Cassava improvement in Ghana, with the support of the International Institute of Tropical Agriculture (IITA), has yielded several varieties. Adoption challenges and susceptibility of some varieties to diseases and pests necessitated this study. New improved varieties are needed to feed the growing cassava-based industry. Marker assisted selected genotypes resistant to pests and diseases developed by the International Center of Tropical Agriculture (CIAT) and evaluated by the Crops Research Institute (CRI) together with farmers and end-users, identified outstanding clones CR52A-31 and CR52A-25 with starch levels between 26-30% and root yields of 50 to 62 t/ha. Genotype CR 42-4 recorded the highest yield of 101.2 t/ha at Ejura location but the second highest yield across all locations. Farmers preferred two other genotypes CR52A-4 and AR14-10. The materials have been crossed to local varieties with unique characteristics for further evaluation. Participatory testing and the use of MAS has accelerated the improvement of cassava and promise to ensure enhance adaptation and adoption.

*Related GCP project– G4008.26: A cassava breeding community of practice in Africa for accelerated production and dissemination of farmer-preferred cassava varieties resistant to pests and diseases (PI: Emmanuel Okogbenin, NRCRI)*

**Notes:**

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*Related GCP project—G7010.01.02: Improving and deploying markers for biotic stresses in cassava (PI: Chiedozie Egesi, NRCRI)*

**Notes:**

This image shows a full page of primary-ruled notebook paper. It features ten sets of horizontal lines across the page. Each set consists of a solid top line, a dashed middle line, and a solid bottom line, providing a guide for letter height and placement. The paper is otherwise blank, with no margins or additional markings.

## 2.5: Drought tolerance performance of cassava genotypes in the savannah ecological zone of Ghana

*Kwabena Acheremu\*, Joseph Adjebeng-Danquah, Samuel Kwadwo Asante, Bright Boakye-Pepurah and Elizabeth Yaa Parkes*

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Eighteen cassava genotypes, introduced from IITA, CIAT and landraces were evaluated for two seasons for physiological traits that contributed to tolerance to drought conditions in Northern Region of Ghana. The location used for the study falls within, the savannah ecological zone, that is noted for its inherent long dry spell and great variability in the distribution and amount of precipitation that could result in the reduction in cassava root yield of 30-60% and even 100% when the crop fails completely. The cassava genotypes were studied for drought tolerant attributes such as; leaf retention, plant height, canopy spread, stem diameter and yield parameters. The genotype CTSIA 48 recorded the highest leaf retention with an average leaf number of 160, and CTSIA 8, the highest canopy spread of 1.134m. The genotype 96/409 recorded the highest root yield of 12.50 t/ha. The genotype 96/409 showed stability in terms of performance in the drought environment for both seasons. The average amount of rainfall during the period of study was 250mm. The genotypes would be used in the improvement of the farmer preferred landraces, to introgress the drought tolerance trait.

*Related GCP project:* Not applicable

**Notes:**

Bharadwaj Chellapilla,\* Shailesh Tripathi, Rajeev Varshney and  
Kumar Jitendra.

Development of breeding material through the deployment of MABC and MARS is urgently required to develop high yielding wilt resistant lines in chickpea, the most important pulse crop of India. At IARI, New Delhi a core set of markers for *fusarium* wilt (TA 96, TA 110) and yield QTLs (TR 56, TA 78, TR 29, TA 47) have been identified through mapping related activities. The strategy being adopted involves in summary, crossing the selected parental genotype Pusa 372 (high yielding, wilt susceptible) with WR 315 (wilt tolerant donor parent) and F1 generated will be backcrossed with the recurrent genotype. The BC1F1 generated will be screened with targeted polymorphic markers to identify heterozygote plants carrying the targeted alleles. The selected BC1F1 plants will be backcrossed with the recurrent genotypes. As per planned schedule of this proposal, the BC3F1 plants will be screened with the markers and the selected plants will be selfed to generate the BC3F2. These plants will be screened to select the homozygous BC3F2 plants carrying the wilt resistance allele (foreground selection) and high yielding QTLs (background selection). These selected plants will be selfed to generate BC3F3 progenies.

Related GCP project: Not applicable

**Notes:**

[illegible]

## 2.7: Improving drought tolerance of Kenyan chickpea variety by introgressing root QTL traits using Marker Assisted Backcross Crossing (MABC)

*Paul Kimurto,\* Moses Oyier, Richard Muliwa, Sarah Songok, Bernard Towett, Erick Cheruiyot, Rajeev Varshney, Pooran Gaur, Thudi Mahender, Gangarao Rao and Said Silim*

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Drought is the leading constraint in chickpea production in Semi arid tropics. Previous efforts to improve drought tolerance have been hindered due to its quantitative nature. Recent research efforts have availed the genetic resources especially molecular markers and root QTL traits conferring resistance to drought. This study introgressed these QTL root traits identified in ICRISAT donor parent genotype ICCV 4958 into Kenyan cultivar ICCV 97105 (recurrent parent) through MABC. Four markers STMS11, GA24, ICCM0249 and TAA170 were used to track QTL traits from one generation to the next. Crosses (ICCV 97105 x ICCV 4958) were made to introgress the drought QTL traits to obtain F1 and planted; DNA extracted and analyzed for QTL presence as heterozygote and backcrossed to the recurrent parent to obtain BC1F1 seeds and process was repeated to finally obtain BC3F1. These will be selfed to obtain BC3F2 and BC2F2, which will be planted in the field and compared with the RP ICCV97105 to confirm the presence of root QTL; determine the improvement in drought tolerance and confirm the agronomic performances of the RP in comparison with newly developed progenies. The seeds will be increased and preserved for further research.

*Related GCP project—TLI-Improving Tropical Legume Productivity for marginal environments in sub-Saharan Africa and South Asia: Phase II [G6010.04: Improve chickpea productivity for marginal environments in sub-Saharan Africa and South Asia (PI: Rajeev K Varshney, ICRISAT)]*

**Notes:**



## 2.9: Development and implementation of genomic and genetic resources for improving chickpea productivity for marginal environments in Sub-Saharan Africa and Asia

Rajeev K Varshney,\* Pooran Gaur, Mahendar Thudi, L Krishnamurthy, NVPR Gangarao, Hari Sharma, Rachit Saxena, Aravindkumar Jukanti, Paul Kimurto, Serah Songok, Richard Mulwa, Asnake Fikre, Shailesh Triapthi, Ch Bharadwaj, Subhojit Datta, SK Chaturvedi, N Nadarajan, MS Sheshashayee, Reyazul Rouf Mir, Pavana Hiremath, Himabindu Kudapa, Sabhyata Bhatia, Abhishek Rathore, Trushar Shah, Hari Upadhyaya, Andrzej Kilian, Akhilesh Tyagi, Mingcheng Luo and Doug Cook

*\*International crops research institute for the semi-arid tropics (ICRISAT), Patancheru, Hyderabad, India;  
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Significant progress has been made to develop and utilize large-scale genomic and genetic resources in chickpea breeding programmes. Large-scale genomic resources including >1600 SSRs, DArT array (>15,000 features), GoldenGate Assay (768 SNPs), KASPar assays (>2000 SNPs), a high-density reference genetic map and several intra-specific genetic and QTL maps have become available. BAC-fingerprinting based whole-genome physical map is also being developed. Several superior lines with improved drought tolerance and insect resistance for Ethiopia, Kenya and India were identified based on phenotyping of the reference set. Employing these lines, development of >70 pre-breeding populations and MAGIC populations is in progress. A genomic region harboring several QTLs for drought tolerance traits contributing >30% phenotypic variation was introgressed into three elite cultivars and >20 promising BC<sub>2</sub>F<sub>3</sub> lines have been identified.

**Notes:**







## Groundnuts

### 2.11: Pattern of flowering and yield components under water stress imposed during reproductive phase in groundnut (*Arachis hypogaea* L.)

*Halilou Oumarou\*, Falalou Hamidou, Vincent Vadez*

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Groundnut typically experiences a range of water stress with a dramatic negative impact on yield when it occurs at reproductive phase. This work investigated the effect of water stress imposed progressively at early flowering stage of ten genotypes of groundnut under glasshouse conditions and contrasting for drought tolerance. Three water regime were imposed: A well-watered treatment where plants were maintained at field capacity, a water stress imposed by reducing the soil water content by 120g daily until the fraction of transpirable soil water (FTSW) was depleted, before re-watering at field capacity, and a water stress imposed until the FTSW dropped to 20%, maintained at these FTSW level for 15 days before rewatering at field capacity. Transpiration, flowering pattern, yield and its components were measured. During 30 days after starting flowering, no significant genotypic variation for transpiration was observed, and tolerant genotypes produced more flowers (61) than the sensitive (17) except JL24 (56). After water stress was released, sensitive genotypes produced more flowers. Under water stress conditions, the flowers abortion in sensitive genotypes (47%) was less than in tolerant (61%) but the pods and seeds weight were significantly ( $P < 0.001$ ) higher in tolerant than in sensitive. This study showed also that the yield and its components were more affected by stress1 than stress2.

*Related GCP project—G6010.01: Improve groundnut productivity for marginal environments in sub-Saharan Africa (PI: Vincent Vadez, ICRISAT)*

**Notes:**

[illegible]

## 2.12: Consensus genetic maps for drought tolerance related traits and foliar diseases resistance traits in cultivated groundnut (*Arachis hypogaea* L.)

*Manish Pandey\*, Rajeev Varshney, Bhimana Gautami, Vanhi  
Sujay, MVC Gowda, T Radhakrishnan, Vincent Vadez, Shyam  
Nigam, Trushar Shah, Hari Upadhyaya, Sachiko Isobe, Guohao He,  
David Bertioli, Steven Knapp and Douglas Cook*

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The realized yield in groundnut in the semi-arid region remains low due to its exposure to drought and foliar diseases. With an objective to identify genomic regions for drought tolerance and foliar disease resistance, two consensus maps were developed using five individual genetic maps based on populations segregating for drought tolerance (TAG 24  $\times$  ICGV 86031, ICGS 44  $\times$  ICGS 76 and ICGS 76  $\times$  CSMG 84-1) and foliar diseases (TAG 24  $\times$  GPBD 4 and TG 26  $\times$  GPBD 4). These maps are comprised of 293 loci (drought tolerance) 225 loci (foliar disease resistance). A comprehensive QTL analysis for drought tolerance has identified 153 main effect QTLs (M-QTLs) and 25 epistatic QTLs (E-QTLs) that are scattered throughout the genome that indicates non-applicability of MABC approach for molecular breeding for drought tolerance. On the other hand, 43 M-QTLs have been identified for resistance to foliar diseases and one genomic region contains major QTL each for late leaf spot and rust resistance with a high phenotypic variation (68-83%). Therefore, MABC approach is being used for molecular breeding for rust resistance and genomic selection approach is being explored for molecular breeding for drought tolerance.

*Related GCP project—G6007.01/G6010.01: Improve groundnut (Arachis hypogaea L) productivity for marginal environments in sub-Saharan Africa (PI: Vincent Vadez, ICRISAT)*

**Notes:**

20



## 2.14: Development of Maize Drought Tolerance in Indonesia:

## Status and Progress

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*\*Indonesian Cereals Research Institute (ICERI), Maros, Indonesia;*

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Maize is very important crop in Indonesia as second staple food crop after rice. Prime varieties and advance technology is required to overcome the climate change, since maize crop is cultivated at dry and acid land in Indonesia. 79% maize crop in Indonesia is cultivated in the dry land; therefore it is required drought tolerant (DT) varieties. Asian maize drought tolerance (AMROUT) is joint project between CIMMYT and GCP facilitate Indonesian researcher to interact with other Asian countries producer, member of AMROUT. The establishment of maize producer network enables Indonesia to exchange the genetic material, experience, and transfer technology in order to accelerate the development of drought tolerant varieties. The aim of the research are to develop new hybrid maize varieties which is early maturity  $\leq 90$  days, high yielding potency ( $> 10$  t/ha) and tolerant to drought. The research project consisted of two activities: First: Phenotyping of AMROUT hybrids and lines under drought tolerant and normal irrigation in Indonesian. We evaluated of 09-DRT-CAGCP-24 population on 2009. The results of evaluation showing that crosses of (CA00310/AMATLC0HS71-1-1-2-1-1-1-B\*6-B- B-B-B)/CML442; (CA14517/P145C4MH7-1-B-1-1-B-1-1-BBBB-B-B-B-B-B)/CML488; (PT963216-B-B-B-B-B-B-B-B-B-B)/DRB-F2-60-1-1-1-BBB and CML429/CML470 have get yield  $> 8$  t/ha, better than the best local check variety. For this year, we evaluate of AMROUT trial under drought condition in ICERI Experimental station at Bajeng, South Sulawesi...

*Related GCP project– G4008.56: Improving drought tolerance for maize in Asia*

AMDROUT: Asian maize drought-tolerance project (PI: Bindiganavile S Vivek, CIMMYT)

**Notes:**



## 2.16: Exploiting the genetic diversity of available maize germplasm using markers to cluster inbred lines collection at KARI Katumani

James G. Gethi\* and Humberto Gomez Paniagua

\*Kenya Agricultural Research Institute (KARI), Machakos;  
jgethi@wananchi.com

Maize is very prone to water stress especially during the flowering stage, and enormous efforts have gone towards developing varieties that can provide reasonable yield in water stress environments. To achieve this, scientists have explored various avenues such as studying the physiology of drought in tropical materials, screening for earliness and search for markers linked to drought QTLs. In the drylands of Kenya, efforts have been directed towards development of drought tolerant materials that flower early to maximize on available moisture and better survive when moisture is limiting. To achieve this, 407 inbred lines developed at KARI and obtained from GCP Project #13, #15 and CIMMYT were genotyped using 46 Simple Sequence Repeat (SSR) markers. The objective was to classify the inbred lines into genetically related groups and help structure the breeding program to take advantage of possible heterotic combinations for drought stress tolerance and therefore develop better products.

The inbred lines were genotyped under the GCP Genotyping Support Service. The SSR markers used covered the whole of the maize genome. The mean Polymorphic Information Content (PIC) of the SSR markers was 0.6115. The mean allele frequency was 0.4651 with a mean allele number of 7. The gene diversity ranged from 0.2270 to 0.9014...

*Related GCP Project—G8009.06.06/Activity 3.2.6: Genotyping Support Service (excludes 3<sup>rd</sup> call, whose implementation will begin in 2011) (PI: Chunlin He, effective October 2010/Humberto Gómez-Paniagua, GCP) (This project follows on from G4007.21, which ended in 2008, and G4009.01, which ended in 2009)*

**Notes:**

[illegible]





## Rice

**2.18: The use of crop growth performance data for characterization and mapping of variability in soil water holding capacity**

*Alhassan Tswako Maji,\* Emmanuel Myimaorga Abo, Mark Nwoye  
Ukwungwu, Samuel Oladele Bakare, Abraham Attah Shaibu,  
Marie Noelle Ndjondjop, and Mohammed.Bashiru*

*\*National Cereals Research Institute (NCRI) Badeggi, PMB 8, Bida,  
Niger State, Nigeria;  
email: tswakoma@gmail.com*

Understanding and managing spatial variability in soils has become one of the main strategies to optimize rice production. The test of uniformity was conducted on 0.68 ha of land divided into 4x4 (16m<sup>2</sup>) main plots, and each main plot sub-divided into 4 sub-plots of 2x2m from which representative plant height, number of tillers, plant fresh weight and dry weight were sampled and measured. The average value of the 4 plants from the main plot was used as the plot value. These values were further transformed to return a maximum value of 1 by dividing all scores by the highest value. The results showed significant variation in soil water holding capacity as a factor of crop growth variation. Of the four growth parameters measured, tiller count gave the list coefficient variation of 3.2%, plant height, 10.8%, plant dry matter, 37.5% and plant fresh weight had the highest coefficient of variation of 147.1. Combined transformed data was used to physically classify and map the plots. The classes have scores of 0.9-1.0, most favourable, 0.7-0.8, favourable, 0.5-0.6, dry and 0.3-0.4, very dry. The results provide rice breeders and agronomist awareness to develop protocol and management practices for drought tolerance phenotyping.

*Related GCP project—G7010.04.01: Improving rice productivity in lowland ecosystems of Burkina Faso, Mali and Nigeria through marker-assisted recurrent selection for drought tolerance and yield potential (PI: Marie Noëlle Ndjiondjop, AfricaRice)*

**Notes:**

This image shows a full page of white paper with horizontal dashed lines, typical of primary-ruled notebook paper. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

*\*Rice Gene Discovery Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand; e-mail: jsiangliw@gmail.com*

*Related GCP project—G4009.09: CoPs – Strengthening rice breeding programmes using genotyping and improving phenotyping capacity for biotic and abiotic stresses in the Mekong region (PI: Jonaliza Lanceras-Siangliw, RGDU-BIOTEC)*

**Notes:**

[illegible]

## 2.20: Root phenotyping for drought tolerance: evaluation of the OryzaSNP panel using a range of methods

Amelia Henry,\* Adam Price, Akira Yamauchi, R Chandra Babu,  
Len Wade, Kenneth McNally

*\*International Rice Research Institute (IRRI), Los Baños,  
Philippines;  
email: A.Henry@cgiar.org*

Rice is highly susceptible to drought stress compared to other crops, which is thought to be attributable in part to a shallow root system. By linking genetic polymorphism to root growth at depth, molecular markers could be identified to improve drought tolerance in rice. In this project, root phenotyping was conducted on the OryzaSNP panel, a collection of 20 diverse cultivars and landraces within the aus, indica, and japonica groups that have been genotyped with over 160,000 SNP markers. Each root phenotyping method was refined for studying rice roots under drought, in order to characterize rice genetic diversity for drought response and root growth at depth. Phenotyping systems included field conditions, 1-m depth lysimeters, 1-m tall transparent-walled rhizotrons, containers with herbicide placement at depth, pinboard root boxes, line-source greenhouse studies, and containers with wax-layers. All studies focused on evaluating deep root growth, but other parameters including water uptake, root branching, and penetration of compacted layers were assessed in some systems. Initial results point to aus lines as promising root trait donors for drought. A collective analysis of these results will be provided to geneticists for identification of molecular markers for deep root growth.

*Related GCP project—G3008.06: Targeting drought-avoidance root traits to enhance rice productivity under water-limited environments (PI: K McNally, IRRI, effective June 2010; previous PI, Rachid Serraj, IRRI)*

**Notes:**

[illegible]

\* Rice & Cash Crop Research Center, National Agriculture & Forestry Research Institute, P.O. box 7170, Vientiane, Lao PDR;  
e-mail: phetmanyseng@gmail.com

*Related GCP project—G3008.09: Breeding drought tolerance for rainfed lowland rice in the Mekong region (PI: Boonrat Jongdee, BRRD)*

## 2.22: MAGIC Rice: production, characterization, and its use in breeding networks

*Hei Leung,\* Nonoy Bandillo, Pauline Andrea Muyco, Chitra Raghavan, Anna Sevilla, Irish Lobina, Michael Thomson, Ramil Mauleon, Rakesh Kumar Singh, Glenn Gregorio and Edilberto Redoña*

*\*Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute, DAPO 7777, Metro Manila, Philippines  
email: h.leung@cgiar.org*

Breeding populations produced through *multi-parent advanced generation inter-crosses* (MAGIC) can be used directly as source materials for development of varieties adapted to different environments. We have developed two MAGIC populations using indica and japonica ecotypes of rice. Each population is comprised of 8 founders that include elite and modern varieties known to exhibit tolerance to a range of biotic and abiotic stresses, high yield potential, and good grain quality. We made additional cycles of intercrossing to increase the level of recombination and expand the diversity (indica x japonica mating). The size of the MAGIC populations ranges from 600 to 2,000 lines. The most advanced population is indica MAGIC with 1,439 S4 lines being advanced to RIL. We will select 400 S2:4 bulk lines for multi-location evaluation in breeding networks in southeast Asia and Africa. Early generation (S3) selection has yielded individuals with tolerance to salinity and drought. Disease screening also suggests transmission of blast resistance from the resistant founder to a majority of the progeny, showing expected pattern of trait transmission. We will apply genotyping-by-sequencing to characterize the RIL. The finely mapped population will provide SNP markers for MAS in future deployment of the MAGIC populations.

*Related GCP project—GCP Project G4008.01: Populations for multiple allelic segregation developed in rice and sorghum through multiple parent intercrossing (PI: Hei Leung, IRRI)*

**Notes:**





*\*Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil;  
email: schaffer@cnpms.embrapa.br; reschaffert@hotmail.com*

Two pairs of isogenic cytoplasmic male sterile lines were derived from a segregating F<sub>5</sub> progeny derived from the cross of an elite B line, BR007B and an Al tolerant line SC283. Four isogenic restorer lines were developed by marker assisted back crossing (MABC) using an elite R line, BR012R and three sources of Al tolerance; SC549, CMSXS225 and SC566-14. The isogenic R lines derived from SC549 and CMSXS225 were developed with six backcrosses to BR012R with selection in nutrient solution with 27μM Al. The isogenic line R line developed from SC566-14 was developed with three backcrosses using molecular markers for *Alt<sub>sr</sub>*. The eight lines and eight resulting isogenic hybrids (3 dwarf grain sorghum hybrids) were characterized in nutrient solution with 0, 11, 20, 27 and 39 μM Al. The results of 3, 5, and 7 day seminal root growth of these isogenic lines and hybrids in nutrient solution validated that these isogenic lines and hybrids can be used as a tool to field validate the effect of zero, one or two Al tolerant alleles on the effect of productivity and stability in environments with acid soils and subsoils with Al toxicity very common in the tropics. These lines were included...

*Related GCP project—G4008.10: Assessment of the breeding value of superior haplotypes for AltSB, a major Al tolerance gene in sorghum: linking upstream genomics to acid soil breeding in Niger and Mali (ALTFIELD) (PI: Robert Schaffert, EMBRAPA)*

Notes:

[illegible]



## 2.26: Phenotyping of panicle characteristics in a sorghum mapping population in Mali

*Dramane Sako,\* Sidy Bekaye Coulibaly, Denis Lespinasse,  
Michel Ragot, Michel Vaksmann, Jean-Francois Rami, Niaba  
Témé*

*\*Institut d'Economie Rurale, Mali and GCP sponsored PhD student at WACCI of Ghana;  
e mail: dramanesako@yahoo.fr*

Sorghum shows striking variation in panicle architecture such as branch number and branch length. The number and growth of branches affects directly grain yield. To analyze the implication of panicle architecture in grain yield, a bi-parental population, composed of 401  $F_4$  families derived from the crossing between Tiandougou and Keninkeni was developed. The whole population was evaluated together with the two parents during the 2010 cropping season at Sotuba research station. One panicle per entry was used to measure panicle traits like panicle length, number of nodes, average number of branches per nodes, number of primary branches, average primary branches length, average number of secondary branches and seed weight per panicle. A t test was used to compare the mean of the families to the mean of the two parents and correlation among panicle traits were analyzed. Seed weight was positively and significantly correlated to the number of primary and secondary branches. Increasing the number of nodes decreases the number of branch per nodes. Primary branch length was negatively correlated to the number of primary branches. This information will be used for QTL detection together with yield and grain quality components in marker assisted recurrent selection (MARS).

*Related GCP project—G4008.48: Improve sorghum productivity in semi-arid environments of Mali through integrated MARS (PI: Jean-François Rami, Agropolis–CIRAD)*

**Notes:**



S Misra,\* S Tetali, S Tamhankar, D Bonnett, M Zaharieva, RR Hanchinal, AS Desai, I Kalappanwar and R Trethowan

*\*Agharkar Research Institute, Pune, Maharashtra, India;  
satishmisra@yahoo.com,  
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Higher genetic diversity and allelic richness in cultivated emmer wheat, as revealed by microsatellite markers (SSRs), coupled with high drought and heat tolerance suggests that this species could be used to improve hexaploid bread wheat. A collection of emmer wheat accessions originating from 35 countries has been established. The genetic diversity structure is described using morphological traits, SSR and DArT markers, and drought and heat tolerance related traits assessed. Around 108 genetically diverse emmer wheat accessions capturing 376 (80%) of the 470 total alleles from the original set were then used to create new AB-genome diversity in hexaploid wheat using various strategies. The strategies are: (i) the emmer reference set was crossed to three *Ae. tauschii* accessions to produce new emmer based synthetic wheat (SHW) and new synthetic back-cross lines (SBL) are being generated by crossing SHW to elite bread wheat; (ii) crosses between emmer wheat and hexaploid bread wheat were made to develop new hexaploid materials with recombined A and B genomes; (iii) durum wheat based SHW were crossed to emmer wheat and emmer based SHW to produce AB-genome recombinants; (iv) durum based synthetics were crossed to and emmer based synthetics. This newly developed diversity is excellent pre-breeding material.

*Related GCP project–G3008.01: New wheat germplasm generated with broadened AB genome diversity (PI: SC Misra, ARI–ICAR)*

**Notes:**

[illegible]



### 2.30: Physiological trait based phenotyping of

## International core set for drought tolerance in wheat

KV Prabhu,\* GP Singh, Neelu Jain, SC Mishra, PC Mishra,  
N K Singh, TR Sharma, Praveen Chuneja, VS Sohu,  
GS Mavi, Biswajeet Mondal, Neha Rai, Shweta Umar,  
Dnyaneshwar Kadam, Lokesh Thawait and AC Pandey

*\*Indian Agricultural Research Institute, New Delhi, India;  
email: kvinodprabhu@rediffmail.com*

India is the world's second largest producer of wheat and Indian wheat production is of critical importance to global food security. Climate change is projected to reduce the amount of water available for irrigated wheat production. It is therefore vitally important that more water-use-efficient farming systems and wheat cultivars are identified. To identify QTLs in wheat to enable wheat to tolerate water deficiency and heat without losing much of productivity, an attempt is made using MARS in Indian locations. A trial comprising 145 entries was evaluated and phenotyping was carried out. Data on germination, days to heading, yield, thousand kernel weight, days to maturity, canopy temperature depression, NDVI and SPAD was taken. Significant correlations were observed between DH and DM, yield and DH (negative), TKW with DH, DM and yield, CT with DM and yield (negative) and canopy CTD with DH and yield and negative correlation with CT.

*Related GCP project—G7010.02.02: Molecular breeding and selection strategies to combine and validate quantitative trait loci for improving water-use efficiency and heat tolerance of wheat in India (PI: Vinod Prabhu, IARI-ICAR)*

**Notes:**

[illegible]

## **Theme 3:** Crop information systems

### 3.1: Construction of Genetic Linkage Maps and Mapping of Quantitative Trait Loci in the Integrated Software Package QTL IciMapping v3.1

Jiankang Wang

*Institute of Crop Science and CIMMYT China Office, Chinese Academy of Agricultural Science (CAAS), No. 12 Zhongguancun South Street, Beijing 100081, China;  
e-mail: wangjk@caas.net.cn; jkwang@cgiar.org*

Linkage maps construction and QTL mapping were once two separate procedures. Some software are available for linkage map construction, whereas others for QTL mapping. Now we have one integrated software package that can do both, i.e., QTL IciMapping v3.1, freely, available from <http://www.isbreeding.net>. Core computing modules in QTL IciMapping were written by Fortran 90/95, and the interface was written by C#. The software runs on Windows XP/Vista/7, with .NET Framework 2.0(x86)/3.0/3.5. To our knowledge, this is the first software which integrates the linkage map construction and QTL mapping, saving researchers a lot of time when they are using QTL mapping to identify the genes of important traits. Four general steps are involved in map construction: Grouping, Ordering, Rippling, and Outputting. Grouping can be based on (i) anchored marker information, (ii) a threshold of LOD score to test linkage relationship, and (iii) a threshold of marker distance. Three ordering algorithms are (i) SER: SERiation, (ii) RECORD: REcombination Counting and ORDERing, and (iii) MF: Multi-Fragment heuristic algorithm. Five rippling criteria are (i) SARF (Sum of Adjacent Recombination Frequencies), (ii) SAD (Sum of Adjacent Distances), (iii) SALOD (Sum of Adjacent LOD scores), (iv) COUNT (number of recombination events), and (v) LogL (Logarithm Likelihood of the marker sequence)...

*Related GCP project–G4008.14: Breeding for drought tolerance with known gene information (PI: Jiankang Wang, CIMMYT)*

**Notes:**

[illegible]





### 3.3: Coverage based consensus calling (CbCC) of short sequence reads and comparison of CbCC-results for the identification of SNPs in chickpea, a crop species without a reference genome

Trushar Shah,\* Sarwar Azam, Vivek Thakur, R. Pradeep, B. Jayashree, A. BhanuPrakash, Andrew D. Farmer, David J. Studholme, Greg D. May, David Edwards, Jonathan D. G. Jones and Rajeev K. Varshney

*\*International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru 502324, Andhra Pradesh, India; e-mail: r.k.varshney@cgiar.org; tm.shah@cgiar.org*

In crop species such as chickpea (*Cicer arietinum* L.) that lacks a reference genome sequence, next generation sequencing (NGS)-based SNP discovery is a challenge with commonly used SNP discovery methods. Therefore, a coverage based consensus calling (CbCC) approach was used with four short reads alignment tools (Maq, Bowtie, Novoalign and SOAP2). By using this approach on 15.7 and 22.1 million Illumina reads for two chickpea genotypes ICC 4958 and ICC 1882, together with the chickpea transcriptome assembly (CaTA), a non-redundant set of 4543 SNPs was identified. Experimental validation of 224 randomly selected SNPs showed the superiority of Maq as 50.0% of SNPs predicted by Maq were true SNPs. Using combinations of two tools, the greatest accuracy (55.7%) was reported for Maq and Bowtie, with a combination of Bowtie, Maq and Novoalign identifying 61.5% true SNPs. SNP prediction accuracy generally increased with increasing reads depth, however, in case of Maq, SNPs predicted at lower read depths (<10) showed greatest accuracy. In addition to identification of large number of SNPs in chickpea, this study provides a benchmark comparison of read depths as well as prediction accuracy of four commonly used tools.

*Related GCP project—G4010.05 Development of integrated SNP mining and utilisation (ISMU) pipeline based on next-generation sequencing (NGS) and high-throughput (HTP) genotyping technologies for facilitating molecular breeding (PIs: Rajeev Varshney/Trushar Shah, ICRISAT)*

**Notes:**

[illegible]





### 3.7: To contribute crop traits lists using the new online

## Crop Ontology Curation and Annotation tool

Luca Matteis, Milko Skofic, Hector Sanchez, Rosemary Shrestha, and Elizabeth Arnaud\*

\* *Bioversity International*;

*e-mail: l.matteis@cgair.org; e.arnaud@cgiar.org*

The Crop Ontology (<http://cropontology.org/>) provides validated names, definitions and relationships for traits of cassava, chickpea, maize, *Musa*, potato, rice, sorghum and wheat. These lists were developed by Bioversity, CIMMYT, CIP, ICRISAT, IITA, IRRI. The sources of breeders' trait names were the International Crop Information System (ICIS), the Bioversity key crop descriptors and the Trait dictionaries developed by the GCP crop communities. An online tool is now available (<http://cropontology-curationtool.org>) for the crop communities to share trait lists, contribute new trait names and download concepts for annotating data. The tool allows users to display and print the lists. By using a login, curators of crop trait lists can comment any term or add online attribute information (e.g. photos). A full ontology can be directly uploaded or created online using the current OBO upload service. The system will support other standard formats like OWL. The ICIS teams will synchronize their Trait Templates with the ontology using the web service API. Any application can send to and receive data from the Curation Tool using this API that provides the ability to create, read, update and delete terms. The site is hosted on Google App Engine and the versioned code is hosted on GitHub.

*Related GCP project—G4011.01: Development, maintenance and use of trait dictionaries and a Crop Ontology to facilitate the annotation of phenotypic data by crop communities (PI: Elizabeth Arnaud, Bioversity)*

Notes:

[illegible]

### 3.8: QuMARS: A QU-GENE application module to simulate marker assisted recurrent selection and genomic selection

Jiankang Wang\*, Scott Chapman and Mark Dieters

*\*Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China;*

e-mail: wangjk@caas.net.cn; jkwang@cgiar.org

The QU-GENE application module QuMARS has been developed to simulate marker assisted recurrent selection (MARS) and genomic selection (GS) in plant breeding. For MARS, selection uses a prediction of molecular markers with significant effects based on the linear regression models. For GS, a BLUP prediction marker effect (without significance test) is used for selection. QuMARS can simulate the three major stages in a MARS or GS breeding program. Stage I: Building a training population and the genotype to phenotype (GP) prediction model. In plants, the training population is normally derived from two fixed parental lines, for example, it can be a doubled haploids population, F2, or F3. Both genotyping and phenotyping need to be conducted in the training population. Phenotyping can be the line per se performance where the breeding objective is to select elite advanced lines, such as in wheat and bean, or the testcross performance where the breeding objective is to select elite hybrids, such as in maize and sorghum. Stage II: Recurrent selection from the training population. (i) To select individuals in the training population, either based on the available phenotypic data or the GP model built in Stage I. (ii) To inter-mate the selected individual to form a new population, grow and genotype the new population...

*Related GCP project—G8009.03.03/ Activity 2.2.3: Develop and deploy simulation tools for complex G-E systems (Activity Leader: Mark Dieters, UO)*

**Notes:**

[illegible]



### 3.10: Molecular Breeding Services at IBP/GCP

Chunlin He\* and Xavier Delannay

*\*Generation Challenge Programme, c/o CIMMYT, Texcoco, Mexico;  
e-mail: c.he@cgiar.org*

SSRs and SNPs have become the preferred markers for marker assisted selection in crop breeding but they are not easily accessible in developing countries. The Genotyping Support Service (GSS) at GCP was created to support breeding programs not only by collaborating with service providers worldwide but also by providing training for data analysis. Since August 2007, GCP has launched a total of 3 calls for research grant applications of GSS projects. A total of 174 applications have been received and 73 of them have been awarded as molecular genotyping projects with SSRs and SNPs as well as DArT markers. The awarded research projects with topics from genetic diversity studies to marker-assisted selections have covered 14 crops from 25 countries. GCP is currently transitioning the marker services from SSR to SNP markers which will be accessible to customers through GDMS at IBP. So far, 1000-2000 SNPs have been converted into KASPar system for SNP genotyping for 7 crops of critical importance to developing countries. With available markers at IBP, the GCP will provide the developing country breeding programs with easier access to more convenient genotyping services to support molecular breeding schemes such as MAS, MABC, MARS, and GWS.

*Related GCP projects—G8009.05 Objective 3.1: Provide access to critical molecular breeding services (PI: X Delannay, GCP) G8009.06 Objective 3.2: Provide assistance with a range of molecular breeding support services (PI: C de Vicente, GCP [up to October 2010])*

**Notes:**

[illegible]

*\*International Center for Tropical Agriculture (CIAT), Cali, Colombia;  
email: g.hyman@cgiar.org*

*Related GCP project—G8009.06.05/ Activity 3.2.5: Phenotyping sites and screening protocols (Activity Leader: Xavier Delannay, GCP)*

Notes:

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### 3.12: Information management and data curation support services of IBP/GCP

*Arllet Portugal,\* Clarissa Pimentel and Graham McLaren*

*\*Generation Challenge Programme, c/o CIMMYT, El Batán, Texcoco, Mexico.*

*e-mail: a.portugal@cgiar.org*

Quality information system is an integral component of molecular breeding. Lack of information systems is one bottleneck in adoption of molecular breeding in developing countries. The Information Management Support Service of GCP was created to provide assistance in installing, configuring the Integrated Breeding (IBP) platform information system for use by specific breeding programmes and for global crop improvement databases. The Data Curation Support Service was created to assist in curating data into the platform information system of these breeding projects and to establish global crop improvement databases that integrate pedigree and genotype data from publicly available sources. Seven workshops and trainings were coordinated from April 2010 to February 2011 through these Services targeting the nine use cases supported by IBP. Details about these workshops are available at <http://ibp.generationcp.org/confluence/display/MBP/Activity+3.2.2+and+3.2.3> Trait Dictionaries and Fieldbook templates are outputs of these workshops and accessible in the said site. Use of handheld device for fast data collection was introduced. Four Honeywell and nine HP iPaq handheld units were distributed. The Information Management Support Service will make the informatics tools developed through IBP available to developing countries and the Data Curation Support Service will facilitate management of quality data for accurate predictive breeding.

*Related GCP projects—G8009.06.02/Activity 3.2.2: Information Management and G8009.06.03/Activity 3.2.3 Data curation (Activity Leader: Arlet Portugal, GCP)*

**Notes:**

[illegible]

**Theme 4:**  
Capacity building and  
Product delivery



*S Beebe, \* M Blair, F Alemayehu, A Asfaw, R Chirwa, L Kalolokesya, P Kimani, G Makunde, F Watweru*

*\*Centro Internacional de Agricultura Tropical (CIAT), Colombia;  
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The GCP has administered a project, Tropical Legumes-I or TL-1, to develop tools for the improvement of drought tolerance in four legume crops including common bean. TL-1 is closely aligned with a sister project, Tropical Legumes-II (TL-2), which focuses on breeding. Both projects have had a significant capacity building component, and coordination between the two projects has allowed training both in field management under TL-2, and formal degree training in TL-1. Thesis projects contribute directly to the common goals of the two projects, and cover topics of: QTL analysis for drought tolerance traits; participatory evaluation in drought prone areas; association mapping for drought tolerance QTL; marker assisted selection for resistance to common bacterial blight. A project on marker assisted recurrent selection is currently underway. Students benefiting from this project proceed from Ethiopia, Kenya, Malawi and Zimbabwe.

*Related GCP project G4009.07.01: TLI students for analysis of drought tolerance in common bean (PI: Steve Beebe, effective October 2010/ Matthew Blair, CIAT)*

Notes:

[illegible]

#### 4.3: Introgression of *Saltol* and fine-mapping of reproductive stage QTLs to develop rice varieties highly tolerant of salt stress

*M. Sazzadur Rahman\*, Michael J. Thomson, Zeba I. Seraj and Abdelbagi M. Ismail*

*\*University of Dhaka, Bangladesh; and Crop & Environmental Sciences Division, International Rice Research Institute (IRRI), Philippines;  
e-mail: s.rahman@irri.org*

Salinity is a major problem for rice production in South Bangladesh, and is being aggravated by natural and human activities, such as the increasing frequencies of cyclones and storms, and the excessive withdrawal of water at the upstream of major rivers. Developing high yielding varieties with greater tolerance of salt stress will help in adapting to these adverse conditions, and to sustain food security. *Saltol* introgression lines developed through the previous GCP projects are being evaluated in field trials, however, and because of the complexity of the tolerance trait, this QTL provides moderate to high tolerance only at the seedling stage. Multiple tolerance genes were also recognized in the *Saltol* region, and to identify the optimum introgression size, varying segments of the *Saltol* locus are being introgressed into BRRI dhan28 from the donor FL378. BC<sub>3</sub>F<sub>2</sub> progenies with segments of four different sizes (1.3, 2.4, 3.3 and 3.7 Mb) were developed, with a clean or minimum donor background. Furthermore, two novel QTLs associated with tolerance at the reproductive stages (Chr 1L and Chr 5S) from Boilam, a salt tolerant landrace from South Bangladesh, were identified and are being fine-mapped to be combined with *Saltol* using marker assisted backcrossing.

*Related GCP project– G4010.04 Enhancing capacity for use of advance genotyping for fine-mapping and pyramiding of major salt tolerant QTLs through MABC for the development of durable saline tolerant rice varieties.(PI: Zeba I Seraj, UoD)*

**Notes:**

[illegible]

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Rice is one of the major food staples in Sub Sahara Africa (SSA). It contributes more than 40 % of gross domestic product (GDP) and 70% of the labour force. The demand for rice surpasses production rate, which create a wide gap being filled by importation that drains the scarce foreign reserve of these nations. Generation Challenge Program (GCP) established a program called "challenge initiative" to address the aforementioned problems using Communities of Practice (CoP) approach in three West African countries (Burkina Faso, Mali and Nigeria) as pilot study. Major activities of CoP are capacity building and support for the implementation of markers assisted selections in the breeding programs of NARS. To achieve these, CoP will provide training for genotyping, data management and analyses among others. Communities of Practice essentially are interactive forum for collaboration and exchange of ideas. This will be led by a coordinator whose responsibility is to facilitate project activities and provide technical support in modern breeding techniques and is assisted by a mentor from each member countries. CoP will hold regular meetings and workshops to discuss joint research activities and share information to strengthen the NARS breeding program. CoP members comprised scientists at senior and junior level of the national research institutions as well, voluntary individual scientists...

*Related GCP project—G7010.04.01: Improving rice productivity in lowland ecosystems of Burkina Faso, Mali and Nigeria through marker-assisted recurrent selection for drought tolerance and yield potential (PI: Marie Noëlle Ndjiondjop, AfricaRice)*

**Notes:**


This image shows a single sheet of white paper with horizontal dashed lines, typical of primary-ruled notebook paper. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.











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