2011 Project updates

Generation Challenge Programme

CULTIVATING PLANT DIVERSITY FOR THE RESOURCE-POOR

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Generation Challenge Programme

2011 Project updates

(incorporating projects completed in 2010 and 2009)

August 2011

Generation Challenge Programme (GCP)
Hosted by CIMMYT
(Centro Internacional de Mejoramiento de Maíz y Trigo;
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This publication has been printed in black-and-white. For colour images, please contact the appropriate Principal Investigator.

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Acronyms and abbreviations

AARC	Awassa Agricultural Research Center, Ethiopia
ABC	Agricultural Biotechnology Center, Gödöllő, Hungary
ABRII	Agriculture Biotechnology Research Institute of Iran
ACCI	African Centre for Crop Improvement, South Africa
ACGT	African Centre for Gene Technologies, South Africa
ACPFG	Australian Centre for Plant Functional Genomics, Pty Ltd
ADOC	allelic diversity for orthologous candidate genes
AGRA	Alliance for a Green Revolution in Africa
AICPMIP	All-India Coordinated Pearl Millet Improvement Project
Al	aluminium
Alt_{SB}	marker diagnostic for aluminium tolerance
APSIM	Agricultural Production Systems Simulator
ARC–Sudan	Agricultural Research Corporation, Sudan
ARI	Agharkar Research Institute, India
ARI(s)	advanced research institute(s)
ARI–HAS	Agricultural Research Institute of the Hungarian Academy of Sciences, Hungary
ARI–Naliendele	Agricultural Research Institute–Naliendele Research Station, Tanzania
ARM	Annual Research Meeting
ARS–Durgapura	Agricultural Research Station, Durgapura, Jaipur, Rajasthan, India
ASTI	CGIAR Agricultural Science and Technology Indicators, Italy
BAC	bacterial artificial chromosome
BAU	Birsa Agricultural University, Ranchi India
BCMV	bean common mosaic virus
BINA	Bangladesh Institute of Nuclear Agriculture
BIOTEC	National Center for Genetic Engineering and Biotechnology, Thailand
Bioversity	Bioversity International
BLB	bacterial leaf blight
BMGF	Bill & Melinda Gates Foundation
BRRD	Bureau of Rice Research and Development, Rice Department, Thailand
BRRI	Bangladesh Rice Research Institute
CAAS	Chinese Academy of Agricultural Sciences
CAPS	cleaved amplified polymorphic sequence (markers)
CARDI	Cambodia Agricultural Research and Development Institute
CAZRI	Central Arid Zone Research Institute, India
CB	conventional breeding
CBI	Crop Breeding Institute, Department of Research for Development, Zimbabwe
cDNA	complementary DNA
CERAAS	Centre d'étude régional pour l'amélioration de l'adaptation à la sécheresse, Senegal
CGIAR	Consultative Group on International Agricultural Research
CGN–WUR	Centre for Genetic Resources–Wageningen University and Research Centre, The Netherlands
CHPRRU	Corn Host Plant Resistance Research Unit, USDA–ARS
CIAT	Centro Internacional de Agricultura Tropical (International Center for Tropical Agriculture)
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo (the International Maize and Wheat Improvement Center)
CIMS	Centro de Inteligencia sobre Mercados Sostenibles of INCAE
CINVESTAV	Centro de Investigación y de Estudios Avanzados, Mexico
CIP	Centro Internacional de la Papa (International Potato Centre)

CIRAD	Centre de coopération internationale en recherche agronomique pour le développement, France
CLDRI	Cuu Long Delta Rice Research Institute, Vietnam
CMTV	Comparative Map and Trait Viewer
CNG	Centre National de Génotypage, Commissariat à l'Energie Atomique, Evry, France
CNRA	Centre National de Recherches Agronomiques, ISRA
CoP	community of practice
Cornell	Cornell University
COS	conserved orthologous sequence
CP	Challenge Programme (of the CGIAR)
CRI–Ghana	Crops Research Institute, Ghana
CRIL	Crop Research Informatics Laboratory (CIMMYT and IRRI)
CRI–Sri Lanka	Coconut Research Institute, Sri Lanka
CRRI	Central Rice Research Institute, India
CRS	Chitedze Research Station, Malawi
CRURRS	Central Rainfed Upland Rice Research Station, India
CSIRO	Commonwealth Scientific and Industrial Research Organisation, Australia
CSSL	chromosome segment substitution line
CSU	Colorado State University, USA
CStuU	Charles Sturt University, Australia
$\Delta^{13}C$	carbon isotope discrimination
DAR	Department of Agricultural Research, Myanmar
DARS	Department of Agriculture Research Services, Malawi
DArT	diversity arrays technology
DArT P/L	Diversity Arrays Technology Pty, Ltd
DMR	Directorate of Maize Research, India
DNA	Deoxyribonucleic acid
DOA–Thailand	Department of Agriculture, Thailand
DPKit	Delivery Plan Kit
DPSPP–EKC	Department of Plant Sciences and Plant Physiology, Eszterházy Károly College, Eger, Hungary
DREB	drought-responsive element binding protein (gene)
DWR	Directorate of Wheat Research, India
DZARC	Debre Zeit Agricultural Research Centre, Ethiopia
ECABREN	Eastern and Central Africa Bean Research Network
EgU	Egerton University, Kenya
EIAR	Ethiopian Institute of Agricultural Research
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária (Brazilian Agricultural Research Corporation)
EMU	Eduardo Mondlane University, Mozambique
ERECTA	a leucine rich repeat receptor-like kinase (gene)
EST	expressed sequence tag
ESU	Ebonyi State University, Nigeria
ETH	Eidgenössische Technische Hochschule, (Swiss Federal Institute of Technology), Zürich
F_1 etc	first filial generation etc
FABI	Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa
Fedearroz	Federación Nacional de Arroceros, Colombia
FOFIFA–DRA	Foibem-Pirenena Mombra ny Fikarohana Ampiharina Amin'ny Fampandrosoana ny eny Ambanivohitra (National Centre. for Applied Research on Rurual Development) Département de la Recherche Agronomique, Madagascar
GCP	Generation Challenge Programme of the CGIAR

GIS	geographic information system(s)
GISH	genomic *in situ* hybridisation
GOST	GreenPhyl Ortholog Search Tool
GRSS	Genetic Resources Support Service
GSS	Genotyping Support Service
GxE	genotype by environment interaction
HAAS	Hebei Academy of Agricultural Sciences, Institute of Dry Farming, China
HAKI	Research Institute for Fisheries, Aquaculture and Irrigation, Hungary
HPC	high-performance computing
HZAU	Huazhong Agricultural University, China
IAMZ	Instituto Agronómico Mediterráneo de Zaragoza, Spain
IARI	Indian Agricultural Research Institute
IA–Tápiószele	Institute for Agrobotany, Tápiószele, Hungary
IBONE	Instituto de Botánica del Nordeste, Argentina
ICABIOGRAD	Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development
ICAR	Indian Council of Agricultural Research
ICARDA	International Centre for Agricultural Research in the Dry Areas
ICASEPS	Indonesian Center for Agro Socio-Economics and Policy Studies, Indonesia
ICERI	Indonesian Cereals Research Institute
ICFORD	Indonesian Center for Food Crops Research and Development
ICIS	International Crop Information System
ICL	Imperial College London, UK
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
ICS–CAAS	Institute of Crop Science, Chinese Academy of Agricultural Sciences
IER	Institut d'économie rurale, Mali
IFPRI	International Food Policy Research Institute
IGD	Institute for Genomic Diversity, Cornell University, USA
IGKV	Indira Gandhi Krishi Vishwa Vidyalaya (Indira Gandhi Agricultural University), India
i-GOST	iterative version of GOST
IIAM	Instituto de Investigação Agrária de Moçambique (Institute for Agricultural Research, Mozambique)
IIPR	Indian Institute of Pulses Research
IITA	International Institute of Tropical Agriculture
ILRI	International Livestock Research Institute
iMAS	Integrated Marker-Assisted Selection System
INCA	Instituto Nacional de Ciencias Agricolas, Cuba
INERA–Burkina Faso	Institut de l'environnement et de recherches agricoles, Burkina Faso
INERA–DRC	Institut national pour l'etude et la recherche agronomiques, democratic Republic of the Congo
INIA–Chile	Instituto de Investigaciónes Agropecuarias, Chile
INIA–Uruguay	Instituto Nacional de Investigación Agropecuaria, Uruguay
INIFAP	Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias, Mexico
INRA	Institut national de la recherche agronomique, France
INRA–Morocco	Institut national de la recherche agronomique, Morocco
INRAN	Institut national de la recherche agronomique du Niger
INTA–Nicaragua	Instituto Nacional de Tecnologia Agropecuaria, Nicaragua
IP	intellectual property
IPB–The Philippines	Institute of Plant Breeding, The Philippines
IPK	Leibniz Institute of Plant Genetics and Crop Plant Research, Germany
IPM CRSP–VPI	Integrated Pest Management Collaborative Research Support Program–Virginia Polytechnic Institute and State University, USA
IPT	isopentenyltransferase (gene)

IRAD	Institut de la recherche agronomique pour le développement, Cameroon
IRC	Interactive Resource Centre
IRD	Institut de recherche pour le développement, France
IRRI	International Rice Research Institute
ISABU	Institut des sciences agronomiques du Burundi
ISAR	Institut des sciences agronomiques du Rwanda
ISRA	Institut sénégalais de recherches agricoles, Senegal
JCVI	James Craig Venter Institute, USA
JIC	John Innes Centre, UK
JIRCAS	Japan International Research Center for Agricultural Sciences
JLNKV	Jawahar Lal Nehru Krishi Vishwavidyalaya, Jabalpur, India:
KARI	Kenya Agricultural Research Institute
kb	Kilobase
KU	Kasetsart University, Thailand
KUL	Katholieke Universiteit Leuven, Belgium
LAAS	Luoyang Academy of Agricultural Sciences, China
LD	linkage disequilibrium
LIMS	Laboratory Information Management System
LUMC	Leiden University Medical Center, The Netherlands
MAB	marker-assisted breeding
MABC	marker-assisted backcrossing
MAGIC	multiparent advanced generation inter-cross
MahU	Mahidol University, Thailand
MARS	marker-assisted recurrent selection
MAS	marker-assisted selection
MAU	Marathwada Agricultural University, India
Mb	megabase
MPIDB	Max Planck Institute for Developmental Biology, Germany
MSV	maize streak virus
MU	Moi University, Kenya
N/A	not applicable
NAARI	Namulonge Agricultural and Animal Research Institute, Uganda
NaCRRI	National Crop Resources Research Institute, Uganda
NAFRI	National Agricultural and Forestry Research Institute, Laos
NAM	nested association mapping
NARI	National Agricultural Research Institute, Eritrea
NARS	national agricultural research system(s)
NaU	Nagoya University, Japan
NAU	Nanjing Agricultural University, China
NCE	no-cost extension
NCGR	National Center for Genome Resources, USA
NCSRC	National Corn and Sorghum Research Center, Thailand
NCSU	North Carolina State University, USA
NDUAT	Narendra Deva University of Agriculture and Technology, India
NERICA	new rice for Africa
NGO	non-governmental organisation
NIAB	National Institute of Agricultural Botany, UK
NIAS	National Institute of Agrobiological Sciences, Japan
NIL	near-isogenic line
NKLCGGE	National Key Lab of Crop Genetics and Germplasm Enhancement, China
NMRI	National Maize Research Institute, Vietnam
No.	number
NPGRC	National Plant Genetic Resources Centre, Tanzania
NRCPB	National Research Centre on Plant Biotechnology, India
NRCRI	National Root and Tuber Crops Research Institute, Nigeria

NRCS	National Research Centre on Sorghum, India
NSFCRC	Nakhon Sawan Field Crops Research Center, Thailand
NU	Ningxia University, China
NWSUAF	Northwest Sci-tech University of Agriculture and Forestry, China
ORE	Organisation for the Rehabilitation of the Environment, Haiti
OSU	Oregon State University, USA
PAU	Punjab Agricultural University, India
PBI–University of Sydney	Plant Breeding Institute–University of Sydney, Australia
PGRCU	Plant Germplasm Resources Conservation Unit, USDA–ARS
PhilRice	Philippine Rice Research Institute
PI	Principal Investigator
Pioneer	Pioneer Hi-Bred International, Inc
POC	Plant Ontology Consortium
PROINPA	Promoción e Investigación de Productos Andinos, Bolivia
PSU	Pennsylvania State University, USA
PU	Purdue University, USA
Pup1	marker diagnostic for phosphorus uptake
QPIF	Queensland Primary Industries and Fisheries, Australia
QTL	quantitative trait locus
QTLxE	QTL by environment interaction
R&D	research and development
RARS	Regional Agricultural Research Station, Nandyal, India
RAU	Rajasthan Agricultural University, India
RCB–IPB	Research Center for Biotechnology, Bogor Agricultural University, Indonesia
RF	The Rockefeller Foundation
RFLP	restriction fragment length polymorphism
RGDU	Rice Gene Discovery Unit, Thailand
RIKEN	Rikagaku Kenkyūsho (Institute of Physical and Chemical Research), Japan
RIL	recombinant inbred lines
RNA	ribonucleic acid
RYMV	rice yellow mottle virus
SAARI	Serere Agricultural and Animal Production Research Institute, Uganda
SAAS	Shanxi Academy of Agricultural Sciences, China
SABRN	Southern Africa Bean Research Network
Saltol	marker diagnostic for salt tolerance
SARI–Ghana	Savannah Agricultural Research Institute, Ghana
SARK	senescence associated receptor protein kinase
SAU	Sichuan Agricultural University, China
SCRI	Scottish Crop Research Institute, UK
SIRDC	Scientific and Industrial Research and Development Centre, Zimbabwe
SNP	single nucleotide polymorphism
SP	Subprogramme
SP1, SP2 etc	Subprogramme 1, Subprogramme 2 etc.
SPL	Subprogramme Leader
SPS	sucrose phosphate synthase (gene)
SPVD	sweet potato virus disease
SSA	Sub-Saharan Africa
SSR	simple sequence repeat
SUoAg	Sokoine University of Agriculture, Tanzania
TAMU	Texas A&M University
TBD	to be determined
TF	task force
TLI	Tropical Legumes I Project
TLII	Tropical Legumes II Project

TNAU	Tamil Nadu Agricultural University, India
TPE	target population of environments
TSL	The Sainsbury Laboratory, UK
TU	Tishreen University, Syria
UAS	University of Agricultural Sciences, India
UBU	Ubon Ratchatani University, Thailand
UCB	Universidade Católica de Brasilia, Brazil
UCG	Universidade Católica de Goiás, Brazil
UdR	Universidad de la Republica, Uruguay
UdB	Università di Bologna, Italy
UdU	Università di Udine, Italy
UGA	University of Georgia, USA
UKZN	University of KwaZulu–Natal, South Africa
UoA	University of Arizona, USA
UoAa	University of Aarhus, Denmark
UoAb	University of Aberdeen, Scotland
UoAl	University of Alberta, Canada
UoC	University of California, USA
UdAC	Universidad Autónoma Chapingo, México
UoD	University of Dhaka, Bangladesh
UoF	University of Frankfurt, Germany
UoGh	University of Ghana
UoH	University of Hohenheim, Germany
UoMi	University of Missouri, USA
UoN	University of Nairobi, Kenya
UoP	University of Pretoria, South Africa
UoQ	University of Queensland Australia
UoT	The University of Tehran, Iran
URGV	Unité de Recherche en Génomique Végétale, France
USDA–ARS	United States Department of Agriculture–Agricultural Research Service, USA
USDA–ARS PGRU	USDA–ARS, Plant Genetic Resources Unit
USP	Universidade de São Paulo, Brazil
UoV	University of Virginia, USA
VBI	Virginia Bioinformatics Institute, VPI
Virginia Tech	see VPI
VPI	Virginia Polytechnic Institute and State University, USA
WACCI	West Africa Centre for Crop Improvement, University of Ghana
WARDA	Africa Rice Center
WMS	Workflow Management System
WUR	Wageningen University and Research Centre, The Netherlands
YAAS	Yunnan Academy of Agricultural Sciences, China
ZU	Zhejiang University, China

2011 PROJECTS

Research Initiative crops

Cassava

1. G3007.03: Genetic and physical mapping resources produced for drought breeding in cassava

September 2007–February 2010; NCE: February 2011

Principal Investigator

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As cassava is fairly tolerant to water stress, it is a preferred crop for poor farmers in Sub-Saharan Africa, South East Asia, and Latin America, where adverse environmental conditions, particularly drought, are common. This project aimed to advance towards accelerating marker-assisted breeding in cassava to further increase its drought tolerance. We took advantage of existing cassava genetic resources, particularly a mapping population generated at the International Center for Tropical Agriculture (CIAT, Cali, Colombia) after crossing two cassava genotypes (MVen77 and MCol1734) that show contrasting drought tolerance phenotypes. The specific goals of the project were to apply genomic technologies and use the existing genetic materials to: 1) construct a bacterial artificial chromosome (BAC)-based physical map of the cassava genome that will accelerate all areas of genomic analysis in cassava, facilitating the development of molecular markers as well as other resources that will ultimately benefit cassava breeding; 2) rely on the cassava physical map to obtain DNA sequence information evenly spread throughout the genome; 3) use this DNA sequence data to identify candidate single nucleotide polymorphism (SNP) markers with a genome-wide distribution; 4) use those SNP markers to assess the genetic diversity of cassava germplasm, construct a genetic map of cassava using the MVen77 and MCol1734 drought mapping population, and integrate the genetic and physical maps, delivering a comprehensive genetic and genomic resource to the cassava breeding community; 5) create a database displaying all the information generated in this project through a user-friendly web interface containing a genome browser, a BLAST server, and a download page; and 6) train National Programs researchers and breeders in new genomics and genome-wide genotyping technologies through a Capacity Building Workshop.

During the course of our project Roche-454 Life Sciences, the US Department of Energy (DOE) Joint Genome Institute (JGI), and the University of Arizona completed a draft assembly of the cassava genome and released it along with its gene annotation in collaboration with our group at the Institute for Genome Sciences. We took advantage of this new resource to identify SNP markers within genes annotated across the genome to complement our physical map-derived SNPs. Gene-derived markers have the advantage of being likely associated with traits of interest. All SNPs were used to genotype a diversity panel of over 200 cassava genotypes provided by the National Programs breeders that attended the Capacity Building Workshop, as well as the MVen77 x MCol1734 mapping population using the Illumina VeraCode GoldenGate genotyping technology and a BeadXpress instrument installed a the University of Pretoria for the purpose of this project.

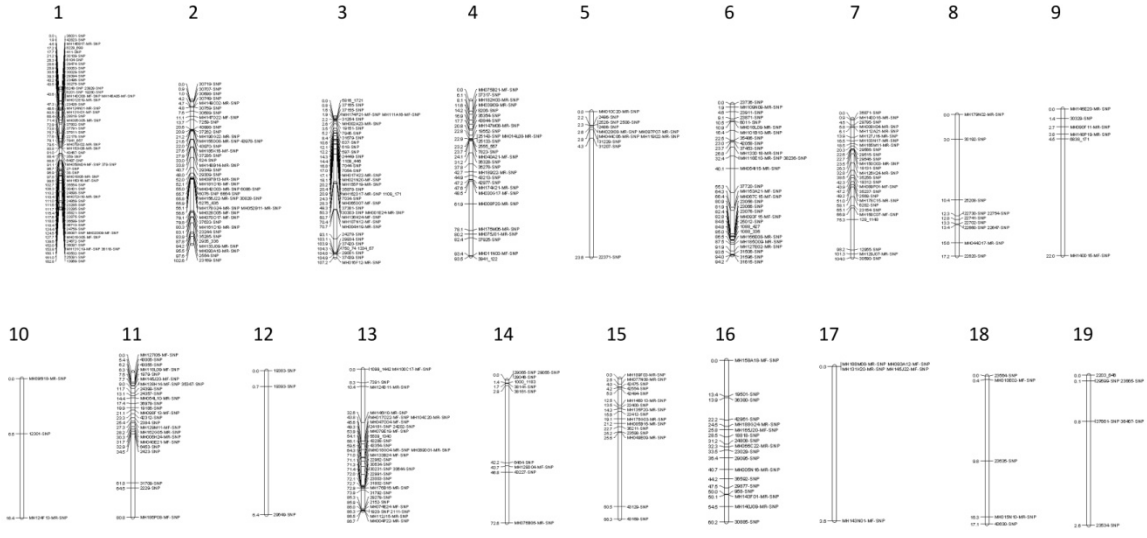
As a result, our project has delivered a BAC-based physical map of cassava spanning 700 Mb (which is consistent with the size of the cassava genome), nearly 800 candidate SNP markers of which, over 700 were validated by genotyping the mapping population and the diversity panel of cassava genotypes, and 556 were polymorphic in the mapping population and were used to construct, to our knowledge, the first SNP-based genetic map of cassava showing 19 linkage groups (Fig 1).

The GCP is having our SNP markers converted to the KASPar system from KBiosciences to make them, along with additional SNP markers from other sources, available to the broad cassava breeding community. Furthermore additional analyses are ongoing in order to finalize manuscripts for publication of the results.

The SNP information is publicly available through FTP downloads and the BAC-end sequence-derived SNPs can be viewed in the genome browser page of the project's website (http://cassava.igs.umaryland.edu). All our data will be integrated with the genome sequence and the Bill & Melinda Gates Foundation project awarded to the University of Arizona.

This project will synergize with the Challenge Initiative (CI) project "Phenotyping cassava for drought tolerance to identify QTLs", which will include phenotypic analyses of the MVen77 x MCol1734 mapping population in different field conditions for different traits related to water stress.

Figure 1



2. G7010.01.01: Development of a genetic resource base for drought and biotic stress improvement in cassava

April 2010–March 2013

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The overall objective of this project is to enhance the utility of the existing GCP cassava reference set by redefining its contents so that it not only represents diversity but also encompasses germplasm with a range of responses to traits of interest such as drought, starch quality, β-carotene content, disease resistance etc. Diversity will be expanded to include more germplasm from southern, eastern and central (SEC) Africa which is currently under-represented. It is anticipated that a trait-based reference set will be of more use to the cassava breeding community than a purely conventional reference set that represents diversity. The project also aims at conserving and exchanging the reference set among participating institutions. In this way, the project aims at delivering useful germplasm to NARS breeding programs in Africa.

Initially 576 cassava varieties will be genotyped for 1,536 SNPs using an Illumina GoldenGate assay (Illumina Inc., San Diego, CA). This will consist of approximately:

- One hundred varieties from SEC Africa, selected from SSR data from over 1,000 varieties, and breeder preferences. SSR diversity was measured using 26 SSR markers visualised on an ABI 3730 capillary sequencer (from SP1 Phase 1). This set of 100 genotypes has been defined and DNA is being accessed, although this is a challenge as currently germplasm from SEC Africa is not conserved in any formal way but exists in NARS breeding programs.
- One hundred IITA germplasm lines containing traits of interest to breeders, such as high β-carotene, drought tolerance, high dry matter etc. This set has been defined and DNA is available.
- One hundred and two IITA germplasm lines that represent broad diversity based on 30 SSR markers (8 ABI and 22 PAGE with silver staining) and agro-morphological traits (from SP1 Phase 1) and together with CIAT germplasm constituted a ‘preliminary reference set’. The revision of this set is nearing completion.

- One hundred CIAT germplasm lines containing traits of interest to breeders, such as high β-carotene, drought tolerance, high dry matter etc.
- One hundred and forty eight CIAT germplasm lines that represent broad diversity based on 30 SSR markers (8 ABI and 22 PAGE with silver staining) (from SP1 Phase 1). This, together with IITA germplasm constituted a 'preliminary reference set'.

From the SNP genotyping results we will select a set of about 200 genotypes that reflect both diversity and breeder preferred traits. This will form the GCP reference set, which, due to the collaborative nature in which it has been defined, should have commitment for its maintenance and distribution by IITA, CIAT and the NARS. This reference set will be dynamic and should be reviewed and updated on a regular basis as new varieties become available. Once defined, 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 project to Ghana, Tanzania and Nigeria. Access to the reference set will allow breeders in these countries to broaden the genetic base of their breeding programs using largely breeder-preferred germplasm. This should allow substantial genetic gain within NARS breeding programs. In addition, the SNP genotyping data should facilitate modern marker-based breeding approaches. The project has defined a 'core' set of germplasm from SEC Africa which provides a priority list of germplasm, based on diversity and breeder information that regional conservation efforts could utilise to structure an initial collection.

3. G7009.10/G7010.01.02: Improving and deploying markers for biotic stresses in cassava

G7009.10: December 2009–February 2010
G7010.02: March 2010–February 2014

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- Donald Danforth Plant Science Centre, St. Louis MO, USA: Martin Fregene

1. Research activities and progress at NRCRI, Nigeria

a. Development of mapping populations for cassava mosaic disease (CMD) resistance

Two mapping populations have been derived from crosses between CMD resistant genotype 97/2205 and a susceptible clone 30555; and between another resistant clone, 96/1089A and 30555. At least 200 F_1 plants segregating for each of the population for CMD have been established in the field for phenotyping and SNP genotyping. Genotyping will commence in August 2011.

b. Screening for polymorphic SNP markers for each of the parents in CMD mapping populations

At least 600 SNP markers found to be polymorphic for each of the 3 parents being used in the CMD mapping populations. Genotyping was done at KBiosciences.

S/No	Genotype	No. of polymorphic SNP markers
1	TMS30555	636
2	TMS95/1089A	623
3	TMS97/2205	619

c. Identification of sources of resistance to cassava bacterial blight (CBB) and cassava green mite (CGM)

Sixty five genotypes of cassava introduced from IITA have been evaluated for response to CBB and CGM. Screenhouse evaluations identified 15% as resistant to CBB. Poor symptom development delayed collection of data for CGM. NRCRI germplasm of about 500 accessions are under field evaluation for response to CBB and CGM. A select few will be screened in the screenhouse to confirm field results.

2. Research activities and progress at CRI, Ghana

a. Identification of sources of resistance to cassava bacterial blight (CBB) and cassava green mite (CGM)

Two hundred and twenty five accessions of cassava were introduced from Plant Genetic Resources Institute, Bonsu to CRI Kumasi for field evaluation. Data collection is still ongoing. A select few will be assessed in the screenhouse to confirm field data. In the current season, the materials will be screened for response to CGM attack.

3. Research activities and progress at ARI, Tanzania

Development of 3 bi-parental populations for validation of cassava brown streak disease (CBSD) SNP markers

Three bi-parental CBSD populations developed for validation of CBSD SNP markers. The families are Namikonga x AR 37-80, Kiroba x 3C83-13 and Muzege x Cheupe. For each of the families at least 200 genotypes are expected to be successfully established for phenotyping and genotyping as the SNP markers become available.

Tangible outputs delivered

Two mapping populations for two new sources of CMD resistance have been established in the field for phenotyping. Polymorphic SNP markers for 3 parents of CMD segregating mapping populations identified. Putative sources of resistance to CBB in Nigeria and Ghana identified and confirmatory tests are underway. Three bi-parental populations for validation of CBSD SNP markers developed and under evaluation in Tanzania.

4. G7009.09/G7010.01.03: Implement MARS Project for drought tolerance in Africa

G7009.09: December 2009–February 2010
G7010.01.03: March 2010–February 2014

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Marker-assisted breeding through MARS has been initiated to improve the efficiency of producing elite germplasm with exceptional performance under drought by identifying useful allele (QTL) combinations and pyramiding (and fixing) multiple sources of genes for drought tolerance into a set of new progenitors. The following project activities were conducted:

Evaluation of parental lines for drought tolerance
Six elite genotypes identified for attributes for drought tolerance and high productivity (good yield, disease resistance, high dry matter, and vigor) in the African germplasm were selected and tested for field performance Kano in the semi-arid agroecology to determine candidate parent lines for use in the development of mapping population to implement MARS for drought tolerance in cassava. The six genotypes were TMS91/0234, TMS92/0057, TMS92/0067, TMS95/0289, TMS92/0326 and TMS98/0581Nigeria. Results indicate that fresh root yield ranged from 16.8 – 31.0t/ha amongst the genotypes evaluated. Genotypes that had fresh root yield above 20t/ha (the minimum average yield target for Nigeria across ecologies) were considered as drought tolerant. The average cassava yield for the best local varieties reported in this ecology is often less than 10t/ha. TMS92/0510 had the least yield of 16.8 tons and was considered drought susceptible. This study showed that except for TMS92/0510, the other genotypes were considered drought tolerant and suitable for use as drought tolerant genotypes for MARS activities

Development of mapping populations
Two types of parental combinations were used for the crosses to develop mapping populations. The first type of pairing was between a drought tolerant and high yielding variety crossed to a second parent with drought susceptibility and low yield, with both parents combining other useful traits of breeding interest (disease resistance and quality traits). The second type of cross include combination between parents which are both drought tolerant and combining other targeted traits. Cassava is highly

heterozygous and is expected to segregate in the mapping population for all traits of interest including drought tolerance. This type of cross has the advantage of maximizing the effect of complementary genes and increasing the frequency of favorable alleles for the development of superior varieties with good yield through MARS. Pollination was successfully carried in 2009 and 2010 resulting in the development of nine mapping populations. The best three populations based on seedling establishment with over 200 genotypes were selected. They are TMS 98/0505 and TMS92/0510; TMS98/0505 x TMS98/0581; and TMS98/0505 x TMS91/02324. The populations are in the multiplication phase to generate enough planting materials for full phenotyping activities later in 2011.

Parental survey for polymorphic SNPs

Parental survey of the three mapping populations developed were carried to identify polymorphic SNP markers from the 1740 SNP markers available. Polymorphism assessment in the parental line lines indicate that an average of 33% were polymorphic in the parent genotypes and would be used in mapping for QTLs. The number of polymorphic markers in the parent is given in Table 1.

Table 1. Polymorphic SNP markers in parent lines of mapping populations

Parents	Number of polymorphic markers
TMS 98/0505	567
TMS92/0510	535
TMS98/0581	522
TMS91/02324	652

Infrastructural development of phenotyping sites in Nigeria and Ghana

Two phenotyping sites were selected for this project. They are Minjibir at Institute of Agricultural Research, Ahmadu Bello University in Kano (Nigeria) and Tamale at SARI in Ghana. Weather stations have been installed at each site. Irrigation systems and perimeter fencing of the trial site are presently at advanced stages to facilitate conduct of MARS experiments.

Tangible Outputs

- Mapping populations developed
- Polymorphic selected for Linkage analysis and QTL mapping

5. G7010.01.04: Phenotyping cassava for drought tolerance to identify QTLs

April 2010–March 2012

Principal Investigator and Lead Institute

Alfredo Alves, EMBRAPA, Alfredo.Alves@ars.usda.gov

NO UPDATE SUBMITTED

Capacity building: Cassava

Community of practice

6. G7010.01.05/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

January 2008–December 2010 (G4008.26)
January 2011–December 2013 (G7010.01.05)

G7010.01.05 report

Principal Investigator

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Summary

Cassava is staple food for over 200 million people in sub-Saharan Africa. Significant improvements have been made through breeding to develop improved varieties which meet the needs and requirements of farmers and other end-users. , Through previous GCP funded projects, markers associated with CMD resistance have been identified and have been used to deploy useful germplasm from the primary center of diversity in the Neo tropics. In order to consolidate on gains made so far the cassava breeding community of practice project was initiated by the SP5 sub-programme of the GCP to facilitate rapid uptake of MAS breeding in Africa. The CoP primarily aims to facilitate the routine application of MAS in breeding programs and to develop relevant schemes that effectively integrates MAS with field – based strategies. Other goals of the GCP include the strengthening of capacity of NARs in modern breeding, development of an efficient network that integrates breeding among NARs breeder and the establishment of web-based database for information sharing including germplasm exchange. Under the CoP, different breeding populations are being developed, and through genotyping activities initiated by NARs in collaboration with CIAT and the GSS. Excellent genotypes developed through MAS have been identified and are currently being evaluated in four countries (Nigeria, Ghana, Tanzania, and Uganda). The member states in the CoP are target countries in the second phase of the GCP which is mainly focused on improving yield in drought prone environment. NARs are very strategic in the development of products to

farmers. The success of this will depend on the capacity or ability of NARs to rapidly take up products from the GCP cassava CI initiatives. Principally, use of more efficient marker systems e.g. SNPs, access to high throughput genotyping platforms and use of efficient breeding schemes such as marker assisted recurrent selection (MARS) require that effective training component is put in place to sustain rapid gains from the CI in Africa. This project therefore seeks to undertake capacity building activities which will give the needed impetus required for NARS to rapidly deploy new tools or technologies from present cassava CI projects in routine molecular breeding of improved varieties for the benefit of poor resource farmers whose livelihood depends on cassava. The proposal would also seek to use these markers to develop useful genetic stocks, breeding populations and elite gene pools in aid of cassava improvement in Africa.

7. 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

January 2008–December 2010

G4008.26 report

Principal Investigator and Lead Institute

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-

The cassava breeding community of practice (CoP) in Africa project has its primary aim the target of improving the capacity of NARs (Ghana, Nigeria, Uganda and Tanzania) in modern breeding strategies with emphasis on the application of molecular marker tools in development of superior cassava varieties. The following major activities were successfully accomplished in the four countries:

1. Sharing germplasm and information

Web based database www. for building linkages between users of cassava improvement technology has been created. Breeder-to-breeder visit for experience sharing and flow of information among NARs were done in the CoP to strengthen breeder network. Backcross population developed for delayed post harvest physiological deterioration and improved protein content using modified advanced backcross scheme and selected via MAS for cassava mosaic disease (CMD) resistance were transferred from CIAT to Africa. Phenotyping results identified genotypes with high protein content of 8 – 14% indicating that high protein content introgressed from

Manihot esculenta sub spp *flabelifolia* in to cassava in the backcross scheme was largely successful.

2. Integration of MAS with field based breeding
MAS developed varieties were validated for CMD and cassava green mite (CGM) resistance in on-farm trials in the four countries. In East Africa, genotypes combining CMD resistance and (cassava brown streak disease) CBSD tolerance in East Africa were developed. Evaluation of 64 families for the response of the CMD2 gene to the disease revealed differential disease expression pattern suggesting that the disease expression of CMD2 genotypes was influenced by genetic background effects. Cassava is highly heterozygous and integrated breeding scheme to reduce high segregation in the seedling nurseries at the early stages of the breeding scheme, and minimize MAS cost was implemented by fixing favorable alleles at target loci. This resulted resulting in the identification of 90 - 384 genotypes homozygous for the six targeted loci screened for both CMD and CGM. These genotypes could be used as parent lines in breeding for CMD and CGM.

3. Strengthening NARS breeders in field based and molecular breeding
A fast track marker-assisted breeding (MAB) strategy was implemented in the NARs for the rapid development and testing of improved CMD resistant varities in the participating countries. Cassava accessions of African and Latin American germplasm including wild derivatives were analyzed with 27 SSR markers for genetic diversity with molecular markers. Cluster analysis produced two broad groups of Nigerian and Latin American germplasm indicating genetic differentiation based on geographical origin. Genetic diversity differentiated sub-groups for each germpalsm set reflecting pedigree differences and breeding history. QTL mapping identified Ten QTLs for high protein content with PVE ranging from 15% and 25%. All the QTLs showed additive gene action Using bulk segregant analysis, nine SSR markers were found associated with early bulking in CMD resistant F_1 populations. Hybridization activities to introgress LA germplasm in to farmer preferrred varieties to develpe superior genotypes in the genetic improvement activities of NARs resulted in the development of F_1, S_1, S_2, S_3 and M_2 populations. Annual workshop was conducted to improve genetic analysis of molecular and phenotypic data including data management using ICIS.

4. Building linkages with end-users
The project strengthened linkages with target stakeholder and end-users of GCP products and outputs through plant participatory breeding and participatory varietal selection involving farmers. Database of secondary and tertiary end-users involving processors/consumers has been generated to improve product delivery efficiency.

Tangible Outputs
Building on a previous funded GCP project, *Development of Low Cost Technologies for pyramiding useful genes from wild relatives of cassava into elite progenitor*s Development of low-cost technologies, the CoP facilitated the release of a MAS developed variety UMUCASS33 (high yielding and CMD resistant) in Nigeria last year while four top elite lines combining both CMD resistance and cassava brown streak disease tolerance (CBSD) tolerance have been nominated in Tanzania for release. The achievements recorded in this project is expected to lead to better organized and efficient breeding initiatives in the NARS programs of Africa for rapid product development and increased food security in the region.

Legumes

Beans

8. G3008.07: Basal root architecture and drought tolerance in common beans

November 2008–October 2011

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Context: The overall goal of this project is to evaluate the utility of Basal Root Growth Angle Plasticity (BRGAP) and Basal Root Whorl Number (BRWN) for enhancing the tolerance of common bean lines to drought and low soil phosphorus availability. The project includes physiological assessment of the utility of these phenes, phenotypic profiling of bean germplasm, and characterization of their genetic control.

Findings and implications:
BRGA plasticity: Over 100 genotypes have been evaluated for BRGA plasticity under low and high phosphorus availability in multiple field locations. A limited number have shown a stable BRGA adjustment in response to low phosphorus levels. A final field trial has been installed in the low P field at PSU. This study will be complemented by lab evaluation of BRGA in pouches. If these studies do not reveal consistent plasticity of BRGA in response to P availability we will conclude that this trait will not be a useful selection criteria for breeding for low P soils. Other traits have been observed in the process of looking for BRGA plasticity that offer greater promise. Root system dimorphism permits a plant to access both nutrients in shallow soil profiles and water in deeper soil. A dimorphic root system could be characterized by extensive adventitious roots with deep basals, shallow basals with a strong highly branched tap root, or extensive adventitious roots with a strong highly branched tap. Phenotyping has been done for a portion of one population and another population will be phenotyped this season at PSU. Field experiments in South Africa evaluated the utility of dimorphic root systems and the experiment will be repeated this season at PSU. If this trait proves useful it would decrease the tradeoff between optimizing water and phosphorus acquisition. Evaluation of this trait using shovelomics means large numbers of accession can be characterized and the selection process can progress rapidly.

BRWN: The utility of BRWN has been evaluated in the laboratory and multiple field environments (PSU, South Arica, Mozambique, Colombia). Greater BRWN substantially enhanced bean growth under drought and low soil P availability in most cases. Since this traits is rapidly phenotyped in young seedlings in the lab, this trait shows promise in bean breeding, and in fact is now being deployed in bean breeding in Mozambique.

Phenotypic profiling of diverse *Phaseolus* germplasm for BRWN and BRGAP has identified promising sources of these traits and opportunities for improvement of commercial grain classes. We are genotyping regions surrounding promising QTL for BRWN. While it was originally foreseen that this would be accomplished with SSR, access to the bean and soy genome sequences permits searching for genes associated with root development.

These results will be of direct benefit in bean breeding, by validating the utility of specific root traits for drought and low P soils, and by identifying genetic control of this trait. Because BRWN is readily phenotyped in young seedlings, its validation as a useful trait would make available a new tool for bean breeding in Africa. Phenotypic selection for BRWN is already being employed in the bean breeding program of IIAM-Mozambique.

Links: We have active links with bean breeders in Mozambique, Malawi, Zambia, Brazil, and Honduras to assist them in deploying root architectural traits in their breeding programs.

Next steps/challenges:
Value of BRGAP: the expression of this plastic trait has been, not surprisingly, variable in the field. We plan a final set of studies with a new population of RILs to determine if it is able enough to be useful. A technical challenge has been the difficulty in obtaining reliable drought stress at our sites in RSA and Colombia. We will continue to explore dimorphic root architectures as an alternative trait.

Value of BRWN: This trait is very promising. We will evaluate it again under drought stress, and combined drought/low P stress in rainout shelters, in the greenhouse, and in field studies in Colombia, Mozambique, Zambia, and South Africa.

9. G4008.11. Dry bean improvement and marker assisted selection for diseases and abiotic stresses in Central America and the Caribbean

January 2008–December 2011; NCE: July 2011

Principal Investigator and Lead Institute

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1. Research activities and progresses at INIFAP.

Activities conducted in the first semester 2011 include:

1.1 a trial under full irrigation and terminal drought during the dry season at CEBAJ, Celaya (Bajio region)., and SENGUA, San Luis de La Paz, Guanajuato (highland region). This trial included 289 F5 derived from the cross of Pinto Villa (Acosta et al., 1995)/Pinto Saltillo (Sanchez et al. 2004), two drought resistant cultivars, Durango race.

A leaf sample from ten different plants per family was taken, dried and sent for SNP genotyping at the UK. In the mean time data have been taken on field grown plants at both locations; data taken so far includes: plant phenology, row height and width, fresh and dry weight of leaf tissue, specific leaf weight and canopy temperature (Table 1). This data are being analyzed and data taken in 2010 is presented in the form of a research note published at the Annual Report of the Bean Improvement Cooperative (Annex 1).

Table 1 Basic statistics for data recorded on 289 F5 families derived from the cross Pinto Villa/Pinto Saltillo. Data recorded across moisture treatments.

Trait	Mean	Std Dev	Skewness	Kurtosis	Bimodality
Vigor – 1 to 5 visual scale	3.3172	0.715	0.059	-0.469	0.392
Flowering – day^{-1}	39.94	1.413	1.072	2.500	0.388
Maturity - day^{-1}	84.79	2.331	0.267	-0.096	0.365
Rep Period -day^{-1}	45.3	2.941	2.213	6.952	0.591
Canopy Temp – Degree C	22.9	0.596	0.344	1.259	0.261
Canopy height – cm	37.1	2.356	0.187	1.789	0.215
Canopy width – cm	35.15	2.412	-0.153	-0.282	0.372
Leaf fresh wt – $mg/28.3mm^2$	79.83	4.728	0.472	2.025	0.242
Leaf dry wt – $mg/28.3\ mm^2$	16.37	1.205	0.173	0.164	0.322
Leaf water content	3.926	0.212	0.207	0.088	0.334
Leaf Specific Wt	8.338	0.614	0-173	0.164	0.322

1.2 A second activity was to test under greenhouse and field conditions at CEBAJ, Celaya, Guanajuato under two moisture treatments: full irrigation and terminal drought stress six red and six black seeded Mesoamerican genotypes that were previously tested for gene expression under stress. The molecular characterization to drought stress via the amplification of specific DNA segments from ESTs sequences previously reported as related to drought stress (Table 2) was reported in 2010. The level of expression of six genes related to drought response was recorded in a set of each, black and red seeded Mesoamerican bean lines. These genotypes were chosen after intensive testing as promising high yielding material with seed of commercial value in the red a black seeded classes from material introduced from CIAT (Beebe et al., 2008). The trial at the greenhouse was conducted during the winter season of 2010/2011 and in the field during the early irrigated planting season of 2011. Our aim is to compare the response under drought stress with the results from the gene expression trial above mentioned.

As we reported before, red seeded genotypes over expressed a larger number of genes than the black seeded (Annual report 2010), and thus are considerer superior. In general, red seeded middle American bean genotypes are considered as more advanced in their genetic improvement than black seeded due to superior adaptation traits including seed yield (Beebe *et al*., 2008). Among the red seeded genotypes SCR 13 was outstanding since it show high levels of expression in five genes, whereas the best black genotype, SEN 70 over expressed four genes. The EST identified as PvS09 that has the identity of LEA 5 show a high relationship with water stress response since it was over expressed in most genotypes under stress conditions (data no shown). ESTs corresponding to clones identified as of cationic transport protein (PvS05), Serin threonin protein phosphatase (PvS06) and chaperone/heat shock protein (PvS07), respectively, show high expression under drought stress in the red genotypes, but no in the black seeded genotypes. The over expression of different genes between the sets of beans tested suggest the existence of different mechanisms of resistance between these two sets of bean genotypes. Therefore, crosses between these two seed classes, and with drought resistant pinto genotypes, must be carried out to accumulate different genes (plant mechanisms) to cope with drought stress.

Table 2. Primer, sequence and reference for five ESTs primers specifically related to drought response. In total, twenty two ESTs primers were tested.

Primer	Sense	Antisense	Accession	Amplicon
PvS02	GGCACGAGGCATTTGTTCAC	GGCAAATTCCCATTGTTCCGGG	AY052627	996
PvS05	GCACAGGCGCAAGAAGTGTCAATG	TGATTCTGTGGTGAGCACAAGGCAGC	AF402773	1146
PvS06	GGAGATCCGTCAACTCTGCGTCAAT	CCAATCACAAGCAACCAACTGGTG	AY220096	1080
PvS07	CATGTCTGACGCAGGACGC	GCGAAGTGACAGCGACAGC	CX129914	304
PvS09	AGGTGAGGTGGCTGCTGCGAAAC	TGGTATCCCCTATCACGCACGCTCC	CX129891	465

Tables 3 and 4 show the stem and branches dry weight and of green leaves of each, eight black and red seeded cultivars grown under irrigation and water stress. Unfortunately, the temperatures inside the greenhouse at noon were higher than those tolerated by beans, near 40^{o}C, and some genotypes did not set pods due to flower and small pod abortion, thus for comparison only dry weigh of mentioned organs are reported. In the sample taken before the flowering stage (Table 3), in addition to differences within cultivars under both moisture conditions, the leaf dry weight average from red cultivars was higher than that of the black cultivars. This leaf dry weight was taken from green photosyntetically active leaf tissue, thus the results suggest a higher stress (moisture and heat) tolerance in the red seeded genotypes.

Table 3. Plant and leaves dry weight of eight black and eight red seeded bean cultivars grown under two moisture treatments in the greenhouse at Celaya, Gto. Mexico. Destructive samples taken at the pre-flowering stage.

Black	Irrigated		Streesed		Red	Irrigated		Streesed	
	PDW	LDW	PDW	LDW		PDW	LDW	PDW	LDW
SEN 70	6.73	1.50	6.57	1.50	SER 118	7.93	6.37	6.33	1.10
SEN 26	8.90	2.83	9.57	2.03	SCR 13	7.77	6.50	7.10	3.43
SCN 7	8.83	2.77	9.83	2.97	SCR 11	7.43	3.37	7.93	2.47
SEN 44	9.20	1.53	8.53	1.93	SCR 17	6.90	5.57	7.47	2.80
E 15-55	6.63	1.00	8.20	1.73	SER 83	8.63	5.30	7.57	2.47
NH 81	7.33	2.37	7.50	1.37	SCR 6	8.63	7.4	7.7	2.8
SEN 56	8.67	2.40	6.83	1.83	SER 18	9.63	6.73	8.23	4.03
N 8025	9.40	2.87	5.97	1.53	INTA	8.60	6.17	7.93	3.50
Ave	8.21	2.16	7.87	1.86		8.19	5.92	7.53	2.82

PDW = Above ground dry weight from three plants; LDW= Leaves dry weight from three plants.

Table 4. Plant and leaves dry weight of eight black and eight red seeded bean cultivars grown under two moisture treatments in the greenhouse at Celaya, Gto. Mexico. Destructive samples taken at the mid-pod filling stage.

	Irrigated		Streesed			Irrigated		Streesed	
Black	PDW	LDW	PDW	LDW	Red	PDW	LDW	PDW	LDW
SEN 70	1.14	1.77	1.08	0.83	SER 118	4.83	3.02	0.83	0.79
SEN 26	1.91	3.11	1.34	0.97	SCR 13	4.99	2.81	1.91	0.97
SCN 7	2.55	3.01	1.60	0.84	SCR 11	3.25	2.80	1.49	0.89
SEN 44	2.33	2.84	1.28	0.89	SCR 17	3.84	2.08	1.69	0.85
EL 15-55	1.15	2.53	1.72	0.96	SER 83	4.20	3.18	1.90	0.83
NH 81	2.09	2.74	1.08	0.55	SCR 6	4.35	3.44	2.00	1.06
SEN 56	2.25	2.07	1.38	0.75	SER 18	4.39	3.43	1.92	1.14
N 8025	3.22	3.69	1.25	0.74	INTA	5.41	3.44	2.40	1.42
Ave	2.08	2.72	1.34	0.82		4.41	3.02	1.77	0.99

PDW = Above ground dry weight from one plant; LDW= Leaves dry weight from one plant…

10. G6007.03/G6010.03: Improve common bean productivity for marginal environments in sub-Saharan Africa

G6007.03: May 2007–April 2010 (TLI Phase I)
G6010.03: May 2010–April 2014 (TLI Phase II) (see separate reports for Phase I and Phase II

Phase II report

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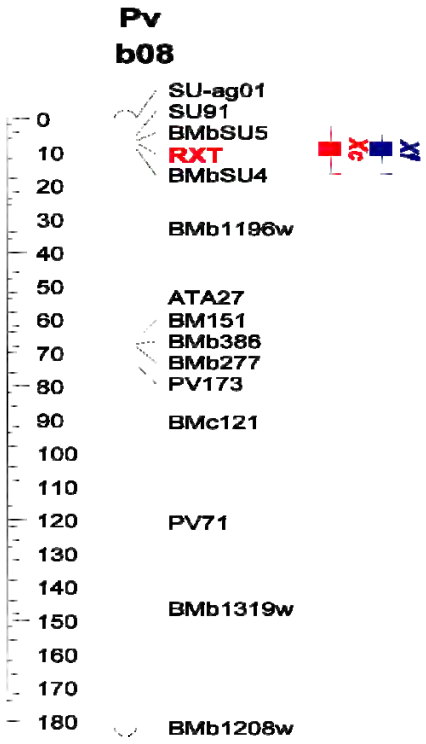
Context: The Tropical Legumes I (TL-I) project seeks to develop genetic tools in support of a sister project, Tropical Legumes II (TL-II), where such tools shall be deployed in varietal development. Outputs therefore take the form of methodologies, physiological understanding leading to selection criteria, or products of pre-breeding that can serve as parental materials. TL-II deals with four legume crops, of which the current report deals with the common bean (*Phaseolus vulgaris* L.) component.

Findings: Physiological studies revealed variability in traits with potential value for drought tolerance. A soil tube experiment of 36 lines was performed with selections from the reference collection in the previous year. Low positive correlations with yield were found with rooting depth at 40 d and with root density at 20-40 cm depth.

A trial of 36 genotypes including elite Andean (large seeded) genotypes was planted with and without irrigation and suffered significant stress in the drought treatment. A novel phenotyping method was tested, employing an infrared camera mounted on a mobile platform, and digital photographs, as opposed to static readings of a hand held infrared "gun." This method permits rapid data taking (important for uniform environmental conditions) of a large foliar area, for subsequent analysis. Three visual scales (degree of wilting, leaf loss and seed fill) were tested on the same trial of Andean (large seeded) types. The lines in the drought treatment showed marked visual differences in leaf fall.

An interspecific line that is derived from *P. acutifolius* and that presents resistance to wilting is being combined with other drought sources, with the expectation of pyramiding of tolerance traits. F2.4 and F3.5 families derived from this source are being planted in 2011 to test the potential of this trait for drought resistance, and to develop visual scoring for wilting. The F2.4 families will be planted in three sites in Africa as observation trials.



TL-I seeks to implement marker assisted selection (MAS) for simply inherited traits as a first step toward more MAS with complex traits. Among families evaluated with the SU91 marker for CBB resistance, 97 lines carried the SU91 marker and expressed resistance. Among 344 lines evaluated for a Zabrotes resistance marker, 89 expressed the marker, and several had very good yield. These lines will be shipped to partners including those with potential of practicing MAS, for evaluation as potential parents and the creation of populations for MAS. A gene based marker (RXT = resistant to Xanthomonas) linked to the SU91 marker was developed from a sequence for a protein involved in resistance to bacteria. It will be adapted to agarose gels and shared with partners. SU91 presents occasional recombination events, and we hope that this new markers will overcome this problem.

Under the sister TL-II project, a regional network of drought researchers in eastern and southern Africa has been established, and this represents a budding community of practice for the implementation of the tools and knowledge developed in TL-I.

Next steps and challenges: Work on MAS for simple resistance traits is well advanced and is being implemented in Colombia and Malawi. Selection for Zabrotes needs to be implemented. The greatest challenge is obtaining high quality phenotypic data for MARS in a timely fashion permit reliable selection of QTL, in an effort that spans two continents.

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11. G6007.03: Improve common bean (*Phaseolus vulgaris* L) productivity for marginal environments in sub-Saharan Africa

May 2007–April 2010

Phase I report

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- DR&SS: Godwill Makunde

Context: The Tropical Legumes I (TL-I) project seeks to develop genetic tools in support of a sister project, Tropical Legumes II (TL-II), where such tools shall be deployed in varietal development. Sources of drought tolerance exist within common bean germplasm but have not been widely deployed across genepools. In this project we laid the groundwork for QTL-based, marker assisted selection (MAS) through germplasm screening (reference collection), marker development (SSRs and SNPs), testing of MAS techniques, QTL identification for drought traits and pre-breeding to develop new populations to move genes from drought tolerant sources into the Andean beans that are preferred in the region.

Findings: A reference collection consisting of 202 genotypes, separated in one Mesoamerican lattice design (11 x 11) and one Andean lattice design (9x9) was evaluated at CIAT-Colombia, SARI-Ethiopia, Univ. Nairobi - Kenya, DART-Malawi, SELIAN-Tanzania and CBI-Zimbabwe. Another set of 202 regional and local varieties was established and distributed among sites within the two regional African networks (ECABREN and SABRN) and was tested at CIAT-Colombia. Finally, landraces of Kenya and Ethiopia were evaluated phenotypically and genotypically as part of the PhD of Asrat Asfaw (SARI/Wageningen). Coordination with the TL2 phenotyping sites was in many cases a key part of the success of this part of the objective.

In marker development for common beans, a total of 1500 simple sequence repeat loci were identified and over 1000 microsatellite markers tested. Over 200 of the markers have been genetically mapped and 400 have been evaluated for polymorphism among parents and genotypes described above. Other marker types tested were a legume ortholog GoldenGate OPA assay of 786 SNPs (with Obj 5), 10 ADOC gene markers, and an additional set of 93 SNPs. As part of genomic resource development, two subtractive and two full-length cDNA libraries were constructed with mRNA from plants stressed or unstressed by drought.

In marker assisted selection for biotic resistance, we developed and tested a set of 30 SSR markers for arcelin-based bruchid resistance derived from wild beans and made over 200 crosses to incorporate this gene into Andean beans with commercial seed types (red mottled, cream mottled and large red beans, especially). Two other biotic

stresses were targeted for pyramiding with each other or with the arcelin gene, namely resistance to bean common mosaic necrosis virus (BCMNV) and common bacterial blight (CBB). The evaluation of three markers for QTL against CBB resistance is the thesis topic of Lizzie Kalolokesya (SABRN/Univ. of Zambia) who along with Godwill Makunde (CBI) has been sponsored by TL2 and has received laboratory training in CIAT-Colombia and South Africa.

In marker assisted selection for abiotic stress resistance, two QTL mapping populations were evaluated in four sites over one or more season (Awassa-Ethiopia, Chitedze-Malawi, CIAT-Colombia and Thika-Kenya) with both drought and non-drought treatments. Root depth and photosynthate mobilization were the two target traits as well as yield information for the two populations and were used in the thesis projects of Asrat Asfaw and MSc candidate Felix Waweru (Univ of Nairobi).

Two full advanced backcross populations were developed by crossing 2 ESA varieties with two Mesoamerican SER lines that are among the most advanced small red beans for drought tolerance and these will all be used in the second phase of the TL1 project.

Next steps and challenges: TL-I phase 2 has already initiated and has been operational for one full year. A separate report has been submitted for the first year.

References:

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Capacity building: Beans

Human resources (support to teams, students, travel grants, workshops)

12. G4009.07.01 TL1 students for analysis of drought tolerance in common bean

October 2009-october 2010; NCE: October 2011

Principal Investigator and Lead Institute

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Collaborating institutes and scientists

- SARI: Ethiopia. Fitsum Alemayehu

- DR&SS: Godwill Makunde
- Lizzie Kalolokesya – SABRN/Univ. of Zambia
-

Context:
As part of the GCP's continuing commitment with capacity building, one PhD fellowship was set aside per crop in phase 2 of the Tropical Legumes I (TL-I) project. Given the high priority of testing the potential of marker assisted recurrent selection (MARS), the research component of the PhD study of Fitsum Alemayehu will be focused on the application of MARS to common bean for the improvement of drought tolerance in Andean beans with commercial grain type. Godwill Makunde is finishing his PhD study in 2011, and Lizzie Kalolokesya completed her study in 2010.

Findings:
A total of 193 lines will be evaluated in multiple sites in Ethiopia and Colombia. These are derived from crosses of sister lines coded as SAB, with CAL 143.

(CAL 143 x SAB 620) x SAB 626
(SAB 628 x CAL 143) x SAB 659
(SAB 628 x CAL 143) x SAB 686

SABs 626, 628, 659 and 686 are all sister lines with a very similar genetic composition, while CAL 143 is a widely adopted variety in six countries in southern Africa. The SAB lines present improved remobilization of photosynthate to grain derived from Mesoamerican race Durango, while CAL 143 has excellent adaptation to nutrient poor soils. The population therefore has potential to combine multiple traits that contribute to drought tolerance, and recurrent selection is especially appropriate.

In support of this study, DNA extraction from seed was successfully tested, to permit flexibility in the timing of genotyping and in the selection of plants for intercrossing. This is a simple but significant achievement that has the potential of facilitating application of MAS with greater flexibility. This is especially important for MARS since intercrossing is practiced based on genotyping of F1 plants, and in determinate Andean bush beans time from DNA sampling to flower would be less than three weeks. Sampling DNA prior to planting will permit selection of seeds with the greatest number of desirable QTL prior to planting.

A recurrent selection population was formed among F3.7 families, and crosses among lines and among resulting F1's have already been created. These are being held for marker evaluation and selection while phenotypic and genotypic data are obtained on the 193 lines.

Fitsum Alemayehu and Godwill Makunde both visited CIAT headquarters from August to December, 2010. They participated in a greenhouse study of rooting depth in parental materials using the soil cylinder technique, and Fitsum gained experience in laboratory technique. This was also the opportunity to discuss progress of both with their professor Dr. Maryke Labuschagne.

Next steps and challenges: The greatest challenge is obtaining high quality phenotypic data for MARS in a timely fashion permit reliable selection of QTL, in an effort that spans two continents. Time will be short to compile all phenotypic data and perform the QTL analysis.

Chickpeas

13. G4008.12: Linking genetic diversity with phenotype for drought tolerance traits through molecular and physiological characterization of a diverse reference collection of chickpea

January 2008–December 2009; NCE: September 2011

Principal Investigator and Lead Institute

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Collaborating institutes and scientists

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- JIRCAS, Japan: Satoshi Tobita and Osamu Ito
- UAS, Bangalore, India: M.S. Sheshshayee

World wide, terminal drought is a key constraint to chickpea productivity. Incorporation of morphological and physiological traits that are strongly associated with drought tolerance into well adapted genetic backgrounds is expected to improve the yield stability. The accessions of chickpea reference collection were chosen to be phenotyped for carbon isotope discrimination ($\Delta^{13}C$), the best opted estimate of transpiration efficiency (TE). These accessions were also phenotyped for specific leaf area (SLA) and SPAD chlorophyll meter readings that are considered as two other proxies for TE. The collaborating centres, University of Agricultural Sciences, Bangalore and ICRISAT, Patancheru, were chosen on the basis of diversity in short duration growing environment and Isotope-ratio mass spectrometry capability of University of Agricultural Sciences, Bangalore and JIRCAS, Japan.

The field experiments conducted both in 2008-09 and 2009-10 at ICRISAT, Patancheru, had shown that the accessions of the reference collection ranged from 35 to 66 days in 50% flowering and 79 to 115 days to maturity and the optimum irrigation application extended the mean maturity time by 15 days. Shoot biomass production ranged from 2800 to 5500 kg ha^{-1} and the grain yields ranged from 1400 to 2800 kg ha^{-1} under terminal drought that was found enhanced with optimum irrigation to 3600 to 8900 in shoot biomass and 700- 2300 in seed yield. Large variations in $\Delta^{13}C$ values were observed that ranged from -0.25.5 to -0.28.0 in 2008-09 and -24.2 to -27.2 in 2009-10 under drought while the range was narrow under optimally irrigated conditions. $\Delta^{13}C$ variation was significantly associated with per day grain productivity and the explained 7% of the variation in 2008-09 while 25% in 2009-10. Also the phenology and the yield components were also largely and significantly associated with $\Delta^{13}C$. SPAD or SLA, the surrogates of TE, were not significantly associated with $\Delta^{13}C$ but SLA and SPAD were significantly and negatively associated with each other. The trials at UAS, Bangalore did not succeed in both the years due a late sowing in 2008-09 and a poor germination in 2009-10. A genotyping using DArT markers resulted in identifying 1157 polymorphic markers on the reference collection. The marker trait association lead to the identification two DArT markers that were closely associated with the $\Delta^{13}C$ trait under terminal drought.

The results and general experience was shared with the future NARS users and collaborators in Tropical Legumes I and II from Africa and Asia in a training cum capacity building workshop conducted between 25-29 May 2010 at JIRCAS, Japan and Hokkaido University, Sapporo, Japan.

Parellel findings in another study had revealed that the QTL for $\Delta^{13}C$ also co-localized the same genomic region where large number of QTLs for various other drought tolerance traits, related to root traits, yield and HI. This offers an advantage for the ongoing molecular breeding efforts in chickpea drought tolerance where an introgression of this genomic region into elite cultivars is expected to improve all the desirable traits in one attempt. Also the root QTL introgressed progenies (BC_3F_3) developed in a parallel study can be an ideal material to look for these markers in the selection process. The results are being written up as journal articles and the data will be uploaded into the GCP central registry after Sep 2011.

14. G6007.04/G6010.04: Improve chickpea productivity for marginal environments in Sub-Saharan Africa and Asia- Phase II

G6007.04: May 2007–April 2010 (TLI Phase I)
G6010.04: May 2010–May 2014 (TLI Phase II)

Principal investigator

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- University of California-Davis, USA: Doug Cook, Ming Cheng Luo

Summary

Chickpea is the world's second largest grown food legume and the developing countries account for over 95% of its production and consumption. Drought is globally the number one constraint to chickpea production, causing yield losses of around 3.7 million tons (out of a total production of 8.6 million tons). TL I Phase II aims at harnessing the resources developed during Phase I for chickpea crop improvement. Eight superior lines were selected based on phenotyping of reference collection and 37 and 44 crosses were made in sub-Saharan Africa and India respectively for developing pre-breeding populations. Further 28 two-way crosses, 14 four-way crosses and seven eight-way crosses were made and F_1s have been shown for developing MAGIC populations (Activity 1). For designing the KASPar assay, 2486 genes containing high confidence SNPs were chosen and by using Marker Services of Integrated Breeding Platform, successful KASPar assays were developed for a total of 2005 genes. Towards development of genome-wide physical map, in

collaboration with National Institute of Plant Genetic Research (NIPGR), New Delhi (S Bhatia and A K Tyagi) and UC-Davis, USA (MingCheng Luo), two new BAC libraries were constructed using *Hin*dIII and *Eco*RI restriction enzymes. To date, 15,744 clones were fingerprinted and 10,368 fingerprints were edited. Fingerprinting of remaining clones is in progress to develop genome wide physical map (Activity 2). For enhancing MABC activities, involving NARS partners as leaders under TL I Phase II, Ms Serah Songok, a PhD student from Egerton University in Kenya, is involved in introgression of QTL for root traits from ICC 4958 into ICCV 97105 and ICCV 95423. In this context, 3 cycles of MABC have been completed and 100 BC_3F_1 seeds were generated in each cross. DZARC in Debre Zeit has completed first backcrossing of Ejere × ICC 4958 and Arerti × ICC 4958 with the recurrent parents (Ejere and Arerti). In case of MARS, that was initiated in the Phase I of TL-I, $F_{3:5}$ progenies from two crosses (JG 11 × ICCV 04112 and JG 130 × ICCV 05107) developed were evaluated at three locations (Debre Zeit in Ethiopia, Koibatek in Kenya and Patancheru in India) under rainfed and irrigated. QTL analysis is in progress for both the populations. A proposal was developed and submitted to the Department of Biotechnology (DBT), Government of India for funding a TL-I complementary project on application of MABC and MARS research to enhance drought tolerance in chickpea in India. The project has been approved with a total budget of about US $ 850,000 over a period of 3 years (Activity 3). A workshop on modern breeding technologies for chickpea improvement was conducted in the Year 1 (October 25 – November 19, 2010) at the ardent request of breeders and collaborators. (http://www.icrisat.org/bt-publicdomain-mas2.htm). One PhD student, Ms Serah Songok, currently working at ICRISAT, has been registered at Egerton University. A second PhD student, Mr Musa Jarso, has registered at Addis Ababa University will commence work shortly on molecular breeding. Another PhD student, Ms Alice Koskie, registered at WACCI would work on MARS activities. Mr Kebede Teshome, PhD student, registered at Haramaya University is currently working at ICRISAT. An MSc student, Mr Abebe Sori, is registered at Haramaya University and Mr Moses Oyier, has been registered at Egerton, and has commenced work at both the university and at ICRISAT. Mr Getachew Tilahun, registered at Addis Ababa University will start to work on MABC for drought tolerance. Efforts to identify and enroll the balance of the targeted number of MSc students are ongoing (Activity 4). Compilation of the marker sequence data, marker genotyping data, mapping data and phenotypic data obtained in Tropical Legumes I Project Phase I is in progress. Data will be curated in appropriate databases by the end of April 2011. Till now 11 datasets were curated and data have become available in local database (Activity 5).

15. G7009.02: Mapping and validation of QTLs associated with drought tolerance traits in chickpea

January 2009–December 2011

Principal Investigator and Lead Institute

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- University of Agricultural Sciences (UAS), Bangalore, India: KP Vishwanatha; MS Sheshashaye
- Regional Agricultural Research Station (RARS), Nandyal, India: Veera Jayalakshmi
- Agricultural Research Station (ARS), Durgapura, Jaipur, Rajasthan, India: SJ Singh
- RAK College of Agriculture (RAKCA), Sehore, Madhya Pradesh, India: M. Yasin

This project builds on Tropical Legume I project, where efforts are being made to improve drought tolerance in chickpea through marker-assisted breeding. In this project, we proposed multilocation evaluation of ICC 4958 × ICC 1882 and ICC 283 × ICC 8261 RILs for grain yield, harvest index and carbon isotope discrimination for mapping and validation of QTLs for these traits.

Mapping of QTLs for root and other traits from ICC 4958 × ICC 1882 RILs
A set of 264 RILs from ICC 4958 (high root biomass) × ICC 1882 (low root biomass) was evaluated for phenology, biomass, yield, harvest index (HI) for two crop seasons (2008/09 and 2009/10). The trial was conducted in alpha lattice design with 2 replications under irrigated and rainfed conditions. There were four locations (Patancheru, Nandyal, Durgapura and Sehore) during 2008/09. The data from Durgapura was not considered for analysis due to poor plant stand. Leaf samples were collected from two locations (Patancheru and Sehore) and analyzed for carbon isotope discrimination (δ13C) at UAS-Bangalore. The RILs were evaluated at 5 locations (2008/09 locations + Hiriyur) during 2009-10. Both sets of trials at Sehore were considered non-stressed due to unexpected rains during the crop season. Leaf samples were collected from all locations, except Sehore, and sent to UAS-Bangalore for analysis of carbon isotope discrimination (δ13C). Correlation coefficients between different traits were worked out separately for each location. Carbon isotope discrimination showed significant positive correlation with HI (r=0.2) at Sehore and with yield (r=0.227) at Patancheru.

Genotypic data was generated for all 358 polymorphic SSR markers (258 markers under TL-I project and 100 markers under this project). A linkage map of ICC 4958 × ICC 1882 RILs was developed in which 241 markers were mapped on to 8 linkage groups spanning a distance of 621.5 cM. The phenotypic data on root traits available for 2 years (2005 and 2007) at ICRISAT and data on other traits collected under this project were used for QTL analysis. Several QTLs were identified for days to 50% flowering, days to maturity, drought tolerance indices, HI, grain yield and related traits. Several QTLs were mapped onto the same genomic region where the root trait QTLs were mapped earlier under TL-I which indicate that the genomic region identified earlier in TL-I project has an important role in conferring drought tolerance.

Mapping of QTLs for root and other traits from ICC 283 × ICC 8261
RILs of ICC 283 (low root length density) × ICC 8261 (high root length density) cross (n=286) were evaluated during 2010/11 at the same five locations and in the

similar way as the RILs of ICC 4958 × ICC 1882 were evaluated in year 2. The trial at Hiriyur was discarded due to poor plant stand.

A total of 313 polymorphic markers were genotyped (213 markers under TL-I project and 100 markers under this project) on ICC 283 × ICC 8261 RILs. A linkage map comprising of 168 markers, spanning a distance of 533.06 cM, covering all eight linkage groups of chickpea was developed. The phenotypic data on root traits available for two years (2006 and 2008) from ICRISAT and data collected under this project on other traits were used for QTL analysis. Several QTLs were identified on LG4 for root traits, HI, yield and related traits (Fig 1). The phenotypic variation for various traits ranged from 2.5% (secondary branches) to 54% (shoot dry weight). Interestingly, most QTLs were mapped onto the same genomic region which was identified from ICC 4958 × ICC 1882 RILs. Thus, this mapping population provided validation of QTLs identified from ICC 4958 × ICC 1882 RILs.

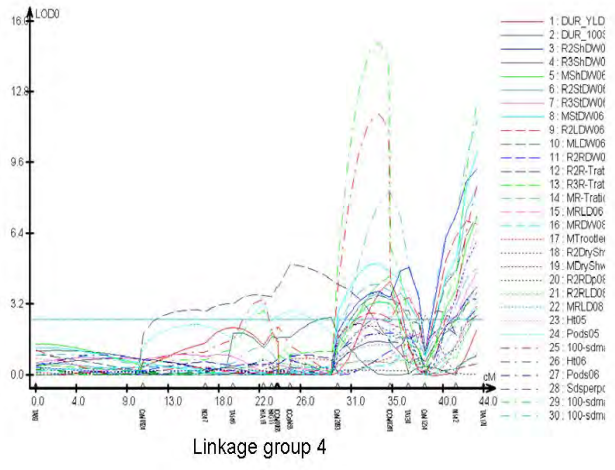

Fig. 1: QTLs for root traits, HI, grain yield and several other traits identified on linkage group 4 in ICC 283 × ICC 8261 RILs.

Tangible outputs delivered: A genomic region controlling root and several other traits (shoot biomass, HI, δ13C) was identified from ICC 4958 × ICC 283 RILs and was validated in ICC 283 × ICC 8261 RILs. This provides strength to the research efforts being made in TL-I project on MABC for introgression of this genomic region from ICC 4958 and ICC 8261 to farmer-preferred cultivars for improving drought tolerance.

16. G7009.06: Development of a SNP platform for molecular breeding in elite material of chickpea

November 2009–October 2010; NCE: October 2011

Principal Investigator

Doug Cook, UC–D; drcook@ucdavis.edu

NO UPDATE SUBMITTED

17. G7010.06.01: Accelerating development of genomic resources and strengthening NARS partner capacities for enhancing adoption of molecular breeding for drought tolerance in chickpea

June 2010–May 2014

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- Ethiopian Institute of Agricultural Research (EIAR), Ethiopia: Asnake Fikre,

Summary
Marker assisted backcrossing (MABC) and marker assisted recurrent selection (MARS) - two most important molecular breeding approaches are gaining importance in the recent past. These are extensively being employed for more precise selection and introgression of desired traits into elite cultivars in variety of crop plants. Chickpea is most important food legume in the arid and semi-arid regions especially in Sub-Saharan Africa (SSA) and South Asia (SA). Drought is one of major constraints to chickpea production. Significant genomic tools like SSRs (simple sequence repeats), SNPs (single nucleotide polymorphisms) and DArT (Diversity Array Technologies) arrays and a hot spot containing QTLs for several drought related traits has been identified in Phase I of Tropical Legumes –I (TL-I). For efficient molecular breeding especially for MABC, while markers closely linked with QTLs are desirable, cost effective marker (e.g. SNP) genotyping platform are critical for successful implementation of MARS and background selection of MABC. Cost-effective KASPar genotyping assays were developed and validated for a set of 2005 high confidence SNPs in collaboration with Integrated Breeding Platform. Efforts are underway for development of genome-wide physical map for chickpea. Two MSc students (one in Kenya and the other in Ethiopia) and one PhD student (Kenya) have already started their work. One workshop “Modern Breeding Methodologies for Chickpea Improvement” was held at ICRISAT, Patancheru. Through this workshop, sixteen scientists representing five countries from Africa and four countries from Asia were trained in use of modern genomics tools for chickpea improvement.

Activity wise progress has been given as follows

Activity 1: Develop genomic resources for enhancing MABC and MARS activities (Genomic resources)

1.1 *Compiling a larger number of informative SNPs in cultivated germplasm and development of integrated SNP arrays for chickpea coordinated*

In the Phase I of TLI and some other projects, a large number of SNPs were identified. For making the use of SNPs in the breeding programme, it is essential that

marker genotyping assays are cost-effective. For this KASPar assays were chosen, a set of 2,486 genes containing high confidence SNPs were chosen and were validated on DNA of 94 genotypes diverse genotypes. As a result a set of 2005 SNP were validated.

1.2 *Development of integrated QTL and physical maps for selected genomic regions (max. 10) for drought tolerance and insect resistance identified in Phase I and*
1.3 *Next generation sequencing of pooled DNA of BACs identified*
Towards development of genome-wide physical map, in collaboration with National Institute of Plant Genetic Research (NIPGR), New Delhi (S Bhatia and A K Tyagi) and UC-Davis, USA (MingCheng Luo), two new BAC libraries were constructed using *Hin*dIII and *Eco*RI restriction enzymes employing pCC1BAC Epicentre vector in DH10b. To date, 15,744 clones were fingerprinted and 10,368 fingerprints were edited. As result of assembling 8,612 clones, 1189 contigs with an average length of 120kb were obtained from 6016 clones. Fingerprinting of remaining clones is in progress to develop genome wide physical map.

Activity 2: Strengthen capacity of NARS partners (Capacity-building)
2.1: *Organisation of a workshop (in collaboration with the chickpea objective of TLII) in the area of modern breeding.*
A workshop on modern breeding technologies for chickpea improvement was held during October 25 – November 19, 2010 at the ardent request of breeders and collaborators. The workshop trained sixteen chickpea scientists from both TLI and TLII initiatives as well as others, twelve from Africa (Ethiopia, Kenya, Tanzania, Malawi, Algeria) and four from Asia (India, Nepal, Bangladesh, Myanmar). (http://www.icrisat.org/bt-publicdomain-mas2.htm).

2.2: *Training of at least 4 MSc and 2 PhD students from NARS institutes in chickpea genomics and breeding.*
One PhD student, Ms Serah Songok, currently working at ICRISAT, has been registered at Egerton University. She has already started work on MABC activities. A second PhD student, Mr Musa Jarso - a breeder from EIAR, has registered at Addis Ababa University will commence work shortly on plant molecular breeding. Another PhD student, Ms Alice Koskie, registered at WACCI would work on MARS. An MSc student, Mr Abebe Sori, is registered at Haramaya University. A second MSc student, Mr Moses Oyier, has been registered at Egerton, and has commenced work at both the university and at ICRISAT. Mr Getachew Tilahun, registered at Addis Ababa University, will start to work on MABC for drought tolerance. Efforts to identify and enroll the balance of the targeted number of MSc students are ongoing.

Capacity building: Chickpeas

Human resources (support to teams, students, travel grants, workshops)

18. G4009.07.03: Marker-assisted backcrossing (MABC) for drought tolerance in chickpea-students for analysis of drought tolerance in chickpea (TLI-Kenyan student)

December 2009–December 2010; NCE: December 2011

Principal Investigator
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Collaborating institutes and scientists

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- ICRISAT, India: Pooran Gaur, Mahendar Thudi

Summary
Chickpea is an important grain legume in South Asia (SA) and sub-Saharan Africa (SSA) especially in eastern and southern Africa. Drought is a major abiotic constraint affecting production of chickpea worldwide. Until recently, breeding efforts to improve drought tolerance have been hindered due to its quantitative genetic basis and poor understanding of the physiological basis of yield in water-limited conditions. Recently at ICRISAT, superior lines with improved drought tolerance have been identified and a large number of molecular markers developed. A genomic region harbouring several QTLs for root traits contributing up to 30% phenotypic variation was identified on linkage group 5 of the chickpea genome under Tropical Legume I (TLI) project. The major goal of this project is to enhance the drought tolerance of elite Kenyan genotypes using the marker-assisted back crossing (MABC) approach. Based on marker polymorphism assay on ten agronomically elite Kenyan chickpea cultivars and ICC 4958 (a drought tolerant variety from India), ICCV 95423, an elite cultivar from Kenya was selected as a recurrent parent. In October 2009, the first cross of the recurrent and donor parent was made to obtain F_1 .In April 2010, on identification of heterozygous F_1s, backcrossing to the recurrent parent to obtain BC_1F_1 was done. In July 2010, after foreground selection with markers flanking to the QTL region, selected BC_1F_1 plants were backcrossed to the recurrent parent to obtain BC_2F_1. In the main 2010 crop season (Oct 2010- Feb 2011), selected BC_2F_1 plants after foreground selection were backcrossed to the recurrent parent and BC_3F_1 seeds were harvested. These seeds will be used for selfing in the 2011 off-season and subsequently BC_3F_2 seeds will be used for seed multiplication in the main 2011 crop season. In parallel, selected BC_3F_2 lines will be phenotyped for root traits to confirm the introgression of the QTL region. Selected lines will be eventually tested in field conditions in Kenya and India to identify the best performing lines for there possible release as drought tolerant varieties.

Activity wise progress has been given as follows

Activity 1: Identification of parental genotypes for marker assisted backcrossing
Ten elite genotypes from Kenya together with ICC 4958 were screened for marker polymorphism with four SSR markers GA24, TAA170, ICCM0249 and STMS11. Based on marker polymorphism between possible combinations of elite cultivars with ICC 4958 (drought tolerant) and results of TL II project, ICCV 95423, a farmer-preferred cultivar, was selected as a target genotype for introgression of the QTL region.

Activity 2: Make cross and first backcross use markers to identify best individuals by applying foreground and background selection
During the 2009 crop season (October 2009- February 2010), 40 seeds of the recurrent parent (ICCV 95423) were sown along with ICC 4958 the donor parent, in the field and crosses were made. 14 F_1 seeds were harvested in February 2010. These F_1 seeds were planted in the greenhouse in the first off-season of 2010 (April 2010-June 2010). DNA was isolated from all F_1 plants and screened with two SSR markers (TAA170 and ICCM0249) flanking to the QTL region to identify true hybrids. Subsequently, two F_1 plants were selected and used for making the first back-crosses. While making the back-crosses, F_1s were used as male and the plants from the recurrent parent were used as female. In the end, 49 BC_1F_1 seeds were harvested.

Activity 3: Make a backcross on MAB selected individuals to generate BC_2F_1; advance 5 BC_2F_1 lines from each individual.
Forty two BC_1F_1 seeds were sown in the second 2010 off-season (July 2010-September 2010). After DNA isolation from the seedlings, foreground selection was done as a diagnostic tool to trace the introgression of the target drought related QTL region in the progenies by indirect selection of closely linked markers TAA170 and ICCM0249. As a result, 10 plants were selected for backcrossing to the recurrent parent. Subsequently, 89 BC_2F_1 seeds were harvested in the month of September 2010.

Activity 4: Backcross each selected line to recurrent parent to create MAB BC_3F_1
In the main 2010 crop season (October 2010- February 2011) 59 BC_2F_1 seeds were sown in the field in November 2010. After foreground selection, 6 plants were selected for further backcrossing. Eventually, 260 BC_3F_1 seeds were harvested from the backcross of ICCV 95423 × ICC 4958 in February 2011.

Community of practice
A Community of Practice for chickpeas was launched at the annual project meeting in May, to be coordinated by Dr Pooran Gaur (ICRISAT) and mentored by Dr Teresa Milan (Universidad de Cordoba), and will initially bring together chickpea breeders and researchers from Algeria, Ethiopia, India and Kenya.

Cowpeas

19. G4008.13: Improving drought tolerance phenotyping in cowpea

January 2008–December 2010, NCE: March 2011

Principal Investigator
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- International Institute of Tropical Agriculture, Kano and Ibadan, Nigeria: Sato Muranaka: Ousmane Boukar

- Institut de l'Environmentet des Recherches Agricole, Saria, Burkina Faso: Issa Drabo
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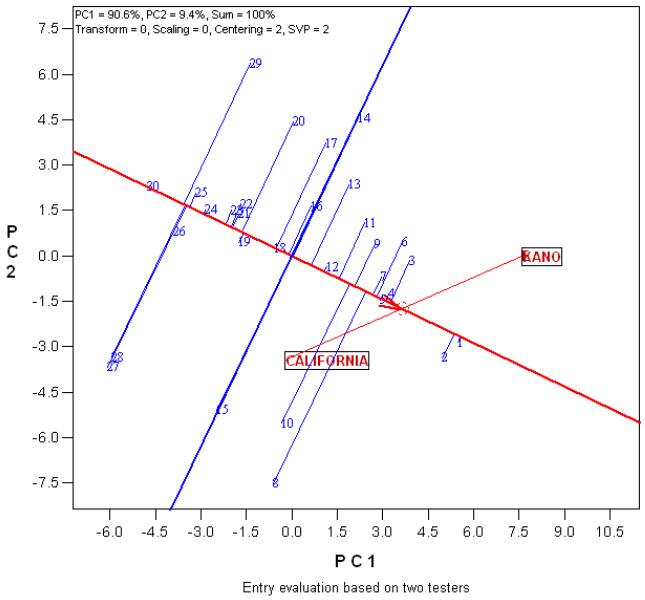
The overall objective of this project was to improve drought tolerance phenotyping practices in cowpea.

Objective 1: Evaluation of drought resistance of 30 early- and 30 medium-cycle cowpea lines under field conditions. 28 field trials were conducted in Senegal, Burkina Faso, Nigeria and California with a common set of 30 early and 30 medium maturing cowpea lines to assess cowpea varietal responses to terminal drought. Terminal drought was imposed by 1) deliberately planting late, close to the end of the normal rainy season; 2) planting at drought-prone locations during the main season; and 3) planting in dry off-season environments under irrigation and withholding irrigation from flowering to maturity. Data collected included biomass at early and late points in the growing season, days to flower and maturity, grain yield, individual seed weight, yield components, drought susceptibility scores and chlorophyll fluorescence (SPAD) ratings. This large data set is currently undergoing analysis at IITA (Figure 1).

Objective 2. Determining the relationship between drought tolerance and shoot and root traits. Root length measurements of 4 cowpea genotypes (IT98K-610, IT98K-555-1, IT99K-241-2 and IT98K-208-5) grown in cylinders in a screenhouse trial were made during Oct. – Nov. 2009 at IITA-Kano. IT98K-205-8 and IT99K-241-2 which showed higher shoot productivity under drought stress in the field, showed a significant increase of root length in deepest segment (900-1200mm) in the cylinders. These results indicate that IT98K-205-8 and IT99K-241-2 were able to increase root mass in the deepest segment (900-1200mm) under drought stress and the deeper root distribution may contribute higher productivity of these lines under drought.

Objective 3. Measure canopy thermal images of cowpea genotypes subjected to drought using a modern thermal imaging system under field and greenhouse conditions. The thermal imaging experiment under field conditions was conducted at Coachella, CA. Eight genotypes with contrasting levels of drought tolerance were evaluated by taking thermal images of each plot. Grain yield was taken at harvest. Preliminary analysis suggests that there were no significant correlation between canopy temperature and grain yield. Possible explanations include:



Entry Codes	Entry Name
1	It95k-1105-5
2	Cb 27
3	It95m-190
4	It85f-3139
5	It84s-2246
6	It95k-1479
7	It99k-407-8
8	It95k-207-15
9	It95k-1491
10	Cb 46
11	It82e-18
12	Yacine
13	It98k-205-8
14	It84s-2049
15	It90k-284-2
16	It85f-867-5
17	It97k-499-39
18	It97k-569-9
19	It99k-241-2
20	Sh 50-17-9
21	Melakh
22	Ife Brown
23	Apagbaala
24	524 B
25	Kvx 61-1
26	Mouride
27	Bambey 21
28	It95m-303
29	It83d-442
30	Ucr P-24

Figure 1. GGE biplot of grain yield reduction by drought stress of 30 early maturing lines

1) limited accuracy of the camera which was +/- 2 ºC or 2% of the reading and may be insufficient to detect small differences between cultivars on a plot by plot basis; 2) the cultivars were at different phenological stages due to differences in maturity, thus confounding the results; and 3) the stomata of all cultivars were already closed. The accuracy of the thermal imaging method was assessed at the seedling stage in a growth chamber, using a wide-angle lens to include multiple cultivars within a single image. This proved to greatly increase the ability to detect significant differences between cultivars that differ by less than 0.2 ºC. There appears to be a 5-day period of time, prior to most visible indications of stress, during which the differences between cultivars are most evident. The current hypothesis is that some varieties shed their unifoliates under stress, close their stomata earlier, more fully, or more continuously to conserve moisture, whereas others continue to fix CO_2 and transpire H_2O, and are therefore cooler. A third experiment was designed to measure photosynthesis and stomatal conductance of cultivars with contrasting levels of drought tolerance in a greenhouse. Stomata of all genotypes evaluated under moderate stress were closed even while visual differences between cultivars continued to become apparent, suggesting that stomatal conductance and transpiration may have relatively little to do with the contrasting levels of drought tolerance among cowpea cultivars.

Tangible outputs delivered

1. Baseline drought tolerance information for selection of appropriate check entries.

Publications during the current reporting period

Muchero, W., J.D. Ehlers, T.J. Close, and P.A. Roberts. 2009. Mapping QTL for drought stress-induced premature senescence and maturity in cowpea [*Vigna unguiculata* (L.) Walp.] Theor. & Appl. Genet.118: 849-863.

20. G6007.02/G7009.05; G6010.02/G7010.07.01: Improving cowpea productivity for marginal environments in Africa

G6007.02: May 2007–April 2010/G7009.05: July 2009–June 2010 (TLI Phase I); G6010.02: May 2010–May 2014/G7010.07.01:May 2010–May 2014 (TLI Phase II)

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- Institut de l'Environmentet des Recherches Agricole, Ouagadougou, Burkina Faso: Issa Drabo: Jean-Baptist Tignegre,
- Institut Senegalaise de Recherches Agricole, Bambey, Senegal: Ndiaga Cisse
- Institut Recherche Agronomie. pour le Developpement, Cameroon: Ousman Boukar (Phase I)
- Eduardo Mondlane University: Rogerio Chiulele (Phase II)

Phase I of this project developed key genomic resources needed to implement modern breeding of cowpea including 1) high-throughput Illumina 1536 GoldenGate SNP genotying assay, 2) a high quality consensus genetic map, 3) high density SNP fingerprints of key genotypes relevant to breeding programs and genetic diversity, 4) phenotypic information leading to the identification of trait markers and QTL, and 5) a very high-quality physical map anchored to the SNP-based consensus genetic map**.** The high-throughput genotyping platform consists of 1536 EST-derived SNPs chosen from a selected subset of about 10,000 SNPs discovered from comparisons of 183,000 EST sequences from 13 cowpea genotypes. The cowpea consensus genetic linkage map (Muchero et al., 2009a), includes 928 markers spanning 11 linkage groups over a total map size of 680 centimorgans (cM). The map was the result of merging genetic maps built from 6 RIL populations that had been genotyped with the 1536 GoldenGate Assay. Fingerprints of 640 genotypes were generated using the 1536 SNP assay and included 370 accessions from the IITA/GCP Reference collection, standard varieties, newly released varieties and the most promising potential parental lines for West African breeding programs. The project phenotyped recombinant inbred line (RIL) populations for several biotic stresses and described QTLs for resistance to foliar (Muchero et al., 2009b) and flower thrips, Fusarium wilt, root-knot nematodes, bacterial blight and to ashy-stem blight (caused by *Macrophomina. phaseolina* (Muchero et al., 2011). Drought tolerance QTL were identified following phenotyping four RILs for drought tolerance and through phenotyping a diverse set of 200 cowpea accessions (Muchero et al., 2010). We also tested the feasibility of outsourcing of genotyping from African NARS to service providers in Canada and the UK and found no significant barriers to workflow.

During the first year of Phase II we are utilizing the resources developed in Phase I for MARS and marker-assisted backcross (MABC) breeding. MABC and phenotyping of MARS populations are currently underway in partner countries. We further improved the consensus genetic map with the incorporation of SNP

genotyping data from a total of 1,293 individuals representing 13 mapping populations. The new consensus map contains 1,107 EST-derived SNP markers (856 bins) on 11 linkage groups (680cM) yielding an improved consensus map with 33% more bins, 19% more markers, and improved marker order when compared to the previous cowpea SNP consensus map. We have begun development of an 8-parent MAGIC population is as a long-term community resource for trait discovery and breeding. To date, single (*(AxB; CxD; ExF; GxH*), double (*ABxCD; EFxGH)* and 4-way crosses have been completed, with the last step involving crossing two streams of >300 individuals (i.e. *300 ABCD x 300 EFGH)* in pairwise fashion to generate >300 unique 8-way (ABCDEFGH) individuals. We collaborated with the GCP and KBiosciences in the development and validation of the GCP Cowpea Genotyping Platform. 1122 of the 1172 SNPs were successfully converted to the KASPAR format by KBiosciences and available to the broader cowpea community through the GCP-Genotyping Support Service.

Tangible outputs delivered in Phase I, first year Phase II

1. High-throughput genotyping platforms as described above
2. High quality consensus genetic maps (Muchero 2009a); April 2011 version http://harvest-web.org/; and physical map (http://phymap.ucdavis.edu/cowpea/).
3. **'Find Polymorphic Cowpea Markers'** tool that generates a matrix summary of the polymorphic markers between any two user-selected cowpea genotypes (http://harvest-web.org/).
4. SNP fingerprints of 640 individuals
5. Markers/QTL for drought tolerance and resistance to foliar and flower thrips, Fusarium wilt, root-knot nematodes, bacterial blight and to ashy-stem blight

References

Muchero W, Diop NN, Bhatt PR, Fenton RD, Wanamaker S, Pottorff M, Hearne S, Cisse N, Fatokun C, Ehlers JD, Roberts PA and Close TJ (2009a). A consensus genetic map of cowpea [*Vigna unguiculata* (L) Walp.] and synteny based on EST-derived SNPs. *Proc. National Acad of Sciences* DOI: 10.1073/pnas.0905886106. http://www.pnas.org/content/early/2009/10/09/0905886106

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Muchero, W., J.D. Ehlers, T.J. Close and P.A. Roberts. 2011. Genic SNP markers and legume synteny reveal candidate genes underlying QTL for *Macrophomina phaseolina* resistance and maturity in cowpea [*Vigna unguiculata (L) Walp*.]. BMC Genomics 12:8.

Capacity building: Cowpeas

Human resources (support to teams, students, travel grants, workshops)

21. G4009.07.02 Capacity-building in modern cowpea breeding

October 2009–October 2010; NCE: October 2011

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- Eduardo Mondlane University: Rogerio Chiulele

Two students (one each from Senegal and Mozambique) were nominated by our collaborating institutes/partners for participation in a PhD program at UC Riverside under partial sponsorship of this proposal. Both students had difficulty obtaining a passing Test of English Language (TOFL) score for admittance to our University when they took this test in 2009 while in their home countries, so we arranged for them to attend an 8-week intensive English course at UCR during the Spring of 2010 (sponsored by USAID training funds that were available to us). Both students completed the course in good standing but again failed to obtain passing TOFL scores and returned to their home countries by June 2010. Both students have subsequently re-taken the TOFL test and the student from Mozambique has recently obtained acceptance to the PhD program at UC Riverside and slated to start at UC Riverside in Jan. 2012. The student from Senegal did not obtain high enough GRE scores for admittance, but has continued to work with the TL-1 project in Senegal and is enrolled in a PhD program at a local University with her training costs covered by the TL-1 (Senegal) component.

Tangible outputs delivered in Phase I, first year Phase II

Two African PhD students with improved English skills.

Community of practice

A joint CoP for cowpeas and soya beans was launched at the Tropical Legumes II annual project meeting at IITA in mid-May 2011, with pioneer membership drawn from Burkina Faso, Cameroon, Kenya, Malawi, Mali, Mozambique, Niger, Nigeria, Senegal, Tanzania, and US. It will be coordinated by Dr Ousmane Boukar (IITA) and mentored by Dr Jeff Ehlers (UC-R).

Groundnuts

22. G4008.06: Single nucleotide polymorphism discovery, validation, and mapping in groundnut

January 2008 to December 2008; NCE: June 2011

Principal Investigators

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Collaborating institutes and scientists

- ICRISAT: David Hoisington; Rupakula Aruna; Rajeev Varshney

1. Research activities and progress at UGA

DNA marker resources have been expanded for molecular breeding applications in groundnut (*Arachis hypogaea* L.). The objective to enhance the infrastructure for translational genomics and molecular breeding research in groundnut has been achieved by massively parallel sequencing of 17 tetraploid genotypes which, by comparison with the reference transcriptome of 'Tifrunner', has provided a database for SNP discovery. Over 350 Mb of sequence from root, leaf, and pod tissues of 17 genotypes was assembled along with Sanger and Roche 454 sequences from the reference 'Tifrunner' transcriptome. 8486 single nucleotide polymorphisms (SNPs) were identified when the data were subjected to moderately stringent filtering to account for a SNP in at least two sequences from a genotype, allele frequency among genotypes, sequence errors at ends of reads, and proximity to neighboring SNPs or indels. An Illumina GoldenGate 1536-SNP array was designed from these 8486 candidate SNPs by prioritizing based on Illumina design score and distance from predicted intron-exon boundaries. The GoldenGate assay was used for genotyping of 80 tetraploid inbred lines, 3 amphidiploids, and several diploid accessions of *Arachis*. Loci could be detected for >95% of the SNP assays indicating successful design using this platform. However, SNPs between tetraploid genotypes were rare unless the tetraploid was synthetic. Nevertheless, the validated SNPs can be used to construct a smaller chip or transferred to an alternate platform for lower throughput assays. Sequence data from non-coding regions of the groundnut genome will be needed to increase the probability of finding nucleotide differences between cultivated genotypes in order to enhance the density of markers that can be used for breeding.

The SNPs identified and validated in this project can be used for genetic mapping of groundnut populations using several SNP assay platforms. The Illumina GoldenGate platform is most suitable for advanced recombinant inbred lines since the primers almost always amplified homeologous regions of the genome and intergenomic "SNPs" were detected as heterozygotes which would interfere with detection of a true heterozygote within a sub-genome. Groundnut breeding programs with the capability for molecular breeding can use the discovered markers initially in QTL linkage analyses and subsequently for marker-assisted selection.

2. Research activities at ICRISAT

The SNP array was used to analyze genotypes of interest to the ICRISAT breeding program where many of the parental lines have been crossed in various combinations. These populations are at various stages of development although some are at the recombinant inbred line stage appropriate for GoldenGate analysis.

Tangible outputs delivered

- 350 Mb of 454-generated reduced representation sequence from 17 genotypes and an assembly of ~211,000 contigs from 21 genotypes, providing a reference transcriptome for tetraploid groundnut.
- Database of over 8000 SNPs identified from these genotypes.
- SNP genotypes (1536 loci) for 80 elite and exotic tetraploid groundnut lines have been generated and can guide mapping population development and analysis.

References

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23. G6007.01/G6010.01: Improve groundnut productivity for marginal environments in sub-Saharan Africa

G6007.01: May 2010–May 2014 (TLI Phase I)
G6010.01: May 2007–April 2010 (TLI Phase II)

Phase II report

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- ISRA, Senegal: Ousmane Ndoye, Issa Faye
- University of Georgia Athens, USA: Andrew Paterson
- CIRAD: Jean-Francois Rami / Daniel Fonceka
- EMBRAPA, Brasil: David Bertioli and Soraya Leal-Bertioli

Building on the achievement from phase 1 of the TLI project, we pursue efforts to increase rust, early and late leaf spots (ELS/LLS), and rosette resistance and drought tolerance in groundnut. Focus is on confirming disease resistance and drought tolerance, adding new diversity for disease resistance from wild relatives, development of SNPs, QTL identification for disease resistance, development of suitable populations for drought, breeding of critical traits and capacity building of partners.

Confirm drought-disease resistance sources - Assess new diversity - A set of 50 genotypes exhibiting high contrast under intermittent drought across years and locations, all with good agronomic characteristics, has been identified for further testing and to pick parental lines for crossing. This list has been complemented by 10 FMPVs lines and well-known breeding lines.
Analysis of the disease resistance evaluation data from the reference set is completed:
Rust Resistance: ICGV 02194, ICG 11426, ICGV 01276, ICGV 02286, and ICG 02446
Rosette Resistance: ICG 14705, ICG 13099, ICG 9449, and ICG 15405
ELS Resistance: ICG 6022, ICG 405, ICG 14466, ICG 6057, ICG 9449 and ICG 12509 (Malawi); ICG 6703; ICG 10036, ICG 10384, and ICG 11219 (Mali), ICG 5663, ICG 4156, ICG 721 and ICG 9905 (Malawi, Mali, and Senegal). Nurseries have been developed for confirmation, and three farmer participatory varietal selection (PVS) trials, have been performed in Eastern-Southern Africa during 2010-11.
Chromosome substitution lines (CSSL) of synthetic AixAd in the background of Fleur11 (122 BC4F3 lines) are being multiplied for distribution. Several new synthetics, developed at ICRISAT, are used in Senegal to develop new AB-QTL populations. Six new synthetics produced in Brazil have also been crossed to cultivated lines.

Develop SNP markers for cultivated groundnut
Genotypes for the SNP study have been selected, considering phenotypic diversity for drought response and general utility by other TLI components (based on the frequencies of usage in breeding crosses). DNA are being exchanged. Activities related to Phase I BAC fingerprinting is complete, but a lower than expected % of fingerprints passed QC.

Map disease resistance QTL - Anchor these QTLs to the physical map
Three populations for rust (JL24 x ICGV 94114, ICGV 93437 x ICGV 94114 and ICGV 93437 x 95342), two populations for ELS (ICGV-SM 95714 x Robut 33-1, ICGV-SM 95714 x ICGV 93437) have been phenotyped. Three populations for rosette (Chalimbana x ICGV-SM 90704, CG7 x ICGV-SM 90704 and JL 24 x ICGV-SM 90704) were one generation behind and will be advanced to F6. Genotyping of 96 SSR markers has been completed in the parental lines of two populations for each disease. Polymorphism varied between 4 and 22 markers being polymorphic out of the 96 being tested.
12 BC1F3 lines, with improved resistance to LLS and agronomic characteristics similar to the cultivated, using synthetic AixAd into Runner-886 have been bred, showing that disease resistance can be improved with synthetic tetraploid from wild peanut.

Development of new disease and drought populations
A number of breeding materials are under development to introgress the different disease resistance in farmer-market preferred varieties. New mapping populations are also being developed for disease resistance, using new resistant sources. Introgression of rust and LLS resistance QTL is also taking place (BC3F2 and BC2F3 lines).
Following the identification of 50 genotypes, good agronomically and contrasting for drought, DNA has been extracted from all these lines, plus 10 popular lines, and 24 SSR markers have been genotyped. A dissimilarity matrix was assembled to identify the most contrasting entries. From this, a total of 22 crosses have been made.

Strengthen capacity of NARS partners
Several young scientists from different participating countries have taken part in training in India and/or are registered as student in local universities. The overall idea is to develop a new generation of young scientists trained to multi-tasking of modern breeding programs (generate good phenotypic data, use markers, manage a breeding program).

Management and storage of data
Drought assessment data of the reference collection have been delivered. Data from the lysimetric trials will follow shortly. Phenotypic data of the three RIL populations that were phenotyped in Phase 1 have been delivered. Marker data, three genetic maps from Phase 1 have been also delivered to the data curator. Data for disease resistance assessment from Phase 1 have been compiled and will be delivered by end 2012.

Next steps or challenges - How the findings will benefit crop improvement
Seed multiplication rate in groundnut is a limit as well as RIL population advancement.

24. G6007.01: Improve groundnut (Arachis hypogaea L) productivity for marginal environments in sub-Saharan Africa

May 2007–April 2010 (Phase I)

Phase I report

Principal Investigator and Lead Institute

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Groundnut (peanut, *Arachis hypogaea* L.) is important for food and cash in Sub-Saharan Africa, grown under very low input and rainfed drought-prone conditions. Rust, early and late leaf spots (ELS/LLS), and rosette can cause 50-60% pod yield loss. Drought losses are estimated to US$500 million/year. The project explored germplasm diversity for new source of disease resistance and drought tolerance, developed molecular tools for faster/efficient breeding and initiated breeding and QTL mapping of resistance/tolerance.

Findings and implications (products and impacts)
Phenotyping for disease resistance (Rosette, Rust, ELA/LLS) - Rosette resistant lines were: ICG 14705, ICG 13099, ICG 9449, and ICG 15405. Rust resistant lines were: ICGV 02194, ICG 11426, ICGV 02286, and ICGV 01276. ELS resistant lines, with a score <5 (1-resistant; 9 susceptible), as good as or better than the resistant checks

(ICG 7878 an ICG (FDRS) 4), were ICG 9037, ICG 5663, ICG721, ICG 5745, ICG 5663 and ICG 8285. Elite nurseries were planted for farmer perception of haulm/grain quality

Phenotyping for yield under drought and drought component traits - The reference collection of groundnut was tested under a standard intermittent stress protocol across locations. Results revealed large genotype-by-year interaction although genotypic effects were also significant. Popular JL24 and ICGVSM87003 had poor pod yield under stress conditions. Thirty lines had higher pod yield under stress than drought adapted popular Fleur 11 and 55-437. Large GxE interaction for pod yield prevailed.

Physical map - Overgo hybridization of genetically mapped DNA markers and BAC fingerprinting of an A genome BAC library were done. Integration of hybridization and fingerprint data will use standardized formats, accessible via a standard 'WebFPC' database. Overgo hybridization data is available on a standalone basis (www.plantgenome.uga.edu/bacman/peanut/probelibraryquery.php).

Sequencing of the long SSR enriched library was completed, 139 functional primer pairs were developed, of which 83 are polymorphic for a panel of 22 diverse cultivated peanut. At ICRISAT, primer aliquots have been procured for 4675 SSR markers. This includes 1316 SSRs available in public domain and 3359 unpublished SSR marker from SJ Knapp UGA, USA (2207 SSRs) and Doug Cook UC-Davis, USA (1152 SSRs).

Develop reference genetic AA and AABB maps - F2 plants derived from an *Arachis duranensis* x *A. stenosperma* cross have been advanced to F5/F6 generation (>100 RILs). A map based on SSRs, candidate genes, and comparative genome markers is available with 494 markers. 34 sequence resistance gene candidates were mapped on the F2 diploid AA map. 17 resistance gene candidates were added to the genetic map. The F2 tetraploid population (elite Runner-886 x synthetic AixAd) has been genotyped with 150 markers.

Phenotype segregating populations and identify QTLs for leaf spots and rust - F2/F3 of the AA mapping population were assayed for late leaf spot resistance. F2/F3 and F5/F6 RIL populations derived from elite cultivated peanut (Runner-886) and the synthetic AixAd were assayed for rust and late leaf spot. QTLs for late leaf spot were identified in the AA map, close to mapped candidate disease resistance genes, defining for the first time, regions of the *Arachis* genome that control LLS resistance.

Mapping of drought related traits - Three populations had limited SSR polymorphism: 202 in RIL of ICGV86031xTAG24, 83 in RIL ICGS44 x ICGS76, 119 in RIL ICGS76 x CSMG84-1 and 84 loci mapped. Only RILs of ICGV86031xTAG24 has decent marker coverage. TE has been phenotyped in that population. 59 QTL, most with small effect, were identified for TE and related traits. Drought tolerance traits in groundnut seem to be governed by main-effect and epistatic QTL each with a small effect. Genome wide marker approaches such as MARS should be effective approaches to breeding.

Development of elite varieties from good x good crosses - Several breeding populations have been advanced in each of the difference disease targeted, for mapping resistance QTL in the phase 2 of TLI.

Next steps or challenges - How the findings will benefit crop improvement
The lack of genetic diversity has been addressed by assessing wide germplasm and developing molecular tools to have a minimum basis. A remaining challenge will be to develop a marker system (SNP) that allows us to move towards MARS (a must in view of the project's results). The transfer of product from TLI to potential users will imply capacity building

Key achievements:

- The identification of new sources of disease resistance and drought tolerance
- Availability of wild introgression from chromosome segment substitution lines
- Large number of SSR markers and progress towards a physical map
- A complementary platform of partners and a good flow of product to TLII
- Genetic maps in diploid and tetraploid species
- Pipeline of backcross products introgressing disease resistance into popular varieties

Capacity building: Groundnuts

Human resources (support to teams, students, travel grants, workshops)

25. G4009.07.04: Capacity-building *à la carte* 2009 – Ensuring 'good' and relevant phenotypic data to feed molecular breeders: the need for long-term training of scientists of NARS partners to TLI Objective 1

December 2009–December 2010; NCE: December 2011

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The Tropical Legume I project is targeting disease resistance and drought tolerance as the major traits limiting crop production of 4 legumes (groundnut, bean, cowpea and chickpea) in Sub-Saharan Africa. Skills to precisely phenotype these traits in a marker-assisted approach are key to the success of breeding in sub-Saharan Africa. For that, there is an urgent need to train a critical mass of plant breeders and technicians to the techniques and protocols that they will need to master to undertake breeding in their locations.

Drought traits phenotyping

Being the key trait targeted at the origin of the GCP, and being very complex, it has received most of the focus in the project. In Niger, Oumarou Halilou has taken part to the assessment of the groundnut reference collection in the field under fully irrigated and intermitted stress conditions. A number of publications are currently under development in relation to that work. In the scope of this work, Oumarou has learnt the logistics of running large field trials (>300 entries, 2 water treatments, 4 replications per treatment and entry), and related sampling. He has learnt about scoring of leaf wilting to assess when re-watering of the drought plot is needed, about sampling in the field, and sub-sampling at harvest to determine shelling percentage, seed number and seed size. In parallel to this, Oumarou have been involved in the assessment of putative component traits. This was his Master thesis from the Abdou Moumouni University in Niamey. He is now registered as a PhD student in the scope of TLI (G2010.1).

Nouhoun Belko, a PhD student registered at the University in Ouagadougou, has come to ICRISAT to assess putative traits related to the adaptation to terminal drought in a number of cowpea genotypes contrasting for drought yield in the field in his assessments made in Senegal. We have trained him to protocols that have been used to assess similar traits in other crops (See Kholova et al., 2010a and 2010b, J Exp. Bot.). In short, tolerant cowpea lines developed smaller leaf area, have lower leaf conductance under both low and high VPD, have leaf conductance being sensitive to high VPD. Therefore leaf conductance differences between tolerant and sensitive lines are higher under high VPD. Canopy temperature measurements were made and showed tight relationship with canopy conductance, provided they are measured at vegetative stage and at the time of the day with highest VPD. Tolerant cowpea lines have also a delayed transpiration decline upon progressive exposure to drought than sensitive lines. A matrix of traits assessed and values in tolerant/sensitive genotypes has been made that can be used as selection indices.

Root phenotyping

This replaced disease phenotyping, which was included at the time of developing the proposal, and which did not show high demand from partners.
A specific demand was made from the Egerton University to have training in root assessment techniques and other possibly interesting aspects in chickpea. Mr Julius Kaunyangi then visited ICRISAT to be trained on drought, especially on root phenotyping. From this training, a small lysimetric facility will be developed at Egerton, instead of the PCV tubes that were initially planned. The lysimetric facility would open to a much wider range of possible use and generate much more informative data. The trainee then learnt how the system works, how to operate it and analyze data in a simple way, and would be capable of transferring it to Egerton subsequently.

Breeding and use of marker

Although this was not initially planned, Nouhoun Belko has taken part of the Center of Excellence in Genomics (CEG) course at ICRISAT headquarters in India, organized in March 2010.

The visit of Julius Kaunyangi also included a training at the Center of Excellence in Genomics (CEG) course at ICRISAT headquarters in India and targeted to chickpea

(25 Oct – 19 Nov 2010). Other trainees (Issa Faye, Falalou Hamidou and others linked to the TLI project) under this activity took part of the CEG course, held 8-19 November 2010 at ICRISAT headquarters in India. Following this, two trainees (Philippo Machamba and Oumaru Halilou) stayed for 4 months to undertake training in phenotyping and breeding.

Links to previous work
In March 2008, a training course on phenotyping was organized at ICRISAT, in which a number of technicians and scientists were trained over 4 weeks on drought phenotyping and related to the Tropical Legume I project (TLI). These technicians and scientists spanned across the 4 crops of the TLI project. Although successful, a course on phenotyping is too short to fully grasp an approach that is complex and not the mere application of "recipes".

Next steps or challenges - How the findings will benefit crop improvement
There is obviously a critical need to train young scientists but also research technician to undertake the tasks of modern breeding programs. This will require long term training, since not all the skills needed can be acquired in short training courses.

Crosscutting activities: Legumes

26. G6010.05: Cross-cutting crop activities (drought phenotyping, data management and capacity building)

May 2010–May 2014 (TLI Phase II)

Principal Investigator and Lead Institute:
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Activity 1: Identification of critical traits to refine selection indices and guide breeding for superior adaptation to drought of TLI crops for targeted environments

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Collaborating institutes and scientists

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Identification of critical traits to refine selection indices and guide breeding for superior adaptation to drought of TLI crops for targeted environments

Transfer of materials – Cowpea seeds from IITA and UC-Riverside have been transferred to India and Senegal. Bean materials have been transferred from CIAT to ICRISAT. The transfer of chickpea seed to CIAT is still pending.

Establishment of facilities - A lysimetric facility has been established in Niger and is almost functional. The design of the cylinder has been adapted to the sparser sowing densities used in the region and tubes are 25 cm diameter and 1.0 m long. A total of 400 lysimeters are available. A lysimetric facility has also been developed at CIAT and will be used for bean and chickpea. The facility contains 120 tubes.

Assessment of plant water use - Tolerant cowpea lines developed smaller leaf area, have lower leaf conductance under both low and high VPD, have leaf conductance being sensitive to high VPD. Therefore leaf conductance differences between tolerant and sensitive lines are higher under high VPD. Canopy temperature measurements were made and showed tight relationship with canopy conductance, provided they are measured at vegetative stage and at the time of the day with highest VPD. Tolerant cowpea lines have also a delayed transpiration decline upon progressive exposure to drought than sensitive lines.
Assessment of similar traits in chickpea showed common traits. Tolerant chickpea lines have a slow growing characteristic at vegetative stage under non-stress conditions, lower leaf conductance at vegetative stage but higher leaf conductance during pod filling, under well-watered conditions, than sensitive lines. Contrary to cowpea, the soil moisture thresholds where transpiration declines is higher in tolerant than in sensitive lines.
Similar work is in progress in groundnut, where it was also found that most tolerant groundnut lines had smaller leaf canopy than most sensitive lines.

Water extraction measurement - 15 entries of cowpea have been tested in the lysimetric system in India, to assess water extraction from the soil profile and rooting traits (length density, depth), and their relationships.
20 genotypes of chickpea, contrasting for seed yield in the field, have been tested for rooting traits and water extraction. Rooting traits and water extraction did not discriminate between tolerant and sensitive lines. Rather, the discrimination was in the pattern of water usage from the soil profile; tolerant lines used less water during vegetative stages than sensitive lines. But, tolerant lines used more water during the reproductive growth stage than sensitive lines.

Partitioning traits – A field trial was conducted at CIAT using 36 promising genotypes and checks to evaluate phenotypic differences in canopy biomass, pod partitioning index, stem biomass reduction and pod partitioning index under drought and irrigated environments. Results showed that superior adaptation to drought was associated with higher pod partitioning index values.

Crop model simulation - A crop simulation model for the 4 legumes of TLII, plus soybean, is now available. This model uses the same structure across the legumes with coefficients that follow the same syntax but have crop-specific values. There is still a need for additional data to parametize the model for crop phenology of popular lines in each specie, especially for common bean and cowpea but the other crops are well enough documented for the simulations to begin. Simulation values have begun in chickpea and will continue during Year 2.

In addition to that, efforts have been made to develop weather datasets using MarkSim, a weather data generator developed at CIAT. It is planned to compare the simulated data generated by MarkSim to real weather data at locations covered by

many weather datasets. Then, the choice will be made to either generate weather data from MarkSim (if correlation with real weather data is good), or to capture real weather data from those locations where weather data are scarce (e.g., West Africa).

Activity 2: Data curation of the Tropical Legumes I project

- The Data Management Coordination Centre for the project has been established at ICRISAT and PVNS Prasad appointed to coordinate data management efforts across the 4 crops and implement the data management strategy. Data managers have also been identified at the two other Crop Lead Centres (IITA for cowpea and CIAT for common bean).
- Data catalogues were initiated based on final reports from Phase I. These were discussed at length during the 2011 annual meeting and responsibilities for the data collation, curation and submission defined with requisite timelines. Catalogues are now available for chickpea, cowpea and groundnuts, but delayed for common bean due to change of PI. The data managers are scheduled to meet in Mexico in July for further refinements.
- The data management strategy was developed – and presented at the 2011 annual meeting in Madrid. Tools and databases for phenotyping and genotyping data (being developed under the IBP project) were also presented and discussed.

Activity 3: Capacity building for sub-Saharan African scientists and project planning

- The launch of TLI Phase II of the project took place from 22nd to 24th August 2010 in Madrid, Spain while the Annual Meeting was held May 4th – 7th 2011, also in Madrid. Both meetings brought together project researchers from CG Centres, NARS and other collaborating institutes. Sustaining practical links between TLI and its sister project TLII is an ongoing effort. However, the passing of TLI research products to TLII may not be fully realised until later in TLI Phase 2 at which time TLII will also be ready to utilise these products. The two projects share breeders and field sites. In groundnut, new sources of disease resistance and drought tolerance identified by TL1 have been tested in farmer participatory selection trials in TLII, while more than 321 cowpea accessions from TLII have been genotyped by TLI. Drought phenotyping data provided by TLII was used in an association analysis. In beans, PhD programmes are being implemented collaboratively by TLI and TLII, and a drought trait identified by TL1 had been implemented for phenotyping by TLII in Ethiopia. Chickpea lines from TLII have been selected for identification of SNPs, and TLII is also providing parental lines for the breeding effort. TLII breeding populations have been genotyped under TLI with markers for root traits and Fusarium wilt resistance. Phenotyping methodologies for drought tolerance developed under TLI were made available to TLII, and training of NARS partners is also done collaboratively.
- Significant beneficial interactions are also expected with the Integrated Breeding Platform Project – particularly in capacity development, access to data management and analysis tools, and access to various breeding services.

Maize

27. G4008.33: Drought tolerance phenotyping of the GCP maize inbred line reference set

January 2008–February 2011; NCE: February 2012

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED

28. G4008.56: Asian maize drought-tolerance (AMDROUT) project

November 2008–October 2013

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Background

Over 80% of the 19 million hectares of maize in South and South-East Asia is grown under rainfed conditions and prone to drought. Addressing the problem of drought can provide the highest technical returns to rainfed maize R&D investments. Significant progress has been made in the development of drought tolerant maize (Bänziger and Araus 2007) and highly elite CIMMYT inbreds are available whose resultant hybrids out yield commercial checks by more than a ton per hectare. Due to donor interests most of this investment has been directed at Africa with insufficient spill-over to Asia or Latin America. Much of germplasm in Africa is white while the Asian requirement is for yellow maize.

Drought tolerance is a highly polygenic trait with involvement of several chromosomal regions and a significant proportion of additive effects. In the private seed industry, marker-assisted recurrent selection (MARS) is increasingly used to accelerate breeding for complex traits, especially grain yield. By increasing the frequency of favourable alleles in breeding populations, MARS enables the doubling of breeding gains. It can be expected that similar approaches could assist in the accelerated introgression of drought tolerance into Asian germplasm.

Approach

This project is applying marker-assisted selection within bi-parental (or pedigree) breeding populations generated using African drought tolerant white source inbreds and elite Asian adapted yellow inbreds. SNP genotyping is being done by Kbiosciences. The outputs from the project include Asian adapted drought tolerant inbreds, molecular marker information associated with drought tolerance, and scientists trained in integrating MARS with applied breeding.

Results

Identification of donor and recipient lines

A Design II of crosses between elite Asian lines and African donors evaluated across 6 locations covering China, India, Indonesia, Philippines, Thailand, and Vietnam showed CML444 to be the best donor for drought tolerance. Other donors of value are CML538, CML440, CML505, CZL0719, and CZL00009. Recipient lines identified to be of value to this project include: CML470, VL1012767, VL108733, VL108729, VL1012764, and CML472.

Genomic estimated breeding values (GEBV)

F2:3 families from bi-parental crosses were test crossed and evaluated.

Population Name	CIMMYT-Asia recipient line	DT donor line	No. F3 families	No. Polymorphic SNPs	Correlation: observed-predicted values	
					Drought	Optimal
AMDROUT1	CML470	CML444	294	353	0.44	0.21
AMDROUT2	VL1012767	CML444	189	391		

MARS projects from African populations have not shown major QTLs for yield under drought (Babu, personal communication). Hence the approach of genome wide selection, where all marker effects are incorporated in a genomic estimated breeding value (GEBV) for selection (Meuwissen et al. 2001), is being explored. This avoids selection bias associated with significance tests, and captures more genetic variability for highly polygenic traits (Bernardo 2008). For the AMDROUT1 population, marker effects for 343 markers along with the GEBVs and predicted values for the entries were determined using R-script for one drought and one optimal location (3 replications each). Correlation between observed and predicted values was 0.44 and 0.21 for drought and optimal locations, respectively. Selection of F3 families for formation of C1 will be made using phenotypic values once data from all sites are received. The GEBVs and marker effects will be used to identifying C1 individuals for forming C2.

Achievements

This project is building on insights gained within various CIMMYT projects that have identified appropriate markers and improved phenotyping and information management techniques and previous Asia-based projects (AMNET) which established drought breeding approaches with selected NARS. The AMDROUT model and its success has already become the basis for additional donor funded projects that look to better integrate drought tolerance in Asian germplasm through public-private partnerships. An example is the Affordable, Accessible, Asian (AAA) Drought Tolerant Maize Project funded by the Syngenta Foundation for Sustainable Agriculture (SFSA). Drought-tolerant CIMMYT lines will be crossed with Syngenta

varieties bred for Asia, applying Syngenta's genetic mapping technology to speed up and refine the selection of high-yielding, drought-tolerant maize varieties.
The first inbred lines from the AMDROUT project are expected to be generated by the end of the 5 year phase of the project (November 2013).

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29. G7010.03.01 Cloning, characterization and validation of *Pup1*/P efficiency in maize

April 2010–March 2014

Principal Investigator

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- Moi University/KARI: Eldoret, Kenya, Sam Gudu
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1. Rationale for the SorghumPup1 project

Cereal production on a significant fraction of the soils in developing countries is limited by P deficiency due to P fixation in the soil as well as low levels of total P. This is particularly true on acid soils which are estimated to comprise as much as 50% of the world's potentially arable lands. P deficiency therefore often coincides with drought and frequently aggravates its negative effects because P-inefficient genotypes typically show a strong reduction in root growth under P deficiency and on acid soils, aluminum (Al) toxicity results in a stunted and damaged root system. Hence, on these Al toxic, low P soils, roots remain shallow and incapable of penetrating deeper soil layers (Foy et al., 1993), resulting in sensitivity to drought and P deficiency. Recently our co-PIs from IRRI and JIRCAS identified *Pup1* as a major QTL located on rice chromosome 12 that underlies phosphorus efficiency and has the potential to increase P acquisition efficiency in other cereals. JIRCAS and IRRI have fine mapped the *Pup1* locus to a ~150 Kb region on chr 12, and 2-4 high quality *Pup1* candidate genes have been identified. The research in this project, similar to the research being conducted on the sorghum *Pup1* project, is using comparative genomics to identify

maize *Pup1* homologs and validate one or more of them as *bona fide* genes underlying tolerance to P deficiency. We will use an association genetics approach to validate the role of maize homologs of rice *Pup1* as P efficiency genes. The validation of one or more *Pup1* homologs in maize will be done within a molecular genetic framework that will also allow us to identify other, novel phosphorus efficiency QTL/genes in maize. This contingency is critical if it turns out that *Pup1* homologs are not functional in P efficiency in maize. This project will set the stage for the development of a maize molecular breeding program targeting low P and/or acidic soils in Kenya and other regions of Africa to improve food security and farmers' income.

2. Research activities and preliminary results

2.1 Bioinformatic search for candidate maize Pup1 genes

Initially, we used a bioinformatics approach to identify Pup1 homologs in maize and sorghum. Our JIRCAS and IRRI co-PIs have a strong candidate gene for PUP1 in rice, PUPK46, and we used the sequence for this gene to screen all the predicted proteins from the genomes of maize (http://www.maizesequence.org), rice (http://rice.plantbiology.msu.edu/), and sorghum (http://www.phytozome.net/sorghum), and these sequences (8 from rice, 5 from maize, and 6 from sorghum), as well as PupK46, were used to construct the phylogenetic tree shown in Figure 1 shown below.

Five predicted maize orthologs of the rice *Pup1* candidate gene were selected based on the sequence similarity with rice *Pup-1*. Primer pairs for each candidate gene will be designed for polymorphism discovery between the L3 and L22, the parents of our maize p efficiency mapping population, in order to map them in the target RIL population.

2.2. QTL/gene mapping for P use efficiency in maize

A genetic linkage maize map was constructed based on 370 SNPs and 50 SSR markers. Over the past two field seasons, 150 maize RILs from the cross of P efficient and inefficient lines (L3xL22) have been evaluated for yield components and P content on low and high P soils. These data are now being analyzed in order to map QTLs for P use efficiency. In addition, other candidate genes previously associated with root architecture in the literature which may play a role in P acquisition in low P soils that we find to be differentially expressed between L3 and L22, will also be mapped in this population.

2.3 Subsequent research steps:

We now will begin to conduct expression analysis of *Pup1* candidates in roots and shoots of the selected maize RILs grown under +/-P conditions. We also will phenotype maize lines from both our association panel and the above mentioned bi-parental mapping population for root traits in 2D as well as yield under low and high P conditions in pots in the greenhouse. This will allow us to look for correlations between root traits and P efficiency. We also will identifying markers (SNPs and indels) within *Pup1* candidate genes across the association panel for subsequent association analysis of candidate genes for *Pup1*/P efficiency

3. Figures.

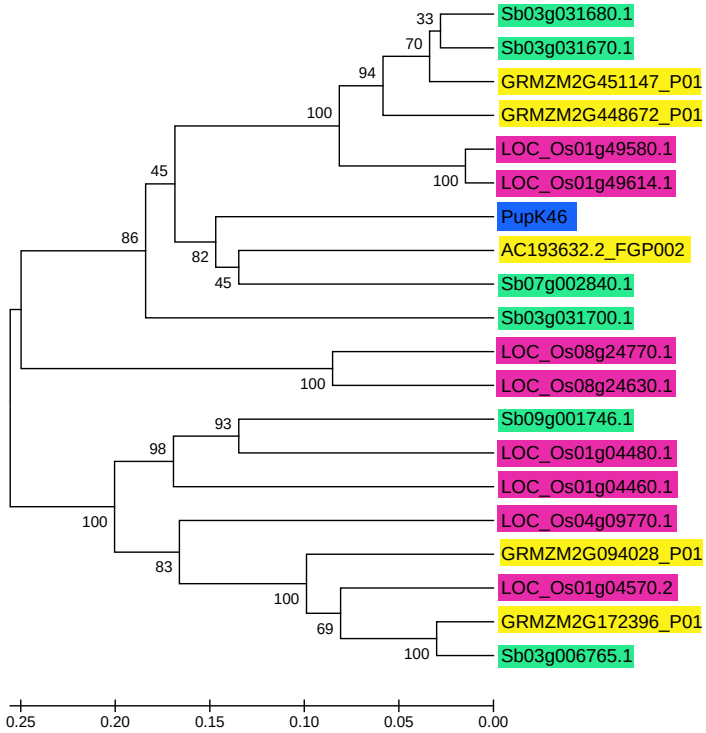

Figure 1. Phylogenetic tree showing the relationships between sorghum, maize and rice orthologs of the rice PUP1 candidate gene, PupK46 (highlighted in blue).

30. G7010.03.02: Validation of *ZmMATE*s as genes underlying major Al tolerance QTLs in maize

April 2010–March 2014

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- USDA-ARS Robert W. Holley Center for Agriculture and Health (US): Leon Kochian, Lyza Maron, Jiping Liu, Miguel Pineros, Ed Buckler
- Moi University (Kenya): Sam Gudu

1. Rationale for the project

Over 50% of the world's potentially arable lands consist of acid soils, where aluminum (Al) toxicity is the primary factor limiting maize yield, one of the world's most important food crop. This problem is particularly important for low input agricultural systems, which includes a large portion of the farmers in Sub-Saharan Africa and as well as small farm holders in other developing country areas. Al tolerance is a quantitatively inherited trait in maize, a crop that displays considerable variation for this trait, as well a highly complex genome organization. Taking advantage of the Al tolerance gene cloned in sorghum (Alt_{SB}) and findings from our

recent research in maize, where two major Al tolerance QTLs were co-localized with Alt_{SB} homologues (*ZmMATE* genes) (Maron et al. 2010) we will characterize and validate functional *ZmMATE* genes or QTLs conferring superior Al tolerance in maize. This strategy will be based on our genetic resources already available as near isogenic lines for both QTLs, segregating populations and crosses between Brazilian sources of Al tolerance and Kenyan adapted germplasm. This germplasm, as well as newly developed crosses, will be subjected to molecular, physiological and field evaluations in order to accomplish the functional validation of candidate genes or QTLs for improving Al tolerance in different tropical maize germplasm. Our Challenge Initiative involves Embrapa, USDA/Cornell University, Moi University and KARI, a research group with a long history of successful partnership on maize and sorghum Al tolerance. The research findings from this project will both greatly increase our understanding of the molecular and genetic basis for Al tolerance in maize, and will provide materials for molecular breeding programs for improving maize production and stability on acid soils in Africa and other developing countries.

2. Research activities and partial results

Validation of functional ZmMATE genes or Al tolerance QTLs in Brazilian maize crosses

Expression profiles of *ZmMATE1* and *ZmMATE2* were evaluated in the Cateto x L53 RIL population. *ZmMATE1* expression was mapped in the same region where the gene was genetically located (Table 1), overlapping with the major Al tolerance QTL identified on chromosome 6. However, the expressed QTL for *ZmMATE2* was located on maize chromosome 3 (Table 1), a completely different position from where the *ZmMATE2* gene was mapped.

Table 1. Expressed QTLs of *ZmMATE1* and *ZmMATE2* mapped in the RILs population by multiple interval mapping.

Gene	*Bin*	*Markers*	*Position*	*LR*	*Effect*	R^2 *(%)*
ZmMATE1	6.00	*ZmMATE1*	26.3	72.55	1.8278	78.65
ZmMATE2	3.08	umc1148	178.5	12.05	0.1534	18.55

Near-isogenic lines for the Al tolerance QTLs were developed using MABC (Cateto as donor and L53 as recurrent line), and characterized for molecular and functional parameters of Al tolerance. NILs for Al tolerance QTL of chromosome 6, flanked the markers *ZmMATE1* and umc1018, presented almost a two fold increase in Al tolerance compared with the recurrent line L53 keeping the *ZmMATE1* expression similar to the donor line, Cateto. This result indicates that the QTL6 harbor genetic factors capable to significantly improve tolerance in maize. However, the NILs for *ZmMATE2* were as Al sensitive as L53, indicating that this gene was not able to improve the tolerance in maize.

Future activities

In order to generate more appropriated materials for field trials, NILs for chromosome 6 were crossed with four maize lines from two major heterotic groups. These crosses and the NILs will be evaluated under different Al saturation in the field during cropping season of 2011. Considering that these genetic stocks are more suitable to functional validation of the Al tolerance effect on yield performance, we will not phenotype the CatetoxL53 RILs under field.

NILs and RILs will be characterized for organic acid exudation and root tip Al content.

Considering that L3 has no polymorphic markers compared with Cateto, we are developing new crosses, which will be submitted to MABC to accelerate the generation of NILs.

All activities using Kenyan materials are depending on the process of transferring the target materials from Kenya to Brazil. This process is under way, and hopefully within a few months we will be able to start the molecular and activities proposed on the project.

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31. G7010.03.05: Marker-assisted breeding for improving phosphorus-use efficiency and tolerance to aluminum toxicity in maize

April 2010–March 2014

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- IRRI: S. Heurer
- USDA-ARS (Cornel University): L. Kochian; L. Maron; M. Pineros; J. Liu

1.0. Research activities

1.1. Screening of Kenyan maize germplasm for Al tolerance in solution culture and for ZmMATE expression in the laboratory

Maize (*Zea mays* L.) is one of the world's most important cereals and is a staple food for many people in developing countries. However, in acid soils, its production is limited by aluminium (Al) toxicity and phosphorus (P) deficiency. In order to address these two related problems, researchers at Moi University and KARI (Kenya), EMRAPA (Brazil) and Cornell University developed over 200 inbred lines. One hundred and seventy five of these lines were evaluated in nutrient culture at 222 µM Al stress and one hundred of them in low P stress (2.0mg/Kg soil) at Sega and

Chepkoilel characterized by acid soil of Kenya. Root growth inhibition measured after 3 days growth in solution culture occurred in 95% of the inbreds although in the most tolerant inbreds, Al treatment induced their root growth (Fig 1). The lines 203B, 203B–14, and CATAL 237/167 X L3–5 were the most Al tolerant having relative net root growth (RNRG) of 119%, 112% and 110% respectively. The lines SCH 3, S 580–17–2–2–5 and HS 723X11-4 were the most sensitive lines with RNRG of only 17%, 20% and 29% respectively. Forty Al tolerant lines having RNRG ≥80% were identified and will be use in our molecular breeding program.

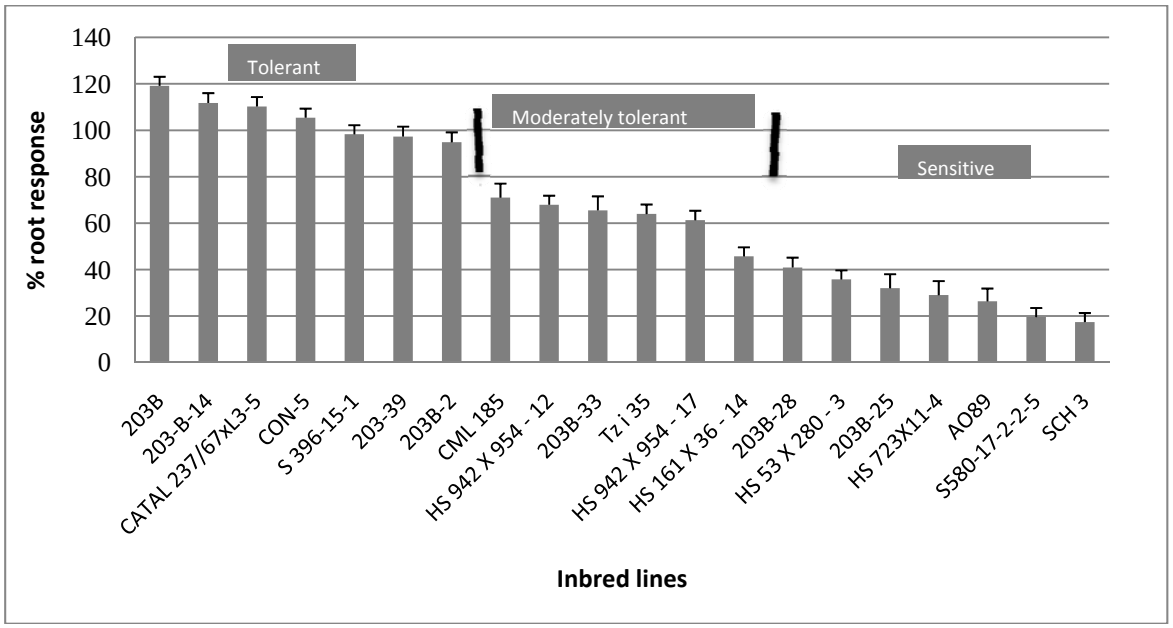

Fig 1: Relative net root growth of selected 20 inbred lines after 3 days of exposure to Al treatment

In terms of P efficiency, KML 036 was the most P-efficient while MUL 229 was the least efficient based on percent yield reduction under low P. Ten lines that are P-efficient exceeding a threshold of 3t/ha grain yield under no P were identified. CON 5 which is among the most Al tolerant germplasm had the highest mean yield of 5 t/ha and 4.6 t/ha in Chepkoilel and Sega respectively under low implying that it would be useful in the development of multistress tolerant maize varieties.

Some of the over one hundred single cross hybrids developed from the inbred lines exhibited Al tolerance equal to or greater than those of the more tolerant parents indicating a positive transgressive inheritance. Fifty eight percent (58%) of the F_1 single crosses were heterotic for tolerance to Al toxicity based on mid-parent heterosis. We identified highly Al tolerant and sensitive Kenyan lines. These lines will be evaluated for *Zm*MATE1 expression by one of Moi University students at EMBRAPA in Brazil later this year.

2.0. Development of Top cross and mapping populations

One hundred and seventy five top cross populations have been developed between the inbred lines and two testers each, adapted to high or medium altitude agro-zones of Kenya. These top cross populations will be used to asses yield performance in the acid soil conditions of western Kenya in the August – December 2011 cropping season.

Mapping populations for both Al and Phosphorus have been developed by crossing contrasting inbred lines in each trait. F1 seeds are available and will be advanced to F2 for genotyping and F2:3 for phenotyping and data obtained will be used to map QTLs controlling tolerance to Al toxicity and P-efficiency.

3.0 Marker assisted selection for genes/QTLs to improve Al tolerance and P-use efficiency in locally adapted maize germplasm

Introgression of *ZmMATE*/Al tolerance QTLs in Brazilian elite lines has been achieved. Two Al sensitive but polymorphic Brazilian maize lines containing SSRs flanking a major Al tolerance QTL were crossed with appropriated donors of the QTL derived from Cateto in order to obtain F1 generation. Two cycles of MABC using markers for forward and backward selection will be undertaken.

Tangible outputs delivered

Forty aluminium tolerant inbred lines have been identified. Ten lines that are P-efficient exceeding a threshold of 3t/ha grain yield under no P (CON 5, Hs 942x954-17, 203B-30, A089, HS 723X110-13, HS L3X5046-8, HS L3X5046-2, 203B-46, 203B-33 and HS 161x36-1). These have been used to develop one hundred and seventy five top cross populations which will be used to asses yield performance in the acid soil conditions of Kenya.

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Rice

32. G3007.05: Detecting and fine-mapping QTLs with major effects on rice yield under drought stress for deployment via marker-aided breeding

August 2007–July 2009; NCE: March 2011

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Context

The project aimed to fine-map, characterize, and mobilize for marker-aided breeding (MAB) the previously detected QTLs and small chromosome segments with large effects on yield under severe drought. Using the identified QTLs the project will develop cultivars with enhanced drought tolerance due to genes introgressed by MAB for participatory evaluation in key target environments and to develop, implement, and disseminate the developed products. At IRRI, together with national partners, strategies for efficiently detecting QTLs with large effects on drought tolerance in a range of genetic backgrounds suitable for deployment in MAB shall be developed.

Findings

Fine mapping of the large effect QTL ($qDTY_{12.1}$) identified in Vandana/Way Rarem population around a 10 cM interval between RM 28048-RM 511 on chromosome 12 (Bernier et al. 2007) showed presence of at least two independent regions (RM1261-28166, RM28048-RM511) affecting grain yield under drought within. RM1261-RM28166 region showed additive effect of 208 kg which is around 50% of the trial mean (417 kg/ha) under stress. In three advanced backcross populations ($BC_3F_{3:4}$) derived from crossing the drought tolerant donor Aday sel. with IR64, four QTLs, $qDTY_{2.1}$, $qDTY_{4.1}$, $qDTY_{9.1}$ and $qDTY_{10.1}$ were identified on rice chromosomes 2,4, 9 and 10 respectively. In populations derived from crossing the drought tolerant donors

N22 with susceptible cultivars Swarna, MTU1010 and IR 64, $qDTY_{1.1}$ on chromosome 1 showed significant effect in all the three genetic backgrounds. Experiments have indicated that $qDTY_{12.1}$ enhanced the grain yield through increased plant water uptake resulting from more effective root architecture. The lines with the QTL had an 18% higher below 30 cm deep root length compared to lines without QTL (Bernier et al. 2009).

$qDTY_{12.1}$ was introgressed in Vandana and $BC_2F_{3:4}$ and $BC_3F_{3:4}$ introgressed near isogenic Vandana lines (NILs) showed yield advantage of 0.5 t/ha under severe and moderate stress reproductive stage drought over moderately drought tolerant cultivar Vandana while maintaining the similar high yield and other morphological traits under irrigated normal situation. The Vandana NILs are 97-99 genotypically similar to Vandana. In IR 64 background, introgressed IR64 NILs with one, two, three and four QTL combinations were developed. The project developed 4 lines with four QTL combinations, 15 lines with three various QTL combinations, 29 lines with two QTLs combinations and 19 lines with different single QTLs. The introgressed IR64 NILs showed improved yield under drought over IR64, yield advantage/similar yield under well-watered conditions, similar phenotypic traits, 93-97 percent genetic similarity to IR64, similar quality traits to IR64 and similar reaction to IR64 for blast and bacterial leaf blight. In large plot screening in 2011 DS, all, except for two lines, showed 1 to 30 percent increased yield under non-stress conditions over IR64. Under stressed conditions, all, except one line, showed 13 to 110 percent increase in yield over IR64.

Next Step
The developed NILs in Vandana and IR64 backgrounds will be tested in India, Nepal, Laos, Tanzania and most promising lines will be identified for release by national system.

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33. G3008.03: Delayed senescence and drought tolerance in rice

November 2008–October 2011

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED

34. G3008.04: Drought from a different perspective: Improved tolerance through phosphorus acquisition

November 2008–October 2011

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1. Cloning of the *Pup1* major gene

The rice quantitative trait locus (QTL) Phosphorus uptake1 (*Pup1*) confers tolerance of phosphorus (P) deficiency but the underlying mechanisms remained unclear since P-uptake related genes and physiological mechanisms could not be identified. In order to gain insight into the function of Pup1, we have sequenced the *Pup1* genomic region in the tolerant donor variety Kasalath and have analyzed the 68 gene models predicted in the region. The data showed that *Pup1* is a hot spot of transposon integration with overall low conservation compared with the Nipponbare reference sequence. Based on detailed sequence and gene expression analyses, a set of genes has been short listed and transgenic plants were generated to study the function of these genes. Overexpression (35S) of one of the analyzed genes, the protein kinase OsPupK46, conferred tolerance to IR64 plants grown in P-deficient soil in combination with a dry-down treatment. A growth and yield advantage was also observed under P-fertilized conditions. OsPupK46 is located within a *Pup1*-specific insertion deletion (INDEL) region and therefore absent from intolerant genotypes. In collaboration with Paolo Pasaresi at the University of Milano, we were able to show that this gene codes for a functional Ser/Thr protein kinase.

Detailed analyses of independent T1 transgenic lines now suggest that *Pup1* confers tolerance of P-deficiency by a vigorous early root growth. Root-scan data of 14 day-old-seedlings grown in hydroponics showed that transgenic plants developed a higher root surface area and total root length compared with non-transgenic controls, under P– and P+ conditions. This was also observed in 11 day-old-seedlings of IR74-*Pup1* NILs. This early advantage enables transgenic plants grown in P-deficient soil to take up more P and other nutrients and to develop a higher tiller number and grain yield compared with Null controls grown in the same pot. Preliminary P content data showed that transgenic plants had a higher internal P use efficiency under P– conditions but not under P+ conditions. This can be explained by a lower P content in grains of transgenic plants.

An Affymetrix microarray analysis of transgenic plants was conducted that confirmed an independent data set derived from an Agilent gene-chip analysis of Nipponbare *Pup1* near isogenic lines (NILs). In both studies, no known P-responsive gene could be identified that was differentially expressed in the *Pup1* materials. The absence of specific P-uptake genes is in agreement with earlier data that failed to identify P-uptake related processes in *Pup1* NILs. From the Affymetrix analysis a set of 29 genes was identified with constitutively lower (21 genes) or higher (8 genes) expression in roots of transgenic plants at different developmental stages grown under stressed and non-stressed conditions. Most of the genes code for upstream regulators for which key functions in hormonal pathways (GA and Jasmonate) and drought-stress response have been shown. Furthermore, many of these genes co-localize with meta QTLs for root traits and drought tolerance. In summery, the data suggest that the *Pup1* protein kinase is the major tolerance gene and that it acts upstream via modulating regulatory pathways thereby enhancing seedling root growth. Early vigorous root growth is a highly desirable trait for rainfed environments and water-saving systems, e.g., alternate wedding and drying. Under P-deficient conditions, a larger surface area and vigorous root growth enhances access to soil endogenous P and its uptake.

2. *Pup1* breeding in Indonesia

At ICABIOGRAD, advanced *Pup1*-breeding lines (BC_2F_5) of three Indonesian upland varieties have been tested for tolerance of blast disease. For the development of the breeding lines, different *Pup1* donors were used of which Kasalath is intolerant of Blast. Since Blast is a prevalent biotic stress in Indonesia, it was important to ensure that the *Pup1* materials are tolerant. The field and greenhouse data now showed that the selected lines were at least as tolerant as the recipient parent with several lines showing a higher tolerance level. An evaluation of agronomic traits of the *Pup1* breeding materials under two different P-fertilizer treatments in a field experiment showed that the introgressed *Pup1* locus conferred a yield advantage to those recipient parent without (Situ Bagendit) or partial presence (Batur) of the tolerant *Pup1* locus but not to the recipient parent that naturally possesses *Pup1* (Dodokan). Selected lines will be subjected to preliminary yield trials in 2011 and to an advanced yield trial in 2012.

35. G3008.06: Targeting drought-avoidance root traits to enhance rice productivity under water-limited environments

November 2008–October 2011; NCE: October 2012

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Root biology is at the forefront of progressing fields to impact agricultural productivity in low-input systems. Since rice research has historically emphasized irrigated environments, and because of the difficulties associated with studying roots, there are large gaps in our knowledge about root traits for drought tolerant rice. A better understanding of the genetic variation in rice for drought response and root traits, as well as practical methods for studying them, are needed by the research community.

1. Phenotyping of root traits in the OryzaSNP panel: The OryzaSNP panel (a set of 20 diverse genotypes that have been mapped for SNP markers) is being collectively phenotyped in a range of conditions by project partners to look at genetic diversity for root response to drought, and a number of root research methods are being refined to accomplish that objective. OryzaSNP roots have been evaluated in the field in TNAU, AfricaRice, and IRRI (Henry et al., 2011), and in controlled environments using methods including the root box and slant box (Nagoya), herbicide screening (Aberdeen), wax-layer method for resistance to penetration (Charles Sturt), and cylinders/lysimeters (IRRI, AfricaRice, Barwale Foundation).

The main products of these efforts are a) the identification of potential donors that have root traits conferring drought tolerance, and b) publication of a manual of root methods based on the advances in protocols achieved through this project. Results from characterization of the OryzaSNP panel from various partners are being compiled into one paper describing genetic variation and environmental response. A review paper on root biology and genetic improvement for drought tolerant rice has been published (Gowda et al., 2011). These products will be most beneficial for researchers needing drought tolerance donors, target traits, and methods for studying roots.

Results thus far have pointed to the aus group as the most promising source of drought donors, showing deep root growth and high drought response index. Therefore a large aus panel was selected from the IRRI Genebank. These lines have been phenotyped under drought in the field during two seasons at IRRI, and 226 aus lines are now being planted in 1000+ lysimeters at IRRI and also in the herbicide screening system at Aberdeen. These results will be used for association mapping after the aus panel genomes have been sequenced, and are expected to result in identification of drought donors, as well as genes/selectable markers for improvement of existing varieties.

2. Evaluation of root QTL NILs and advanced breeding lines: The second main group of activities of this project is targeted more toward use by farmers. We have characterized lines including IR64xAday Sel NILs (Venu et al., 2011), Bala/Azucena/Kalinga NILs, ARB root QTL lines, and IR64 NILs (Kato et al., 2011)

under field drought conditions. Molecular analysis of the IR64 NILs is also being conducted under this project. We are screening 10 advanced breeding lines at five NARES sites in India, and of these 2 partner sites (Raipur and Hazaribag) have been supplied with equipment and training for root sampling. Major products are a) markers for breeders, b) identification of which environments these advanced lines are most suitable for increasing yields under drought, and c) candidate drought tolerant varieties for release in India.

3. Next steps: For the remainder of the project, we will complete our experiments and convene at a project workshop to be held September 12-15 2011 at IRRI. At the workshop, project partners will present research results, meet with a statistician to plan the compilation of our results for a collective paper on our root phenotyping of the OryzaSNP panel, and meet with the IRRI editor about publishing our root methods manual. Young scientists from India, Iraq, and Benin are also being included in this workshop to help promote the future generation of root researchers.

4. Conclusions: There is still a lot to be learned about root traits that can confer drought tolerance in rice. Deep rootedness is one possible mechanism, but there are other root parameters (lateral root growth, anatomy), that have been identified in this project which may be beneficial under drought, depending on environmental conditions. These results point to the importance of characterizing the target drought environment to understand which drought prone rice growing regions would be the best targets for each drought tolerant line developed by breeders. Results from this project will help empower rice researchers to realize the huge genetic potential of rice for root traits that can be effective for drought stress, while providing practical, refined methods to promote screening for root traits in local germplasm. Work with such genetically well-characterized lines as the OryzaSNP and aus panels will result in genes and marker identification to accelerate incorporation of promising traits/QTLs into local varieties.

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36. G3008.09: Breeding drought tolerance for rainfed lowland rice in the Mekong region

November 2008–October 2011

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Summary
The main objective of this project is to develop strategies and protocol for selection of drought tolerant genotypes by using diverse populations which have been developed by us in previous projects. A key hypothesis is that addition of aerobic condition in selection of rainfed lowland rice increases the efficiency of breeding as rainfed lowland rice experiences not only flooded and drought conditions but also aerobic condition where soil water content is high but not saturated. This study is conducted in Thailand, Laos and Cambodia. The outcome of this work, in addition to developing strategies for selecting drought tolerance, is to indentify drought tolerant genotypes that can be released as commercial varieties, to identify traits conferring adaptation to aerobic condition, to confirm putative secondary drought adaptation traits, and to develop GIS maps that identify drought prone areas. The approaches used in rice breeding programs are conventional, modeling and farmer participatory.

Control field drought screening was conducted on station in 2009 for selecting lines with good performance under different water availability conditions for their further evaluation in multi-location trials in wet season 2010. In wet season 2009, total of 300 lines were screened at 2 locations in Thailand, 227 and 80 lines at 1 location each in Laos and Cambodia, respectively. Field screening comprised of 3 conditions, irrigated flood, irrigated aerobic, and drought stress at flowering stage. Under irrigated conditions, selection was based on grain yield and filled grain percentage. Under drought condition, selection was based on grain yield, delay in flowering and filled grain percentage. In Thailand 40 lines were selected, and in both Laos and Cambodia 26 lines were selected. Selected lines were grouped into 3 groups based on their performance under different conditions; good under all 3 conditions, good under both irrigated conditions, and good under flood and drought conditions.

In wet season 2010, there were 2 activities, multi-location trial in farmer field and control field drought screening on-station. For multi-location trial, lines selected from control field drought screening were evaluated. There were 7 sites in each country which were selected based on the results from the project's GIS- mapping activity.

Variety preference evaluation by farmers for adoption in agronomic characters was also conducted in Thailand and Laos at close to maturity stage. Our selection of lines from multi-location trials was based on their consistent performance across sites in grain yield, resistant to diseases and farmer votes. In Thailand, 25 lines were selected and 20 lines each in Laos and Cambodia. These selected lines are being evaluated again in farmer fields in wet season 2011. In additional to the selected lines a set of 10 common lines were also evaluated in the multi-location trial to investigate their performance across 3 countries. These 10 common lines were selected based on their performance under control field screening in wet season 2010, from 3 groups; good under all 3 conditions, good under both irrigated conditions, and good under flood and drought conditions.

In wet season 2010, control field drought screening on-station was conducted at 2 locations in Thailand, 1 location each in Laos and Cambodia. In Thailand, 70 lines including 40 lines used in multi-location trial and 30 lines selected from wet season 2010 experiment were screened under line source sprinkler system. 70 lines in Laos and 100 lines in Cambodia were screened under 3 different conditions; flood, aerobic and drought stress at flowering stage.

There was reduction in grain yield under aerobic in comparison with flood conditions. Across counties, years and experiments, it was found that grain yield under flood condition was higher than aerobic condition in all experiments. It was observed that there was reduction in panicle grain weight rather than panicle number. The difference in grain yield between flood and aerobic conditions depended on the duration of aerobic condition. The longer period of aerobic condition, the larger reduction in yield. This evidence suggests that adaptation to both flood and aerobic conditions might be required for rainfed lowland rice line under unfavorable condition.

Water availability in rainfed lowlands in the Mekong region has been identified across Laos, NE Thailand and Cambodia by combining rice crop model simulation results and geographical information system (GIS) mapping software. The crop growth model developed by Fukai et al. (1995) has been modified with the addition of a water balance model (Inthavong et al 2011). The water balance model estimates daily water loss from evapotranspiration and deep drainage, the latter being a function of soil clay content (Tsubo et al. 2007). The model estimates the start and end of the growing season and consequently the length of the rice growing season for any location with appropriate weather and soil input data. Following Inthavong et al. (2008), GIS maps of various drought patterns (particularly early and late season drought) in the rice growing regions of Laos, NE Thailand and Cambodia have been made. These maps have been used to identify the areas where early season drought and late season drought are likely to have serious effects on rice yield. The results have been used for identification of target environments where particular genotypes and particular drought tolerance traits may be useful to increase grain yield.

37. G4006.01: Developing strategies for allele mining within large collections

January 2006–July 2008; project under review in 2011

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NO UPDATE SUBMITTED

38. G4008.05: Connecting performance under drought with genotypes through phenotype associations

January 2008-December 2010, NCE: June 2012

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-

Context
Water stress is frequently the main limitation of rice productivity and yield stability in rainfed systems. Most “mega-varieties” that are grown over vast areas of South and Southeast Asia is highly susceptible to water deficits. Yet, within the primary rice gene pool resides a large amount of genetic diversity for abiotic stress tolerance. Rapid advances in molecular biology provide great potential to harness this genetic diversity within rice but, to fully exploit this information, by relating allelic variation to agronomic performance, an in-depth phenonomics initiative is necessary. By developing a standardized, high-throughput, precise phenotyping strategy, employed across a range of drought environments, valuable data sets on performance under field drought stress on a large reference set of accessions can be generated and alleles associated with the traits measured and on this basis, potential markers for marker-assisted selection (MAS) for agronomic performance under water deficit.

Findings
At IRRI, compared with a control, under reproductive-stage drought stress, mean plant height declined by 33%, grain yield decreased by 79% (indicating severity of drought stress) and harvest index fell by 42 compared with a control. However, some

of the accessions of medium duration showed high tolerance of drought, with 72 accessions yielded more than 2,000 kg/ha under reproductive-stage drought stress at IRRI under situations when more than 300 accessions yielded less than 1,500 kg/ha. Similarly, in Thailand, vis-à-vis a control, under reproductive-stage drought stress, a mean reduction in plant height of 14% was observed, whereas this was 11% for mean grain yield. At Central Rice Research Institute (CRRI), Cuttack in India, the grain yield decreased significantly under reproductive-stage stress. At CRRI, compared with control, under reproductive stage stress, mean plant height declined by 14 % and grain yield decreased by 46% compared with a control. However, some of the accessions of early duration showed high tolerance of drought, with 76 accessions yielding more than 1,000 kg/ha under reproductive-stage drought stress at CRRI under situations when only 8 accessions yielded less than 1,000 kg/ha. Similarly for medium duration accession of 80 days or more than 80 days to 50% flowering, a number of accessions had more than 1000 kg/ha grain yield. Genotype X environment interactions will be studied utilizing the data generated in the project.

At CIRAD (Montpellier, France), experiments indicated key role of DR as a constitutive and adaptive trait in the maintenance of early vigor across a severe, short drought event. Both under water supplied and stress situations, a sensitivity analysis (quantification of the impact of model genotypic parameters on simulated variables) performed with Ecomeristem (model simulating rice plant morphogenesis depending on genotypic parameters and environmental conditions: water, light, temperature, air humidity; Luquet et al. 2006) confirmed the key role of DR on seedling early vigor. In a second time, the parameters characterizing the morphogenetic typology of a genotype in Ecomeristem were optimized (i.e. estimated by fitting model simulations to corresponding observations) for each of the 200 japonica genotypes, firstly, under non limiting conditions. Model parameters were *plasto:* plastochron, *Ict*: tillering sensitivity to plant internal carbohydrate availability (IC=daily plant internal supply/demand C ratio), *MGR*: meristem growth rate defining the potential size of successive organs. The positive relationship observed experimentally between DR and RGR was confirmed by that between optimized *plasto* and measured or simulated RGR. The model appears relevant to consider the functional role of DR in rice early vigor and the genetic diversity expressed at this level. For sugar content vs. morphogenetic pattern, starch concentration in source leaves was negatively ($P<0.01$) correlated to DR, suggesting the existence of a bold vs. conservative strategy of rice seedlings that would either invest C assimilates in new, structural organs or store it. Similarly starch in sink leaves was positively and negatively correlated respectively to tillering and organ size ($°P<0.05$), confirming the trade off of seedlings between organ size, number and carbohydrate storage. Interestingly, carbohydrate patterns confirm the functional analysis of source-sink relationships pointed out through morphogenetic data on the 203 genotypes. For transpiration rate response to FTSW, for several genotypes, no breakpoint was found and genotypes seemed to reduce their transpiration from FTSW 0.9. These results may be due to the high ETP lived by some of the plants in the greenhouse. They will have to be further explored and compared.

Challenges

Imposing desired level of drought stress under natural screening has been a challenge and often drought screening trials failed to be exposed to desired level of stress in wet season.

39. G4008.45: Populations for multiple allelic segregation developed through nested intercrossing in rice

August 2008–July 2010; NCE: July 2011

Principal Investigator

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1. Research activities and progresses

1.1 Selection of parental lines

A total of 88 elite parental rice lines chosen among a collection of promising breeding materials were planted out at CIAT (48 lines) and AfricaRice (40 lines) in pots in the screen house. With the aim of checking the purity of the parental lines, fresh leaves samples of four replicates of each parent and every F1 plant were collected for DNA extraction; the extraction was carried out using the protocol described in Lorieux, 2002.

At CIAT, a diversity survey of 48 tropical *japonica* candidate lines for parents of the metapopulation was done at CIAT using a set of 24 SSR markers evenly distributed on the twelve rice chromosomes. The data were analyzed using the Darwin 5.0 and NTSYS programs, and the SAS statistical package (Multiple Correspondence Analysis), in order to identify a final subset of fifteen lines that would maximize the genetic diversity (Table 1).

At AfricaRice, 21 polymorphic SSR markers, well distributed on the rice genetic map, were used to assess the level of purity within the 40 lines. Lines that showed 100% similarity for all the markers were declared as pure. The diversity was evaluated by UPGMA clustering using the NTSYS program (Figure 1). The study of 37 out of the 41 parents gave 53 alleles with an average of 2.5 alleles per locus.

1.2 Production of F1 hybrids

F_1 hybrids were produced by crossing the *indica* IR64 accession as female with all 88 candidate lines. Ten F_1 seeds were sown per retained combination, and were checked for heterozygosity using 3 or 4 SSR markers (Figure 2).

1.3 Production of F2 lines

The F_1s were brought to the field (AfricaRice) or grown in the screen house (CIAT) in order to be selfed and to produce the F_2 seeds that represent the starting point of the Single Seed Descent (SSD) process.

At CIAT, at least 400 F_2 seeds were obtained for each combination. Of each F_2 population, 400 seeds were sowed in order to make sure that we obtain final F_7 population sizes of at least 200 individuals through the SSD process.

Table 2 shows a list of the identified F_1 hybrids and the number of F_2 seeds produced at AfricaRice. New crosses have been done for the cross combinations with no certified hybrids. DNA analysis is ongoing to certify more hybrids.

1.4 Production of SSD lines
We are currently producing the F5 and F6 seeds – depending on the cross – at both sites for the selected 20 crosses (10 at CIAT and 10 at AfricaRice). We expect to sow the F5 or F6 plants between August and October 2010. The final F7 seeds should be available by february 2012.

2. Tangible outputs delivered
The main output of this project is obviously the genetic material itself, represented by the NAM metapopulation. We think that the NAM population will provide the rice research community with a highly efficient and powerful genetic resource that will eventually allow to fully take advantage of the numerous genomic tools that are now available for this species. Accurate, powerful, multi-allelic QTL detection and fine/ultrafine mapping of QTLs for many important agronomic traits are expected from future studies based on this resource.

We expect that GCP and external partners, will develop various phenotyping projects on drought tolerance and other traits, based on the resource created in this project.

3. Challenges
Genome sequencing of parental lines of the NAM metapopulation will certainly increase very much the potential of this genetic material, in allowing us to perform a better inference of the ancient recombination events based on complete genomic information instead of partial (SNP) coverage.

40. G7009.07: Cloning, characterization and validation of Alt_{SB}/Al tolerance in rice

October 2009–October 2012

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1. Rationale for the SorghumPup1 project
On acid soils Al toxicity results in rapid damage to and growth inhibition of root systems, which leads to significant yield reductions due to poor uptake of water and nutrients. Rice is the most Al tolerant cereal, yet Al toxicity is still a major limitation to rice production in both rainfed lowlands and uplands. In this proposal we will take advantage of our recently cloned sorghum Al tolerance gene that is a member of the

MATE family of organic solute transporters, to identify rice homologs that are candidate tolerance genes. In rice, we have conducted a computational analysis of the MATE family and have identified 5 MATE genes that are co-localized with previously identified Al tolerance QTL. In this project we will test them as candidate Al tolerance genes using T-DNA rice knockout lines. We also have been carrying out complementary approaches as our preliminary findings indicate the rice MATE orthologs at best play a minor role in rice Al tolerance. Hence we are fine-scale mapping and cloning of a novel major rice Al tolerance QTL; this QTL appears to be due to a transcription actor, art1, which regulates the Al-inducible expression of other rice Al tolerance genes. Also, we have completed whole genome association mapping of rice Al tolerance, which has identified a number of novel rice genomic regions harboring Al tolerance loci. We already are suing these results for the cloning of other new rice Al tolerance genes.

2. Research Activities and Results

2.1. GWA and QTL analysis of rice Al tolerance. Rice is significantly more Al tolerant than other cereal crop, yet mechanisms of rice Al tolerance are largely unknown and no genes underlying natural variation have been reported. We screened 383 diverse rice accessions and then conducted a genome-wide association (GWA) study as well as QTL mapping in two bi-parental populations of Al tolerance. For the GWA analysis, subpopulation structure explained 57% of the phenotypic variation and the mean Al tolerance in Japonica was twice that of Indica. As depicted in Figure 1, forty-eight regions associated with Al tolerance were identified by GWA analysis, most of which were subpopulation-specific. Four of these regions co-localized with a priori candidate genes and two highly significant regions co-localized with previously identified QTLs. Three regions corresponding to induced Al sensitive rice mutants (ART1, STAR2, Nrat1) were identified through bi-parental QTL mapping or GWA to be involved in natural variation for Al tolerance. Haplotype analysis around the Nrat1 gene identified susceptible and tolerant haplotypes explaining 40% of the Al tolerance variation within the aus subpopulation and sequence analysis of Nrat1 identified a trio of non-synonymous mutations predictive of Al sensitivity in our diversity panel. GWA analysis discovered more phenotype-genotype associations and provided higher resolution, but QTL mapping identified critical rare and/or subpopulation-specific alleles not detected by GWA analysis. Mapping using Indica/Japonica populations identified QTLs associated with transgressive variation where alleles from a susceptible aus or indica parent enhanced Al tolerance in a tolerant Japonica background. This work supports the hypothesis that selectively introgressing alleles across subpopulations is an efficient approach for trait enhancement in plant breeding programs and demonstrates the fundamental importance of subpopulation in interpreting and manipulating the genetics of complex traits in rice.

2.2 Next Research Steps.

- Have identified both novel and previously identified QTL. The largest novel QTL is on chr 12 and explains 25% of variation in an IR64xAzucena population. Fine scale mapping of this QTL is nearly completed and has identified a strong Al tolerance candidate gene, *art1*, which is an Al-dependent transcription factor (STOP1 homolog).
- Will determine if rice Nrat1 important in Al sensitivity in *aus* subpopulations. This will involve finding out if the sensitive alleles of this gene encode an Nramp transporter that is less effective at mediating Al uptake. This will test hypothesis that Al tolerance is conferred by reducing cell wall Al levels.

- Are beginning the development of Al tolerance NILs based on the identified QTLs and the newly identified loci from whole genome association mapping to quantify the contribution of individual loci and as a resource for improving rice Al tolerance via breeding approaches.

3. Figures

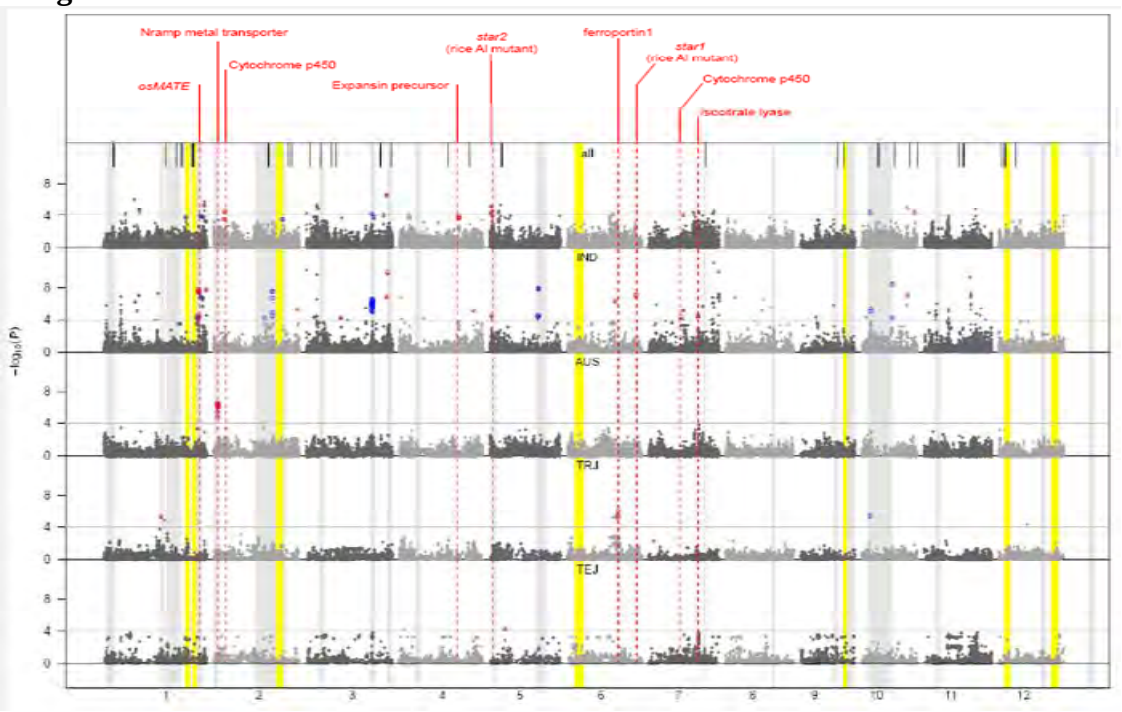

Figure 1. GWA analysis across (all) and within subpopulations (IND=*indica*; AUS=*aus*; TRJ=*tropical japonica*; TEJ=*temperate japonica*). *A priori* candidate genes are listed across the top, with those identified within 200kb of significant SNPs colored red. Color bands indicate bi-parental QTL positions from previous reports (grey) or from this study (yellow). SNP color indicates co-localization with QTLs (blue) or candidate genes (red).

4. Publications

Famoso AN, Clark RT, Shaff JE, Craft E, McCouch SR, Kochian LV. 2010. Development of a novel aluminum tolerance phenotyping platform used for comparisons of cereal aluminum tolerance and investigations into rice aluminum tolerance mechanisms. Plant Physiol.153: 1678–169

Famoso AN, Zhao K, Clark RT, Tung C-W, Wright MH, Bustamante C, Kochian LV, McCouch SR. 2011. Genetic architecture of aluminum tolerance in rice (*O. sativa*) determined through genome-wide association analysis and QTL mapping. PLoS Genetics (In Press).

41. G7010.03.04: Improved rice cultivars for Asian problem soils: Pyramiding of major genes/ QTLs for tolerance to phosphorous deficiency and aluminium toxicity

November 2009–October 2013

Principal Investigator and Lead Institute
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1. Progress with *Pup1* marker development and variety development
The major quantitative trait locus (QTL) Phosphorus uptake1 (*Pup1*) confers tolerance of phosphorus deficiency in soil and is currently one of the most promising QTLs for the development of tolerant rice (Oryza sativa L.) varieties. To facilitate targeted introgression of *Pup1* into intolerant varieties, the gene models predicted in the *Pup1* region in the donor variety Kasalath were used to develop gene-based molecular markers that are evenly distributed over the fine-mapped 278-kb QTL region. To validate the gene models and optimize the markers, gene expression analyses and partial allelic sequencing were conducted. The markers were tested in more than 80 diverse rice accessions revealing three main groups with different *Pup1* allele constitution. Accessions with tolerant (group I) and intolerant (group III) *Pup1* alleles were distinguished from genotypes with Kasalath alleles at some of the analyzed loci (partial *Pup1*; group II). A germplasm survey additionally confirmed earlier data showing that *Pup1* is largely absent from irrigated rice varieties but conserved in varieties and breeding lines adapted to rainfed, drought-prone environments. A core set of *Pup1* markers has been defined, and sequence polymorphisms suitable for single nucleotide polymorphism marker development for high-throughput genotyping were identified. Following a marker-assisted backcrossing approach, *Pup1* was introgressed into two irrigated rice varieties and three Indonesian upland varieties. First phenotypic evaluations of the introgression lines suggest that *Pup1* is effective in different genetic backgrounds and environments and that it has the potential to significantly enhance grain yield under field conditions. The data have been shared with Asian and African NARES and will be published in a Plant Physiology special issue in July. The data have also been shared with EMBRAPA to facilitate cloning of the *Pup1* orthologous gene from Sorghum.

2. Developing rice with multiple stress tolerance
Phosphorus deficiency is often accompanied by other abiotic stresses, e.g., drought and aluminum (Al) toxicity, and it is therefore essential to develop rice with multiple stress tolerance. At IRRI, well-defined drought tolerant indica breeding lines are now available that will be crossed with the newly developed *Pup1* indica materials to pyramid drought and P-deficiency tolerance. The mapping of Al-toxicity tolerance in the tolerant Indonesian rice variety Dupa has been initiated at IRRI in collaboration with ICABIOGRAD. Seeds of the mapping population (200 F3; Dupa x ITA131) will

be provided by ICABIOGRAD later this year. At IRRI, a PhD student from Indonesia is optimizing a hydroponics-based screening system using tolerant and intolerant check varieties.

42. 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

Duration: April 2010–October 2014

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1 Progress at AfricaRice

1.1 Evaluation of F_3 population for general adaptability in rainfed lowland ecology

High genetic variability was observed amongthe 495 F_3(IR64 x WAB638-1) progenies for days to first panicle initiation, number of tillers, and plant height. The severity of symptoms of iron toxicity was low. Stem borer infestation was generally low on most entries. No gall midge infestation was recorded under natural infestation.

1.2 Production of $F_{3:4}$and$F_{3:5}$seeds

The $F_{3:4}$ seeds of 1886 families constituting the four MARS populations were collected by NARS in their countries and brought to AfricaRice for seed increase and production of $F_{3:5}$ seedsin the field. The MARS population named IR64xB6144F-MR-6-0-0 was chosen by the breeder during the field monitoring tour and will be used for genotyping and phenotyping experiments. Two additional populations - IR64xIRAT104 and IR64xFAR035 - were also selected and will be used for drought phenotyping and QTLs detection.

1.3 Genotyping of the MARS parental lines using SNP markers

A total of 805 informative SNP markers were identified between parental lines (IR64 used as female and BW348-1, B6144F-MR-6-0-0, FARO 35, WAB638-1 used as male) of the four MARS populations.

1.4 Data gathering and information management

The Rice CI Database IRIS-Rice CI was setup.Nomenclature and standards were establishedfor F_1, F_2and1886F_3lines derived from the four cross combinations ARC1, ARC2, ARC3 and ARC4 (see Table1). Workbooks were created and transmitted to partners for data recording. A feasibility study was carried out using handheld recorders received from GCP to facilitate collection and recording of agronomic data. A website was createdfor the Rice CI project with the following link for public access http://www.africarice.org/rice-challenge/ and the link for the project members and collaboratorshttps://cgxchange.org/login/auth.htm

1.5Capacity enlargement through training courses

Six NARS researchers (two per country) attended a three-weektraining on the maintenance of infrastructureequipmentof the drought phenotyping platform. In addition, three NARS researchers spent three months at AfricaRice and were exposed to the molecular techniques, the use of IRIS database, electronic fieldbook as well as theoretical courses related to drought phenotyping. The NARS breeders also participated inthe monitoring tour toIbadan where all the populations were multiplied for seed increase. During the monitoring tour, a MARS population named IR64 x B6144F-MR-0-0 was selected to be phenotyped for yield potential the 2011 wet season as well as drought tolerance in the 2011/2012 dry season.

2. Infrastructure improvement at NARS

2.1 Establishment of the drought phenotyping platform in West Africa

The sites for phenotyping were carefully identified and geo-referenced in Logorola (Mali), Banfora (Burkina) and Baddegi (Nigeria). Wells were dug and the water pipelines are being installed. The soil physical and chemical characteristics are being analysed. One weather automatic station was provided and also installed in each country. A gravimetric irrigation system for plots irrigation during the dry season was identified. In Baddegi, the rehabilitation of the identified plots isbeing implemented.

3 Progress at IER

3.1 Evaluation of F_3 populations for general adaptability in lowland and production of $F_{3:4}$ seeds

Among 493 F_3(IR64 x B6144F-MR-6-0-0) progenies evaluated,35 families out of 493 suffered severe disease attack and were discarded. Plants in the remaining families have good adaptability to the lowland ecosystems of Longorola. The $F_{3:4}$ seeds were sent to AfricaRice for the production of $F_{3:5}$ seeds.

4 Progress at INERA

4.1Evaluation of F_3 populations for general adaptability in lowland and production of $F_{3:4}$ seeds

Iron toxicity was less pronounced on at least 5-10% ofthe 486 F_3(IR64 x BW348-1) progenies evaluated in the valley fringe and valley bottom. . African Rice Gall Midge attack was also observed on two to three F_3families. The $F_{3:4}$ seeds were sent to AfricaRice for the production of $F_{3:5}$ seeds.

5 Progress at NCRI

5.1. Evaluation of F_3 populations for general adaptability in lowland and production of F_4 seeds

African Rice Gall Midge attack was recorded on most of the 414 F_3(IR64 x FARO 35) progenies, but no signs of serious disease. Otherwise, the plants were welladapted to the lowland ecosystem. The $F_{3:4}$ seeds were sent to AfricaRice for the production of $F_{3:5}$ seeds.

Table 1 Summary of the mean values of traits measured on 500 progenies of the four F_3 MARS population evaluated in Mali, Burkina Faso and Nigeria (Baddegi and Ibadan).

Crosses	Plant height (cm)	50% Flowering (days)	Panicle length (cm)	Number of tillers
ARC 1 (IR64 x BW348-1)	115.7±19.15	91±5	27.1±3.27	59.9±18.8
ARC 2 (IR64 x B6144F-MR-6-0-0)	102.7±11.45	86±7	22.3±2.64	20±7
ARC 3 (IR64 x FARO 35)	71.4±9	92±10	21±2	52±16
ARC 4 (IR64 x WAB638-1)	129±20	90±4	32±4	11±3

6. Progress at CIRAD

6.1 Adaptation of SARRAH model to the project objectives

SAMARA V2 was developed for this project and about 50 new modules were written. Consequently, a number of cultural practices can now be simulated.

7. Challenges

The establishment of the drought phenotyping platform in West Africa is still a challenge to the implementation of the Rice CI project.

Rice: Capacity building

Human resources (support to teams, students, travel grants, workshops)

43. G4009.02.01: Study of Burkina Faso rice landraces diversity and breeding for resistance to *r*ice *y*ellow *m*ottle *v*irus (RYMV)

March 2009–February 2011

Principal Investigator

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1. Molecular characterization of Burkina Faso rice landraces

1.1 Diversity analysis

The two cultivated rice species *Oryza glaberrima* and *O. sativa* were encountered in the collection. The diversity indexes of the two species were compared for the 22 SSR loci. The diversity observed in *O. sativa* was higher compared to that of *O. glaberrima*. The mean PIC was 0.53 in *O. sativa* and 0.25 in *O. glaberrima*. Similarly, the allele number of *O. sativa* (8.4) was twice as high as that of *O. glaberrima* (4.0). Among the 22 markers, only seven (RM1227; RM215; RM237; RM447; RM474; RM5 and RM514) had more than four alleles in *O. glaberrima* accessions, whereas in *O. sativa* accessions, only four loci (RM124; RM338; RM452 and RM484) had less than five alleles. These four loci also showed lower allelic diversity in the overall population. Only two SSRs (RM237 and RM474) had their PIC values higher in *O. glaberrima* than in *O. sativa*.

1.2 Core collection establishment

A core set, with a limited allelic diversity reduction and a higher number of sample reduction, was implemented separately for the *O. sativa* and *O. glaberrima* accessions, using the software package DARwin. A core set of 13 individuals was obtained for *O. glaberrima*, while 39 individuals were identified for *O. sativa*. The two were pooled together and a unique core of 52 accessions accounting for 89% of the alleles of the whole collection was established.

2. Search for new RYMV resistance genes or alleles among the rice accessions

The screening of the accessions of the collection with the isolate B27 showed that the symptomless accessions represented only 2.2% of the collection. The moderately resistant plants showing mild symptoms, accounted for 5.4% of the collection. More than 92% of the accessions evaluated were susceptible accessions clustered with distinct mottle symptoms and height reduction. The screening of the collection showed the vulnerability of *O. sativa* accessions over *O. glaberrima* accessions. None of the *O. sativa* plants of the collection were symptomless 14 days post infection while ten *O. glaberrima* showed a delay of symptom expression when screened with the isolate BF1. The resistant *O. glaberrima* accessions were evaluated with the $Rymv_1$ allele-specific markers. This showed that their resistance is not based on the same alleles as TOG5681, TOG5672 and TOG5674.

Tangible outputs delivered

A core collection of 52 individuals including O. glaberrima and O. sativa representing the diversity of the whole collection was established. Moreover, ten O. glaberrima accessions and one O. sativa accession were proved to be tolerant to RYMV. The tolerant O. sativa accession BM24 has been recommended to be included in the development of durable RYMV resistant varieties.

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44. G4010.01.01: Identification of novel QTLs for salinity tolerance and pyramiding with submergence tolerance to develop improved rice varieties for Bangladesh

March 2010–March 2013

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Summary
Salinity is a major problem particularly in coastal regions where rice-based farming systems predominate. Salt stress is a major constraint for rice production in these areas because of the high sensitivity of modern rice varieties. Farmers have grown traditional rice landraces adapted to salt-affected areas for generations despite their numerous undesirable traits, including long duration, low yield and poor grain quality. Thus negative characters in traditional varieties and the numerous and complex traits involved in salinity tolerance remains major challenges for conventional breeding to make significant progress; and has led to increased interest in molecular breeding methods (Yamaguchi and Blumwald 2005; Ismail et al. 2007; Thomson et al. 2010). Identification of novel QTLs for salinity tolerance in rice from native landraces is necessary for use in molecular breeding to fast track the development of salt tolerant, high yielding varieties. The rice genotypes Kutipatnai and Ashfoll (Indica) are Bangladeshi landraces tolerant of salt stress at vegetative stages and Azucena (Tropical japonica) and IR64 are popular variety of Philippines, and are sensitive to salinity. These genotypes were used for developing the mapping populations being used in this study. F3 seeds of Kutipatnai / Azucena are being harvested form selected F2 plants. Crosses between Ashfoll/IR64 were completed and F1 seeds has been

harvested. Parental survey has been done among Kutipatnai, Azucena, Jataibalam, Asfoll, IR64 and IR29, and 104 SSR and Indel Markers has been selected as polymorphic between parental lines for each populations. These markers are subsequently using for genotyping the mapping populations.

Flash flooding or submergence is also a major problem in rainfed ecosystems in the coastal region of Bangladesh. More than 2 million ha area in Bangladesh and 15 million ha in South and South East Asia are affected by flash floods. Coastal saline areas invariably experience frequent submergence for short periods with saline or freshwater during wet season. Oftentimes, the stagnating water is saline to various degrees and exerts greater stress to rice than non-saline water when submergence occur at early growth stages of rice crop. The ideal rice genotype for these areas needs to combine dual tolerance of salinity and submergence for better adaptability. Incorporation of salinity and submergence tolerance into the same HYV will be more effective for rice cultivation in coastal region. BR11 is a mega rice variety cultivated in rainfed lowland (T Aman) season in Bangladesh. BR11-Saltol and BR11-Sub1 are already developed; these genotypes are being used as donor to combine both of these genes into the genetic background of BR11 .Crosses have been made between BR11-Saltol and BR11-Sub1 and F2 seeds has been harvested form the selected F1 plants of the BR11-Saltol and BR11-Sub1 . Genotyping of about 350 F2 plants was completed and 16 Plants has been selected from them based on their homozygocity for Saltol and SUB1 QTLs. Phenotyping and further characterization will be pursued after harvesting the F3 seeds.

Conclusion

Identification of novel QTL will help to develop promising salinity tolerant varieties in future and pyramiding Saltol and SUB1 QTLs in BR11 will develop promising variety for the submergence prone salt affected areas of Bangladesh.

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Yamaguchi Y, Blumwald E. 2005. Developing salt-tolerant crop plants: challenges and opportunities. Trends Plant Sci.; 10:615–20.

Community of practice

45. G4007.03/G4009.09: CoPs – Strengthening rice breeding programmes using genotyping and improving phenotyping capacity for biotic and abiotic stresses in Mekong region

G4007.03: January 2007–December 2008; NCE: October 2009
G4009.09: November 2009–October 2012

Principle Investigator and Lead Institute
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Collaborating institutes and scientists
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Mekong mega varieties were improved in terms of biotic and abiotic stresses using marker-assisted backcrossing (MAB). During phases I and II (2004 – 2009), single trait was added to each variety. Myanmar varieties namely Manawthukha (MNT) and Sin Thwe Latt (STL) were improved in terms of grain quality and aroma and salinity tolerance, respectively. Promising lines of MNT and STL are soon to be released to Myanmar farmers. Grain quality and aroma of Thadokkham 1 (TDK1) and CAR3 from Laos and Cambodia, respectively was the main target for improvement during phases I and II. The aromatic lines of TDK1 and CAR3 will be planted in farmer's field this wet season 2011. In phase III, new traits are now being added to lines that were advanced in the previous phases of the Mekong project. Bacterial blight (BB) resistance and submergence tolerance are also improved in MNT and STL, respectively. Fixed lines of aromatic MNT with BB resistance will be completed this year while salinity and submergence tolerant STL will be available at the end of 2012. On the other hand Laos and Cambodia will plant aromatic TDK1 and CAR3 in farmer's field this year. Blast resistance (BL) and submergence tolerance (Sub) will be added to aromatic TDK1 and the fixed homozygous lines will be selected in wet season 2011. Submergence tolerance and brown planthopper resistance (Bph) are now being added to aromatic CAR3 and F1 aromatic CAR3 with Sub and Bph will be available this wet season. In order to validate the new traits that were added, phenotyping facilities were developed in partner institutes. By improving both genotyping and phenotyping capacity of partner institutes in Mekong region, breeding programs are hastened and farmers are soon to benefit from the products of this project.

Sorghum

46. G3008.05: Discovery and development of alleles contributing to sorghum drought tolerance

November 2008–October 2011

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NO UPDATE SUBMITTED

47. G4008.10: Assessment of the breeding value of superior haplotypes for *Alt_{SB}*, a major *Al* tolerance gene in sorghum: linking upstream genomics to acid soil breeding in Niger and Mali (ALTFIELD)

January 2008–December 2010; NCE: December 2011

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED

48. G4008.02: Phenotyping sorghum reference set for drought tolerance

January 2008–December 2010; NCE: December 2011

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- ICRISAT, Nairobi, Kenya: MA Mgonja
- UAS Dharwad, India: PM Salimath
- KARI Machakos, Kenya: CK Karari
- NPGRC Arusha, Tanzania: L.N.D. Mapunda in place of Late Dr W Ntundu
- IER Mali: Sidi Bekaye Coulibaly
- ISRA/CERAAS, Thies, Senegal: N Cisse

Research activities and progress at ICRISAT and collaborators
Based upon the two years evaluation data (2008-09 and 2009-2010) of sorghum reference set at various locations for stay-green character, SCMR, TE and rate of

water loss per unit of leaf area under terminal drought conditions, the promising lines were selected by NARS partners for respective locations. These lines along with control cultivars were evaluated at 2-3 locations by each NARS partner in Asia and Africa for stover/grain yield and other component traits.

ICRISAT, Patancheru, 100 lines were selected and divided into seven maturity groups (Group 1 having 8 accessions, Group 2 46 accessions, Group 3 20 accessions, Group 4 11 accessions, Group 5 6 accessions, Group 6 5accessions, and Group 7 4 accessions). These 100 lines and three control cultivars, ISs 2205, 18758, and 33844 were evaluated in split-plot design with three replications under irrigated and post-flowering water-stress conditions. Data were recorded for 4 qualitative (midrib color, plant pigmentation, basal tillers and nodal tillers), and 11 quantitative (days to 50% flowering, plant height, panicle excertion, ear head length, ear head width, SCMR, number of heads, straw weight, head weight and grain weight) traits.

Dharwad, 30 promising lines and three controls (ISs 2205, 18748, and 33844) were evaluated in randomized block design with four replications at four locations (Dharwad, Bijapur, Indi and Bailhongal). However, the growth and stand of the entries in the trials conducted at Bijapur and Indi was not satisfactory and the experiments were rejected. The data were recorded for quantitative traits (days to 50% flowering, ear head length, ear head width, ear head weight, SCMR at DTF, grain yield per plant, SCMR at maturity, number of basal tillers and 100-seed weight) at Dharwad and Bailahongal locations.

Senegal, 14 selected lines and two controls were evaluated in randomized complete block design with two replications at three locations (Bambey, Nioro, and Darou). Significant differences were observed for time to 50% flowering, plant height, overall agronomic and mold scores, panicles size. Seedling vigor was good in all 3 sites, Namaka and CMS63 being the most vigorous. Based on plant and panicle size and grain quality, overall agronomic performance was considered poor for introduced genotypes, however, these genotypes had good mold score while the local control cultivars were susceptible. The overall mean grain yield was 991 kg ha^{-1}. No significant difference was observed between genotypes. However some entries (ISs15443, 20700, 651(902)656, 14414, 8685) yielded over 1t ha^{-1}. CE180-33 obtained the highest performance with 1.7 t ha^{-1}. Based on yield IS 15443 produced a mean yield of over 1t ha^{-1}, similar to the local control CE 151-262, across three locations. A second year of multilocation trial will be necessary to conclude about the potential of introduced genotypes as most of them performed as well as the local control cultivars with yield above 1000 kg ha^{-1}.

Mali, 12 lines and 3 controls (B 35, Séguifa, and Jakumbè) were evaluated in RCBD with four replications for post-flowering drought stress at Cinzana Agricultural Research Station and Bema Agricultural Research Sub-Station. Data were recorded for ten quantitative traits. Five lines, ISs 452 (411) 510, 393 (421) 659, 3963, 22287, and 24009 have been identified as tolerant to post-flowering drought stress based on the number of green leaves at physiological maturity and leaf chlorophyll index. These lines performed well in the two locations and have the same level of tolerance as the tolerant control B35. The tolerant varieties showed lower grain yield indicating that tolerance to post-flowering drought stress may be detrimental to grain production. However, the tolerance can help in reaching food security in drought prone zones.

Kenya, 22 lines and three controls (KARI Mtama-1, Gadam, ZSV-3) were evaluated in three replications at three locations (Kampi ya Mawe, Masongaleni, and Kiboko) for 17 morpho-agronomic traits.

Tanzania, due to death of our collaborator Dr Ntundu, the experiment was not conducted.

Data recorded at different locations is being analyzed to identify promising drought tolerant lines specific to each location as well as promising drought tolerant lines suitable for cultivation across wide locations.

49. G4008.46: Populations for multiple allelic segregation developed in rice and sorghum through multiple parent intercrossing

August 2008–July 2010; NCE: February 2011; project under review

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED

50. G4008.48: Improving sorghum productivity in semi-arid environments of Mali through integrated MARS

July 2008–July 2013

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- CIRAD/IER, Mali: Michel Vaksmann
- Syngenta Seeds, France: Denis Lespinasse, Michel Ragot.

The Sorghum MARS project proposes to associate recent approaches on sorghum breeding that have been developed at IER and methodologies for marker assisted recurrent selection (MARS) that have proven to provide significant improvement of breeding efficiency for complex traits, especially in the case of maize.

Two bi-parental populations targeting two different environments of sorghum cropping area in Mali have been developed from two complementary elite varieties. QTL analysis and recurrent crossing of progenies monitored with molecular markers are being used to cumulate favorable alleles toward different ideotypes for a range of important traits.

The first population (coded 114) was produced from the cross between Tiandougou and Keninkeni. Tiandougou is a breeding line from IER and a short caudatum-guinea type. It is a dual-purpose variety with high yield and some level of stay-green. Keninkeni is a recent breeding line, short guinea type obtained through a recurrent selection programme involving many progenitors. The second population (coded 118) was obtained from the cross between Tiandougou and Lata3. Lata 3 is a breeding line from ICRISAT. It is a guinea type with intermediate height and tillering. Both populations have been advanced to the F3 generation (400 individuals) for genotyping and QTL analysis. $F_{3:4}$ and $F_{3:5}$ seeds have been produced for family based field evaluation of F3 individuals.

The phenotyping of the population 114 has been installed in three locations and 2 conditions (6 experiments) during the 2010 rainy season (June-November):

- Sotuba: 2 sowing dates (18/06, 13/07)
- Cinzana: 2 sowing dates and soil conditions (25/06, 17/07)
- Farako: 2 sowing dates (25/06, 10/07)

The traits recorded on the different locations are primarily focused on productivity, yield components, flowering, and plant and panicle morphology. In addition, more detailed measurements will be realized on panicle architecture and grain quality in one location.

The evaluation of the second population (118), following a similar protocol is ongoing for the 2011 rainy season, on the same locations.

The QTL analysis of the first population has been completed based on a SSR/SNP genetic map of 200 markers. QTL based indices are being constructed based on this information. The best F4 families are identified based on the best combinations toward several genotypic ideotypes (methodology developed by Syngenta). During the 2011 season, 10 to 20 F4 families will be sown (40 to 50 individuals/family) and genotyped. Based on the genotypic information of individual F4 plants the crosses to be conducted in September will be identified.
The same process will be conducted in 2012 with the second population while a new cycle of crosses between progenies will be conducted for the first population.

In terms of capacity building, two staff members of IER are involved in training activities in connection with the project and will achieve their thesis project as part of the MARS project, with a focus on the genetic control of panicle architecture and on grain quality in sorghum. Concerning infrastructures, the MARS activities have benefited from the GCP CB reinforcement targeted to Challenge Initiatives. In this context, a field area of 4 ha has been rehabilitated at the Sotuba and Cinzana research stations: automatic weather station, fencing, irrigation, and field equipment. A refrigerating unit dedicated to the storage of seeds produced by the project has also been installed at Sotuba.

The integration of genotyping technology and QTL discovery in the breeding program requires significant modification of the breeding process. A tight interaction between breeders, genotyping service, and geneticists in charge of data analysis and crossing schemes development is thus critical to the success of the approach.

51. G7009.04: Development and evaluation of drought-adapted sorghum germplasm for Africa and Australia

January 2009–December 2011

Principal Investigator and Lead Institute

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- Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Bamako, Mali : Michel Vaksmann

This project aims to improve drought adaptation and productivity by combining traits from Australian and Malian sorghums. One such trait that we have extensively studied in Australia is stay-green which enhances grain yield under post-anthesis drought in 3-dwarf sorghums. Preliminary studies have been undertaken to evaluate whether this trait is also effective in taller and photoperiod-sensitive sorghum typically grown in Mali. Isogenic 2-dwarf versus 3-dwarf pair comparisons in a stay-green and senescent background were grown for two seasons (2009 & 2010) at a rain-out shelter facility in north-eastern Australia. Under stressed conditions, the stay-green pair maintained more green leaf area (Fig. 1) and kept growing after flowering, resulting in a yield benefit compared with the senescent pair (Fig. 2). Height did not counteract the benefits of stay-green. On the contrary, under stress, the tall version of the stay-green pair yielded significantly more than the short version (Fig. 2). While this yield advantage was independent of lodging, stem mass during grain filling increased in the stay-green pair and decreased in the senescent pair, highlighting the role of stay-green in lodging resistance. In a further study (2011), crop water use was assessed in two tall genotypes varying in stay-green. Preliminary analysis of this data indicates that the tall stay-green line extracted more water during grain filling compared with the tall senescent line. F2-populations from crosses between Malian and Australian lines have been grown and evaluated with the aim of introgressing the stay-green trait into photoperiod sensitive Malian lines. In addition, a photoperiod-sensitive, tall mapping population is being developed to identify the most important QTL for grain yield and stay-green in a background relevant to sorghum in Mali.

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Borrell AK, Jordan DJ, Mullet J, Henzell RG and Hammer GL (2006). Drought Adaptation in Sorghum. In (ed. J.-M. Ribaut) Drought Adaptation in Cereals. Haworth Press Inc., New York. ISBN-13:978-1-56022-277-4 (Hard Cover) and ISBN-13:978-1-56022-278-1 (Soft Cover).

Illustrations and figures

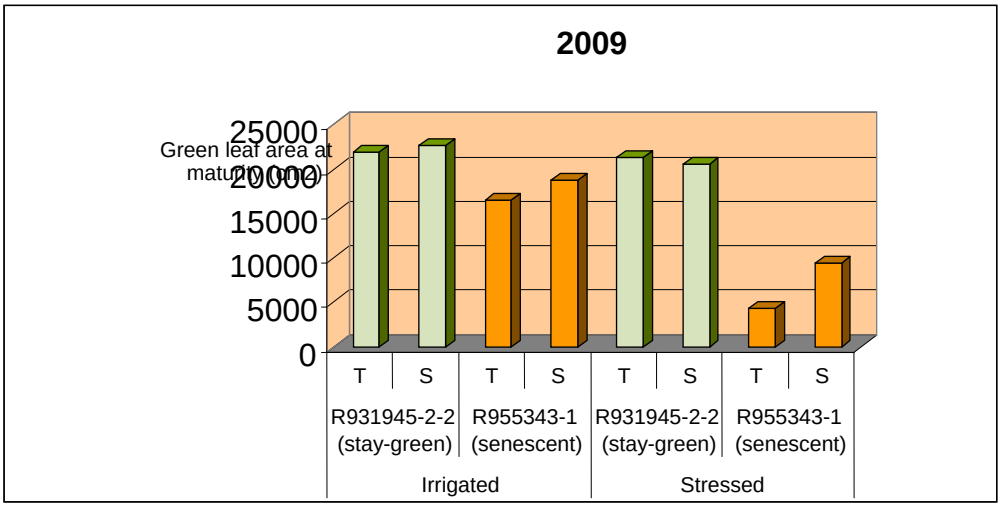

Figure 1. Remaining green leaf area at maturity in the 2009 rain-out shelter study.

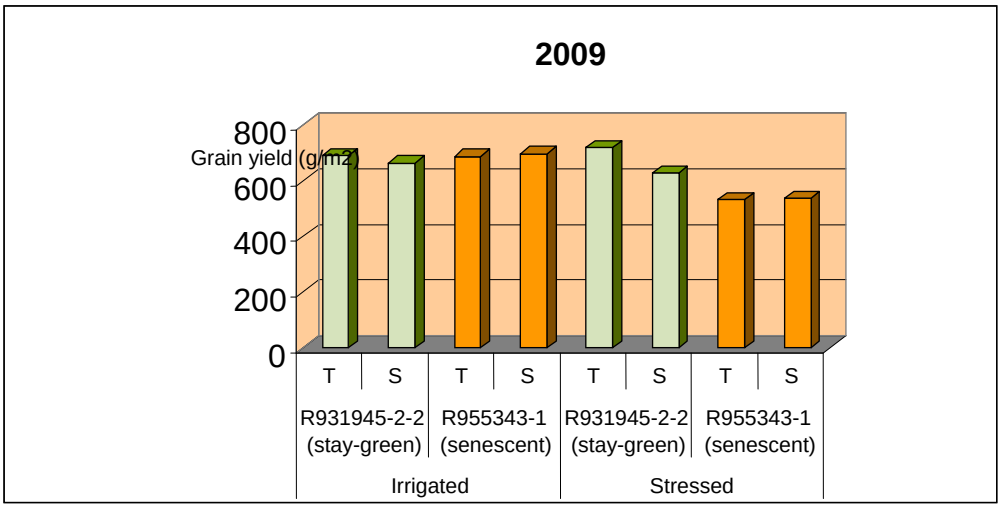

Figure 2. Grain yield in the 2009 rain-out shelter study.

52. G7010.03.03: Establishing a molecular breeding program based on the aluminum tolerance genes, *Alt$_{SB}$*, and the P efficiency QTL, *Pup-1*, for increasing sorghum production in sub-Saharan Africa

April 2010–March 2014

Principal Investigator

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-

Context:

Adaptation to low soil fertility is one main advantage of sorghum in many parts of Africa. Low soil P availability is an inherent problem for most dryland savannah soils in Sub-Saharan Africa, often compounded by Al-toxicity, in higher rainfall areas. Within the ALTSORGHUM project, we conducted association mapping for AltSB and identified SNPs and indels highly associated with Al tolerance

Findings and implication:

1. Markers for AltSB alleles have been identified, and are presently converted to the KASPar system (http://www.kbioscience.co.uk/), used by the GCP genotyping service. An initial set of breeding lines from INRAN's and EMBRAPA's breeding programs has been evaluated with these markers. This needs to be confirmed with the full range of parental lines used to create the breeding populations in each country, using the converted markers.
2. All partnering breeding programs have initiated the development of new breeding populations combining varieties with known sources of AltSB alleles, as well as known adaptation to low phosphorus availability, as observed during field trials in each country. All teams are using the most adapted populations of sorghum available, segregating for the ms3 –gene, as a source of male sterility.
3. In Kenya, the lines segregating for male sterility (ms3) received from Mali were used to generate 25 five crosses with lines contributing drought, Striga and soil acidity tolerance during the first season of planting in 2010, at KARI- Kibos station in Kenya. The parental lines included four P-efficient lines: O2, L6, N140d and C5 (O2XC1); five drought tolerant lines; five aluminium tolerant lines (SC566, SC283, O2XC1, A11XM45 and S58) and eleven striga tolerant lines. Selfing of the F1's was done in the second season. Currently, the selfed lines are being random mated to form a population combines multiple stress tolerances.
4. In Mali and Niger a series of experiments in fields with soil low in available phosphorus have been conducted, and various analytical tools have been used to optimize genotype differentiation under such necessarily heterogeneous conditions. All teams have been trained in using the procedure available in the Genstat software for spatial analysis of trends in experimental fields, to improve the differentiation between genotypes.
5. The Mali team has trained one student on the implementation of the hydroponic screening method for quantifying AL-tolerance in sorghum. The team is presently acquiring the materials to set up the system in Mali.

Next steps/challenges:

1. Finalizing two generations of recombination of inter-mated sorghum parental lines in isolated fields for building the base populations is not guaranteed in all places. ICRISAT is supporting INRAN in this effort.
2. Field testing for adaptation to low P conditions requires more than one year of evaluation. Thus the first results of the project may only be indicative.
3. Partners require training to be able to use the genotypic data appropriately for breeding.

53. G7010.03.06: Improving phosphorus efficiency in sorghum by the identification and validation of sorghum homologs for *Pup1*, a major QTL underlying phosphorus uptake in rice (SorghumPup1)

April 2010–March 2014

Principal Investigator and Lead Institute

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- Boyce Thompson Institute – Cornell University (US): Zhangjun Fei
- Institute for Genomic Diversity – Cornell University (US): Steve Kresovich, Martha Hamblin, Sharon Mitchell, Theresa Fulton
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- Moi University (Kenya): Sam Gudu
- ICRISAT – Bamako (Mali): Eva Weltzien, Fred Rattunde and Willmar Leiser
- JIRCAS (Japan): Matthias Wissuwa
- IRRI (The Philippines): Sigrid Heuer

1. Rationale for the SorghumPup1 project

Low productivity due to soil constraints and a lack of properly adapted crop cultivars 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 efficiency and has the potential to increase P acquisition efficiency in other cereals. JIRCAS and IRRI fine mapped the *Pup1* locus to a ~150 Kb region on chr 12, and 2-4 high quality *Pup1* candidate genes have been identified. We are establishing a framework based on comparative genomics to identify sorghum *Pup1* homologs and validate their role as *bona fide* genes underlying tolerance to P deficiency. This research is based primarily on association analysis to identify statistically significant associations between allelic variation at *Pup1*candidate genes and P efficiency assessed both in the field and under controlled conditions in the laboratory and greenhouse. The Al tolerance gene, Alt_{SB}, is an Al-induced root citrate efflux transporter and citrate can mobilize P that is fixed in the

soil clay fraction and increase its availability for root P uptake. Therefore, we will use the same approach to study a possible synergistic role of Alt_{SB} in increasing P uptake into sorghum roots. Findings of the SorghumPup1 project will be deployed into a molecular breeding platform within the SorghumMB project. Thus, this project sets the foundation for a molecular breeding program targeting marginal soil areas in southern Mali, Niger and Kenya and other areas of Sub-Saharan Africa to improve food security and farmer's income.

2. Research activities and partial results

2.1 Bioinformatic search for candidate Pup1 genes in sorghum

In our 2010 annual report we showed results for a bioinformatic and phylogenetic analysis for *Pup1* homologs in sorghum, which comprise a large gene family. We designed primers for SNP discovery within candidate genes using Primer-blast (http://blast.ncbi.nlm.nih.gov/) so that selected family members can be individualized. Primer specificity was also checked manually by inspection of candidate gene sequence alignments. Table 1 and Figure 1 summarize our approach.

The following activities are being undertaken:

- SNP discovery within candidate genes.
- Phenotyping of the US association in the field for P efficiency. The 2010 experiment is completed and the 2011 experiment is underway.
- Phenotyping of a subset of the US association panel in the field for P efficiency in Japan (JIRCAS).
- Phenotyping of the US association for root traits under low P (2D). The entire panel has been phenotyped and the data is being analyzed.

Our next immediate steps are:

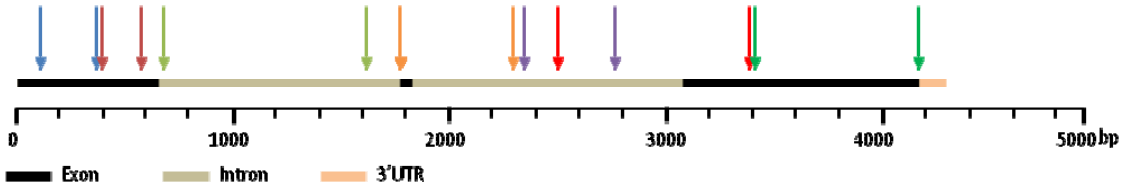
- Expression analysis of *Pup1* candidates in roots and shoots of 6 sorghum lines subjected to control conditions and P deficiency.
- Phenotyping the US association panel for root traits (3D) and in pots in the greenhouse for P efficiency.
- Obtain genotypic data for SNPs and indels within *Pup1* candidate genes across the association panel.
- Association analysis with traits related to P efficiency and root traits.

3. Figures and tables

Table 1: *Pup1* homolologs in sorghum that have been selected for SNP discovery. The DNA and protein sequences for rice *Pup1* were used to search the sorghum genome (http://www.phytozome.net/sorghum) for homologs.

Hit	Location	Size (bp)	BLASTN		BLASTP		# Primer Pairs
			E-value	Identity	E-value	Identity	
Sb07g002840	chr 7: 3011700 - 3016004	4304	0.0	76%	6.00E-143	73%	7
Sb03g031670	chr 3: 60080358 - 60084458	4100	2.00E-123	71%	1.00E-129	70%	4
Sb03g031680	chr 3: 60085127 - 60088181	3054	3.00E-121	71%	2.00E-130	70%	4
Sb07g000727	chr 7: 572957 - 579734	6777	1.00E-107	76%	8.00E-76	74%	8
Sb03g031700	chr 3: 60110148 - 60113362	3214	9.00E-78	68%	1.00E-115	63%	3
Sb03g031690	chr 3: 60103142 - 60107812	4670	7.00E-73	73%	6.00E-76	69%	6
Sb03g006765	chr 3: 7009497 – 7012497	3001	2.00E-37	83%	2.00E-108	55%	4

Figure 1: Positions for the primers designed for the best *Pup1* hit in sorghum (Sb07g002840) using Primer-blast (http://blast.ncbi.nlm.nih.gov/). Pairs of arrows with the same color denote primer pairs.



54. G7010.05.01: Enhancing sorghum grain yield and quality for the Sudano-Sahelian zone of West Africa using the backcross nested association mapping (BCNAM) approach

July 2010–June 2014

Principal Investigator and Lead Institute
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Collaborating Institutes and Scientists

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- **CIRAD:** Centre de Coopération Internationale en Recherche Agronomique pour le Développement: Jean François Rami, Michel Vaksmann (IER /CIRAD).
- **ICRISAT:** International Crop Research Institute for the Semi-Arid Tropics: E. Weltzein, Fred Rattunde, Ibrahima Sissoko.

Sorghum improvement in Africa goes through a wide range of harsh and highly variable environments. Local landraces have developed specific adaptation to the biotic and abiotic environments with excellent grain qualities and poor yield potential. To improve the yield potential while maintaining key important traits of the local varieties, the BCNAM approach was initiated to broaden the genetic base of three locally adapted elite recurrent parents, Lata, Grinkan and Keninkeni to develop BC1F4 populations that undergo genotypic and phenotypic trait evaluations. Each recurrent parent was crossed to 10 specific donor parents (SDP) and 10 common donor parents (CDP). The expected number of populations is twenty for ICRISAT and 40 for IER with at least 100 individuals per population.

These populations will be derived from a modified backcross populations that will be of long-term value in relating sorghum traits to their corresponding genes. The planned population structure will facilitate the QTL mapping of range of traits conditioning productivity, adaptation, and preferred grain quality traits.

This study will result in a set of connected progenies expected to have highest value in terms of breeding and of mapping genome regions bearing favourable alleles in the right genomic background. The genotypic and phenotypic evaluation of each population will be done to correlate favourable allele contributions to the phenotypic effects.

Parental lines showed among them genetic variation, thus leading us to the type of population to develop. Genetic sterile lines of the three recurrent parents are available for further use.

Parental lines were evaluated across two latitudes at IER in 2010. Early results show that lines are earlier in lower (Farako) than in higher latitudes (Bamako).
Variations in key traits were observed among parental lines on sugar content, juicy level, midrib color, time to flag leaf appearance, coefficient of sensitivity to day length, thousand seed weight, numbers of seeds per panicle and plant height. These yield components will be confirmed in 2011. Fifty four populations with an average of 100 BC1F4 lines per population are being developed of which 20 are from ICRISAT and 34 from IER. These populations are currently being advanced at BC1F2 level in the current cropping season. Precautions were taken at the BC1F2 level for maturity and plant height in respect to the recurrent parent. BC1F3 lines will be produced in 2011 offseason. Genotypic data will be collected from BC1F3 while phenotypic data will come from BC1F4 lines across three different environments in Mali in 2012.

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All people who have contributed to the current results.

Wheat

55. G3008.01: New wheat germplasm generated with broadened AB genome diversity

November 2008–October 2011

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Main achievements:

1. Research activities and progresses at CIMMYT, Mexico:
An initial collection of global dicoccum diversity was assembled including accessions originating from 38 countries, provided by CIMMYT and ICARDA genebanks. Molecular diversity analysis was performed on this collection using a set of 35 highly polymorphic SSRs. Previous reports have indicated how the molecular diversity groupings largely reflect geographic origin. A subset of 108 emmer wheat accessions was selected from the initial global collection of 308 accessions based on agronomic type, taxonomic characterization and diversity of geographic origin. The selected accessions captured 376 (80%) of the 470 total of alleles thus representing most of the diversity of the original set. A crossing program was undertaken to produce synthetic hexaploids wheats by crossing the subset of 108 dicoccums with three different *Ae. tauschii* accessions. So far in total 115 dicoccum synthetics are produced. Molecular diversity analysis indicates that the subset of 108 reasonably well covers the diversity of full set of 308. Seed of all new synthetics is now being arranged for distribution to project collaborators or further multiplied where in insufficient seed quantities are currently available. About fifty seven direct crosses were also made between *T. dicoccum* accessions and durum or bread wheat. Seed of crosses will be shared between project collaborators for its use in wheat improvement programme.

2. Research activities and progresses at Plant Breeding Institute, University of Sydney:

At PBI, University of Sydney, crosses have been made between emmer wheat and hexaploid bread wheats. Seeds were obtained from almost 50 crossing combinations. The hexaploids used for crosses were the durum based synthetic (Sokoll), the emmer based synthetic (*T. dicoccon* P194625/*Ae. tauschii* 372), Indian cultivars (PBW502, PBW550, DBW16, DBW17) and the drought tolerant CIMMYT lines Berkut and Waxwing*2/Kiritati. Backcrosses have been made to hexaploid wheat and double haploids are under development using the derived BC_1F_1. The F_2 is being increased for further assessment.

Bread wheat/dicoccon F_1 seeds from 53 successful combinations were obtained in CIMMYT and grown in Ciudad Obregon for selection and further backcrossing.

3. Research activities and progress at Agharkar Research Institute, Pune, India.
At Agharkar Research Institute, primary durum based and emmer based synthetic wheats were inter crossed in the research farm situated at Hol. One hundred seventy

six lines are developed out of around 30 cross combinations. They are at F_5 generation planted at IARI, Wellington, for generation advancement.

One hundred and seventy six families in F_4 generation generated from inter crossing between durum and dicoccum based synthetics were crossed with elite Indian bread wheat varieties *viz.* MACS-6222, NIAW-302 and GW-322. In all 257 cross combinations were made during the peak flowering season. Seventy crosses having more number of seeds lines are being grown at Wellington during off season. Rest of the cross seeds will be grown in normal season for generation advancement.

Continuing the molecular diversity analysis of Indian Dicoccum germplasm, in addition to the 33 polymorphic SSR markers studied earlier some more SSR markers were screened so as to have minimum two polymorphic markers per chromosome. Out of 27 additional SSR markers screened, 15 were polymorphic. A total of 204 alleles were detected at 53 SSR loci with the average polymorphic information content (PIC) of 0.35 per locus and mean Rp value of 1.

4. University of Agricultural Sciences, Dharawad

At the University of Agricultural Sciences Dharwad, 102 lines (which include durum based synthetics and emmer based synthetics with parents) were received from CIMMYT and ARI. To favour recombination within the AB genome, durum and emmer based synthetics were crossed to diverse emmer wheat accessions. Thirty F_1's derived from the crossing synthetics were backcrossed again with dicoccum parents. In 2010-11, again crossing of genetically diverse durum and dicoccum based SHW were initiated. Seeds from 66 cross combinations were harvested. In diallele crossing programme, 20 crosses were attempted and seed setting was observed in 13 crosses.

Two hundred twenty two hybrids generated (95 F_1's, 53F_2's, 21 F_1BC_1's and 53 F_3's) will be characterized for physiological traits and a screening of these lines against major foliar diseases. Few new crosses involving promising germplasm lines for disease and agronomic performance will be performed.

Next steps and challenges

1. Seed of direct crosses and synthetics generated at CIMMYT will be shared between project collaborators.
2. Synthetic Back cross line generation by crossing drought tolerant bread wheat varieties with newly prepared Synthetic Hybrid Wheats.

56. G4008.03: Precision phenotyping of the GCP spring wheat reference sample for drought

January 2006–December 2010; NCE: March 2011

Principal Investigator

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- CIMMYT-IRAN, DARI: J. Kamali,
- SPII, Iran: AR. Nikzad, S. Tahmasbi, S. Sarikhani

1. GCP wheat reference samples, seed multiplication and purification

The GCP wheat references samples (spring, winter and durum wheat) were seed multiplied during 2008 and 2010 in Mexico. In this project, emphasis was given to the spring wheat reference sample; accessions were also purified, seed from single spikes was collected, multiplied twice and has been stored to be available on request from the CIMMYT germplasm bank. Excessively early and late heading accessions were excluded for the formation of the GCP association mapping panel including 193 accessions.

2. Phenotyping for drought-adaptive traits in multi-location trials

Four field trials were carried out in the 2008/2009 growing season at the CENEB experimental station in Cd. Obregon, Mexico, at the Sidi El Aidi experimental, 50 km south of Casablanca, Morocco and at the Hasan Abad field station, Darab, Iran. At Cd. Obregon two trials differing in planting date were conducted. The following traits were measured: Early ground cover (EGC), ear emergence (EARE), days to anthesis (ANTH), days to maturity (MAT), plant height (PH), waxy leaves (WAX), leaf pubescence (LPUB) and rolling (LROL), canopy temperature at the vegetative (CTv) and grainfill (CTg) stage, stem carbohydrates (CHO), chlorophyll content at anthesis (CHLanth), normalized difference vegetative index at the vegetative (NDVIv) and grainfill stage (NDVIg), grain number (GM^{-2}), percent grain filling (%GF), thousand kernel weight (TGW), and grain yield (GY). For each environment and across environments (Table 1), phenotypic data were analyzed by restricted maximum likelihood (REML) to fit a mixed model. MAT was used as covariate to adjust for differences in phenology.

3. Genotyping with DArT and STS markers for *Vrn, Ppd* and *Rht* genes

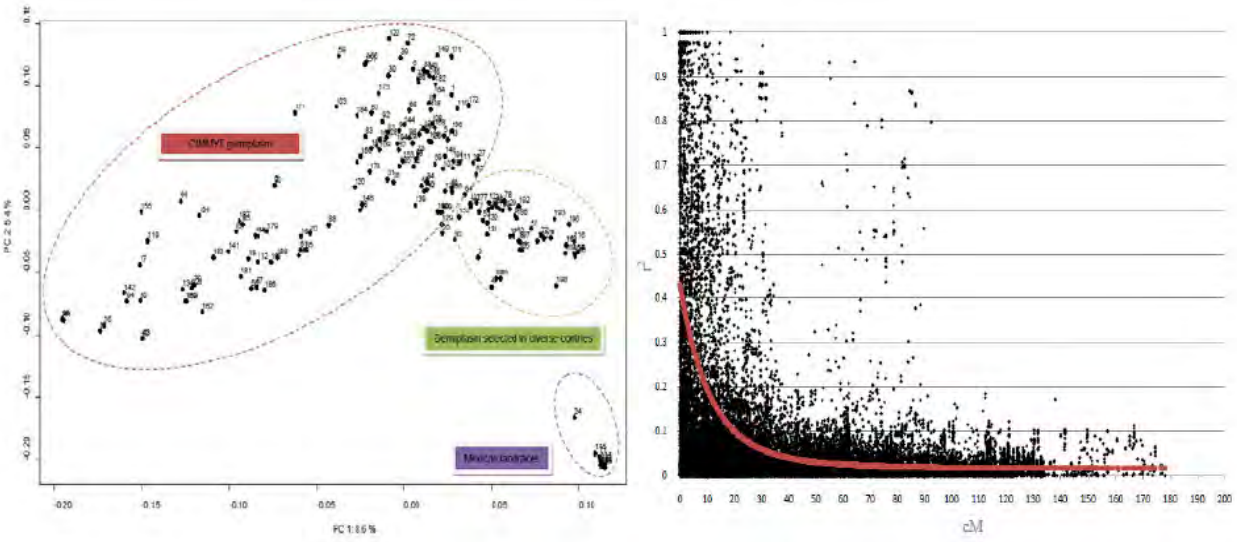
Whole genome marker polymorphism was generated via DArT profiling by Triticarte Pty. Ltd. (Canberra, Australia; http://www.Triticarte.com.au). A total of 2855 DArT markers were polymorphic,1905 DArT marker were located on a integrated map provided by Triticarte Pty. Ltd. Accessions were also genotyped with markers linked to *Vrn, Ppd* and *Rht* genes. The genetic structure of the panel was investigated with principal component analysis (PCA) according to Price (2006) using the software package R (www.r-project.org). Highly correlated DArT markers (R^2> 0.99) and

Table 1. Anova results. Number of location, genetic variance, least square difference, mean, coefficient of variation and heritability. Not all traits are represented.

	MAT	PH	CHLanth	CTv	CTg	GM^{-2}	TKW
No of Loc	4	4	3	3	4	2	4
σ^2 Entry	4.23	84.44	2.53	0.11	0.07	984166.46	8.67
σ^2 Loc x Entry	6.07	21.82	2.54	0.03	0.19	522510.92	2.97
GMEAN	118.41	81.06	47.01	23.78	29.13	6372.30	30.47
LSD	3.89	9.07	3.88	1.09	1.07	1867.78	3.33
CV	1.68	5.70	4.21	2.33	1.87	14.91	5.56
Heritability	0.69	0.89	0.57	0.44	0.32	0.68	0.86

markers with very low frequency (<5%) were excluded. The first 10 **e**igenvectors of the PCA were subsequently used as *Q* population structure coefficients for association analyses. Pairwise linkage disequilibrium (LD) was calculated by means of the square of the correlation coefficient of allele pairs between two loci (r^2) according to Weir (1996). Only mapped DArT with a higher than 5% frequency were considered. Significance *p* values of LD for marker pairs was determined with the χ^2 Test. Via PCA three main germplasm groups were defined. Mexican landraces in the panel clustered most separated followed by germplasm released in various countries such as Australia, China or India (Fig. 1). CIMMYT germplasm represented a large diverse germplasm group. LD decreased with increasing genetic distance, an indication of LD maintained by genetic linkage. The LD decline was similar (~20 cM) than in previous whole genome studies in wheat estimating a LD decline within a average distance of 10 to 30 cM (Crossa et al. 2007, Dreisigacker et a. 2011).

Fig 1. First two principle component analyses and LD plot



4. Marker trait associations

Association analyses were carried out in Tassel 3.0 software (http://maizegenetics.net). The significance of marker phenotype associations was tested with (i) the fixed general linear model (GLM) including the *Q* population structure coefficients as covariates and (ii) the mixed linear model (MLM) including the Q population structure coefficient and the 191 x 191 kinship matrix. Each environment was first analyzed separately and then environments were combined. Marker trait associations were considered reliable when the significance was detected with the two associations tests mentioned above. Thresholds of *P* 0.005 and 0.001 were considered. Association mapping revealed the influence of several chromosome regions on the variability of adaptive, physiological and yield component traits under drought stress. A number of the regions have already been described in bi-parental populations (e.g., in Pinto et al. 2010, Diab et al. 2008). The project contributed to access their relevance and has highlighted additional novel QTL. The GCP panel provided additional valuable information as relatively few QTL for physiological and drought adapted traits have been identified to date (Rebetzke et al. 2008; Reynolds and Tuberosa 2008), fewer still have been utilized in breeding.

Table 2. Location of significant DArT markers associated with phenotypic values across environments. Not all traits are represented.

Chr	MAT	PH	CHLanth	CTg	CTv	GM^{-2}	TKW
1A							
1B		wPt.7242 (29.6) wPt.1818 (66.4)			wPt.5801 (23.1)	wPt.7359 (11.3)	
2B	tPt.1663 (6.2)	wPt.4527 (33.8) RHT1		wPt.1964 (29.3)	wPt.742929 (126.9)		
2D	Ppd-D1				Ppd-D1		
3A			wPt.741190 (38.8)				
3D							wPt.741032 (33.8)
4A		wPt.1091 (59.6)		wPt.6502 (89.9)			
4B							wPt.1400 (38.1)
5A		wPt.798459 (48.4) wPt.798702 (48.4)					wPt.6462 (124.9)
5B		wPt.2305 (24.4)			wPt.9782 (130.3)		
5D	Vrn-D1						
6A						wPt.3965 (23.6)	
6B					wPt.4867 (12.7) wPt.2424 (58.1) wPt.6208 (66.0)		
7B		wPt.4120 (220.0)					
-		wPt.8866		wPt.6748 wPt.743218		tPt.8109 wPt.0489 wPt.664378 wPt.8462	wPt.0530

References

Crossa J, Burgueno J, Dreisigacker S, et al. 2007. Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177, 1889–1913.

Dreisigacker S, Shewayrga H, Crossa J, et al. 2011. Genetic structures of the CIMMYT international yield trial targeted to irrigated environments. Mol Breeding, DOI: 10.1007/s11032-011-9569-7.

Price AL, Patterson NJ, Plenge RM et al. 2006. Principal components analysis corrects for stratification in genome-wide association studies. Nature Genet. 38, 904–909.

Pinto RS, Reynolds MP, Mathews KL, et al. 2010. Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theor Appl Genet 121, 1001–1021.

Diab AA, Kantety RV, Ozturk NZ, et al. 2008. Drought-inducible genes and differentially expressed sequence tags associated with components of drought tolerance in durum wheat. Sci Res Essay 3:009–026.

Reynolds M, Tuberosa R. 2008. Translational research impacting on crop productivity in drought-prone environments. Curr Opin Plant Biol 11:171–179.

57. G3008.08: Breeder-friendly high-throughput phenotypic tools to select for adaptive traits in drought environments

November 2008–October 2011; NCE: June 2013

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED

58. G7009.01: Examining natural variation in the transcriptional regulation of drought responses in wheat

January 2009–December 2011

Principal Investigator and Lead Institute
Peter Langridge, ACPFG; peter.langridge@acpfg.com.au

NO UPDATE SUBMITTED

59. G7010.02.01: Wheat breeding and selection strategies to combine and validate QTLs for WUE and heat tolerance in China

April 2010–March 2014

Principal Investigator and Leading Institute
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Collaborating institutes and scientists

- Institute of Crop Science, CAAS: Zhonghu He, Xinmin Chen, Xinguo Mao, Ang Li, Xiaoping Chang
- Institute of Dry Farming, HAAS: Xiumin Chen, Kejiang Li, Wenchen Qiao
- Institute of Crop Science, SAAS: Meirong Sun, Xiurong Li, Yongfeng Chai, Junling Zhang
- Institute of Nuclear and Biological Technologies, XAAS: Zhenlu Wu, Zheru Fan, Yueqiang Zhang, Jianfeng Li
- CIMMYT, EL Batan, Mexico: Matthew Reynolds
- Plant Breeding Institute, University of Sydney, Australia: Richard Trethowan

Good phenotypic data underpins genotyping and much of the breeding process. This project aims to assemble and integrate into breeding programs genetic diversity for WUE (water use efficiency) and heat tolerance from key sets of genetic resources. We propose combining existing QTLs for performance under moisture stress in elite Chinese backgrounds using a combination of empirical selection for yield, marker selection for genomic regions and selection for relevant physiological traits. The

expectation is that the frequency of favorable alleles for tolerance to moisture stress will be improved.

In 2010, the first year for our project, we preliminarily observed the international core set in the fields at six sites, selected 12 Chinese elite cultivars as the cross parents, made a series of crosses using Chinese elite cultivars and international core set as parents, identified and validated some QTL markers, and their favorable alleles related to drought or heat tolerance in Chinese wheat populations. The scientists and graduate students involved in the project were trained in phenotyping and wheat CI data management for gene discovery and physiological breeding, respectively. Based on the work in the previous year, we carried forward the activities.

(1) Selecting a core set of WPHYSGP

The morphological and physiological traits, adaptability to the local environments and the grain yield of 145 CIMMYT nursery WPHYSGP were evaluated in the growing season of 2010-2011 at multi-location, including three facultative wheat sites (Yuncheng and Linfen of Shanxi, Hengshui of Hebei), three winter wheat sites (Beijing, Changzhi in Shanxi, and Guyuan in Hebei) and a site in spring wheat region (Urumqi of Xingjiang). Among them, two winter wheat sites, Changzhi and Guyuan, a facultative wheat site Linfen were supplementary. The accessions were sowed in the spring and will be harvested in the coming August in Urumqi and Guyuan. According to the phenotypes in this season and consulting the results last year, 25 elite accessions were selected from WPHYSGP (Table 1), which will be further evaluated and used in the project activities as the prior plant materials.

Table 1 Elite accessions selected from WPHYSGP

No.	Plot	No.	Plot	No.	Plot
1	9607	10	9637	19	9681
2	9625	11	9643	20	9696
3	9628	12	9646	21	9698
4	9692	13	9649	22	9700
5	9738	14	9655	23	9725
6	9746	15	9656	24	9728
7	9611	16	9658	25	9742
8	9614	17	9662		
9	9633	18	9669		

(2) Crosses using the WPHYSGP and/or local cultivars as parents

A total of 9 populations were selected from more than 40 crosses derived from Chinese cultivars (Table 2), 32 populations of BC_1F_1 from 424 crosses made between the WPHYSGP and local cultivars based on the agronomic traits and adaptability to the environments. These populations will be regenerated and/or evaluated in the fields. More than 800 crosses were made between the WPHYSGP and local cultivars in this growing season. Among them, about 300 crosses are BC_1F_1. These populations will be identified for their agronomic traits and environment adaptation in the field in the next growing season.

Table 2 Populations derived from crosses of WPHYSGP and/or local cultivars

No.	Cross	Generation	Population
1	Jingdong 8 × Aikang 58	$F_{2:3}$	260 line
2	(Chang 4738 × Linhan 7061) × Chang 4738	BC_2F_1	> 400 grain
3	Yannong 19 × Yunhan 618	F_5	395 plant
4	Yannong 19 × Yunhan 719	F_3	373 plant
5	Hengguan 35 × Jifeng 3703	F_2	320 plant
6	Hengguan 35 × Shi 03-5285	F_2	286 plant
7	Zhonghan 110 × Chang 4738	F_2	240 plant
8	Xinchun 6 × Xinchun 14 (i.e. ATTILA)	F_5	~ 600 plant
9	Xinchun 6 ×20e-20	F_3	> 300 plant

(3) QTL markers confirmation and development

Four backcross populations, [(Lumai 14 × Jinmai 47) × Lumai 14] and [(Lumai 14 × Chang 6878) × Lumai 14] in the generation of BC_3F_5, [(Lumai 14 × Xifeng 20) × Lumai 14] and [(Jinmai 47 × Xifeng 20) × Jinmai 47] in BC_3F_4, have been phenotyped under both drought stress and well-watered conditions. The genetic effects of introgressed genes/QTLs are being detecting by molecular markers. QTLs for drought and heat tolerant traits, such as early vigor, canopy temperature, stay-green, chlorophyll fluorescence and associated traits, plant height, seed setting, grain yield and thousand grain weight have been developed or validated in genetic populations and/or natural populations.

The particular challenge for us is the spring character of WPHYSGP, which greatly limited their utilization in the winter wheat regions in China. Therefore, the most crosses were made only based on the flowering meet in the first two years. The core set of WPHYSGP selected are the accessions with cold tolerance, good morphology and similar flowering time with the local cultivars. We will use them as the prior parent materials for making crosses in the coming season.

60. Molecular breeding and selection strategies to combine and validate QTLs for improving WUE and heat tolerance in India

July 2010–June 2014

Principal Investigator
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Head, Division of Genetics, Indian Agricultural Research Institute; New Delhi, India. Tel: (91)11-25841481; Fax: (91) 11-25841481

Collaborating institutes and scientists

- National Research Centre for Plant Biotechnology, New Delhi, India: NK Singh
- Punjab Agricultural University, Ludhiana, India: Praveen Chuneja
- Agharkar Research Institute, Pune, India: SC Mishra
- JNKVV, Regional Station, Powarkheda, India: PC Mishra
- CIMMYT, Mexico. M. Reynolds

2.1.1 Phenotyping of the Wheat Physiological Germplasm Screening Nursery (WPHYSGP) at four locations

IWPHYSGP Trial comprising 145 entries was evaluated and phenotyping was carried out. Data on germination, Days to heading, Yield, Thousand Kernel Weight, Days to Mature, Canopy temperature, Canopy temperature Depression, NDVI Index and SPAD was taken. Descriptive statistics of the entries are given as below:

AGROBASE: D E S C R I P T I V E S T A T I S T I C S

VARIABLE	AVERAGE	MINIMUM	MAXIMUM	HERITABILITY
GERM	88.664	40	95	0.59
DH	66.154	50	84	0.94
YLD	179.243	66	373	0.64
TKW	31.404	20	44	0.76
DM	102.822	80	125	0.81
CT	28.259	24.6	31.2	-
CTD	4.373	1.8	8.1	0.28
NDVI	0.747	0.58	0.88	0.33
SPAD	53.588	43.7	62.3	0.51

Significant correlations were observed between days to heading and days to maturity, yield and days to heading (negative), thousand kernel weight with days to heading, days to maturity and yield, canopy temperature with days to maturity and yield (negative) and canopy temperature depression with days heading and yield and negative correlation with canopy temperature.

2.1.2 **Phenotyping of the populations derived from selected F_2s:** During offseason 2010-11 single plant selection of F_2 derived F_3 progenies from each of the twenty five crosses between four centres were planted and phenotyped for various drought tolerance related traits. Data were recorded for *grain yield, chlorophyll content, canopy temperature depression, early ground cover, NDVI, relative water content* and other phenological characters

These progenies have been harvested as bulk. DNA from the parental lines are being analysed for parental polymorphism survey with microsatellite markers.

2.1.3 **ICIS software implementation on GCP material: G**enealogical data of six crosses uploaded into ICIS up to F_4 generation. International core set from CIMMYT has also been uploaded along with local crossing block and other segregating populations.

2.1.4 **Recruitment of contractual staff in the project**: Three SRF's have been recruited at Delhi centre. At other centres, the process of the recruitment of the contractual staff is in progress.

2.1.5 **Introgression of known QTLs in elite Indian lines**
With an aim to mobilize drought tolerance or the QTLs into elite Indian backgrounds through marker assisted backcross breeding selected donor lines such as Excalibur, Krichauff and Babax were crossed to HD2967 and PBW343+*Lr24-Lr28-Yr10-Yr1; Donors* HD 2987, HI 1500, C 306, HD 2781 were crossed to recurrent parents GW 322 and HD 2733 in two locations.

2.1.6 **Human Resource Development Programme: Training of Indian Scientists in physiological trait based Phenotyping protocols:** Four Indian scientists

namely Dr. GP Singh (IARI), Dr. G S Mavi (PAU), Dr P C Mishra (Powarkheda) and Dr. SC Mishra (ARI, Pune) were trained with Dr Matthew Reynolds during May (GPS) and November 2009 (others).

2.1.7 **Training on ICIS at IARI, New Delhi from September 13-16, 2010:** Training was jointly organized by Indian Agricultural Research Institute, New Delhi and Generation Challenge Programme from September 13-16, 2010. 36 participants from India and GCP participated in the training. This training was directed towards management of pedigrees, phenotypic data and both background and foreground genotype a local standalone ICIS will be established at each breeding node.

2.2 Key Products Developed by the Project:

1. 15-20 wheat lines adapted to Indian conditions with superior drought/heat tolerance developed using molecular markers.
2. 3-5 traits and 5-8 QTLs recommended for wheat breeding in India.
3. Four mega varieties with improved WUE and Heat tolerance which have the potential of covering about 24 million hectares and minimize the loss due to heat or drought or both to an extent which goes up to 20-50% productivity/day

Cross-crop projects

61. G3008.02: Improving grain yield on acid soils by the identification of genetic factors underlying drought and aluminium tolerance in maize and sorghum

November 2008–October 2011

Principal Investigator
Leon Kochian, USDA-ARS Robert Holley Center for Agriculture and Health, Cornell University, Ithaca, NY 14853

Collaborating institutes and scientists

- Embrapa Maize and Sorghum, Sete Lagoas, Brazil: Jurandir Magalhães; Claudia Guimaraes; Robert Schaffert; Sidney Parentoni; Vera Alves; Maria José Vasconcelos
- USDA-ARS/Cornell**:** Lyza Maron; Miguel Pineros; Jiping Liu; Randy Clark; Ed Buckler; Jon Shaff
- Moi University/KARI, Eldoret, Kenya: Sam Gudu
- Institute for Genomic Diversity, Cornell University: Stephen Kresovich; Sharon Mitchell; Martha Hamblin

1. Rationale for project

This project builds upon our progress on the cloning of the major aluminum tolerance gene in sorghum, the identification of several major maize Al tolerance QTL, and the identification of candidate genes for the two largest QTL. Hence we are poised to significantly advance our program on improving the acid soil tolerance of maize and sorghum. We have also expanded our program to begin to investigate the molecular determinants for root-related aspects of drought tolerance, in order to improve agronomic performance of crops whose root systems are severely restricted by Al toxicity and hence are more susceptible to yield reductions from drought stress on acid soils.

2. Research activities and results

2.1 Findings. Genetic resources (mapping panels) for both maize and sorghum are being phenotyped for Al tolerance (nutrient solution) and drought tolerance (field). In the previous year, only “per se” seeds from the maize inbred line panel were available. A group of 188 inbred lines were evaluated “per se” under drought and irrigated conditions at the Janaúba screening site. A 70% reduction in mean grain yield was observed under drought stress compared to irrigated conditions for these maize inbreds. Broad sense heritability for grain yield was 0.50 and 0.54 under irrigated and drought conditions, respectively. Four maize inbred lines with high levels of drought tolerance were identified. Also, 199 maize inbred lines from the panel were crossed with two testers (inbreds L3 and 228-3). These single cross hybrids were evaluated at Janaúba and Teresina screening sites in 2010. Data for stay green (1 to 5 scale) and chlorophyll meter scores from Janaúba drought trials indicated that L3 crosses tend to be greener under drought stress than L 228-3 crosses. A mild correlation was observed between measures of stay green and chlorophyll scores in both groups. Also, the 225 sorghum line IGD association panel has been

phenotyped for drought tolerance in the screening sites at Janaúba and Teresina in 2010 – 2011 and the data is now being analysed.

Aluminum tolerance has been determined in 79 maize inbred lines from our association panel. It was possible to identify new lines that are as Al tolerant as the standard for maize Al tolerance (Cateto). Genotytping of the maize panel with SNPs markers is expected to be concluded by September, 2011. Maize near isogenic lines have been generated to validate Al tolerance QTLs that co-locate with orthologs of the sorghum Al tolerance gene, *SbMATE*, which is responsible for the Alt_{SB} locus. Results indicate that the transference of the maize QTL on chromosome 6 from Cateto to the susceptible line L53 significantly increased Al tolerance in the NILs. Additionally, we have verified that *ZmMATE1* is the candidate gene responsible for the major Al tolerance QTL on chr 6QTL. Results and genotypes identified in this project have been shared with partners in Kenya to begin assessing the interplay between drought and Al tolerance in Africa.

2.2 Next research steps.

- We are finishing the second year drought field trials for maize and sorghum;
- We are working now on the analysis of 2D and 3D root system architecture in sorghum and still need to move onto maize;
- We are just finishing RNA sequencing in sorghum using next generation sequencing and are beginning to computationally analyse the data;
- In collaboration with Ed Buckler's lab, we are doing genotyping by resequencing of the sorghum association panel and when this is finished, will complete genome-wide analysis of sorghum Al tolerance and drought tolerance;
- We are finishing genotyping by resequencing of Embrapa's maize RIL mapping panel, which will greatly improve the resolution of the genetic map for this population.

3. Figures

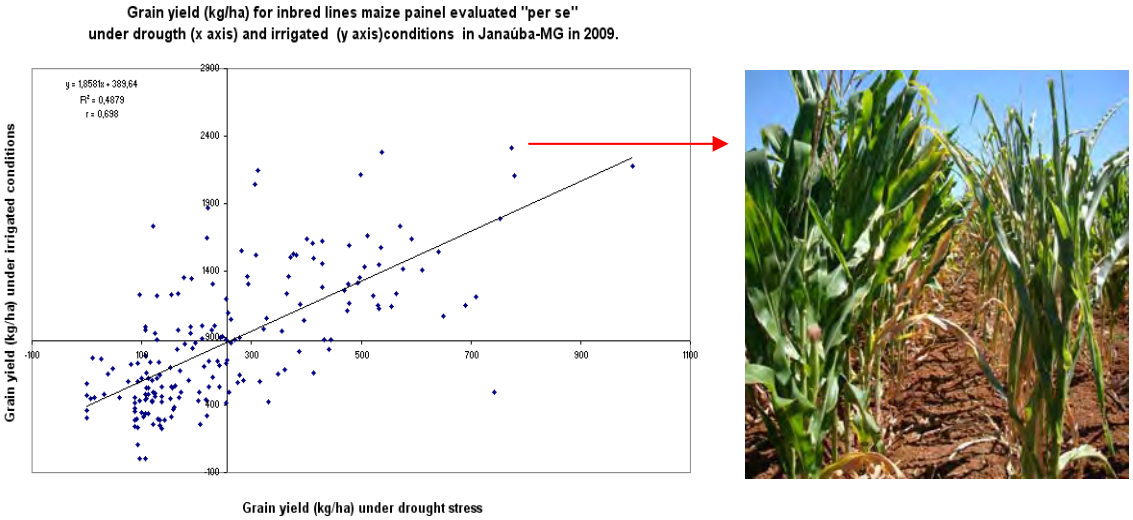

Figure 1. Grain yield under drought stress (x axis) and under full irrigated condition (y axis) for 188 maize inbreds from EMBRAPA´s panel evaluated in Janaúba-MG, 2009. Red arrow links position of a drought tolerant inbred line in the graphic with its picture in the drought stress trial (right side)

4. Publications

Maron LG, Piñeros MA, Guimarães CT, Magalhaes JV, Pleiman JF, Mao C, Shaff JE, Belicuas SNJ, Kochian LV. 2010. Two functionally distinct members of the MATE (multidrug and toxic compound extrusion) family of transporters potentially underlie two major Al tolerance QTL in maize. Plant Journal 61: 728-740.

Krill AM, Kirst M, Kochian LV, Buckler ES, Hoekenga OA. 2010. Association and linkage analysis of aluminum tolerance genes in maize. PLoS One 5: 1-11.

Other crops

Millet

62. G4008.07: Improving molecular tools for pearl millet

January 2008–December 2009

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED; PROJECT UNDER REVIEW

Potatoes

63. G4008.15: Developing potato cultivars adapted to Southern Africa countries

January 2008–December 2009; NCE: November 2011

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED

Sweet potatoes

64. G4007.07: Marker-assisted selection for sweet potato virus disease (SPVD) resistance in sweetpotato germplasm and breeding populations

August 2007−July 2010; NCE: June 2011

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED

Crosscutting activities

Capacity building

Human resources (support to teams, faculty, students, travel grants, workshops)

65. G4006.36: Capacity-building and research project (Academic position in molecular breeding supported)

September 2006–December 2011

Principal Investigator and Lead Institute
Mark Laing, (laing@ukzn.ac.za) African Centre for Crop Improvement (ACCI), University of Kwa Zulu Natal, PBX01, Scottsville, Pietermaritzburg 3209 South Africa

Collaborating scientists:
- Jedidah W. Danson (dansonj@ukzn.ac.za) University of Kwa Zulu Natal, PBX01, Scottsville, Pietermaritzburg, South Africa

This year 2011, ACCI's molecular breeding component is conducting research in 9 GCP countries namely: Ethiopia, Kenya, Malawi, Mozambique, Rwanda, Tanzania, South Africa, Uganda and Zambia. There are (11) eleven 2nd year students and (10) ten 1st year students. The project had initially 5 objectives: Objective 1: Teach Biotechnology to PhD students at UKZN-AACI. 2: Supervise PhD Research students in Biotechnology at ACCI. 3: Develop a functional toolkit of biotechnology procedures for plant breeders in Africa. 4: Assist in developing genotyping centers. 5: Assist in institutional support of biotechnology for 6 African Universities. 6: Assist in developing a dynamic inventory of biotechnology capacity in southern and eastern Africa.

Completed task among the six is number 3: An FTA toolkit was optimized and is in use in the field for students who have a molecular component. The toolkit has also been utilized previously in (2)-4th year students research in Uganda and Kenya using rice samples. The FTA cards technology from Whatman was optimized at ACCI and introduced to students. The technique is a big advantage as it does not require liquid nitrogen during sampling. The samples on FTA cards are stable for 2+ years. For task number 4, there have been no genotyping centers as yet developed. The resources are limiting and we continue to use GCP recommended centers. Other tasks facing limitations due to funds availability are such as support for African universities (5), and developing an inventory of biotechnology capacity in eastern and southern Africa (6).

Ongoing tasks: It is now in the 3rd year (2009, 2010, 2011) that biotechnology has been taught consistently at ACCI with the available position funded by GCP. There are several students under supervision. 2 in 4th year (6 students applied but only 2 got GCP funding), 3 in 3rd year and there may be 6-10 in 2nd year depending on the outcome of the proposals now under development.

The main challenge or priority remains to get funding for the students to conduct molecular breeding research in relation to the conventional breeding activities.

66. G4008.37: Training plant breeders at the West Africa Centre for Crop Improvement

March 2008–February 2014

Principal Investigatorand Lead Institute
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Fax (233) 21-520604

Collaborating institutes and scientists

- Biotechnology Centre, University of Ghana; Kwame Offei
- College of Agriculture and Life Sciences, Cornell University; Vernon Gracen

The West Africa Centre for Crop Improvement (WACCI), a partnership between the University of Ghana (UG) and Cornell University, was established, with funding from the Alliance for a Green Revolution in Africa (AGRA) in June 2007, to train 40 Plant Breeders in a PhD programme at the University of Ghana. In 2008, the Centre obtained an additional grant from the Generation Challenge Programme (GCP) to train four additional students. The WACCI PhD is a 5-year programme, students undertake two years of coursework in the University of Ghana and three years of field research in their research institutions.

Currently, the Programme is in the fourth year and has enrolled a total of thirty-six students from seven West and Central African countries (Burkina Faso, Cameroon, Ghana, Kenya, Niger, Nigeria and Mali). Four out of the thirty-six students are supported by grants from the Generation Challenge Programme (GCP).

List of Students on GCP Grant

No.	Name	Country	Institute
1	Dramane Sako	Mali	Institut d'Economie Rurale (IER)
2	Thompson Ruth N.A.	Ghana	CSIR- Crops Research Institute (CRI)
3	Joseph Benoit Teyioue Batieno	Burkina Faso	Institut de l'Environnment et de Recherches Agricoles (INERA)
4	Joseph Adjebeng-Danquah	Ghana	Savanna Agricultural Research Institute (SARI)

Two of the students have successfully completed their coursework and are currently in their home institutes conducting their thesis research. Below is a list of the students, their thesis topics and supervisory committees.

List of Students in the field (Third Year)

No.	Name	Crop	Thesis Topic	Supervisory Committee
1	Dramane Sako	Sorghum	Quantitative Trait Loci Analysis for Yield Components and Panicle Architecture in Sorghum	E.Y. Danquah, V. Gracen , S. K. Offei, Niaba Teme*

2	Thompson Ruth N.A.	Cassava	Genetic variability and inheritance of Postharvest Physiological Deterioration Tolerance in local and improved populations	S. K. Offei, K. Asante, E.Y. Danquah, Joe Aduening Manu *

*In-Country Supervisor

The other two students on GCP support are in the second year taking modular courses. Both are currently working on their mini projects in their home institutions. Mini projects are preliminary research studies carried out before the commencement of their major project. Below is a list of students, their proposed crops and proposed topics.

List of Students in the second year

No.	Name	Crop	Proposed Thesis Topic
1	Joseph Benoit Teyioue Batieno	Cowpea	Genomic and classic selection to improve drought tolerance in cowpea
2	Joseph Adjebeng-Danquah	Cassava	Marker-assisted selection for improving cassava yields in drought prone environments of Ghana

Training of the four students at the PhD Level is going on as planned and hopefully by December 2014, all four students would have submitted their dissertations for examination.

Human resources

A data management workshop specifically for TLI followed immediately after the launch meeting, and TLI breeders and data managers have participated in other workshops organised by the Integrated Breeding Platform project. Human resource capacity building embedded in the specific crop objectives is making good progress. More details are in respective crop sections, while infrastructure support and development is reported in the Capacity building section of 'Crosscutting activities'.

Infrastructure

A lysimetric system and a manually movable rain out shelter have been installed at the ICRISAT Sahelian Centre in Niger. At Egerton University in Kenya, a rain-out shelter and a greenhouse have been installed at the main university campus, and an irrigation system, weather station and fencing installed at the affiliated Koibatek Dryland Farmers Training Centre where most of the chickpea field trials will take place, together with plot rehabilitation. Similar work has been completed at the Naliendele Research Station, Tanzania. This infrastructure support for field sites is serving both TLI and TLII as they share these sites.

Learning materials

67. G4009.08: Plant breeding: Concepts & methods: A learning module

November 2009–October 2010; NCE: June 2011

Principal Investigator and Lead Institute
Theresa Fulton, Institute for Genomic Diversity, Cornell University, tf12@cornell.edu, 130C Biotechnology Building, Ithaca, NY 14853 USA, ph: 1-607-255-4323, fax: 1-607-255-6249

Collaborating institutes and scientists

- CropGen International, Robert Koebner

A new learning module has been completed, on the topic of Plant Breeding: Concepts & Methods. This module complements existing GCP materials, such as Marker Assisted Breeding (http://www.generationcp.org/mab/). It includes topics such as inheritance, quantitative variation, plant breeding methods, and new technologies. The materials were developed in the form of powerpoint slides, were reviewed by several Cornell University faculty and a visiting scientist from Ghana, revised and submitted to GCP for formatting. A draft version is now available online at http://www.generationcp.org/plantbreeding/ for final proofreading.

Crop information

68. G4008.32: Quality management procedures in GCP research laboratories promoted

July 2008–June 2009; NCE: June2011

Principal Investigator:
Julian Smith, International Development, Food and Environment Research Institute, Sand Hutton, York, UK YO41 1LZ; Julian.smith@fera.gsi.gov.uk, tel +44(0)1904 462746

David Galsworthy, Quality Manager, Fera. Derek Tomlinson, International Development, Fera

Collaborating institute(s) and scientist(s):
- Jeffrey Ehlers, University of California Riverside (UC-R); jeff.ehlers@ucr.edu
- Pooran Gaur, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT); p.gaur@cgiar
- Marie-Noelle Ndjiondjop, Africa Rice Center; m.ndjiondjop@cgiar.org
- Emmanuel Okogbenin, National Root Crops Research Institute; eokogbenin@yahoo.com, e.Okogbenin@cgiar.org
- Chunlin He, Breeding services Manger, GCP; c.he@cgiar.org
- Arvind Kumar, IRRI; a.kumar@cgiar.org
- Richard Trethowan, Sydney University; richard.trethowan@sydney.edu.au

Project context:
To assess opportunity for positioning quality assurance measures along a generic plant breeding pipeline to optimize delivery of desired project deliverables and their communication by the GCP.

Findings and implications:
The focus of the project was changed in line with GCP direction toward outsourcing molecular typing. A new focus was given to achieve the following objectives

1. To identify and characterize the top-tier processes that best describe a generic plant breeding pipeline from point of germplasm accession through to characterization and breeding to performance trials and release.
2. To identify quality assurance points within the top-tier processes and develop an overall quality management frame (QMF) for the breeding pipelines of the GCP.
3. To document major exception that may be expected as relate to particular crops and breeding systems.
4. To engage with GCP members to share and review proposed QMF and refine systems accordingly.

Progress to date
- A web forum has been developed and populated with subject areas
    - https://secure.fera.defra.gov.uk/gcp/index.cfm?

- A outline of a plant breeding pipeline has been drafted as identifies top tier processes and potential intervention points
    - https://secure.fera.defra.gov.uk/gcp/secure/documentStore.cfm?id=7
- A outline of a implementation plan has been drafted for discussion
- https://secure.fera.defra.gov.uk/gcp/secure/documentStore.cfm?id=7
- A half day workshop was hosted at the IPB meeting at Wageningen, 31st May 2011 to identify and discuss some critical intervention points
- A presentation was provided at the IPB meeting on quality assurance and the 1-Quality@GCP project
- Fera investigators gained from the participants of IPB meeting a fuller understanding of the GCP and the limits within which a QMF may operate
- Whilst participants at the IBP noted the desirability of a quality system, a repeated comment was on the effort required for implementation when time and funding were constrained; i.e. how to attain the 'activation energy' of the system.
- The case was put by Fera that for institutes it would be increasingly an expectation for accessing funding that institutes had demonstrable quality assurance – that quality assurance was business critical, as already evident at Fera.

Next steps and challenges

- Based on the outcomes of the IBP meeting, Fera needs to revisit some of its expectation of the QMF for the GCP and to refine with the Working Group.
- A critical need is to characterize the interaction of the GCP and the project as the implementation unit.
- This will most likely require broader recognition of choice in approach to design and implementation of plant breeding pipeline attributes, alongside more compliance/justification on the path chosen.
- A major consideration is how to measure the fit-for-purpose nature of the attributes of a pathway (eg equipment records, molecular analysis and data handling, field trail design, data handling systems, skills and competency recording) where these attributes themselves are not within a quality context.
- A quality assurance system will place he burden of demonstrating compliance on the institute/project working under GCP support.
- The final outcome of the 1-Quality@GCP consultation is to be presented at the GCP annual meeting at Hyderabad, September 2011.

69. G4010.05: Development of Integrated SNP Mining and Utilization (ISMU) pipeline based on next generation sequencing (NGS) and high-throughput (HTP) genotyping technologies for facilitating molecular breeding

September 2010–August 2011

Principal Investigator and Lead Institute

Rajeev Varshney and Trushar Shah,

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Collaborating institutes and scientists

- Scottish Crop Research Institute (SCRI) : David Marshall; Iain Milne
- International Crop Research Institute for the Semi-Arid Tropics (ICRISAT): Abhishek Rathore, Sarwar Azam, A BhanuPrakash, R Pradeep
- University of Queensland: Dave Edwards
- National Center for Genomic Resources (NCGR): Greg May; Andrew Farmer

Next generation sequencing (NGS) technologies are revolutionizing crop genomics, including our understanding of genome diversity, development of mapping resources, and studies of ecological and evolutionary biology. By lowering costs and increasing the rate of sequence acquisition, these technologies are causing researchers to re-think how crop genomes and transcriptomes are analyzed. One of the most convenient approaches is to discover single nucleotide polymorphisms (SNPs) in thousands of genes by sequencing and re-sequencing of genome / transcriptome with NGS platforms. Analysis of large-scale NGS data however is a serious challenge to the crop genomics community especially in under-resourced crops like chickpea and pigeonpea where reference genome sequence is not available. This project deals with identification and optimization of appropriate tool/ approach for analyzing the NGS (Illumina GA reads) for identification of the variants or polymorphisms between genotypes of an under-resourced crop species like chickpea.

To discuss issues related to tools, criteria, an international workshop was organized. Presentations and discussions are available at http://www.icrisat.org/bt-publicdomain-secondngs.htm as a resource for research community **(Activity 1)**. In brief, in consultation with several international experts through a workshop, four commonly used tools namely Maq, NovoAlign, SOAP2 and Bowtie were selected. Unlike probability based statistical approaches for consensus calling and by comparison with a reference sequence, a Coverage based Consensus Calling (CbCC) approach was applied with four commonly used short read alignment tools (Maq, Bowtie, Novoalign and SOAP2) on 15.7 and 22.1 million Illumina reads for chickpea genotypes ICC 4958 and ICC 1882 were aligned with the chickpea trancriptome assembly (CaTA). Using CbCC results for these two genotypes, a non-redundant set of 4466 SNPs was identified. Experimental validation of 224 randomly selected SNPs showed the superiority of Maq among individual tools, 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 a large number of SNPs in chickpea, this study provides a benchmark comparison of tools as well as read depths for four commonly used tools for NGS SNP discovery in a crop species without a reference genome sequence. **(Activity 5)**

To convert *in silico* identified SNPs into genotyping assays, a perl script that calculates Assay design (ADT) score has been included in the pipeline (http://hpc.icrisat.cgiar.org/NGS/, the first version was developed in the earlier version of the proposal). ADT score is actually a method for predicting the successful creation of custom genotyping assays (specific for GoldenGate technology from Illumina). (**Activity 2)**

To improve the functionalities in the existing pipeline, the Flapjack programme (http://bioinf.scri.ac.uk/flapjack/) of SCRI was modified to provide direct web-based functions at SCRI. Furthermore, in collaboration with SCRI, Flapjack has been included in the pipeline for graphical genotyping tool for visualization and analysis of the genotyping data for the purpose of selecting the suitable genotypes for diversity analysis and selecting the best parental genotypes for marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS). (**Activity 3)**

The developed pipeline has been well documented and all documentation will be made available soon in public domain. The documentation outlines the steps to be followed in the pipeline. End users can use this pipeline easily with help of documents even though they may not be familiar with /Linux/Unix background. (**Activity 4)**

A standalone version of available pipeline is also being prepared and packaged in CD/DVD to distribute to the plant research community. Standalone version will be helpful for those researchers who have resources like computational facilities, sequencing data but do not have expertise in NGS data analysis. The pipeline is open source and can be developed further by the community. (**Activity 6)**

A demonstration of developed pipeline is being scheduled in the General Research Meeting of GCP in Hyderabad. (**Activity 7)**

References

Flapjack http://bioinf.scri.ac.uk/flapjack/

Yan J, Yang X, Shah T, Sánchez H, Li J, et al. High-throughput SNP genotyping with the Golden Gate assay in maize. *Mol Breed* 2009 DOI 10.1007/s11032-009-9343-2

Thakur V, Varshney R (2010) Challenges and strategies for next generation sequencing (NGS) data analysis. *J Comput Sci Syst Biol* 3:040-042

Varshney RK, Nayak SN, May GD, Jackson SA (2009) Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends Biotechnol* 27:522-30

70. G4010.06: Enhancement and implementation of the crop ontology for data integration and data interoperability

January–December 2010; NCE: December 2011

Principal Investigator and Lead Institute
Elizabeth Arnaud, Bioversity International

Collaborating institutes and scientists

- Bioversity International : Lucas Matteis, Milko Skofic
- CIMMYT: Rosemary Shrestha, Crop ontology coordinator, Hector Sanchez, Arlett Portugal
- CIP: Simon Reinhardt
- IRRI : Mauleon Ramil, Jeffrey Detras
- IITA: Moshood Bakare, Peter Kulakow with the Cassava breeders' group:
- ICRISAT – Trushar Shah
- IRRI : Mauleon Ramil, Chengzhi Liang, Nikki Borja

Self funded collaborators

- Oregon University : Pankaj Jawal, Laurel Cooper
- Manchester University : Norman Morrison, David Hancock

Summary
Within the scope of previous work from 2008-2010, this project continues the incremental validation and refinement of the Crop Ontology (http://cropontology.org) which contains controlled and structured vocabularies related to crop-specific traits for height GCP mandated crops (Cassava, Chickpea, Maize, *Musa*, Potato, Rice and rice mutants, Sorghum, Wheat) as multi-crop passport data and Bioversity descriptors. All the terms used in the ICIS method, property and scale have been compiled in the Ontology allowing to integrate it into the ICIS model. Trait dictionaries are being developed within the framework of the Integrated Breeding Platform (IBP), activity 3.2.2 and 3.2.3 and are available on the IBP wiki. Platforms like the Integrated Breeding Platform, SeeD, GENESYS and GRIN-Global are invited to provide terms to the Ontology and annotate data. This project has the 3 following objectives: (i) expanding the raw ontologies to include protocols, scales (units) and quality standards, and make them accessible in a solid database; (ii) developing an easy-to-use curation/data annotation tool for updating the database; and (iii) identifying teams of curators willing and able to do the curation.

1. Expansion of the Crop-specific Trait Ontologies and Trait Dictionaries

The data produced by phenotyping studies need to be annotated using the controlled vocabulary offered by the trait dictionaries and the Ontology to facilitate their integration into the multicrop platforms (IBP, GENESYS, GRIN-Global, SeeD). Therefore, the work involves adding Crop-specific Trait Ontologies for other mandated crops like groundnut, cowpea, millets, as well as adding a necessary concept for methods of trait measurement and experiments to enable the mapping of ontology terms onto measured, stored or published variables. Trait names are collected either directly from researchers or through submissions using the trait template. The comparison and mapping with the key descriptors developed for GENESYS will continue. In addition, the GCP ontology will be integrated with the Plant Ontology (PO) and the Gramene (Trait Ontology, TO; Environment Ontology, EO) to develop a common, internationally-shared crop trait and anatomy ontology.

2. An online Crop ontology curation and phenotyping data annotation tool

Data annotations can be used to identify the process through which trait data has been obtained and add documentation on the data set itself, as well as the data provenance. It will allow researchers to query keywords that are related to traits, plant structure, growth stages and molecular functions, and access the associated GCP phenotyping and genotyping data sets such as germplasm, crop physiology, geographic information, genes and Quantitative Trait Loci (QTL). To reach that stage, the Crop Ontology must be made available for an easy integration into data-entry user interfaces as pick lists to facilitate data annotation. The online prototype being developed (http://cropontology.appspot.com/) aims at facilitating the ontology curation and decentralized data annotation through direct access or through information systems such as the IBP, ICIS, SeeD or GENESYS,. The data annotation will the Crop Ontology terms based on the trait dictionaries and expanded within the protocols, scales (units) and quality standards. The prototype currently enable to browse the ontology, search terms, display the full information and tree structure, produce a report in PDF format, and to login for adding/deleting terms, adding attributes to one term, uploading a full ontology in OBO and even creating online one ontology.

3. Crop groups developing and curating their crop-specific ontology

During the ICIS developer workshop held in February in Hyderabad, scientists and data managers expressed great interest in the Crop Ontology curation tool, the trait dictionaries and want to get their traits into it. A demonstration of the electronic fieldbook of ICIS made in the IBP workshop held in June in Wageningen proved the usefulness of the dictionaries and the controlled vocabularies for data capture and upload. The community of practice for data management launched in the IBP workshop will support the use of the ontology and trait dictionaries as best practices in data management. Specific sessions to demonstrate the Crop ontology curation/annotation and the Trait dictionaries will be held with the crop groups during the GCP General Research meeting, in September, Hyderabad.

The existing GCP funded Crop Ontology webpage (http://ontology.generationcp.org) is still maintained and the acquired knowledge and methodology is disseminated through training workshops, conferences, and papers. The crop ontology team is regularly invited to share their experience and present the product in workshop on plant phenotype during the Annual Plant and Animal Genomics Conference, San Diego US and was invited to the first international workshop of the NSF-Funded Phenotype Network RCN.

Within this project, Bioversity currently provides basic maintenance on the Central Registry to simply facilitate data upload and access to the data sets for cleaning, versioning and rationalization. The central registry will be migrated on the cloud.

Diversity

71. G4007.01: Genotyping validation of the GCP reference sets

January 2007–December 2008; NCE: Oct 2011

Principal Investigator
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Collaborating institutes and scientists
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- Hari D. Upadhyaya, Tom Hash, ICRISAT
- Ken McNally, IRRI
- Marc Ghislain, Wolfgang Gruneberg, CIP
- Jill Cairns, Susanne Dreisigacker, CIMMYT
- Martin Fregene, Steve Beebe, CIAT
- Michael Baum, ICARDA
- Nicolas Roux, Bioversity
- Robert Asiedu, Sarah Hearne, IITA
- Jeff Ehlers, UCR

The objective of the project is to validate and certify (through re-genotyping by an external genotyping facility) the genotypic information attached to the reference set defined in SP1 from the initial composite set genotyping data. This genotyping validation project is connected to the management of the genetic material constituting the reference sets. As an output, stabilized materials specifically handled as genetic stocks by gene bank curators and associated to validated genetic diversity data will be available.
DNA has been received for the reference sets of 18 species among the 21 species targeted in the project (Table 1). DNA for Yam did not pass the quality control checks and did not provide reliable amplification in genotyping test steps.

For the remaining 17 species the genotyping data have been produced for 15 to 23 SSR with a number of accessions varying between 114 and 362.

The validation of genotyping data is a challenging exercise as the comparison of the two datasets must deal with various and intricate sources of differences: occasional genotyping error, incorrect binning of the SSR raw data in one or the other of the two datasets, drift between the two different germplasms analyzed in a context of genetic heterogeneity of the accession, outcrossing, mislabeling error, error in sample tracking (DNA plate inversion, etc.).
Validation data and curated (for misnaming or bad formatting) original data have been stored in a local database application (Sagacity). This database allows comparison of different genotyping results at different levels (sample, germplasm, accession). An R-package has been developed to extract data from this database and automatically run multiple comparisons of two or more datasets.

The analysis pipeline results in a subset of accessions and loci for which there is an acceptable level of agreement between the two datasets.

From these data a fingerprinting tool kit is developed for each species, to enable technical transfers between laboratories and subsequent comparisons of new genotyping. It is composed of a set of 8 reliable accessions genotyped with the same markers and representing a correct allelic range for the whole set of microsatellite markers.

A validation report is being generated for each species and delivered to the original laboratory and to genbank curators in charge of the multiplication and distribution of the reference sets.

Both validation and original data will be available online through the GenDiversity application and connected to the Singer system for an integrated access to Reference set data.

Table 1

Species	DNA received for reference set	No of samples	Genotyping complete	Analysis completed – Report editing
Barley	Y	294	Y	Y – ongoing
Chickpea	Y	300	Y	Y
Pigeon pea	Y	300	Y	Y – ongoing
Sorghum	Y	350	Y	Y
Coconut	Y	362	Y	Y
Groundnut	Y	300	Y	Y
Finger millet	Y	300	Y	Y – awaiting new version of original dataset
Musa	Y	124	Y	Y
Maize	Y	236	Y	Y
Wheat	Y	330	Y	Y – ongoing
Common Bean	Y	192	Y	Y – ongoing
Cowpea	Y	360	Y	Y
Lentil	Y	114	Y	Y – ongoing
Fababean	Y	152	Y	Y – ongoing
Foxtail millet	Y	200	Y	Y – ongoing
Pearl Millet	Y	300	Y	Y – ongoing
Rice	Y	284	Y	Y
Yam	Y	342	N – Problems encountered on DNA quality	-
Cassava	N	-	-	-
Potato	N	-	-	-
Sweet potato	N	-	-	-

Product delivery

72. G4010.02: Potential benefits of marker-assisted selection technologies on wheat, sorghum, cassava, and rice, and of the Integrated Breeding Platform

May 2010–December 2011

Principal Investigator and Lead Institute
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Collaborating institutes and scientists

- International Food Policy Research Institute, IFPRI: Stan Wood

1. Context

The objectives of the project are to: 1) assess the potential benefits and risks of failure of ongoing Challenge Initiative projects on wheat (India, China), sorghum (BCNAM and MARS projects, Mali), cassava (Nigeria, Ghana, Tanzania), rice (Mali, Burkina Faso, Nigeria); and 2) assess the potential benefits of Molecular Breeding Platform Services, activities, and applications for selected Challenge Initiative (CI) user cases.

2. Findings and Implications of Objective 1 – assessing the potential benefits versus risks of failure

The impact assessment predicted the cumulative and discounted net economic benefits for each project over 20 years from the start of the project. These benefits were projected based on the situation with the targeted traits as compared to the most likely situation without the traits. A summary of the estimated economic benefits of the various crop/country projects are summarized in Table 1. Benefits were calculated using scientist projections on yield changes, chances of research success, and adoption rates obtained through questionnaires. Data on production, prices, and other economic parameters were obtained from published sources. The results indicate that each of the GCP projects is projected to generate several million in net economic benefits over 20 years and the chances of research success range from 30 to 55 percent depending on the project. The factors that are the most uncertain are changes in yields, adoption rates, and probabilities of success. As a rough approximation if any of those three factors are cut in half (doubled), projected overall benefits are halved (doubled). Knowing this fact allows GCP management to alter key assumptions to examine the benefit implications. A completed report, which explains each project's technological pathway, methods used, data gathered, and results was provided to GCP management.

Table 1 – Findings for Objective 1

Crop	Country	Economic Benefits of projected yield changes (US, Million $)			Probability of Success (%)	Projected Adoption Rate (%)
		Most Likely	*Minimum*	*Maximum*		
Rice	Burkina Faso	12.4	2.9	22.0	30	35
	Mali	71.0	21.0	121.3	30	25
	Nigeria	978.9	471.4	1,491.5	30	40
Sorghum	Mali-BCNAM	27.8	11.4	36.1	50	20
	Mali-MARS	32.3	12.7	42.1	50	20
Wheat	China	6,532.2	4,713.3	13,844.7	55	40
	India	986.2	590.6	2,972.5	40	20
Cassava Project 1	Ghana	1443.3	-	-	20	20
	Nigeria	590.0	-	-	20	20
	Tanzania	118.0	-	-	20	20
Cassava project 2	Ghana	277.3	-	-	40	25
	Nigeria	1598.8	-	-	40	30
Cassava Project 3	Ghana	184.4	-	-	30	20
	Nigeria	950.4	-	-	30	25
	Tanzania	119.3	-	-	30	20

3. Next Steps – Objective 2

Next steps focus on Objective 2, assessing the potential economic benefits of the Integrated Breeding Platform (IBP) for the eight GCP projects mentioned above. We are identifying the technological impact pathways and comparing economic impacts of the projects with and without the IBP. We have developed project specific questionnaires that were e-mailed to the PIs and collaborating scientists to learn: (1) how the tools and services of the IBP have been used, (2) constraints to using the IBP, (3) additional tools/services that could be offered by the IBP, (4) if the IBP did not exist what would be substituted and could molecular breeding be conducted, and (5) if the IBP is expected to shorten the duration, increase the odds of success, and decrease the overall cost of the project as compared to completing the project without the help of the IBP. We have received completed questionnaires from the PIs of each project and are still receiving questionnaires back from collaborating scientists. We have begun preliminary economic analysis with the data received thus far. Along with the new questionnaire data, we are utilizing data obtained for Objective 1, such as data on price elasticities, prices, trade, and quantity produced. Conducting this analysis should be helpful for the GCP management and donors in assessing the value of the IBP.

Services

G8009 Integrated Breeding Platform

July 2009–July 2014

Project Manager
Graham McLaren, GCP; g.mclaren@cgiar.org

Collaborating institutes

- AAFC
- Agropolis (including CIRAD, INRA and ICIS development team)
- BI
- CAAS
- CIAT
- CIMMYT
- CSIRO
- CU
- EMBRAPA
- ICRISAT
- IRRI
- IRRI–CRIL
- KUL
- other CGIAR germplasm centres
- SGRP
- UQ
- WUR

Component 1: Integrated breeding portal and helpdesk

July 2009–July 2014

73. G8009.01 Objective 1.1: Establish and manage the molecular Breeding Platform

Activity Leader and Lead Institute
Graham McLaren, GCP; g.mclaren@cgiar.org

a. G8009.01.01/Activity 1.1.1: Establish and manage the Integrated Breeding Platform

July 2009–July 2010

Activity Leader and Lead Institute
Activity Leader: Graham McLaren, GCP; g.mclaren@cgiar.org

-

- The overall management of the IBP Project was overhauled during the year to provide more hands-on and proactive management of this complex project. Details are given in Section II.
- Given that the IBP will be one of the most important legacies of the GCP, and its sustainability is central to the GCP transition, the fundamental elements of the Business Plan developed in the first year of the project

were incorporated into the GCP Transition Strategy which has been approved by the GCP Consortium, including eight crop centers and has the support of the CGIAR Consortium.
- A project brief, a bookmark and various posters were developed in the course of the year. A brochure is planned, to present IBP to a general audience in non-technical language. Several public awareness pieces linked to the cassava user case were also written that articulate the needs, capacities and expectations of IBP users.

b. G8009.01.02/Activity 1.1.2 Develop and deploy the Integrated Breeding Portal

July 2009–July 2014

Activity Leaders and Lead Institute
Grhahm McLaren/Fred Okono, GCP; f.okono@cgiar.org

- The conceptualisation and design work for the portal is now essentially complete, and development work is underway at the Texas Advanced Computing Centre as part of the collaboration with iPlant Collaborative. The portal is accessible via the domain integratedbreeding.net and secure access has been ensured given the private nature of some of the activities and transactions that are intended to be carried out through the portal. iPlant will host the portal, which will facilitate a seamless interaction with both crop databases and the web-based configurable workflow system to be developed under Objective 2.2.

c. G8009.01.03/Activity 1.1.3 Establish integrated breeding helpdesk and coordinate training and communication activities

July 2009–July 2014

Activity Leader and Lead Institute
Graham McLaren/Carmen de Vicente (up to October 2010), GCP; g.mclaren@cgiar.org

-
- Support to users is provided via email correspondence between Service Managers of the platform and users. This helpdesk is most active for marker services, data management and curation, design and analysis and phenotyping sites.
- Partners involved in the user cases were trained in the use of IBP tools for the management of pedigrees, generation of fieldbooks and curation of crop databases. Short training courses were organised to run concurrent with other GCP research and planning meetings, at international, regional and country levels. This training is important for creating a critical mass of users of the IBP tools that will support each other. One on one user support continued to be provided by email and chat through the helpdesk.
- The IBP annual project meeting was held from 1st to the 3rd June 2011 in Wageningen, Netherlands. There were seventy-one participants from user cases, developers, managers and advisors attended the meeting at which

the main event was demonstration and evaluation of the informatics tools developed so far for the platform. Users were generally pleased with the tools provided and with the direction of development for the future.

d. G8009.01.04/Activity 1.1.4 Establish and support crop molecular breeding communities of practice

July 2009–July 2014

Activity Leader and Lead Institute
Ndeye Ndack Diop/Carmen de Vicente (up to October 2010), GCP; NN.Diop@cgiar.org

- Two new crop Communities of Practice (CoPs) were launched this year. The CoP for chickpeas is being coordinated by Dr Pooran Gaur (ICRISAT) and mentored by Dr Teresa Milan (Universidad de Cordoba), and will initially bring together chickpea scientists from Algeria, Ethiopia, India and Kenya. The joint CoP for cowpeas and soya beans has pioneer members drawn from Senegal, Mali, Burkina Faso, Niger, Nigeria, Cameroon, Kenya, Mozambique, Tanzania, Malawi and the US. It will be coordinated by Dr Ousmane Boukar (IITA) and mentored by Dr Jeff Ehlers (UC–Riverside).
- At the IBP annual project meeting in June 2011 two mentors were identified for the Data Managers CoP – Dr Arturo Franco (CIAT) and Dr Elizabeth Arnaud (Bioversity). Dr Ibnou Dieng (Africa Rice Center) and Dr Manoj Kumar Singh (National Research Centre on Plant Biotechnology/ Indian Agricultural Research Institute) will serve as co-ordinators. This CoP was launched in February 2010 at the inaugural meeting of the IBP. Efforts have been initiated to expand this CoP to partners from African NARS.
- The IBP MT see the CoPs as the future stakeholders of the platform and so has supported the upgrading of phenotyping sites for several NARS partners. This work has been carried out through the Phenotyping Sites and Protocols service (See Activity 3.2.5).

Component 2: Information system

July 2009–July 2014

74. G8009.02 Objective 2.1: Make existing tools for data management and breeding logistics available to molecular breeding projects through the MBP

Principal Investigator and Lead Institute
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The main focus of this objective is to provide access to, and support for, existing data management and breeding logistics tools for users of the platform who need these applications before the Configurable Workflow System (CWS) is available. The CWS is being developed in a modular way so that applications can work with existing ones

before the whole system is complete. There was also demand for access to existing genotypic and phenotypic analysis tools. These have been assembled into a Supplementary Toolbox which is available via the IBP Wiki.

a. G8009.02.01/Activity 2.1.1 Identify, deploy and support tools facilitating management of germplasm lists, pedigrees, intellectual property and other passport data

July 2009–June 2012

Activity Leader and Lead Institute

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Collaborating institutes and scientists

- International Rice Research Institute: Guoyou Ye

1. Activity 2.1.1

Existing ICIS tools are available to meet many of the germplasm management needs, and can be distributed to users by FTP or on CDs so they can enter their germplasm into the databases. However, these tools are being modified to meet any nonstandard naming conventions, and to handle any information beyond their scope. The challenge is to not only meet the needs of the use cases in this project, but also those of breeding programmes around the world and across different crops.

With the focus on ensuring quality pedigree information in the pedigree database, existing data validation tools are being improved and new ones developed as required.

Help and support for these tools will be available both within the tools, and through training materials.

In Year 2 of this Activity, all use cases had existing ICIS tools deployed and Data Managers were trained on how to use the tools to enter their pedigree data. Bugs and feature requests were posted to CropForge (https://cropforge.org/), as required.

2. Activities at IRRI

IRRI is handling the development of the current ICIS tools for pedigree management. As bug report and feature requests are posted on CropForge, the ICIS development team is responsible for addressing the issues and releasing updated versions. Due to turnover of senior programming staff on the ICIS development team, responses to bug reports and feature requests were impaired.

Tangible outputs delivered

New ICIS tools were released as required.

Next Steps and challenges
We would like to improve the communication between IBP development teams to ensure that use cases needs are being met; IRRI has been working on a new website for ICIS (http://cropwiki.irri.org/icisweb/), which will focus more on helping users and less on technical details for developers.

b. G8009.02.02/Activity 2.1.2 Identify, deploy and support tools for management of phenotypic characterisation and evaluation

July 2009–June 2012

Activity Leader and Lead Institute
Hector Sanchez/Guy Davenport [up to August 2010], CIMMYT; h.sanchez@cgiar.org

- Three tools are supported for management of phenotypic characterization, and evaluation: IMIS Maize Fieldbook, ICIS Workbook, and The FieldLog. The FieldLog tool version 2.0 for recording information in the field was improved and support was provided to users.

c. G8009.02.03/Activity 2.1.3 Identify, deploy and support tools for management of genotypic characterisation

July 2009–June 2012

Activity Leader and Lead Institute
Trushar Shah, ICRISAT; TM.Shah@cgiar.org

- Work continued on enhancements to the existing Laboratory Information Management System (LIMS) developed at ICRISAT. Major efforts concentrated on improving the efficiency of the workflow by introducing barcode scanning utilities within the LIMS and a number of additional reports requested by users were developed
- Two new implementations of the LIMS system were supported at the headquarters of the World Vegetable Centre (AVRDC) in Taiwan and at the Molecular Biology Laboratory, Dept. of Genetics & Plant Breeding, Chaudhary Charan Singh University, Meerut, India.

75. G8009.03 Objective 2.2: Develop a configurable breeding workflow system (CWS) for the IBP

The new Objective 2.2 combines the methodological research objective and the software engineering objective (2.2 and 2.3 of the original proposal) into a single objective with a much tighter focus on practical breeding applications. This reorganization is a result of lessons learned, highlighted in section III and management changed described in section II. In particular there is a new Implementation Team established to oversee the design, development and testing of the applications of the CWS in collaboration with dedicated user teams. User defined

targets for the CWS Applications were established by the Implementation Team in January 2011 and progress against these targets for year 2 is indicated in Table 1.

Table 1. Functionalities and progress for the Stand-alone CWS

Breeding Manager Application. The Breeding manager supports breeding logistics and breeding population development Key: Application 1. Target • Progress (with links)	**Data Management Application** The Data Manager supports germplasm screening, and characterization, field trials and genotyping	**Analytical Pipeline** The Analytical pipeline covers analysis of phenotype and genotype data to support breeding decisions	**Decision Support Tools** The decision support tools facilitate strategic and tactical breeding decisions including parent selection, line selection and crossing schemes
Breeding Manager • No Target in Year 2 Seed Inventory Manager • No Target in Year 2 Genealogy manager 1. Import basic germplasm lists from files • α version using existing tools evaluated by users. Changes recommended Query Tools 2. Basic dataset retrieval with export to excel or CSV files. • β version available in IB Fieldbook β 5	Trial Fieldbook 1. Produce basic trial fieldbooks from templates, germplasm lists and basic designs 2. Label printing and export/import to hand-held devises 3. Managing trial locations. 4. Analyse trial data exported directly from the FieldBook through a menu interface to R. • All targets met in the IB fieldbook β 5, tested by users at four workshops (WS) Environmental Chr. • No Target in Year 2 Genotyping data 5. Genotyping database with loading and retrieving tools • Prototype Genotype Data Management System (GDMS) developed and demonstrated at 2 WS.	Experimental design 1. Generate basic designs with R • RCBD and Alpha lattice available in IB fieldbook β 5, tested by users at four WS. • Designs also available in α pipeline RCropStat Phenotype analysis 2. Genetic variance analysis, single site and multi-site analysis in GenStat and R • All available with training material and tested in 2 WS. QTL analysis 3. QTL and QTLxE analysis tools with tutorials available in Genstat and R • All available with training material and tested in 2 WS. Selection Indices 4. Phenotype Selection Indices available in R. • Available and tested in CIMMYT	MB Design Tool 1. MBDT available for planning MB experiment with data inputs from local files. • ISMAB developed and tested in 2 WS MAS decision tool 2. Marker aided selection processing facilities based on major known genes added to MBDT • Available in ISMAB MARS decisions 3. OptiMAS – a tool for tracking and assembling favorable alleles. • OptiMAS tool developed and tested in 2 WS. Simulation Tools 4. Stand-alone versions of simulation programs available for use with manuals and training material • QuLine, QuHybrid and QuMARS released with manuals.

a. G8009.03.01/Activity 2.2.1 Develop and deploy an Integrated Breeding WorkBench administration &and configuration application

July 2009–July 2014

Activity Leader and Lead Institute
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- Installation tools have been refined for crop information databases and extended to allow either Access or MySQL back ends. These are available for download for nine crops – rice, wheat, sorghum, maize, cowpea, chickpea, groundnut, common bean and cassava. A prototype IB Workbench has been developed from legacy code. This supports customisation of project resources and allows legacy applications and new CWS applications to be listed and launched from a customisable menu system.
- A prototype Trait Template tool has been developed in conjunction with the IB FieldBook application (Activity 2.2.3), but many templates have been produced simply by filling in an excel form.
- Various upgrades to the ICIS 5.5 database schema have been proposed for improving handling of the trait dictionary and the roles of different persons in breeding projects. The ICRIS schema was reviewed by partners from ICRISAT and IRRI during the ICIS developers' workshop in February 2011 and various improvements were suggested for inclusion in the first version of the IB Genotype Data Management System (GDMS).

b. G8009.03.02/Activity 2.2.2 Develop and deploy an integrated breeding management application

July 2009–July 2014

Activity Leader and Lead Institute
Graham McLaren, GCP; g.mclaren@cgiar.org

- Functionalities and workflows have been developed for the Seed Inventory Tool and the Germplasm List Management Tool for characterization, crossing and pedigree nurseries.
- A number of widely-used genealogy nomenclature rules have been collected and implemented for rice, maize, and wheat. Functionalities for the Pedigree Import Tool have been specified and discussed with users.
- **A** new database schema (MDMS) and middleware have been designed to store and retrieve phenotypic (breeding trial) data these set up a solid foundation in developing a study browser for querying and retrieving phenotypic data in both web-based and standalone systems.

c. G8009.03.03/Activity 2.2.3 Develop and deploy an integrated breeding field trial management application

July 2009–July 2014

Activity Leader and Lead Institute
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- IB Fieldbook β version 4 was released in June after several prototypes were tested by users who provided ideas for improvement. The design functionalities, mockup and alpha version were reviewed by users from the user cases, and the beta versions were tested by users at four separate workshops during the year. It allows users to merge germplasm lists and trait templates with generated field designs to produce Fieldbooks which can be printed or exported to hand-held devices for data collection.
- The Java based Fieldbook was developed in response to user demand for an easy to use system incorporating the best features of the CIMMYT IMIS Fieldbook and the ICIS Workbook, both Excel based applications which are difficult to expand and maintain. This is the first application of the Configurable Workflow System to be developed following a new strategy involving user input at each stage of the development process and allowing more centralized and focused software development by autonomous teams coordinated by the Implementation Team. Functionality of β version 5 is indicated in Table 1. This new management model (section II) was put in place in response to Lessons Learned (section III).
- Voluminous weather data from automatic weather stations are becoming increasingly common at user case breeding sites. This data has long term value for secondary use beyond current breeding activities. A strategy is being developed for the collection and storage of this data in the AgTrials Repository of the CCAFS[1] program where it can be used for breeding activities and will be available for climate change research.

d. G8009.04.01/Activity 2.2.4 Develop and deploy an integrated breeding genotypic data management system

July 2009–July 2014

Activity Leader and Lead Institute
Graham McLaren, GCP; g.mclaren@cgiar.org

- The ICRIS genotyping database schema was reviewed and revised with the help of collaborators from IRRI and the GCP who were involved in the earlier efforts of designing a gentoyping system. The resulting Genotyping Data Management System (GDMS) has been developed in Java using a browser-based front end. Efforts have been made to package the system so that it is available as a stand-alone system for users who do not have sufficient Internet access. A prototype of the system (using

[1] http://ccafs.cgiar.org/

medium-throughput SNP markers) was demonstrated to users at two GCP meetings.

e. G8009.04.02/Activity 2.2.5 Develop and deploy an integrated breeding analytical pipeline

July 2009–July 2014

Activity Leader and Lead Institute
Delphine Fleury, ACPFG/GCP; D.Fleury@cgiar.org

- Two parallel approaches are being taken for the development of the Analytical Pipeline. Firstly, state of the art statistical methodology is being developed and incorporated into the commercial GenStat Statistical Package. This is available to our user cases free of charge during the life of the project through a new collaboration with (VSNi[2]). It will also be packaged by VSNi into a workflow called Breeding View which should be available free for researchers in developing countries beyond the project.
- Secondly, following the original plan of the project, these statistical methods are being implemented as scripts using the R statistical language, avoiding as far as possible the use of mixed model methodology which is not readily available in the public domain.
- A package, named R-CropStat, with a user-friendly, menu-driven interface has been developed as prototype for the menu interface and data manipulation modules of the R based Analytical Pipeline. The analysis scripts will be incorporated into this system.
- Molecular marker selection indices have been developed and made available in R with data sets for testing. Two molecular selection indices were programmed, the Lande and Thompson selection index and the Molecular Eigen Selection Index. Both are used in bi-parental populations when mapping QTLs.

f. G8009.04.03/Activity 2.2.6 Develop and deploy an integrated breeding decision support system

July 2009–July 2014

Activity Leader and Lead Institute
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- An application for graphical genotyping, ideotype design and decision support for Marker Assisted Backcrossing (ISMAB) has been developed. This was demonstrated at a hands-on session during the annual meeting of the IBP project. The feedback received was that the tool was user friendly and intuitive however there were a number of bugs and issues with the sensitivity of the graphical window that needed to be addressed.

[2] http://www.vsni.co.uk/

- Th OptiMAS decision support tool was released in June. This application estimates the probabilities of parental allele transmission, at selected QTL, during generations of selection in bi- and multi-allelic populations and allows users to select candidates and determine a list of crosses to be made which will rapidly increase the frequency of favourable alleles in MARS breeding projects.
- Powerful simulation tools to support integrated breeding have been improved and released. QuMARS which can simulate MARS and GW programs has been incorporated into the QuGene user interface and fully documented in a comprehensive users' manual. Easy to use simulation tools have also been incorporated into the QTL mapping software IciMapping v3.1. These allow users to compare different mapping methods, and answer tactical questions such as the effects of marker density and population size on mapping power.
- A prototype cross prediction tool, based on the cross prediction algorithms was developed using Eclipse RCP. New algorithms for making crossing plans based on known-gene genotypic values were developed and published. Efforts have been made to test how to use specified prediction models in calculating genotypic values on the fly instead of using a large input file with genotypic values of all possible genotypes.

Component 3: Services

July 2009–July 2014

76. G8009.01 Objective 3.1. Provide access to critical molecular breeding services

Principal Investigator and Lead Institute
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a. G8009.05.02/Activity 3.1.1: Genetic Resources Support Service

July 2009–July 2014

Activity Leader and Lead Institute
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- Validation of germplasm reference sets of 19 crops continues. Differences have been observed between the original and validation dataset. The intricacy of various sources of difference and the availability of only two sets of data with no access to original raw data has made comparison challenging. However, re-genotyping of 18 crops (Cassava pending) is completed. An analysis pipeline to compare original and validation has been developed.
- Validated reference sets and Microsatellite Kits for sorghum and chickpea are available from ICRISAT and CIRAD. The reference sets of these crops will be used in a pilot program to evaluate demand, protocols for maintenance, sustainability and quality assurance. A Singer-based ordering

portal for the reference sets has been developed by Bioversity. Other sets will be catalogued and accessible through the portal as they become available.
- Difficulties in validation upon which all other activities depend requires a major adjustment of all timelines but particularly for Activities 2 (Elaborate a global policy and processes with other partners for the identification of the genetic resources, the maintenance of the genetic stocks and their distribution) and 3 (Develop a service integrated in the global germplasm system ensuring maintenance and distribution of genetic stocks for the priority GCP crops).

b. G8009.05.02/Activity 3.1.2 Marker services

July 2009–July 2014

Activity Leader and Lead Institute

Chunlin He/Humberto Gómez-Paniagua (up to October 2010), GCP;
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- Five service providers have been retained in the course of the project year – KBioscience, BecA, DNA Landmarks, ICRISAT and DArT. KBioscience is the only service provider for high throughput SNP genotyping; BecA, ICRISAT and DNA Landmarks are the providers for SSR genotyping while DArT provides the service for DArT markers. The legal master agreements and the genotyping service request agreement (GSRA) with KBioscience and DNA Landmarks, were updated and renewed in the course of the year.
- In the course of the year, 5 service projects were completed, 1 is in progess, 2 have been scheduled and 1 is pending. 8 potential customers have been recommended to use these marker services. Some other clients have contacted the service providers directly as is suitable for experienced users, but will still benefit from IBP conditions and use SNP and SSR markers developed by GCP.
- By using the KASPar system, we have developed valid sets of 1000-2000 SNPs for maize, cowpea, chickpea, pigeonpea, rice, cassava and sorghum. Conversion of SNPs for the AltSB gene of sorghum is in progress.

c. G8009.05.03/Activity 3.1.3 Trait and metabolite services

July 2009–July 2014

Activity Leader and Lead Institute

Xavier Delannay, GCP; x.delannay@cgiar.org

- Beyond preliminary queries on potential client needs and potential service providers, this activity has not been actively pursued. The user cases that are the primary test cases of the proposed IBP services have so far not had a need for the service, and the potential usefulness of the service vis-à-vis the complexities of providing it will be further assessed.

77. G8009.06 Objective 3.2: Provide assistance with a range of molecular breeding support services

a. G8009.06.01/Activity 3.2.1: Breeding Plan development

July 2009–July 2014

Activity Leader and Lead Institute
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- The general molecular breeding workflows for MAS, MABC, MARS have been updated and shared on the IBP wiki. The workflows include the steps of parental selection, population advancement, marker genotyping and phenotyping in the field, data analysis and decision making etc. The development of a cost-benefit analysis tool awaits the completion of simulation tools for specific crops for specific breeding workflows/schemes.

b. G8009.06.02/Activity 3.2.2 Information Management

July 2009–July 2014

Activity Leader and Lead Institute
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- A training workshop for the data managers of the Cassava Research Initiative was held in Accra, Ghana June 22-24, 2010. Training materials were written based on the breeding workflow of the research initiative. Peter Kulakow, the Senior Cassava Breeder at IITA, and his technical staff subsequently visited GCP for further discussions on the Cassava Ontology, trial templates and handheld devices.
- A data management workshop was held for both the data managers and breeders of the Tropical Legumes I Project August 25-27, 2010. The workshop surfaced various challenges faced by the different crop groups that were addressed both at the meeting and in the period following it.
- Two training workshops were organised for the Wheat Research Initiative, in India September 13-16, 2010 and in China October 25-28, 2010. The MS Access version of International Wheat Information System (IWIS3) was installed for the users in India while an updated mySQL version was installed for the partners in China.
- The data management strategy for TL1 was discussed at a dedicated one and a half day session at the project annual meeting in Madrid May 4- 7 2011. The new IB Fieldbook was presented and tested. Feedback was collected to inform further development. The databases for chickpea (IChIS), cowpea (IVIS), bean (IPhIS) and groundnut (IGnIS) were updated to work with the new tool.
- Hand-held computers were distributed to IBP users for evaluation. The Rice and Cassava Research Initiatives subsequently bought additional units for their use from their overall positive experience with the test devices. Challenges identified included difficulties synchronizing data with office-based PCs; short battery life; and small screen and keyboard.

c. G8009.06.03/Activity 3.2.3 Data curation

July 2009–July 2014

Activity Leader and Lead Institute
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- With advice from GCP staff, IITA advanced the curation of the cassava trait ontology and their germplasm information. Trait dictionaries of chickpea, cowpea, bean and groundnuts were established.
- The use of the IB Fieldbook and how to transfer it to a handheld device was explored at two workshops. The sharing of field book templates will help establish standard measurement of traits across different programs with similar objectives.
- A Data Management Workshop for West Africa was held at IITA, Nigeria, February 7-11, 2011 for data managers and breeders of cowpea, rice, sorghum and cassava. It was attended by 30 data managers and breeders of cassava, cowpea, rice and sorghum.
- As reported in Activity 1.1.4, the Community of Practice for Data Managers was boosted with the appointment of co-ordinators and mentors to facilitate knowledge sharing and mutual learning.

d. G8009.06.04/ Activity 3.2.4 Design and analysis

July 2009–July 2014

Activity Leader and Lead Institute
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- Existing training material on the use of the GenStat analytical pipeline was updated to incorporate new developments released in the latest version of GenStat (and in future to be incorporated in the Breeding View interface). In parallel, a set of tutorials similar to those for GenStat were developed for the analytical facilities in the R software that was produced under project Activity 2.2.5.
- Two courses have been delivered, the first one at ESALQ (*Escola Superior de Agricultura Luiz de Queiroz*) at Piracicaba, Brazil, in December 2010 and a second in Wageningen from 6th to 10th of June 2011. The latter course was attended by 65 participants, approximately half from the GCP, drawn from NARS, academia and the private plant breeding sector in Europe.
- Some requests for data analysis support from user cases were met, and more intense interactions are expected in the near future as the volume of user case data increases.

e. G8009.06.05/ Activity 3.2.5 Phenotyping sites and screening protocols

July 2009–July 2014

Activity Leader and Lead Institute
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- The activities this period have centered on the improvement of infrastructure and capacity of NARES partners to conduct the precision phenotyping required by IBP use cases and related GCP-funded projects. IBP had a consultant engineer visit each site to assess infrastructure needs and two training courses were organized for station mangers to ensure quality site management for phenotyping.
- A total of 20 sites in 8 African countries (Burkina Faso, Ghana, Mali, Mozambique, Niger, Nigeria, Kenya and Tanzania), and 7 sites in 2 Asian countries (China and India) received funding for field infrastructure improvements during the reporting period. Improvements covered weather stations, irrigation systems, fencing, heat and rain shelters, and land clearing and rehabilitation to enable quality field experimentation at those sites.

f. G8009.06.06/ Activity 3.2.6 Genotyping Support Service

July 2009–July 2014

Activity Leader and Lead Institute
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- Sixty-three proposals were received in response to the 3rd GSS Call, of which a total of 16 were selected for support – covering 9 different crops across 12 countries. The same service providers that were contracted for Marker Services (Activity 3.1.2) were utilized for GSS.
- All the service requests from the 1st GSS Call have been completed. From the 2nd GSS Call, 11 have been completed, 5 are in progress, 1 has been scheduled and 4 are awaiting availability of SNPs. From the 3rd GSS Call, 1 has been completed, 13 are in progress and 2 are awaiting availability of SNPs.
- It is anticipated that a critical mass of GSS beneficiaries will have accumulated by the end of 2011 for a viable data analysis and interpretation workshop. In the interim, one-on-one customised advice was given to some of the beneficiaries.

g. G8009.06.07/ Activity 3.2.7 IP and Policy Helpdesk

July 2009–July 2014

Activity Leader and Lead Institute
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- Demand for IP and Policy helpline services has been very limited as in the previous year. The few queries there have been received were referred to relevant experts. The need for this service is anticipated to grow as more unique materials are developed though molecular breeding or for critical traits as the user cases mature.

PROJECTS COMPLETED IN 2010

Research Initiative crops

Legumes

Cowpeas

78. G4008.17: Application of marker-assisted selection for *Striga* resistance in cowpea

January 2008–December 2009; NCE: December 2010

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutions and scientists
- INERA–Burkina Faso: Jeremy T Ouedraogo; Issa Drabo
- IITA: Boukar Ousmane
-

Summary
The three objectives of this project were to (i) identify breeding priorities to develop *Striga* resistant varieties; (ii) obtain new sources of *Striga*-resistant varieties for Burkina Faso and Niger; and (iii) implement MAS method for breeding programmes.

In order to identify breeding priorities, participatory rural appraisal (PRA) and farmer participatory variety selection (FPVS) sessions were organized at seven (7) targeted *Striga* hot-spots of Niger. In Niger, the project has confirmed farmer's preferences for IT00K-1148 and IT90K-372-2-1 due to their good agronomic traits, though these lines are susceptible to the dominant *Striga* race in Niger. *Via* FPVS, new farmer's preferred lines such as KVX30-309-6G and TN256-87 were identified, but these lines also lacked the gene for resistance to *Striga*. Genotype IT98K-205-8 and IT99K-573-2-1 were selected by farmers for their resistance to *Striga*, early maturity and high yield potential. IT98K-205-8 was the most evaluated line, especially in 2009, due to its extra-early maturing trait (harvested within 65 days). Since farmers were facing unstable rainfalls in 2009, they found that it could produce grain even under unfavourable conditions. These two *Striga*-resistant genotypes can be useful genetic sources for breeding *Striga*-resistant varieties, with farmers' preferred traits identified in a PRA and a FPVS.

In Burkina Faso, the PRA revealed that farmers prefer varieties with large-sized, white-coloured grain a rough texture. The landrace-like type of cowpea variety was the farmer's ideotype.

In addition to the field trials pot-screening trials were conducted at INRAN-Maradi station and confirmed that *Striga* race *SG3*, dominant in northern Nigeria was also dominant across Maradi and Zinder areas in Niger. In Burkina Faso, the pot-screening for Striga resistance involved more than hundred varieties including resistant and susceptible checks.

At IITA-Kano, six new combinations of *Striga* susceptible (IT00K-1148, IT93K-372-1-2, IT89KD-574-57, TN256-80) and resistant parents (IT98K-205-8 and IT98K-409-4) have been developed and backcrossed with susceptible parents to develop F1BC2 and F1BC3 populations. Existing SCAR marker Mah-se2 showed high marker efficiency at F1BC2 (88%) and F1BC3 (96%) for the evaluation of the resistance to *Striga* and had a potential for MAB, by doubling the percentage of resistant individuals at F1BC2 and F1BC3 generations. However, both existing SCAR markers could not show polymorphism on the crosses made between susceptible parents IT00K-1148 and IT93K-372-1-2 and resistant parents, IT98K-205-8 and IT98K-409-4. Therefore they could not be used for marker-assisted breeding (MAB) on these developed populations. Developed F2BC2 populations were grown in the *Striga* infested fields and selected for early maturing, *Striga* and disease resistance, and seed characteristics. The selected F3BC2 populations were used to develop cowpea varieties, which meet farmer's preferences as identified by the PRA and the FPVS.

Key Products Developed by the Project

1. Validated marker(s) for MAS of SG1 (Burkina Faso) and SG3 (Niger) resistance gene in LG1
2. Validated marker(s) for MAS of SG1 resistance gene in LG6
3. Farmer's participatory selected advance lines for Burkina Faso and Niger Republics.
4. Selected SG1 and SG3 resistant genotypes for further breeding in Burkina Faso and Niger
5. Development of MAS Protocols for *Striga* resistance in a rapid population breeding

Cowpeas: capacity building

Capacity building à la carte

79. G4008.43: Improve cowpea productivity for marginal environments in Mozambique

July 2008–June 2010

Principal Investigator and Lead Institute

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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- UoC–Riverside: Jeff Ehlers; Timothy Close; Philip Roberts
- PSU: Jonathan Lynch

Summary
The present report indicates the activities conducted from July 2008 to September 2010 according to the objectives of the project. The project had three objectives: (1) to provide baseline information on drought tolerance screening of 30 early and 30 medium cycle cowpea varieties and to assess the importance of genotype x environment interactions for grain yield under drought in Mozambique; (2) to assess the genetic variability of drought tolerance in 300 Mozambican cowpea landraces and (3) to develop 10 breeding populations suitable for marker-assisted-selection or marker assisted recurrent selection. Parallel to these objectives, the project aimed to contribute for capacity building on drought tolerance screening of genotypes with different growing cycle, assessment of genotype by environment interaction for grain yield under drought, large scale screening for drought, design and implementation of MAS-based program in collaboration with mentors at UC Riverside. In objective 1, 30 early and 30 medium cycle cowpea genotypes were evaluated for terminal drought in one location during off-season in 2008 (May – October 2008), in 2 locations during the main and off-season in 2009 and in two locations during the main season in 2010 (January - May 2010). This objective aimed to: (1) identify drought tolerant and susceptible checks for future studies, (2) determine the genotype by environment interaction and (3) assess the usefulness of off-season screening to target main season screening for drought tolerance. In objective 2, 216 genotypes were evaluated for drought tolerance in one location during the off-season in 2008 (May – October 2008) for assessing the genetic variability of drought tolerance. The major objective of this study was to characterize Mozambican cowpea germplasm for drought tolerance.

Preliminary results indicated that genetic variability for drought tolerance exists amongst the tested germplasm. As a result, drought tolerant and susceptible checks were identified. Genotype by environments interaction exists but stable genotypes across locations, seasons and water regimes were identified that can be recommended for cultivation under these and other similar environments. Off-season screening was uncorrelated with main season indicating that off-season should only be used for advancing the materials but not for selection or screening. The results on large scale screening of 216 genotypes adapted to Mozambique indicated that genetic variability for drought tolerance exists. As a result, 84 best performing genotypes were further evaluated for drought tolerance during three seasons (2009 main and off-season and 2010 main season) and genotypes with stable performance across seasons and locations were identified. In objective 3, ten breeding populations were developed through hybridizing 2 drought tolerant genotypes introduced from the University of California Riverside with local germplasm. The F1 materials were advance to F2 and the F2 to F3 generation. These materials are available for marker recurrent selection and will be sent to K-biosciences for genotyping.

Constraints that affected the work include:

1. Shortage of irrigation pumps in Chókwè Research Station, the drought site. Thus, an irrigation pump is needed for improving the quality of drought research in the site.
2. Lack of soil moisture monitoring devices. Moisture measurement devices to monitor soil moisture are required.
3. Lack of seed storage conditions. A fridge is required for seed storage.
4. Lack of vehicles for conducting the activities at University. A vehicle is required.

Conclusions
This project was first of this nature in the country. Conducting this project created great opportunity for Mozambique to generating information regarding drought tolerance potential in cowpea. This project offered an opportunity for capacity building in drought tolerance screening, determining genotype by environment interaction under drought and, learn about the application of MAS breeding. In addition, it created an opportunity for networking with other people conducting similar work in Africa and United States of America. However, there are constraints affecting the quality of the work such as limited availability of the irrigation pump, lack of seed storage facilities, lack of soil monitoring devices, and lack of vehicle for conducting the activities. Therefore, a request is being made to GCP to provide funding for acquiring these facilities to ensure that the Tropical Legume I project, currently taking place can run successfully.

Key Products: 1. Drought tolerant check varieties identified; 2. Efficient tools for drought tolerance screening; 3. Genetic populations suitable for MARS and varietal development for Mozambique
Output delivered
(1) Baseline drought tolerance information for 30 early and 30 medium maturity cowpea lines was established and drought tolerant and susceptible checks were identified from the 30 early and 30 late maturing genotypes.
(2) Off-season drought performance was not correlated to main season drought performance which indicates that off-season should not be used for evaluation but for advancing the materials when necessary.
(3) There was no clear relationship between number of roots and drought tolerance on cowpea. This suggest the use of rapid rooting assay need further investigation on cowpea.
(4) Two hundred sixteen (216) genotypes were characterized for drought tolerance
(5) Ten (10) breeding populations were developed. These populations were advanced to F3 where MARS will be applied.
(6) Breeder was trained in the use of Marker Recurrent Selection

80. G4008.39.02: Capacity-building *à la carte* 2008–Improving capacity for phenotyping for abiotic and biotic stress in Burkina Faso

April 2008−April 2010

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED; PROJECT REPORT PENDING UPLOAD

81. G4008.39.03: Capacity-building *à la carte* 2008–Improving capacity for phenotyping and biotic stress in Senegal

April 2008–April 2010; NCE: December 2010

Principal Investigator
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Collaborating institutes and scientists

- University of California Riverside (UCR): N. Ndack Diop; Philip Roberts; Tim Close; Jeff Ehlers

Cowpea is a major grain and fodder crop in Senegal, and one of the few crops adapted to the poor soils, low rainfall and high temperatures found in most of the country. Despite its rustiticity, productivity is decreasing due to drought spells and the pressure from pests. With the funding of the GCP-TL1 project targeting development of improved genomic resources in tropical legumes including cowpea with emphasis on drought tolerance, it is important that capacity exists to properly phenotype germplasm and genetic populations for abiotic stresses (drought and heat) and biotic ones (thrips, nematodes, *Fusarium* wilt, and bacterial blight). The precise and accurate phenotyping will allow to take advantage of molecular markers being identified under the TL-1 project. The objectives of this project is to streighten capacity for abiotic and biotic phenotyping. Two drought phenotyping trials including each 30 genotypes (early and medium maturing) were conducted. The trial included two w*ater regimes:* control (well irrigated conditions) and water stressed (withholding irrigation at flowering stage, about 50 % of the genotypes). In the early maturing group, the following genotypes have been found with similar relative chlorophyll content and small differences in canopy temperatures after 6 weeks of stress for 2 years: IT95M-190, IT98K1111-1, and IT97K-499-39. While in the medium maturity, genotypes with similar characteristics are: IT89KD-288, IT93K-503-1, IT98K-428-3, IT98K-317-2, and IT98K-128-2. Crosses were made between cowpea lines possessing drought tolerance, resistance to flower thrips, and good agronomic performance. The F4 generation of one of them (Mouride x IT84S-2246) was tested for reaction to terminal drought under irrigation. This trial was part of a pilot study to test an outsourcing model for genotyping for Marker Assisted Breeding. Leaf tissue of 95 F4 families and parents, dehydrated with silica gel, was successfully shipped from Senegal to DNA LandMarks, Inc. (Quebec, Canada) and genotyped with a Sequenom platform that followed assay design instructions from UC–Riverside. Comparison with phenotypic data is intended to show presence of previously identified molecular markers (SNPs) linked to targeted abiotic resistance traits, and to agronomic traits, including grain yield and individual grain weight.

Groundnuts

82. G4008.49: Enhancing groundnut (*Arachis hypogaea L.*) genetic diversity and speeding its utilisation in breeding for improving drought tolerance

August 2008–July 2009; NCE: May 2010

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NO UPDATE SUBMITTED; PROJECT REPORT PENDING UPLOAD

Crosscutting activities: Legumes

83. G4009.06: Illumina genotyping of SNPs in legume mapping populations and germplasm

November 2009–October 2010

Principal Investigator and Lead Institute
Doug Cook, UC–D; drcook@ucdavis.edu

Collaborating institutes

- CIAT
- EMBRAPA
- ICRISAT

EXTRACT FROM FINAL TECHNICAL REPORT

Summary
With combined funding from TL1 and the US National Science Foundation, we completed the identification and validation of single nucleotide polymorphisms in a range of legume crop species, including species of interest to the Generation Challenge Program. These species are chickpea, cowpea, common bean and groundnut. In total, 22,827 SNPs were validated and used to design an Illumina GoldenGate genotyping assay to map ~2,400 loci (averaging 600 loci in each species).

The purpose of this Commissioned Research Project Proposal was to extend the SNP discovery activities, which are now complete, to genotyping SNPs in mapping populations and germplasm from each species. Genotyping represented the last major task of TLI Objective 5, and thus this grant allowed us to complete activities that were described and endorsed in the original TL1 proposal to the Bill and Melinda Gates Foundation

Conclusion
Outcomes of this work includes medium-density genetic maps for common bean, chickpea, diploid peanut and cowpea. Moreover, we developed a comparative network is that delimits a framework genome structure for the common ancestors of the warm season and cool season legumes, respectively, as well as that of their common ancestor of ~59 MYA. We can now align each species to this paleogenome, and by this intermediate link we can align each modern day crop genome with greater accuracy to one another.

Potential applications include transfer of trait and gene information between species with greater accuracy. We have been pursuing this paradigm in the case of disease resistance genes and recently used a combination of comparative approaches and species-specific resources to rapidly identify genome regions carrying functional disease resistance genes against cowpea mosaic virus of cowpea and Fusarium wilt of pigeonpea. This has led to genetic markers within and surrounding the causal NB-LRR disease resistance gene clusters, thus providing landmarks sufficient for marker assisted selection activities in breeding programs, and ultimately to clone and functionally validate causal alleles.

We have also begun to evaluate the possibility of cross-species transfer of QTL information for abiotic stress tolerance (drought and salinity). Our test case is genetic and phenotypic information in Medicago and soybean, where relatively extensive analyses have been conducted.

Quantifiable Outputs
1. Molecular genetic map of chickpea.
2. Comparative genomic analysis of chickpea relative to other crop and model legumes.
3. Genotype data for a subset of germplasm mini-core accessions in chickpea.
4. Molecular genetic map of common bean.
5. Comparative genomic analysis of common bean relative to other crop and model legumes.
6. Genotype data for a subset of germplasm mini-core accessions in common bean.
7. Molecular genetic map of groundnut.
8. Comparative genomic analysis of groundnut relative to other crop and model legumes.
9. Genotype data for a subset of germplasm mini-core accessions in groundnut.
10. Molecular genetic map of cowpea.
11. Comparative genomic analysis of cowpea relative to other crop and model legumes.
12. Genotype data for a subset of germplasm mini-core accessions in cowpea.

84. G6007.05: Develop cross-species resources for comparative biology in tropical crop legumes

May 2007–April 2010

Principal Investigator and Lead Institute
Doug Cook, UC−D; drcook@ucdavis.edu

Collaborating institutes
- UCB
- UGA

EXTRACT FROM REPORT TO BMGF (JUNE 2010)

Activities for Objective 5
Develop cross-species resources for comparative genomics in tropical crop legumes

I. Goal, Objectives, and Activities
Activity 1.
The primary goal is to develop genetic maps based on well-conserved genome landmarks (orthologous genes) so that genome regions with shared ancestry can be reliably identified, even in species that have diverged as many as 59 million years ago (MYA)—the time frame separating groundnut from cowpea, common bean, and chickpea. The goal of analysing 500 conserved genes in each target species was exceeded by sequencing about 1,200 genes in each species and developing genetic map positions for 2,118 genes. This is an average of 530 per species.

The derived comparative network includes 1,100 conserved genes and integrates genome locations across nine legume species, including those that are the focus of TLI (i.e. groundnut, cowpea, common bean, and chickpea), TLII–Phase 1 (pigeonpea), and other funding sources (Medicago truncatula, Lotus japonicus, soybean, and lentils). Because this comparative network spans more than 900 Mbp of soybean and 300 Mbp of the Medicago truncatula gene space, it offers considerable opportunity for exploiting knowledge of these characterised genomes to discover genes in the TLI target species and conduct molecular breeding.

One outcome of the comparative network is a framework genome structure for the common
ancestors of warm-season and cool-season legumes, and their common ancestor of about 59 MYA. We can now align each species to this paleogenome and, through this intermediate link, align, with greater accuracy, the modern-day crop genomes with each other. Disease resistance is a common constraint to legume production worldwide. Hence, if we combine robust comparative maps that are also populated by candidate genes for disease resistance, we can accelerate the transition from trait phenotype to molecular genotype.

We validated this approach in both cowpea and pigeonpea; combined genetic and in silico analyses allowed rapid identification of the physical map contigs that contain functional disease resistance genes. This work would previously have taken years; instead, it now takes weeks. In cowpea, we are cloning the resistance locus for the cowpea mosaic virus, and working with Ndiaga Cissé (a member of the Objective 2 team) in Senegal to develop tools for MAS.

An ancillary activity was to integrate genetic and physical map resources (BAC clones or physical maps) in cowpea, chickpea, and groundnut. This was accomplished in cowpea and chickpea by analysing BAC-end-derived simple-sequence repeats (i.e. BES-SSRs). It was also accomplished in groundnut by using BES-SSRs and hybridising about 800 orthologous marker sequences directly to filters containing clones from the groundnut physical map developed under Objective 1. We also developed numerous additional simple-sequence-repeat markers in groundnut and tested their efficacy as genetic markers in both diploid and tetraploid backgrounds.

Activity 2.
Groundnut is an allotetraploid with a very narrow genetic base. Historically, difficulties in developing polymorphic DNA markers, and the complexities of tetraploid genetics hindered breeding and the development of genetic and genomic tools for groundnut. A ‘triangle’ of populations – one AA genome, one BB genome, and one AABB population, all of which incorporate wild polymorphism – was used to overcome these historical limitations, and to introduce new alleles into the groundnut crop.

To ensure continuity of work carried out in TLI and its transferability to work in other laboratories, the populations were all taken to the RIL level, and ‘immortalised’. The most relevant population for this work was the AA genome population, which displays the highest polymorphism. This same population was used to anchor the BAC-based physical map of the AA genome of groundnut that was created in Objective 1. The result of comparative mapping and marker development in Objective 5, together with the work in Objective 1, is a groundnut map that bridges maps

created in groundnut breeding with a unified genetic framework for legumes and with the physical BAC-based map created in Objective 1.

To summarise, at the beginning of TLI, groundnut genetics and genomics lagged behind other species. By the end of the Project, groundnut is well set for a genome-sequencing project.

Activity 3.
Comparative BAC sequencing is shedding much light on general patterns of legume genome differentiation, and specific fates of selected, functionally important regions. The BACs showing the highest concentration of putative disease resistance genes in Arachis appear to correspond to clustered derivatives of the CMR-1 virus resistance gene of Phaseolus, which functions across plant families and is up-regulated in a non-virus-specific manner. Parts of this region reveal gene synteny and colinearity in the legumes Medicago and Glycine, and non-legume dicots Vitis (grape) and Arabidopsis. Additional BACs in the pipeline will shed similar light on the evolution of drought responses, legume-rhizobium symbiosis, storage proteins and associated allergenicity, and the timing of major evolutionary events in the legume family tree.

II. Accomplishments
Our work demonstrated the validity of the three assumptions described in Section II (page 2) and provided proof of the usefulness of cross-genome comparisons by cloning the disease resistance determinant for a major viral pathogen of cowpea. Our top five accomplishments were therefore the following:

- A detailed comparative genetic and genomic analysis, linking cowpea, chickpea, common bean, and groundnut, not only to each other, but also to the full genome sequences of *Medicago*, *Lotus*, and soybean, and to genetic maps for pigeonpea and lentils.
- Developed species-specific gene-based genetic maps for cowpea, chickpea, groundnut, and common bean.
- Identified thousands of SSRs in chickpea, cowpea, and groundnut; and worked with collaborators on related TLI objectives to analyse these molecular markers.
- Sequenced about 42,000 BAC-end sequences in diploid groundnut, and identified and tested 1,152 new SSR markers for this species.
- Used a combination of genome synteny and BAC-clone sequencing to characterise genome regions of agronomic importance, including disease resistance loci in groundnut and cowpea.
-

III. Impact
Objective 5 collaborated extensively with the Objectives for chickpea, common bean, and groundnut led to develop new genomic and genetic resources, including gene-based genetic maps, collections of characterised SSR markers, and BAC library resources. Given the Objective's relatively small budget, the achievements are disproportionately large, and will lead to commensurate impact on NARS breeding programmes.

IV. Lessons Learnt
Thoughtful selection of genotypes was vital to *Objective 5*'s success. Also essential was the Objective's team willingness to be creative and to modify strategies,

particularly when new discoveries indicated alternative directions and the availability of novel technologies.

Capacity building

85. G6007.06: Provide training and capacity-building for SSA scientists

May 2007–April 2010

Principal Investigator and Lead Institute
Carmen de Vicente, GCP (up to October 2010)

Collaborating institutes

- UCB
- UGA

EXTRACT FROM REPORT TO BMGF (JUNE 2010)

I. Goal, Objectives, and Activities

Activity 1.
The Year 3 annual project meeting was conducted at the end of Year 2 and as such reported in the project annual report in May 2009.

A training course, attended by 19 NARS researchers, was conducted during 29 June–3 July 2009 at the Mediterranean Agronomic Institute of Zaragoza (IAMZ, its Spanish acronym) of the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM), Spain. It introduced the researchers to advanced statistical procedures that improve the accuracy with which breeders can assess genotypic performances. The course was carried out in collaboration with the Biometris Unit of Wageningen University and Research Centre. The topics covered included genetic linkage mapping and linkage disequilibrium (LD) mapping; LD mapping, using mixed models; phenotypic data analysis; QTL and QTLxE mapping; and genetic diversity analysis, based on molecular markers. Of the 19 course participants, 16 were TLI and TLII breeders from Benin, Burkina Faso, Ethiopia, India, Kenya, Malawi, Mozambique, Niger, Senegal, Tanzania, and Zimbabwe.

Activity 2.
Partners in Asia and Africa received support for the infrastructure of local facilities, as follows: computer equipment was provided to INERA–Burkina Faso and CBI–Zimbabwe; field equipment to ISRA–Senegal, LZARDI–Tanzania, NARI–Tanzania, and Egerton University– Kenya; and laboratory equipment to SARI–Ethiopia, DARS–Malawi, and IIPR–India.

II. Accomplishments
A launch meeting in Year 1 and two annual reporting and planning meetings in the following years contributed to building a community of legume breeders in Africa and Asia around advanced research institutions and the CGIAR centres. Delivery Plans for each Objective identified the research products delivery pathway to ensure short- and medium-term impact. They also identified existing capacity bottlenecks to adoption of research products and actions needed to overcome them.

- Two training courses, one on phenotyping for drought and the other on data analysis of phenotypic and genotypic data, were conducted to enhance capacity of NARS partners and contribute to their full benefit of the project.
- After careful assessment within teams, infrastructure needs (computer, field, and laboratory) were fulfilled to assist participation in the Project.
- Extra funds were secured to cover human resource development for NARS partners in each Objective.
-

III. Impact

Through the efforts of the Objective 6 team, a community of African and Asian legume breeders and their institutions are prepared to engage in crop improvement, using modern technologies. Links between members of this community will continue to strengthen in the Project's new phase. New members also are expected to join, as the benefits of the new approaches are increasingly made known to larger audiences.

IV. Lessons Learned

Objective 6 proved the value of partnerships between advanced research institutions, NARS, and the CGIAR centres. However, for partnerships to succeed, all members must be equally involved and informed of plans, activities, roles, and responsibilities. Strategies to ensure that communication lines exist at all times between principal investigators and other partners in the team need to take into account the difficulties that arise from limited computer and internet access, as well as travel agendas.

Fulfilling infrastructure needs must be well planned in advance. It requires close supervision and advice from principal investigators and, where necessary, the willingness of CGIAR centres to act as intermediaries to purchase and procure equipment that is available only from abroad or where partners do not have foreign-currency bank accounts. It is clear that, without proper infrastructure upgrades, the involvement of NARS institutions in research will always be limited. Likewise, any project involving NARS must include a plan to identify human capacity bottlenecks and provide adequate training and development activities to ensure that NARS fully participate in the project and subsequently adopt new technologies.

Maize

86. G4007.04: Association mapping of downy mildew resistance in elite maize inbred lines in Thailand

August 2007–July 2009; NCE: December 2010

Principal Investigator and Lead Institutes

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Collaborating institutes and scientists

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Summary

Maize is one of five major crops grown in the uplands of Thailand, which is predominantly used for animal feed, with 80-100% production being sold to commercial poultry and livestock feed mills. It is a highly commercial crop, handled by an extensive network of merchants. Maize sold as animal feed is mainly used domestically, and only a small fraction is exported. Meanwhile, about 5-20% of all maize grown in Thailand is consumed as food, either as white corn or sweet corn. Downy mildew caused by the fungus *Peronosclerospora sorghi* (Weston & Uppal) C.G. Shaw, is one of the most destructive diseases of maize in Thailand. Genetic resistance is a cost-effective and environmentally safe alternative in controlling the downy mildew disease. The objective of this project is to use the association analysis that is a method relies on linkage disequilibrium to study the relationship between phenotypic variation in maize genome for the dissection of downy mildew resistance and genetic polymorphism.

Sixty maize inbred lines from public and private sectors were screened to the spreader-row technique for field inoculation at two locations (NCSRC-IICRD KU, NFR) (Table 1). Percentage disease is determined by the ratio of the total number of plants with systemic infection to the total number of plants multiplied by 100 at NCSR and NFR. Analysis of variance mean squares values for DMR score of 60 maize inbred lines grown at two locations (Fig 1). The analysis of variance show significant variation among entries (Table 2). There was a significant location effect

as well as a significant entry by location effect. The analysis of variance results were used to measure broad sense heritability that showed 0.50. Sixty maize inbred lines were genotyped for 48 SSR markers on all maize chromosomes (Table 3). All of 48 markers produced a total of 489 alleles among 60 entries (Fig 2). The alleles varied from 2 to 29 with an average of 10 alleles per locus. A dendrogram was generating using the UPGMA algorithm with GD matrix that all of the entries could be group in to three clusters (Fig 3). Population structure was estimated using the model based approach as implemented in the software program STRUCTURE. The number of subpopulation (K) was identified likelihood value. However, it was difficult to determine the optimal number of subgroups, since the posterior or probabilities for the number of clusters increased steadily (Fig 4). Association analysis identified marker trait association (P-adj < 0.05). Three significant SSR/trait associations were detected with the Q GLM model over 2 locations (Table 4). These marker loci could explain 38.25–70.93% of the total variation.

Genotypic data for association analysis came from polymorphisms identified in candidate gene sequences. Nine candidate genes (Table 5) were chosen based on chromosomal location around SSR markers associated with DMR resistance. Association between polymorphisms and phenotypic values were performed by the General Linear Model (GLM) analyses in TASSEL. The principal component analysis produced by TASSEL was included as covariate in the analysis to control for populations structure. The polymorphisms were determined as significant for p-adj Marker (based on 10000 permutations) equal to 0.05 or less.

LD between pairs of sites (SNPs) was evaluated by using TASSEL Version 2.1. LD was estimated by squared allele-frequency correlations (r^2), and standardized disequilibrium coefficients (*D'*). The loci were considered to be in significant LD if *P* <0.001. For PIC15 gene, one in the exon (+455 G/C) was significantly associated with location 1 (Table2). For the r_chr2 gene, one in the intron (+616 T/C) and three in exon (+830 G/A, +842 C/T and +941 T/C) were significantly associated with location 2 (Table 6). For TIDP3390 gene, one in the intron (+346 G/A) was significantly associated with location 1 (Table 6). For LD analysis, the three candidate genes have the large red blocks of haplotypes along the diagonal of the triangle plot this indicate the high level of LD between the loci in the blocks, meaning that there has been a limited or no recombination (Fig 5, 6 and 7). In addition, the nonlinear logarithmic trend line indicated that LD decay of three candidate genes is declined slowly (Fig 8, 9 and 10).

Findings

1 Three SSR markers associated with DMR resistance

2 Three candidate genes had significant results from association analysis.

Conclusions

Nine candidate genes were used for association analysis, 3 candidate genes had significant results from association analysis. The LD decay of three candidate genes indicated that LD declined slowly.

Deviations from work plan

The activities have been adjusted due to the late approval of proposal for the project. The activities in appendix A are the same as the previous proposal. The

appendix B has been changed to a new table. Because the maize planting season is during April or May and properly field inoculation is on June or July in Thailand. Then the activies for downy mildew inoculation have to postpone.

Conclusion

1. Three SSR markers associated with DMR resistance
2. Three candidate genes had significant results from association analysis.

Quantifiable outputs

- Three SSR markers associated with DMR resistance
- Three candidate genes had significant results from association analysis.

Capacity building: Maize

87. G4007.13.04: Capacity-building *à la carte* 2007– Characterisation of maize germplasm found in Ghana, using the bulking technique

July 2007–July 2009; NCE: July 2010 (not documented)

Principal Investigator and Lead Institute

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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

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- Universidad de la Republica/IITA, Jorge D. Franco
- CSIR-Crops Research Institute, Kumasi; Manfred Ewool, Research Scientist

Supporting scientists

- CSIR/CRI–Ghana: Maxwell Darko Asante; Ruth Thompson

Summary

Towards the realization of the objectives of the above program a nationwide collection of Ghanaian maize landraces was carried out from October to December 2007. Collections were made from all the agro-ecological zones; comprising the Coastal savanna in the South-East, High Forest in the South-West and the middle zone, Forest transition in mid-north and the Guinea savanna in the northern parts of Ghana. In all about 580 accessions of varied diversity were collected and this exceeded our expectation. These were initially saved at the CRI genebank and some sent to CIMMYT for genotypic characterization. The means of possession by which these farmers came by these landraces were ascertained. These were by through inheritance from their fathers, grand fathers and friends with some attributing source to their ancestors association with early European Missionaries and also from neighboring countries (especially those from the border towns and villages).

This was followed up with a four month training the ABC laboratory at CIMMYT, Mexico. The training at CIMMYT covered topics in Marker assisted selection (MAS) as well as an introduction to programming used in the analysis genetic data namely R, STRUCTURE and POWERMARKER and the creation of core subsets. The training in MAS involved techniques in plant (seed and leaf) DNA extraction, DNA amplification using PCR and the use of the gel electrophoresis as well as scoring and analysis of gels. SSR markers linked to MSV resistant alleles were used in this training. Field phenotyping of Ghanaian maize landraces was also done. Phenotypic data were taken with reference to Bioversity International descriptors for maize (1991). Genotyping of Ghanaian landraces was also done. Twenty SSR markers spanning the maize genome was used for genotyping. Twenty four populations comprising 20 Ghanaian maize landraces and 4 controls; two CIMMYT inbred lines and 2 landraces, one each from Peru and Guatemala, were used for the genotyping, as they are routinely done in all such analyses at CIMMYT. Each population consisted of 15 individuals and the bulk fingerprinting technique (Dubreuil et al., 2006) was used. Genotyper data were analysed with STRUCTURE version 2.2 and POWERMARKER version 3.25. The Powermarker analysis grouped the Ghanaian maize landraces into two main groups which were distantly related to the South American landraces and the CIMMYT inbred lines which were used as controls. These clusters were the northern group (Guinea savannah zone) and the southern group (forest and coastal savannah). Ashanti which is centrally located in Ghana belong to both clusters. Analysis with STRUCTURE was done with K=5 (which corresponds to setting the number of clusters to 5). This analysis showed non-discrete nature of the Ghanaian landraces. However, five broad groups were identified. This genetic characterization data will provide useful information for utilizing these populations in genomic studies and breeding efforts, and as a proof of concept to expand the search for useful alleles in diverse germplasm.

In the phenotyping of the Ghanaian landraces under drought and irrigation a few of local accessions were marked that can be used to develop drought resistant/tolerant maize in Ghana. In addition some basic laboratory and field infrastructure were procured to boost research activity at the CSIR-Crops Research Institute.

Conclusion

The work on the project was finished with minor deviations from the original plan. The following expected outputs from the proposal have now been met:

The project successfully characterized Ghanaian maize landraces genotypically using SSR markers. The distribution and the relationships between 20 populations of these landraces were established. Generally, there are two major groups that also correspond to the two major ecologies pertaining in the north and south of Ghana. However there are also several closely related landraces within each group. Modes of acquisition of landraces have also been documented, suggesting introductions from neighbouring African countries and European merchants/missionaries and seed exchange among local farmers. The project also established the non-discrete nature of the Ghanaian maize landraces. Principal coordinate analysis of selected landraces identified about 5-7 of these accessions for further evaluation for drought tolerance in the subsequent years. A core subset of Ghanaian maize landraces will soon be created which will be used for our breeding programmes including those with resistance to the maize streak virus disease. Most importantly, the human resource capacity of

CSIR-CRI received a boost and at the same time some basic laboratory equipment and field infrastructure have been made available.

Finally, we are grateful to GCP and particularly, Carmen de Vicente for her immense support towards the implementation of planned objectives.

Final products to be used by plant scientists outside the project:

1. Over 580 Ghanaian maize landraces collected
2. Genotyping of Ghanaian maize landraces completed
3. (Capacity building):Training in marker assisted selection completed
4. Equipment for MAS procured
5. Creation of a core sub set of Ghanaian maize landraces completed
6. Improved infrastructure for drought work completed
7. Phenotyping of Ghanaian landraces for drought almost completed

88. G4008.19: Incorporation of an MSV-resistance gene in Mozambican maize varieties, mediated by use of MAS

January 2008–December 2010

Principal Investigator and Lead Institute

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Collaboratoring institutes and scientists

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- University of KwaZulu-Natal: John Derera; Jedida Danson; Sharmane Naidoo

1. Introduction and Justification

Maize (*Zea mays L.*) is grown in all the agro-ecological zones of Mozambique and is the major staple food. Maize productivity is greatly threatened by downy mildew (DM) especially in lowland areas in the southern region, and maize streak virus disease which is prevalent in all maize production areas. Grain yield losses of up to 100% can be incurred when MSV and/or DM susceptible varieties are grown. Therefore varieties for deployment in Mozambique should carry both MSV and DM resistance genes. The project objective is to develop high yielding maize varieties possessing resistance to both maize streak virus and downy mildew for the lowland environments in Mozambique.

2. Research Activities at the University of KwaZulu-Natal

The project entailed transferring a gene cluster for maize streak virus resistance from CML505 and CML509 (donor lines) into a Mozambican adapted line, LP23 which is known to have down mildew resistance. A total of 118 F_3 Recombinant Inbred Lines were derived from the F_2 populations of the crosses CML505 x LP23 and CML509 x LP23 at Makhathini Research Station in South Africa. DNA marker assisted selection techniques were employed to track the gene cluster in the progeny. The lines were

planted out in the tunnel at University of KwaZulu-Natal campus in South Africa. Flinders Technology Associates (FTA^{TM}) is a simple paper-based technology, which proved to be an efficient means to move DNA samples from remote regions without jeopardizing the integrity of the DNA used. DNA extraction and purification from FTA cards was optimized and standardized. Amplification and HRM analysis was done using a Rotor-Gene 6000 instrument and genotypes were automatically assigned by the Rotor-Gene software.

3. Greenhouse and Field Testing Activities

The MSV resistance of the progeny maize was confirmed under artificial inoculation conditions in the greenhouse at CIMMYT Research Station in Zimbabwe. The field testing of the progeny lines for MSV resistance was carried out under natural conditions at Chokwe Research Station in Mozambique. At Chokwe the spreader-row artificial inoculation technique was also applied to screen the progeny inbred lines for downy mildew resistance. At the same time resistant lines were self-pollinated to advance the generations to the F4.

4. Results and Implications

Of the 118 samples genotyped, 35 exhibited resistance to both MSV and downy mildew under field conditions. These lines are being fixed by self-pollination (from F4 to F8) at Makhathini Research Station and at CERU in South Africa, and at Chokwe in Mozambique. The lines will be used to improve the existing maize hybrids in Mozambique for MSV and DM resistance with implication on food security. Potential testers for use in making new hybrids with these lines have been identified, and their genetic diversity is being confirmed by SNP molecular markers. The hybrids and open pollinated varieties will be developed between these testers and the lines, for the benefit of the small-scale commercial and subsistence farmers in MSV and DM-prone lowland environments in Mozambique.

Rice

89. G3007.01: Base broadened of current crop diversity in rice using interspecific bridges with African rice

August 2007–October 2009; NCE: December 2010

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NO UPDATE SUBMITTED; PROJECT REPORT PENDING UPLOAD

90. G4008.08: Transcriptome analysis of near-isogenic rice lines to identify expression signatures and gene combinations conferring tolerance to drought stress

January 2008–December 2009; NCE: December 2010

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists
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International Rice Research Institute: Venuprasad Ramaiah, Arvind Kumar, Ramil Mauleon, Violeta Bartolome, Rachid Serraj, Hei Leung

Summary
Three approaches including phenotyping, genotyping and transcriptome analyses were taken to understand the molecular mechanism of two pairs of near isogenic lines (NILs) exhibiting large difference in their yield response to drought stress at reproductive stage. NILs were originally established for drought and tungro resistance through the backcrossing of Aday-Sel (drought-tolerant) to IR64. IR77298-14-1-2 was the highest yielding and IR77298-5-6 was the lowest yield among three IR77298 NILs tested in 12 field trials from 2003 to 2006. Two series of NILs --IR77298-5-6 and IR77298-14-1-2-- and their drought tolerant progeny IR77298-5-6-18 and IR77298-14-1-2-10 and drought susceptible progeny IR77298-5-6-11 and IR77298-14-1-2-13 together with IR64, as their recurrent parent, were used as plant materials in this project.

Each pair of NILs showed difference in biomass under water stressed conditions in the phytotron and the trend of biomass reduction was similar to that observed under field conditions. Compared with IR77298-5-6-18, IR77298-14-1-2-10 showed much higher tolerance to the drought stress treatment (0.5FTSW: Fraction of Transpirable

Soil Water). Ratio of water uptake was measured among IR64 and NILs. Water uptake difference between IR77298-14-1-2-13 and IR77298-14-1-2-10 was not so large, while IR77298-5-6-18 showed larger increase of water uptake relative to IR77298-5-6-11.

Genotyping data produced in IRRI by the chip-based genotyping analysis suggested that there was no common introgressed DNA region from Aday-Sel to IR77298-5-6 and IR77298-14-1-2 lines. By comparison between IR77298-14-1-2-10 (highly tolerant) and IR77298-14-1-2-13 (susceptible), five regions on three chromosomes showed differences Template GCP Final Technical Report Updated October 2008 in DNA introgression. They are, Chr2: 6-11Mb, 18-22Mb, Chr8: 3-19Mb, Chr11: 3-4Mb, 16-18Mb regions. In the case of IR77298-5-6-18 (moderately tolerant) and IR77298-5-6-11 (susceptible), only one region on Chr9: 14-16Mb showed a difference in DNA introgression. SNP-based genotyping analysis using the Illumina Golden gate system revealed possible introgressed regions on chromosome 5 to 12.

Gene expression analysis in leaves, panicles and roots of NILs based on the 44K oligoarray system was performed in NIAS. Global gene expression analysis revealed that many well-known stress responsive genes are activated in the non-stress condition in IR77298-14-1-2-10 (highly tolerant). Gene family based analysis revealed that drastic up- and down-regulation of transporter encoding genes was specifically observed also in IR77298-14-1-2-10 (highly tolerant).

Differentially expressed genes on the possible introgressed regions between the sister NILs also gave us a very interesting list of genes that can now be genetically validated through segregation analysis using progeny derived from inter--NIL crosses. OsGLP genes, one of the recently recognized as muti-stress responsive genes, show the differential expression between IR77298-14-1-2-10 and IR77298-14-1-2-13. Additional stress-responsive genes showing differential expression between the sister NILs were also found. These genes are subjected to bioinformatic analysis to determine their cis-regulatory relationship.

Combination of genotyping and transcriptome analysis, we have succeeded to narrow down the members of genes to be targeted.

Conclusions

- For understanding of the molecular mechanism of drought tolerance many near isogenic lines (NILs) have been established in IRRI. Among them, we have focused on the BC3F2-derived lines (IR77298-14-1-2 and IR77298-5-6) by backcrossing Aday Sel to IR64. The former showed the highest drought tolerance in yield and the latter showed the lowest drought tolerance among three NILs (including IR77298-12-7) in 12 field trials conducted from 2003 to 2006 in IRRI.
- Phenotyping experiments, including transpiration rate assay and the water uptake measurement, revealed that IR77298-5-6-18 had the lowest transpiration rate and the biggest change of water uptake under phytotron conditions. .
- Genotyping chip analysis and SNP-based genotyping analysis revealed the possible introgressed regions of four NILs.

- Transcriptome analysis revealed that the drought tolerant NIL (IR77298-14-1-2-10) has activated expression of many stress-responsive genes even in the non-stressed condition. This result suggests one of the possible molecular mechanisms against drought stress might be a priming (or pre-conditioning) of expression of the stress responsive genes.
- A short list of candidate genes (including OsGLP) that are drought-responsive are found in the possible introgressed regions in the NIL. Thus, by combining genotyping and transcriptome analysis using NILs, we have a means to rapidly narrow down the genes that may play important roles in conferring drought tolerance.

Final products to be used by plant scientists outside the project:

1. Provide empirical evidence that expression signatures are causally related to QTL phenotype for drought-tolerant detected in near-isogenic lines.
2. Breeding-ready NILs with defined chromosomal regions contributing drought tolerance
3. A panel of candidate genes as potential markers for selection in other breeding lines in drought breeding network
4. A high-quality transcriptome dataset (~44K genes) for two genotypic backgrounds under control, mild and severe drought stress; providing a rich resource for data mining.

Rice: Capacity building

Capacity building a la carte

91. G4007.13.05: Capacity-building *à la carte* 2007–An integrated proteomics and genomics approach to discover salt tolerance genes

July 2007–July 2009; NCE: August 2010

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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

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- Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Mohammad-Reza Hajirezaei
-

Summary
Rice is the primary staple food for more than 2 billion people in Asia, Africa and Latin America. Of the total calories consumed globally, 23% are supplied by rice. It is also an excellent model cereal for molecular biology and genetics research, because grass genomes share a large degree of synteny and rice genome is smaller than other cereals. Salinity is a major factor limiting rice production worldwide. It causes water

deficit, ion toxicity, and nutrient deficiency leading to molecular damage, growth and yield reduction and even plant death. Rice is generally considered to be sensitive to salinity. The analysis of stress-responsiveness in plants is an important route to the discovery of genes conferring stress tolerance and their use in breeding programs. Proteomic analysis provides a broad view of plant responses to stress at the level of proteins. To further understand the mechanism of plant response to salinity, we employed a proteomic approach to profile the protein changes of rice leaf and root under salt stress. We analyzed total and nuclear proteome of rice tolerant (FL478) and sensitive (IR29) lines backed up with metabolome analysis. Plants were grown in Yoshida nutrient solution in trays and after 25 days, plants were treated by 100 mM NaCl for 16. Our results showed that total dry weight leaf was decreased by salt stress starting from day 10. At 16 days after treatment this reduction was about 33% and 11% in susceptible (IR29) and tolerance (FL478) genotypes and clearly showed the different between two genotypes in response to salinity therefore we collected the samples for proteomics analysis at day 16.

The proteome and metabolome analyses were performed at ABRII and IPK, respectively, leading to identification of 104 salt-responsive proteins in rice leaf and root differentially expressed in two genotypes. The metabolimcs analysis also revealed different pattern of metabolomes in two genotypes in response to stress.

Conclusion

We applied proteomic and metabolomic approaches to analyze the differential abundance of proteome and metabolome of two contrasting rice genotypes under salt stress. Our results clearly showed that several many proteins and metabolites changed differently in two genotypes paving the way to identify candidate mechanisms controlling salt tolerance of rice.

Our results suggest that proteomics and metabolomics approach can be a powerful method to broaden our knowledge about plant–environment interaction at proteome and metabolome level. The number of stress responsive proteins ($P < 0.05$) were higher in the sensitive genotype, IR29, than in the tolerant genotype, FL478. The results provide evidence that salinity causes a redirection in protein synthesis, to a much greater extent in salt sensitive genotype. While this work provides experimental evidence on the contribution of several candidate proteins and mechanisms in salinity tolerance in rice, further investigation is required to precisely clarify the function of these proteins in salinity tolerance in planta.

More work is also needed to determine the relationship of timing of proteomic changes and salinity level. Proteome analysis of additional cultivars with different levels of salt tolerance may help to better understand rice behaviour under low temperature conditions. The difference in expression pattern can be examined genetically by further studies on mapping population derived from the cross between contrasting lines.

Final products to be used by plant scientists outside the project:

Key Products Developed by the Project

1. Training 3 staff for performing proteomics, and metabolomics
2. Identifying novel candidate genes and pathways involved in rice tolerance to salinity

92. G4007.13.06: Capacity-building à la carte 2007−Enhancing capacity of ICABIOGRAD in phenotyping and molecular analysis to develop elite rice lines suitable to Indonesian uplands

July 2007−July 2009; NCE: March 2010

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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

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- IRRI, Casiana Vera Cruz

Summary

Within a no-cost extension period of the GCP CB a la Carte, we had completed the construction of Rice Blast Nursery including one head house at ICABIOGRAD. The Head house of Rice Blast nursery was divided to three rooms; room one for preparing seed materials, room two for preparation and storage for blast isolates, and room 3 for seed storage of breeding materials. Those two storage rooms were furnished with air conditioner. A part of the available fund have also been utilized to improved Rice blast inoculation and incubation building including planting tools (pots, containers etc), adding four more sprinklers in the moist room. We are very gratefully to GCP, because these all facilities had greatly improved our capacity of testing plant materials for rice blast. They are not only can be use by GCP projects but also needed by other activities related plant improvement to rice blast at ICABIOGRAD. We conducted phenotypic evaluation by artificial inoculation with dominant blast races and genotypic evaluation using SSR primers for BC4F1-49 plants and BC4F1-162 plants at ICABIOGRAD. The selected plants were backcrossed to Way Rarem to get BC5F1 seeds. The pictures of rice blast nursery are in the bottom.

Conclusion

We have made good progress in all activities of the Capacity Building project that are directly relevant for application and product development.

- The completion of facilities for “Rice blast inoculation and Incubation rooms”.
- The completion of facilities for “ Rice blast nurseries including head house”
- The compilation of Laboratories equipment (One laptop computer, two vertical electrophoresis including power supply and accessories plus gel-doc cybrsafe filter, one refrigerated centrifuge.
- PhD Programme for manpower improvement is in progress (She is in the state of completion her research dissertation)
- Advanced backcross lines of two lines of Way Rarem and Oryzica Llanos-5 (from GCP-SP2 Project) are being developed and seeds of the most advanced lines (BC6F3) will become available next year.
- The molecular markers are being improved to get SSR or SNP markers that could recognize OL5 alleles in resistant advanced population (lines).

- The data obtained from these experiments are encouraging and we are optimistic that NILs of the Way Rarem and Oryzica Llanos 5 breeding lines will help to improve upland rice production in Indonesia.

Final products to be used by plant scientists outside the project:

1. Six SSR or SNIP marker developed for resistance to Blast disease
2. Five NILs of the Way Rarem and Oryzica Llanos 5 breeding lines with enhanced resistance to Blast disease

93. G4008.39.01: Capacity-building à la carte 2008–Enhancing MAS capacity for salt-stress rice breeding in Bangladesh

April 2008−April 2009; NCE: March 2010 (not documented)

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EXTRACT FROM FINAL TECHNICAL REPORT

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Summary
The upgrading of marker-assisted selection (MAS) laboratory in the Plant Breeding Division of Bangladesh Rice Research Institute (BRRI) was of vital importance to increase the efficiency of breeding for varietal improvement targeted to problem soils through the application of molecular markers. This project provided funding for the purchase and delivery of major equipments needed to complete the MAS laboratory at BRRI, and also co-sponsored a MAS workshop held in the new laboratory, facilitated by IRRI co-PIs. Sixteen scientists from National Agricultural Research Extension Systems (NARES) institutions including BRRI, Bangladesh Institute of Nuclear Agriculture (BINA) and Dhaka University (DU) were trained on MAS technology. Principal Investigator, Dr. M A Salam attended the GCP ARM meeting in 2008 in Bangkok, Thailand as the principal investigator of the GCP capacity building project. Under the travel grants of this project, one of the scientists of this project will be attending 14th Australasian Plant Breeding (APB) Conference and 11th Society for the Advancement of Breeding Research in Asia and Oceania (SABRAO) Conference 2009 from August 10-14 in Cairns, Australia to present the paper titled, “Coastal saline environment and rice variety development in Bangladesh.”

Conclusions

GCP assistance helped BRRI in enhancing breeding capacity and utilizing MAS technology. This project also helped strengthen the collaboration with national centers, as BINA and DU and with IRRI. BRRI now is capable of incorporated MABC in breeding programs on regular bases.

Sorghum

94. G3007.04: Tailoring superior alleles for abiotic stress genes for deployment into breeding programmes: A case study based on association analysis of Alt_{SB}, a major aluminium tolerance gene in sorghum (ALTSORGHUM)

August 2007–December 2009; NCE: December 2010

Principal Investigator and Lead Institute

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EXTRACT FROM FINAL TECHNICAL REPORT

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- INRAN: Soumana Souley; Maman Nouri; Magagi Abdou; Adam Kiari; Fatouma Beidari
-

Summary

Aluminum toxicity represents a major agricultural constraint in large regions of acid soils throughout the world. Based on the isolation of AltSB, a major aluminum tolerance gene in sorghum, we applied association analysis as a bridge to integrate our findings to sorghum acid soil breeding programs worldwide. Operationally, we looked for superior AltSB haplotypes in diverse association panels, isolated their allelic effects in near-isogenic lines, and defined elite haplotypes using SNPs and indels associated with Al tolerance, which were used for allele mining in breeding panels. We have found that high Al tolerance is extremely rare in sorghum as only 6% of the accessions in the panel, which belonged largely to the guinea race, were highly tolerant to Al toxicity. Our association studies allowed us to identify polymorphisms associated with Al tolerance with apparently variable linkage disequilibrium among sites. Low frequency alleles at associated loci can discriminate elite haplotypes, which would otherwise require extensive phenotyping to be identified since those are found at a rather low frequency in the association panel. Co-dominant, easy to use PCR assays were developed for haplotype selection for AltSB. Development of NILs (BC3F3 lines) harboring different AltSB alleles was completed and we phenotyped NILs developed from the top tolerant donors for Al tolerance in nutrient solution. We used our tag markers for allele mining in the US sorghum panel and, more recently, in two African panels, belonging to INRAN (Niger) and ICRISAT - Bamako. The tag markers were efficient to mine low frequency AltSB haplotypes in the US and ICRISAT – Bamako panels and selected lines were validated as Al tolerant in nutrient solution. For the INRAN panel, genetic background effects apparently resulted in

reduced phenotypic expression of AltSB. In that case, we started a marker assisted backcross breeding program to introgress the AltSB allele from SC566 into selected landraces; our data suggest that NILs with the SC566 allele retain a substantial portion of Al tolerance observed in SC566. We have also developed a 1536-SNP chip as a community resource and genotyped with these markers our combined association panel in addition to the parents of the MARS population developed by Dr. Jean François Rami. We also genotyped the parents of the BCNAM population in the Sorghum Drought Tolerance CI.

Conclusions

Impact of our finding for improving Al tolerance in Africa

With association analysis we have been able to generate tag markers that are useful to recover Al tolerant accessions directly in the breeding germplasm. This is possible because they represent either the causative polymorphisms of phenotypic differences encoded by *AltSB* or are in linkage disequilibrium with the former. However, Al tolerance in sorghum has a strong relationship with population structure and this was the subject of a new paper accepted in PLoS One. Therefore, allele mining is a feasible approach in sorghum but this should be done with a solid knowledge of population structure in the target germplasm.

Although we can identify highly Al tolerant accessions upon allele mining, genetic background effects are a reality for *AltSB*. Therefore, at this point at least some phenotyping is needed for final confirmation. However, with our DNA-bulking approach we can quickly genotype germplasm in the order of hundreds or even thousands. This can be done in-house with our tag assays our preferably within the Integrated Breeding Platform at high throughput.

Once elite *AltSB* haplotypes are identified, our data indicated that a traditional marker assisted backcross program based on *AltSB* only may result in extensive partial transfer of Al tolerance to the recurrent parents for some *AltSB* alleles. Within other projects we are studying in detail the nature of these genetic background effects and we have already been able to show them to derive from the loss of trans-acting factors (e.g. transcription factors) that activate *AltSB* expression. We have also added *AltSB* expression data to the association panel are undertaking whole genome expression profiling based on RNA-seq to identify candidate genes for these trans-factors. Our final goal is to isolate these factors and use MAS at auxiliary loci to improve the efficiency of our allele mining approach.

Our data also suggests that phenotypic effects at *AltSB* result from the interaction of cis-acting sequences (e.g. binding sequences) and trans-factors. As such, we have been able to identify two donors, SC283 and SC566, with which the partial transfer of Al tolerance to other genetic backgrounds appears not to be limiting. Because of that we are using SC566 as the *AltSB* donor to the INRAN recurrent parents and we expect to complete phenotypic characterization within the next weeks for the 90SN-7 introgression line. We will send the introgressed version of 90SN-7 back to INRAN upon confirmation of the Al tolerance phenotype.

Update to the Final Report Submitted in November 2010

SNP conversion to the KASPar system:

We worked with two SNP sets, the first one (384) was developed previously by IGD (Cornell) and the second one (1536) was developed within our project. For the 384

set, 310 SNPs yielded good quality data and were thus candidates for conversion. We conducted a population structure analysis on approximately 550 sorghum accessions and then calculated the minor allele frequencies (MAF) for each SNP locus within each subpopulation defined with STRUCTURE. Our previous studies based on SSR markers showed patterns of population structure that were consistent with both racial and geographic origins. The mean MAF was 0.21 across the whole panel. Our rationale was to keep loci that show reasonable MAF (f>0.05) within supopulations as a SNP set that can be broadly applicable to different germplasm sets is desirable.

For the 1536 SNP set, 1291 yielded good quality data. The mean MAF was 0.31.Only 12 loci showed MAF<0.05 and 65 had MAF<0.1. The physical distribution of these SNPs was also evaluated. We selected a total of 1601 SNPs that are now being converted to the KASPar system and will be available for marker activities in sorghum. Marker assisted introgression of *AltSB* into INRAN landraces

We have finished producing BC2F3 progeny having 90SN-7 as recurrent parent and SC566 as the *AltSB* donor. These progeny are now being phenotyped for Al tolerance in nutrient solution along with the parents. We will update the GCP with these results. If the introgression line confirms acceptable recovery of the donor Al tolerance, we will repatriate these seeds to Africa for field testing within the ALTFIELD project.

For NR360, after foreground selection we undertook selection with CTG29, which is tightly linked to *AltSB*. We have been able to select only 6 progeny showing single recombination events between *AltSB* and CTG29, which were thus homozogyous for the recurrent parent allele at CTG29. However, we were not able to backcross these progeny to NR360 due lack of flowering synchrony between the male and female parents. We have initiated a backcross breeding program with IRAT-204 and have finished the first cycle of marker assisted selection following a similar strategy to that used for 90SN-7. We are now generating BC2F1 progeny, which will be advanced to BC2F3 fixed for *AltSB*.

Quantifiable Outputs:

1. Seeds for over 500 accessions multiplied and available for association studies.
2. Development of a public database for sharing molecular marker information, including summary statistics tables and matrices of population structure and relatedness.
3. All panels (LR-Cornell, LR-CIRAD and IL-Embrapa) characterized for Al tolerance in nutrient solution
4. Subset of the association panel characterized for aluminum accumulation in root apices.
5. Subset of the association panel characterized for organic acid exudation.
6. Subset of the association panel characterized for *AltSB* expression
7. NILs characterized for aluminum tolerance in nutrient solution
8. Sorghum accessions characterized for Al tolerance in nutrient solution (size depending on the Al tolerance and haplotype results).
9. SNP markers for the *AltSB* containing region (~25 Kb)
10. SNP markers for six candidate Al tolerance genes (4-5 Kb)
11. Haplotype-based markers for aluminum tolerance alleles
12. Genotypes for candidate Al tolerance genes determined in the association panel
13. LD decay around *AltSB* and candidate loci determined

14. Candidate Al tolerance genes and *AltSB* locus tested for association to Al tolerance
15. Generation of BC3F3 NILs harboring elite *AltSB* haplotypes
16. Development of low-cost SNP assays (number depending on the number of elite haplotypes found in the association panel)
17. Subset of the INRAN collection characterized for aluminum tolerance in nutrient solution
18. One well-adapted landrace and one INRAN breeding line converted with superior *AltSB* haplotypes

Wheat

95. G4007.06: Integrating marker-assisted selection into the conventional breeding procedure for improvement of wheat (*Triticum aestivum* L.) in the drought-prone areas of Northern China

August 2007–July 2010

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Developing wheat cultivars with improved drought tolerance (DT) is an efficient way to stabilize wheat production and alleviate food insecurity in China and the world. However, the genetic improvement of DT is a challenging task because of the complexity of these traits in crop plants, environmental factors, and their interactions. Although conventional breeding approach has been successful in breeding for drought tolerance in crops, there is still problem to select genotypes for DT. The project intend to (1) integrate MAS into wheat conventional breeding program and introduce quantitative trait loci (QTLs) responsible for DT in widely cultivated cultivars, (2) screen wheat germplasm resources and offspring of breeding by MAS, and (3) develop a shared practice and collection of resources, information, technology and methodology in wheat molecular breeding for DT. The major intended outputs are (1) to identify and validate 8~10 target DT QTLs and characterize diverse elite Chinese wheat backgrounds in the target environments, to develop 10~15 candidate accessions carrying target genes/markers and elite Chinese wheat backgrounds from the ILs for molecular breeding; (2) to breed 50~60 stable lines carrying target genes/markers using MAS.

Mapping QTLs for plant height developmental behaviours in wheat
A list of traits related with drought tolerance was dissected. One example is the plant height (PH), a crucial quantitative trait related to yield potential in crop plants. Its full expression can be inhibited by a limited water supply. We mapped QTLs for PH developmental behaviours in wheat by two genetic populations, a doubled haploid (DH) population, and a recombinant inbred line (RIL) population. The genetic basis of the PH developmental behaviours was assessed in 10 environments (year × site × water regime combinations) by unconditional and conditional QTL analyses in a mixed linear model. A few QTLs, such as *QPh.cgb-6B.7*, showed high adaptability for water-limited environments. Many QTLs, including four A-QTLs (*QPh.cgb-2D.1*,

QPh.cgb-4B.1, *QPh.cgb-4D.1*, and *QPh.cgb-5A.7*) coincident with previously identified reduced height (*Rht*) genes (*Rht8*, *Rht1*, *Rht2*, and *Rht9*), interacted with more than one other QTL, indicating that the genetic architecture underlying PH development is a network of genes with additive and epistatic effects (Wu et al., 2010).

Validating QTLs for plant height developmental behaviours in wheat

Using a historic collection with 270 accessions and backcross lines, total of six genomic regions covering PH QTL clusters on different chromosomes identified from the genetic populations were used as the candidate QTLs for DH. Five additive QTLs and eight pairs of epistatic QTLs significantly affecting plant height development were detected by unconditional QTL mapping method. Six additive QTLs and four pairs of epistatic QTLs were validated using conditional mapping approach. Among them, three additive QTLs and three pairs of epistatic QTLs were common QTLs detected by both methods. Three QTLs were expressed under both drought and well-watered conditions. The 52 accessions with *Xbarc109-4B*$_{220}$ were taller than those of plants carrying other alleles in periods 2 and 3, except under well-watered conditions in period 3. Greater drought tolerance at maturity was associated with *Xgwm133103-4D* (10 accessions) and *Xwmc112238-4D* (37 accessions), the allelic effects were 0.033 and 0.009, respectively. Accessions with *Xgwm133-4D*$_{119}$ (10 accessions) and *Xwmc112-4D*$_{230}$ (26 accessions) had less drought tolerance than accessions with other alleles at maturity (Table 1). A list of association was identified in the regions of gene *Rht*, indicating a consistency of association analysis with linkage mapping (Zhang et al., 2011).

Table 1. Phenotypic effects of marker alleles significantly associated with DTC in plant height development[a]

Trait	Locus	Allele size (bp)	Allele effect	Trait	Locus	Allele size (bp)	Allele effect
DTC_m[b]	*Xgwm133-*	*103*	0.033		*Xgwm495-4B*	*155*	0.052
		113	-0.031			*159*	-0.051
		119	-0.068			*179*	-0.042
	Xwmc112-4D	*229*	-0.008	DTC_4	*Xgwm18-1B*	*183*	0.016
		230	-0.026			*185*	-0.007
		238	0.009			*187*	0.006
DTC_1	*Xgwm126-5A*	*192*	0.073		*Xbarc187-1B*	*255*	0.052
		194	0.159			*259*	0.028
		198	0.118			*264*	-0.026
DTC_2	*Xcfd43-2D*	*164*	-0.081		*Xbarc228-2D*	*172*	-0.016
		166	-0.083			*174*	-0.044
		168	-0.064			*177*	-0.033
	Xbarc109-4B	*229*	-0.107		*Xwmc41-2D*	*150*	-0.075
		232	-0.098			*153*	-0.064
		235	-0.131			*162*	-0.014
DTC_3	*Xcfd43-2D*	*164*	0.020				
		166	0.018				
		168	0.016				

[a] Three alleles with the largest effects for each locus are shown; [b] DTC_m: drought tolerance coefficient of plant height at maturity; DTC_1, DTC_2, DTC_3 and DTC_4: drought tolerance coefficients of conditional plant heights in periods 1, 2, 3 and 4.

Selected lines carrying target genes/markers

A total of 68 backcross lines of BC_3F_{3-5} were used to validate the QTLs detected in the genetic populations and historic collection. Some lines pyramiding multi-allele with effect of increasing or decreasing plant height exhibited a superiority over the opposite lines. For example, three lines of BC_3F_{4-5}, 10TZ28 and 10TZ33 derived from a backcross [(Lumai 14/Shanhan 8676)/Lumai 14]BC_3F_5, and 10TZ38 from [(Lumai 14/Xifeng 20)/Lumai 14]BC_3F_4 with target alleles of QTLs and agronomic traits were identified in the field experiments in Hebei Province, Shanxi Province and Beijing in the last two grown years. They have been selected by breeders to submit to the national field experiments in 2012 autumn under limited water and rainfed conditions, respectively.

The present data are useful for wheat genetic manipulations through molecular marker-assisted selection (MAS), and provides new insights into understanding the genetic mechanism and regulation network underlying the development of plant height in crops. This case, mapping and validating QTLs for plant height developmental behaviours in wheat indicates the possibility of molecular breeding for plant complex quantitative traits.

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Cross-crop projects

96. G4007.05: Bridging genomics, genetic resources and breeding to improve wheat and barley production in Morocco

January 2007–December 2009; NCE: December 2010

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1. Research activities and progresses at INRA-Morocco and its partners

This project aims at utilizing resources (reference sets, genomic) and tools (genomic) developed by GCP, CG-Centers, ARIs, and NARS for improving wheat and barley. We followed a forward genetics approach, where, we screened germplasm collections (reference sets, bread wheat collections provided by GCP, Mediterranean durum wheat collection and durum wheat collections from ICARDA) to identify variability and used some of the variability for development of mapping populations and conventional and marker-assisted breeding. In 2008, screening of bread wheat grmplasm resulted in identification of 7 lines resistance to Hessian fly. Screening of durum germplasm led to identification of 11 lines resistant to leaf and yellow rusts, and 16 lines resistant to root-rots. In barley, 28 lines identified as resistant to gal midge and 9 are to net-blotch disease. During 2009, screening of barley germplasm resulted in identification of 6, 20, 25 and 75 lines resistance to Barley Stem Gal-midge, the Hessian fly, net bloch and powdery mildew diseases. During 2010, ME bread wheat germplasm collated by GCP was screened for root rot and *Septoria tritici* bloch and identified 32 and 9 resistant lines respectively. These resistance sources had been deployed in the breeding program to broaden the genetic base.

A set of germplasm relevant to the regional breeding programs with known tagged markers were procured for their use in marker-assisted section (MAS) program. These traits include, leaf, yellow and stem rust resistance, quality traits such as high grain protein content, and glutine strength. The crossing programs for MAS are currently at various stages, aiming mainly on pyramiding genes. Doubled haploid technique was integrated into MAS, to speed up the varietal developmental process. During the project period, MAS was implemented for traits such as leaf and yellow rusts (Lr34/Yr18), high-temperature adult-plant (HTAP) stripe rust resistance gene Yr36, high grain protein content gene, rye chromosome 1R (1RS: known to provide resistance to insects, diseases and reported improvements in yield potential and water-use efficiency), dwarfing genes (Rht8, RhtB1 and D1). Glutenine strength (Glue genes) and drought (from Dharwar dry background using Xwmc89 marker). One example of MAS program intended to incorporate high grain protein content, yellow rust resistance, and rye chromosome segment 1RS (the short arm of rye chromosome 1R which provides resistance to insects, diseases and reported improvements in yield potential and water-use efficiency) into a cultivar. A cross was made between Tillila (which a well adapted variety of Morocco, with 1RS) and Yecora rojo-Gpc-B1 (which

has genes for Yr36, high grain protein content, dwarfing genes RhtB1 and D1). MAS for high grain protein content and Yr36 was applied at BC1F1 stage. During 2009, the anthers from selected BC1F1 individuals were used for production of ~ 1000 haploids. The resulting haploids were tested for presence of 1RS, high protein content/Yr 36 genes using MAS. Simultaneously part of the BC1F1 were backcrossed to 'Tilila' to generate BC2F1 generation. The select haploids were diplotized using cholchicine, the resulting DH were back ground selected using SSRs and produced 70 doubled haploids (DH). These selected DH were seed increased under green house condition. These DH will be planted in the field during coming year for evaluation under field conditions. Similarly, other crosses were developed with varies targets. The target of these breeding strategies for the other crosses were to produce wheat lines derived from an elite lines (Moroccan /or Australian cultivars), with superior dough properties and durable rust resistance donated from 'Annuello'. Molecular markers were used to screen a BC1F1 population produced from a cross between the recurrent parent (most of the cases Moroccan elite) and the donor parent ('Annuello'or/Dharwar dry, etc.) for the presence of rust resistance genes (Lr34/Yr18 and Lr46/Yr290), drought QTL (Dharwar dry), etc. Following this, marker-assisted selection will be applied to haploid plants, prior to chromosome doubling with cochicine in 2011. The group has also initiated MAS activities based on marker-assisted recurrent selection. The molecular breeding program established through this GCP grant will be continued future and group is looking forward to mobilize new grants from the suitable donors.

During entire project period, 15 and 9 students/scientists have completed their individual training and Master Degree thesis, respectively. The project resulted in 11 publications in the form of symposium poster/oral communications (10) and journal article (1). Others are in preparation.

In conclusion, this project helped INRA Morocco to integrate various disciplines in involved in wheat improvement and in implementing the molecular breeding program, which is unique in the country and the North Africa region. The identified useful genetic diversity in the germplasm were deployed in the breeding programs to widen the genetic base of wheat and barley. Similar approach and techniques will be adapted for other crop species in future.

Tangible outputs delivered
The resistant wheat lines to various biotic stresses identified through screening germplasm and breat wheat lines derived through MAS and DH. Trained students and researchers in molecular marker applications.

97. G4007.23: Field evaluation of wheat–barley introgression lines under different water regimes

December 2007–December 2010

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1. Research activities and progresses at ARI-HAS and collaborators

The present project aimed to use the wheat/barley addition, substitution and translocation lines developed in Martonvásár to determine how the added barley chromosome (segments) influence various agronomic traits (drought, salt and Al-tolerance) in wheat. It was planned to confirm the results achieved by earlier mapping data or to find new chromosome regions responsible for parameters connected with drought-, salt and Al-tolerance.

Table 1. The yield of the 2H, 3H, 4H, 6HS and 7H Mv9kr1/Igri and 4H, 6H, 7H Asakaze komugi/Manas disomic addition lines, the 4H(4D) substitution line and the 3HS.3BL, 2DS.2DL-1HS, 6BL.6BS-4H and 7DL.7DS-5HS translocation lines in the field.

Genotype	Amount of seeds harvested in Martonvásár (Hungary) [kg]			Amount of seeds harvested in Keszthely (Hungary) [kg]	
	2008	2009	2010	2009	2010
2H Mv9kr1/Igri addition line	1.30	0.05	2,38	0.53	0,46
3H Mv9kr1/Igri addition line	1.56	0.13	2,19	0.58	0,02
4H Mv9kr1/Igri addition line	0.85	0.07	2,02	0.34	0,19
6HS Mv9kr1/Igri addition line	-	-	0,21	-	-
7H Mv9kr1/Igri addition line	-	0,04	0,14	-	-
4H(4D) substitution line	4.10	0,28	1,85	0,50	0,41
3HS.3BL centric fusion line	6.06	2,52	2,17	1,34	0,79
2DS.2DL-1HS translocation line	0.15	0,02	0,07	0,06	0,07
7DL.7DS-5HS translocation line	5.8	0,87	4,7	1,1	0,62
6BL.6BS-4H translocation line	-	0,02	1,60	0,02	0,39
4H Asakazekomugi/Manas add. line	0.11	0,1	0,10	0,6	0,60
6H Asakazekomugi/Manas add. Line	-	0,12	0,12	-	-
7H Asakazekomugi/Manas add. Line	-	0,22	0,12	-	-

Tangible outputs delivered

1. More than 0.5 kg seeds per year were produced of the 2H, 3H, 4H Mv9 kr1/Igri disomic addition lines, the 4H(4D) substitution line, the 3HS.3BL, 7DL.7DS-5HS and the 6BL.6BS-4H translocation line (Table 1). A small amount of seeds (144 g) were produced on the 2DS.2DL-1HS translocation line because of its low fertility. The 4H, 6H, 7H Asakaze komugi/Manas and the 6HS, 7H Mv9 kr1/Igri addition lines were also multiplied in the field.

2. More than 30 plants were selected of the 2H, 4H, 6H, 7H Asakaze komugi/Manas disomic additions, using GISH and FISH and were grown in the phytotron in Martonvasar. Eight disomic additions were selected from the 3H monosomic additions using GISH.

3. A 4HL.5DL homozygous wheat/barley centric fusion was selected and multiplied from the progenies after crossing the 4H(4D) substitution line with the CS ph1b mutant.

4. New wheat/barley translocations have been selected from the progenies of the Asakaze komugi × Manas hybrid.

5. Physical mapping of three wheat/barley translocation lines (5HS.7D, 4BS.7HL, 4D.5HS) were carried out with combination of GISH and molecular markers.

6. Phenotyping was carried out on the wheat and barley parental lines, the 2H, 3H and 4H Mv9 kr1/Igri disomic addition lines, 4H Asakaze komugi/Manas disomic addition, 2H Asakaze komugi/Betzes, 6H Mv9 kr1/Betzes disomic additions, the 4H(4D) substitution line and the 3HS.3BL, 2DS.2DL-1HS, 6BS.6BL-4H and 7DL.7DS-5HS translocation lines in dry conditions in the field using rain shelters in Martonvásár and in China

7. Determination of the salt tolerance of the 2H, 3H, 4H Mv9 kr1/Igri and the 4H, 7H Asakaze komugi/Manas disomic addition lines, the 4H(4D) substitution line and the 3HS.3BL, 2DS.2DL-1HS and 7DL.7DS-5HS translocation lines together with the parental wheat and barley cultivars has been carried out in Eger. According to our studies the 7H Asakazekomugi/Manas addition line is tolerant to moderate (200 mM NaCl L-1) salinity. The 2H, 3H and 4H Mv9kr1/Igri addition lines are highly salt sensitive.

8. Determination of the Al- tolerance of the progenies of the Asakaze komugi × Manas hybrids has been carried out in Martonvásár. Manas had a good Al-tolerance in the Al tolerance test, but the wheat parent Asakaze komugi also had good Al tolerance, thus all the tested Asakazekomugi/Manas addition lines (4H, 6H, 7H) had good Al tolerance

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98. G4008.01: Populations for multiple allelic segregation developed in rice and sorghum through multiple parent intercrossing

December 2008–December 2009; NCE: December 2010

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EXTRACT FROM FINAL TECHNICAL REPORT

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Summary
Aim of the MAGIC is to increase the linkage disequilibrium (LD) decay and the precision with which genetic markers are linked to quantitative trait loci responsible for multiple traits within a population. In fact MAGIC is a two way extension of traditional method of searching for marker-trait correlations in the segregating progeny of crosses between two parents. Firstly, the mapping population is established by intercrossing multiple founder lines. A MAGIC population is therefore genetically diverse thus more QTL can be detected within one population. Secondly the population is cycled through several extra generations of crossing. Each extra generation mills the genetic contribution from the founder lines finer and considerably decrease the linkage disequilibrium. QTL are therefore located with greater accuracy and are of more use in plant breeding and genetical research.

We propose to establish MAGIC populations in rice as a permanent resource for identification of QTL and extraction of useful pre-breeding lines for target environments. We initiated the crossing program using two mating pools consisting of indica and japonica genotypes representing the two different eco-geographic races of rice.The founder lines include traditional and modern varieties known to exhibit tolerance to a suite of biotic and abiotic stresses, wide-adaptation, high-yield potential, and good grain quality. The broad diversity represented by these two populations provides considerable scope for use in Asia and Africa.

By the end of the first phase of the MAGIC project (2008-10, with a 1-year no-cost extension), we completed (a) all the 2-way, 4-way, and 8-way crosses of the indica population and (b) all the 2-way and 4-way crosses and 90% of the 8-way crosses of the japonica population (due to asynchrony in flowering of some japonica genotypes).

For both populations, we started the selfing cycles to develop recombinant inbred lines (RILs). In addition, we have established (a) 50 SSR and 1,534 SNP markers to determine the genetic relatedness of the founder lines; produced dendrograms

depicting the similarity among genotypes; and (b) a set of SSR markers to monitor line purity, hybridity, and progress of the mating cycles.

Beyond the target objectives of the GCP, we have pursued additional cycles of intercrossing to increase the recombination (intercrossing 8 ways) and expanding the diversity (crossing between the indica and japonica MAGIC pools). By June 2011, we have obtained the following populations:

- Indica MAGIC (original from 35 8-way crosses) Harvested 1,437 S4 lines and 602 S3 lines (these S2 lines are due to the inclusion of late-maturing lines)
- Indica MAGIC Plus (from the second round of intrapopulation mating of 175 8-way F_1s) Harvested 2,000 S2 lines (derived from selfing of at least 12 seeds per F_1, >12 seeds of 1/175 F_1 and so on)
- Japonica MAGIC (original from 27/35 8-way crosses) Harvested 567 S2 lines
- Global MAGIC (indica × japonica interpopulation mating) Obtained seeds from 128/175 F_1s (10–20 seeds per F_1 line) 1,402 S1 lines

These materials enable us to pursue a second of the Rice MAGIC project which focuses on the characterization and deployment of the indica MAGIC population. The proposal was submitted to GCP on August 12, 2011 for consideration.

Conclusions and Outlook

By June 2011, we obtained four MAGIC populations, beyond the original expected outputs.

- Indica MAGIC (original from 35 8-way crosses) Harvested 1,437 S4 lines and 602 S3 lines (these S2 lines are due to the inclusion of late-maturing lines)
- Indica MAGIC Plus (from the second round of intrapopulation mating of 175 8-way F_1s). Harvested 2,000 S2 lines (derived from selfing of at least 12 seeds per F_1, >12 seeds of 1/175 F_1 and so on)
- Japonica MAGIC (original from 27/35 8-way crosses). Harvested 567 S2 lines
- Global MAGIC (indica × japonica interpopulation mating). Obtained seeds from 128/175 F_1s (10–20 seeds per F_1 line)

Our experience in developing the MAGIC populations has been positive in that we learn about the critical stages and the amount of work involved in developing the genetic materials. Judging from the phenotypes of S1 and S2 lines (still segregating), we have captured the diversity. It gives us confidence that the two MAGIC populations have considerable potential for increasing efficiency to combine desirable traits in highly adaptive genetic background. With high resolution genotyping (ultimately whole-genome sequencing), the MAGIC populations can be highly valuable for rapid QTL mapping and functional assignment in highly resolved genomes (at gene or gene cluster level). Best of all, these are breeding ready genetic stocks from which adaptive varieties can be extracted.

As the next step forward to advance and utilize the MAGIC populations, we are considering two intervention points (as illustrated in Figure 4 showing the advancement of the indica population)

1. Extraction of heterogeneous inbred family (HIF) lines from the S2 or S3 population.
2. Anther culture to develop double haploid lines using F1 plants from the extra-mating cycles.

Finally, we are considering whole-genome sequencing to establish a permanent genotype database for the highly recombined inbred lines. As reported above, the founder lines have already been subjected to SNP genotyping with a 1536 chip to generate whole-genome graphical genotypes anchored to the rice physical map. This is expected to provide 300-500 well-spaced polymorphic markers for any pair of parental lines. In view of the declining cost in whole-genome sequencing, we will perform low-depth (0.5 to 1X depth) of the RIL derived from the extra-mating cycle of the indica population. Based on the estimate of 34 recombination breakpoints per RIL derived from a single meiosis (Huang et al. 2009), we expect to have approximately 170,000 recombination captured in 1,000 lines after 5 rounds of meiosis. This will yield an average of recombination at the average of 2-3 kb. Although these rough estimates need to be empirically determined, it demonstrates the potential of the MAGIC populations for dissecting QTLs and breeding, and contributing to the long-term goal of assigning functions to the whole rice genome.

Quantifiable outputs:

1. Suitable crossing scheme(s) to develop the populations – *done*
2. Selected agro-ecological environments defined for each crop - *done*
3. Eight founders identified for each MAGIC population - *done*
4. Stock seed for each population – *done*
5. Stock seed from an advanced generation of each population. *Done for indica at S3 genereatio. Will be done for japonica at S2 generation by October 2011.*
6. 2000 S2 progenies from two populations – *initially done for indica with 386 SNP marker panel. Will repeat genotyping using Genotyping-by-sequencing (GBS) method. S2 lines of japonica will be available in October2011. Selected progeny will be genotyped using GBS.*
7. A marker-validated pedigree for progeny—*done*
8. Baseline agronomic phenotype data on up 16 founder lines for rice – *done*
9. Genotype data from a genome-wide set of markers on up to 16 founder lines, samples of 50 plants from the initial generation of each MAGIC population and on 2000 S1 or S2 progenies— *Genetic diversity analysis of 16 founder lines was determined using panel of 50 standard SSR markers spread across throughout the genome. Founder set were run on 1536 SNP platform at Cornell Univ. S3 progeny of indica population was analyzed with SNP markers.*

Other crops

Barley

99. G3007.02: Genomic dissection of tolerance to drought stress in wild barley

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Summary
We combined genomics and genetic resources available in barley to identify regions of the genome that confer drought tolerance and morphological variation. We genotyped a set of Recombinant Chromosome Substitution Lines (RCSLs) derived from a cultivated recurrent parent and a naturally drought and salt tolerant wild donor from the Fertile Crescent, (RCSLs see Matus and Hayes 2003) with 1536 SNPs developed for barley (Close et al., 2009). Of the 1536 SNPs assayed 765 were polymorphic with an average of 107 SNPs per chromosome and 1 SNP every 1.6cM. The RCSLs were phenotyped in 3 environments in Chile (Santa Rosa irrigated, Santa Rosa dry and Cauquenes dry) and 1 environment in Syria (Tel Hadya). QTL analysis was performed using the genotypic data and agronomic and drought trait scores over two seasons. Sixty one QTLs were identified for 14 traits, 28 at Santa Rosa irrigated site, 18 at Santa Rosa dry, 14 at Cauquenes and 1 at Tel Hadya. Several QTLs were identified which have positive effects from the introgressed wild barley donor allele, those RCSLs with the introgressed segment showing significantly higher yields under drought than those without the wild barley introgressions. Associated SNPs were aligned with rice and many of the homologous genes identified are known to be involved in drought tolerance and yield in a range of plant species including barley and wheat. As well as the RCSLs we tapped into a set of agro-ecologically adapted lines termed the Syrian and Jordanian Landrace Collection (SJLC) maintained at ICARDA. These were also genotyped using 1536 SNPs and using historical trait data collected from ICARDA we performed an association analysis. Of 17 significant

associations detected, 8 were associated with yield characteristics, 5 with plant height and 4 with flowering time. Several of these significant associations were around known candidate regions identified in previous studies, such as flowering genes on 2H ***(Comadran* et al., *2010 submitted).*** The significantly associated SNP loci were aligned to rice chromosomes and homologies to candidate genes identified. From these alignments, 13 primers pairs were designed to larger regions containing potential candidate genes and sequenced in a subset of landraces. The number of SNPs identified varied from 1 to 44, and were organised into between 2 and 8 haplotypes with corresponding haplotype diversity values of 0.166 to 0.746. These new polymorphisms were then screened for restriction enzyme recognition sites and where possible CAPs (Cleaved Amplified Products) markers were designed to these regions. These were validated in a complete germplasm set and the association analysis repeated. 6 remained significantly associated with the trait.

Conclusion

In this project we have identified genomic regions that have been introgressed from wild barley into an elite cultivated line that confer a significant increase in yield under droughted conditions. Based on this information we have then surveyed a collection of landraces to evaluate the extent of molecular haplotype diversity in these regions under the assumption that this will be representative of functional diversity. We have not yet tested this assumption. The very high quality genotypic data along with the biological resources that we have characterised (esp. RCSLs) form an essential resource for the identification of the genes underlying the recorded phenotypes, e.g. through the generation of increasingly higher resolution QTL-NILs via backcrossing to Harrington, selfing and genotyping the progeny with markers that we now know define the region. Getting to this stage was an anticipated output of the project. Gene identification was beyond its scope but if deemed appropriate could now progress rapidly - but would require additional resources.

Our project conclusively demonstrated the power and efficiency of highly parallel SNP-based marker analysis for germplasm characterisation and genetic analyses – at least in barley. Our positive experiences convince us that there are opportunities to use such platforms to develop new and innovative ways of breeding plants and understanding how networks of genes and alleles contribute positively and / or negatively to key plant traits. Using some of the trait QTL and haplotype diversity information generated in this project may be one way to proceed, but we believe that the practicalities of doing this needs to be carefully thought through as there now may be more efficient alternatives (e.g. genomic selection - GS). Also, as a note of caution, it is clear from the data we have generated in both this GCP funded project and in additional projects with our groups (where we have examined wild and landrace populations) that SNP ascertainment bias is likely to be a serious impediment to the extended use of the current barley SNP platform for the analysis of highly diverse genetic materials such as those commonly evaluated within the CGIAR (e.g. for germplasm characterisation, GWAS or GS). Consequently, more informative and representative analysis platforms still need to be sought / developed for diverse material (a situation likely to be common to all GCP focus species). While this could be easily done using existing technologies, it would again require further investment.

Final products to be used by plant scientists outside the project:

1. QTLs identified for drought in barley.
2. Small regions of wild barley identified showing increased yield under drought.
3. Candidate genes identified from homologous regions in rice.
4. SNP regions identified from SJLC association panel.
5. Candidates identified from SJLC and markers developed and validated.

Pigeonpeas

100. G4008.47: Developing genomic resources for pigeonpea using next generation sequencing technologies

August 2008–July 2010; NCE: December 2010

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Summary

Pigeonpea (*Cajanus cajan* L.), an important legume crop in Indian subcontinent, ranks sixth in area and production. However, the productivity of pigeonpea crop in semi-arid regions is less than 750 kg/ha due to exposure of the crop with several diseases. Biotechnological tools especially molecular markers have been proven very useful for improving the breeding efficiency in several major crop species, however, few genomic resources and low level of genetic diversity in pigeonpea germplasm is another bottleneck to varietal improvement. To achieve the goal of generating large expressed sequence tag (EST) resources, Roche/FLX 454 sequencing was carried out on a normalized cDNA pool prepared from 31 tissues produced 494,353 short transcript reads (STRs). 150.8 million Illumina sequence tags were generated from 10 pigeonpea genotypes. For identification of SNPs, tags for two genotypes of a given mapping population were aligned with 127,754 TAs (the pigeonpea transcriptome assembly). The number of SNPs in an individual cross ranged from 704 to 6,263. In total, 12,141 SNPs were identified across these genotypes. In terms of developing the marker platforms, CAPS markers could not be validated at a good rated. As a result, a total of 1,834 SNPs were attempted for KASPar assays and successful assays were developed for 1,616 SNPs and tested on a set of 94 genotypes. In addition, four genetic maps were developed based on SSR markers and by using these maps, in addition to two earlier maps, a consensus genetic linkage map was developed that includes a total of 339 SSR marker loci. Activities wise progress made in this project have been given as follows:

Activity 1: Develop pigeonpea EST resources using 454-FLX
Based on its phenology and the utility in breeding programs Pusa Ageti (ICP 28) was chosen for developing these genomic/transcriptomic resources. Deep sequencing was undertaken on cDNAs pools of 31 different developmental stages. Roche/FLX454 sequencing of this normalized cDNA pool generated 494,353 short transcript reads (STRs). Publicly available ESTs and newly generated datasets were analyzed separately and in combination. In order to develop a transcriptome reference in pigeonpea, 505,170 Roche/454 STRs and Sanger ESTs were assembled in combination to yield a total of 127,754 pigeonpea transcript assemblies (CcTAs).

Activity 2: SNP development through Solexa-based transcriptome sequencing from 10 pigeonpea genotypes
For identification of SNPs, tags for two genotypes of a given mapping population were aligned with 127,754 TAs and variants were identified using the Alpheus program of NCGR. The number of SNPs in an individual cross ranged from 704 (BSMR 736 × TAT 10) to 6,263 (ICPL 87119 × ICPL 87091). In total, 12,141 SNPs were identified; however, only six SNPs were found in common across three populations (ICPL 20096 × ICPL 332, ICP 7035 × TTB7 and BSMR 736 × TAT 10) (Dubey et al. 2011).

Activity 3: Genetically map within existing mapping populations SNPs
Due to non availability of suitable restriction sites, CAPS assays could be designed for only 116 SNPs. A very low level i.e., only 10 SNPs out of 116 SNPs showed expected results. As a result, these efforts were abandoned. For developing the Illumina GoldenGate assays, ADT scores were calculated and submitted to Illumina pipleline. However because of inordinate delay in receiving reagents, it was decided not to undertake development of GoldenGate assays. As a result, a total of 1,834 SNPs were attempted for KASPar assays and successful assays were developed for 1,616 SNPs and tested on a set of 94 genotypes. In addition, four genetic maps were developed based on SSR markers and by using these maps, in addition to two earlier maps, a consensus genetic linkage map was developed that includes a total of 339 SSR marker loci.

Activity 4: Develop a consensus map for pigeonpea
Genotyping data generated for four intra-specific mapping populations (ICPB 2049 × ICPL 99050, ICPA 2039 × ICPR 2447, ICPA 2043 × ICPR 3467 and ICPA 2043 × ICPR 2671) together with earlier published two genetic linkage maps (ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035) were used for developing the consensus genetic map in cultivated pigeonpea. Segregation data for 348 markers obtained on 6 different mapping populations was used for merging multiple genetic maps. All the common markers collectively led to the synthesis of a consensus map comprising 339 loci profiled on 11 LGs and covering a map distance of 1,058.98 cM (Bohra et al. communicated).

References
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Sweet potatoes

101. G4008.09: Genomic resources and mapping populations for sweet potato developed to enable trait/gene identification

January 2008, End: December 2009; NCE: June 2010

Principal Investigator and Lead Institute

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To improve access by breeders to the diversity contained in the world potato collection, we have established a Sweetpotato Composite Genotype Set (CGS) consisting of 472 accessions that represent both, the diversity of sweetpotato and the most important agronomical and nutritional traits. The population structure of this set was characterized with molecular markers including microsatellites and DArT. The CGS is available for distribution in the form of disease-free in vitro plants.

High throughput sequencing of normalized cDNA libraries from leaves and stems produced a large amount of gene sequence information. The sequences have been processed, merged with sweetpotato EST sequences available from databases, annotated and published as Sweetpotato Gene Index. The sequences were mined for microsatellites and submitted to amplification and polymorphism detection. Tthe optimal amplification conditions for 195 new microsatellite markers were established and the degree of polymorphism and the number of identified alleles was determined in a test panel of six biodiverse hexaploid sweetpotato accessions and in two diploid sweetpotato mapping parents.

COSII and DArT marker for sweetpotato have been developed. The available DArT sweetpotato microarray contains 1089 probes that revealed polymorphic in 94 clones of the CGS. Out of 413 tested COS II primer pairs 138 successfully amplified a fragment of sweetpotato DNA. The COS II fragments were separated by single-stranded conformation polymorphism electrophoresis and amplified fragments were verified through sequencing.

The development of a diploid reference map for sweetpotato is ongoing. Two accessions of *Ipomoe trifida,* a near relative if sweetpotato, have been crossed and more than 200 progenies were grown and 150 individuals were established as *in vitro* plants as a mapping population. Genotyping of this population with SSR markers was initiated with the microsatellites developed int eh project.. The polymorphism rate of SSR markers in this population amounted only to 20%, requiring the addition of more markers, such as COS II and DArT, to produce a genetic map. DArT genotyping will be accomplished by the end of the third quarter of 2011 to develop the diploid map. COS will be added to the map by SSCP analysis in the progeny.

The project results were diffused using different avenues: All sequence and annotation data as well of the marker and genotyping data were depeosited on-line at the GCP data repository. The full Sweetpotato Gene Index including a blast search option is available at http://www.cipotato.org/sweetpotato_gene_index. The microsatellite marker development and application was performed in a joint effort with our project partners from SSA (Uganda and Mozambique). Visits of our project partners at CIP and joint completion of the marker work provided the opportunity for direct "technology transfer" to breeders in the target region. The Sweetpotato Gene Index and the new SSR markers were published in a peer-reviewed journal (Schafleitner et al., 2010).

The outputs of this project are already in use in several laboratories. The SSR markers are applied at CIP, in breeding programs in Mozambique and Uganda, at North Carolina State University and at INIA Uruguay. Further application can be anticipated as the microsatellite marker data including primer sequences, SSR motive and amplification conditions are publically available and map locations known. To our knowledge, the Sweetpotato Gene Index is in use at CIP and at Louisiana State University for genomics works on sweetpotato.

References

Schafleitner R, Tincopa LR, Palomino O, Rossel G, Robles RF, Alagon R, Rivera C, Quispe C, Rojas L, Pacheco JA, Solis J, Cerna D, Kim JY, Hou J, Simon R (2010). A sweetpotato gene index established by de novo assembly of pyrosequencing and Sanger sequences and mining for gene-based microsatellite markers. *BMC Genomics* 11:604 (doi: 10.1186/1471-2164-11-604).

Crosscutting activities

Breeding

G4008.34 Environmental assessment for phenotyping network

March 2008–December 2009; NCE: June 2010

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

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- Reinaldo Lucio Gomide, EMBRAPA, Brazil
- Peter Jones, Waen Associates and CIAT, Colombia

Summary
This environmental assessment for the phenotyping network was commissioned by the management team of the Generation Challenge Program in its effort to develop a network of sites where genotypes developed by the GCP would be evaluated in the process of developing products for farmers in developing countries. Our task was to support a larger effort to determine sites that would receive investments from the GCP, sites where the possibility of evaluating technologies could provide results to advance GCP's overall crop improvement goals. This project supported the ongoing development of the GCP phenotyping network, characterized trial sites for use in project planning and looked at the relationship between trial sites in an effort to assess the overall portfolio of sites where evaluation work might be carried out.

The project started with an initial characterization of a large number of trial sites that have been traditional testing sites of the CGIAR and the national agricultural research systems (NARS). One difficulty in dealing with many of the sites is that they are perhaps in not as good condition as they have been in the past. Trial work among the international community has not been as active as in the past for various reasons. Nevertheless, a group of about 80 sites was identified by the GCP as candidates for inclusion in the network. Much of the data about the sites was collected through a field campaign by GCP consultants. An effort was made to georeference all of the sites and link any information that we had about the sites to a point map of their locations. A rapid appraisal of geographic characteristics and climatic conditions for each of the sites was developed. This appraisal was partly done by overlaying the map of trial sites locations with a whole series of other information on climate, soils, and other biophysical characteristics. The FAO tool LOCLIM was used to develop agroclimatic summaries for each of the sites. These basic summaries and appraisals are available through links to an online mapping system called Generation Atlas.

More detailed analyses were performed for smaller groups of trial sites that were determined to be of most importance to the GCP's ongoing initiatives. These included a HomologueTM analysis showing the footprint of similar climates for each trial site.

These footprints give an idea of potential zones with similar climates that could be the focus of new rounds of testing or release of materials. For this smaller group of sites there was a drought stress analysis carried out using the BUDGET modelling software. These results showed crop water stress throughout the growing season and for off-season periods. These data can be used to plan drought phenotyping experiments at trial sites.

A third group of analyses were carried out to better understand the relationship between trial sites. These included a cluster analysis of over 500 sites in Latin America, Asia and Africa. The analysis suggests how multi environment trials could be developed to cover a range of environments in order to attain broad adaptation. The analysis also suggests very similar sites, some of which could be eliminated in order to save resources when doing large international trials. An application was developed that used the HomologueTM data to create a huge matrix of similarity indices for over 500 trial sites. The application allows the user to select any site and view the top 10 sites with similar climate characteristics across the network of trial sites. The application also includes the HomologueTM map in the background to give the user an interactive environment for exploring the trial site network.

Through the online application to share information from this project and through new initiatives to extend the project, this work has the potential to sustain itself well into the future. All of the data and reports produced by the project have been stored online inhe Generation Atlas application. The great potential of this application is that it can help us put the trial work in its larger environmental and social context, with the aim of helping us reach GCP end users. This project spawned two new initiatives – the Africa trial sites project and a CCAFS project for evaluating agricultural technology in the context of climate change.

Conclusions

This project supported the development of the GCP phenotyping network, characterized trial sites across Latin America, Africa and Asia and examined the relationship between the sites in an effort to provide planning information for trial work. The phenotyping network is an idea that has evolved since its inception in 2006/2007. The focus of the project has changed in a parallel fashion, to a more focused number of sites where the GCP is expected to carry out experimental work in the coming years.

Project researchers have a much better appreciation for the difficulty of characterizing sites and for developing a network that is robust enough to support the kind of technology evaluation work that is so much needed among the international agricultural research community. Despite huge advances in communications since the year 2000, getting a wide number of people on board to participate in a network is no simple task, especially in many countries that are still catching up in the world of information and communication technologies.

This project spawned some new initiatives that have great potential in further supporting the goals of the phenotyping network. One of these resulted from technological difficulties in sharing the results of this project. Instead of putting project data on CDs, we found a way to focus the results and data sharing aspects of the project on interactive online map servers. These have great potential, not only for

the phenotyping network but for many other aspects of GCP work. Indeed, the management team of the GCP may consider a smaller effort to georeference or geo-enable the entire GCP program under the Generation Atlas concept. For example, every project within the GCP workflow could be located on the Atlas. Every participating institution and the location of every office of GCP collaborating researchers could be visually displayed through the Atlas. We have already begun to georeference core collections of CGIAR germplasm accessions through a pilot project done with ICRISAT. This work could be extended to other crops throughout the system.

A second and third new initiative that was a direct outcome of this phenotyping network project was the Africa trial sites project and its extension with the CCAFS program of the CGIAR. These projects can be thought of as extending the life of the work that we have done with the phenotyping network. While the Africa trial sites project was meant as a pilot project, it has evolved into the agricultural technology evaluation program at trial sites for CCAFS. One example of continuing collaboration was the inclusion of trial site characterization and agricultural technology performance at trial sites in the proposal of the CGIAR research program on climate change. This development guarantees some additional funding from CCAFS to continue developing information on trial sites and managing information on the performance of varieties and other agricultural technologies.

Quantifiable Outputs

1. Tables and graphs of environmental and climate data by candidate FPP
2. Maps of FPP candidate sites overlayed on environmental data
3. Software: Customized ArcReader Map Viewer and maps, graphics and tables from FAO_LocClim analysis.
4. Site profiles of selected FPP's (limited list): HomologueTM maps, drought stress indicators (SWB modeling)
5. Statistical correlation model between FPP's and level 1 LPP's
6. Improved spatial soil water balance algorithm
7. Final project report

Capacity building

102. G4009.01 Genotyping Support Service

December 2008–January 2010

Principal Investigator and Lead Institute
Humberto Gómez Paniagua, GCP

EXTRACT FROM FINAL TECHNICAL REPORT

Collaboratoring institutes and scientists

- Xavier Delannay, GCP
- M Carmen de Vicente, GCP

Summary
The GSS facilitates access to modern breeding technologies for developing world agricultural research & development organisations. It offers world-class cost-efficient genotyping services, including support and training in analysis for proper interpretation of genotypic and phenotypic data.

In its 2008 call (2nd open call of the GSS), 78 applications were received out of which 42 were preselected and shared with the selection committee. Pre-selection was based on completeness of application, fulfilment of requirements of the call (crops, countries, etc…) and responsiveness of applicant to provide further details for a fair assessment of the proposal. A list of accepted proposals (32) resulted from the average grading received from each committee member. The cut-off to include proposals in the list was decided according to the available funding.

The 32 requests were as follows: Barley 1, Coconut 1, Sorghum 1, Sweet potato 2, Yam 1, Rice 2, Cassava 3, Musa 3, Phaseolus 3, Potato 4, Maize 5 and Cowpea 6. These applications were received from Brazil 1, Ecuador 1, Malaysia 1, Mexico 1, Mozambique 1, Nigeria 1, Peru 1, Sri Lanka 1, Thailand 1, Uruguay 1, Bolivia 2, Bulgaria 2, Ethiopia 2, Kenya 2, Philippines 2, Chile 3, India 4 and Ghana 5. Twenty-two of these applications aimed to conduct molecular characterization of germplasm and 10 addressed plant breeding issues.

The workshop for the first call (2007) took place as planned on January 2009.

A new call for proposals opened in October this year, and it is scheduled to close beginning January 2010.

Conclusion
This is a project in progress. In the 2nd call there was an increasing demand of services. This indicates that the GSS fills indeed a real need of developing country partners in an efficient manner.

Communications with applicants and other interested parties as well as the experience accumulated could be used to promote the GSS and the newly launched Marker Services to a wider audience.

Several contrasting situations were found in the 2nd call and innovative solutions devised to address them, all resulting in a much larger workload. Activities are proceeding with normality, except for the delays caused by the administrative procedures. The GSS is becoming more agile and effective as new experience is being accumulated.

Key Products Developed by the Project:

1. Administrative instruments and financial procedures.
2. More products in the project technical report.

Human resources (students, support to teams, travel grants, workshops)

G4009.02.04: Travel Grant – International Symposium on Genomics of Plant Genetic Resources

February–May 2010

Principal Investigator and Lead Institute

Roberto Tuberosa, UNIBO; roberto.tuberosa@unibo.it

EXTRACT FROM FINAL TECHNICAL REPORT

Supported by: Generation Challenge Programme and other sponsors (see Congress website)
Congress Chairperson: Emile Frison (Bioversity, Rome, Italy)
Venue: Bologna Fairgrounds
Workshop Date: April 24 to 27, 2010

General information

The second congress on Genomics of Plant Genetic Resources (GPGR2) held in Bologna, Italy, from April 24 to 27, 2010 and co-organized by Bioversity International, the IPK and the University of Bologna, followed the first edition organized in 2005 in Beijing, China. The objective of GPGR2 has been to critically evaluate how the recent advances in genomics have improved our capacity to harness plant genetic resources for improving crop productivity and nutritional quality. The unifying picture that emerges from over 90 oral and 300 poster presentations is that genomics is becoming increasingly important for selecting superior genotypes. Hereafter, additional details are reported for some of the presentations.

In the opening lecture, Emile Frison analysed how genomics contributes to strengthen a global plant genetic resources system able to more effectively address issues such as managing *ex-situ* collections, sample duplication, representation of crop wild relatives, etc. Properly addressing these issues will require extended partnerships and a more global information system. The congress keynote lecture of Gebisa Ejeta, recipient of the 2009 World Food Prize, reviewed the role of genetics and genetic resources to improve human livelihood. Examples were drawn from plant biodiversity manipulations against major plant epidemics, including the speaker's own outstanding research on sorghum tolerance to Striga that has brought benefit to millions in Africa.

Peter Langridge reviewed how the adoption of genomics-assisted breeding has increased the flexibility of breeding programs and has contributed to improve the stability of wheat and barley production under conditions of abiotic and biotic constraints.

The pivotal contribution of genome sequencing for investigating plant evolution, crop domestication, biodiversity analysis and/or marker discovery was highlighted by presentations on Arabidopsis (Martin Koornneef), Brachypodium (Alan Schulman), tomato (Giorgio Valle), soybean (Suk-Ha Lee), apple (Riccardo Velasco, Silvio Salvi), grapevine (Michele Morgante), rice (Scott Jackson, Masahiro Yano), barley (Robbie Waugh, Takao Komatsuda, Karl Schmid) and wheat (Jerome Salse, Martin Ganal, Tzion Fahima, Beat Keller).

Joe Thome reviewed the results of HarvestPlus, the project funded by the Bill & Melinda Gates Foundation on micronutrient deficiencies in the human diet. To address this problem, HarvestPlus deploys both natural and artificially induced variation to increase iron, zinc and provitamin A content in crops. Remarkable results have been achieved via transgenic strategies and marker-assisted breeding. Carmen de Vicente summarized how in the Generation Challenge Programme, advances in molecular genetics allowed for the release of a new generation of plants, especially for orphan crops. The GCP has developed a molecular marker toolkit, which aims to provide easy access to existing information on molecular markers used in breeding programs. Additionally, the Genotyping Support Service (GSS) of the GCP offers cost-efficient genotyping services, both for fingerprinting and analysis of genetic diversity and for molecular breeding.

Enrico Porceddu and Ed Runge presented two oustanding programs (PhD in Agrobiodiversity and Monsanto's Beachell-Borlaug International Scholars, respectively) for training young scientists in the application of genomics to crop improvement. Along this line, an engaging round-table discussion among experts from the public and private sectors addressed the compelling issue of how to educate the next generation of plant breeders.

In the closing presentation, Andreas Graner masterfully analysed the future challenges in exploiting plant biodiversity. The vast diversity resting on the shelves of genebanks has been tapped into only marginally. Both the improvement in phenotypic analysis and the generation and deployment of genetic information will be instrumental to more effectively harness germplasm diversity. Positional cloning of the genes that underlie agronomic traits will help to unveil their allelic diversity by systematically mining genebank collections for novel alleles. When the access to genes is hampered by low recombination, GM approaches will facilitate the transfer of genes, especially from wild relatives into adapted breeding lines.

Details of the Congress and all abstracts are available at the Congress website (www.gpgr2.com). The June 2011 issue of the journal Plant Genetic Resources presents a special volume that collects 53 manuscripts from the invited speakers and the contributed abstracts. GPGR2 was attended by over 400 participants representing 53 countries. The GPGR2 organizers Emile Frison, Bioversity International, Italy; Andreas Graner, IPK, Germany; Roberto Tuberosa, University of Bologna, Italy) wish to thank the GCP and all other sponsors for their generous financial support.

G4010.01.02: Travel Grant – ICPBM3 International Training Workshop on Plant Molecular Breeding

August–September 2010

Principal Investigator and Lead Institute

- Carmen de Vicente, GCP
- Zhikang Li, CAAS–MST

EXTRACT FROM FINAL TECHNICAL REPORT

General Information

Thank you for Generation Challenge Programme's financial support to the International Training Workshop for Plant Molecular Breeding Technique. The 12-day workshop was successfully held between August 30th and September 10th, 2010 at the Institute of Crop Sciences of Chinese Academy of Agricultural Sciences. Participation to the ICPMB conference was part of the program, where the delegates could interact and hear the presentations of the most up-to-date molecular sciences from world-renowned scientists of this field. A formal Workshop opening ceremony took place on the August 30th. Dr Dongxin Feng, Deputy Director of the Department of International Cooperation (CAAS), Dr Shumin Wang, Deputy Director of Institute of Crop Sciences (CAAS), Dr Zhikang Li, Chairperson for the International Training Workshop for Plant Molecular Breeding Technique and Dr Graham McLaren, Subprogramme 4 leader of Generation Challenge Programme, delivered opening speech to welcome the delegates. Twenty delegates from thirteen countries attended this Workshop. Among the twenty delegates, eleven were given financial support to attend the Workshop and ICPMB conference.

During the Workshop

The workshop was a combination of lectures and practical use of QTL mapping and statistic software, facilitated by the senior staff of the Institute of Crop's Sciences of CAAS, Dr Graham McLaren of GCP, Dr Jauhar Ali and Dr Michael Thomson of IRRI. Delegates also had the opportunity to see the rice grown using molecular breeding techniques at the CAAS trial site at Changping district. There were two outings held- once to the Great Wall of China and another outing to the Forbidden City. During their two-week in Beijing. Delegates have stayed at the CAAS guest house, where many local facilities could be easily accessed. Some delegates also experienced the use of local subway. Valuable feedbacks were received from delegates. Their opinion would greatly help us to refine our future training course.

Learning materials and resources

103. G4007.20: Managing the Generation Challenge Programme in a post-International Treaty World: a proposal fro a technical traning workshop and related materials

August 2007−August 2008; NCE: October 2010

Principal Investigator and Lead Institute

Michael Halewood, Bioversity International, m.halewood@cgiar.org

EXTRACT FROM FINAL TECHNICAL REPORT

Introduction/Background

The Generation Challenge Program's work involves, among other things, pooling plant genetic resources for food and agriculture (PGRFA) across international borders to assemble collections for research. The products of the research of the Generation Challenge Program (GCP) will be distributed to a wide range of users around the world, following strategic product delivery plans. The GCP Consortium Agreement was finalized in May 2004; it establishes rules for consortium members, partners and recipients of GCP research products concerning, among other things, access and benefit-sharing, intellectual property protection, humanitarian non-enforcement of IPR rights against those using Challenge Program intellectual property for the benefit of the so-called 'resource poor', and so on.

The International Treaty on Plant Genetic Resources for Food and Agriculture (the Treaty) came into force in 2004. However, many of the legal rights and obligations associated with the Treaty's multilateral system of access and benefit-sharing (MLS) were agreed-to only in June 2006, when the Governing Body of the Treaty adopted the standard material transfer agreement (SMTA). The MLS provides the basis for the international movements of 64 crops and forages listed in Annex 1 of the Treaty. The Treaty's MLS was created in response to difficulties – both experienced and projected – faced by researchers around the world in gaining access to, and providing, PGRFA. The Treaty provides a transparent, low-cost legal basis for the exchange of PGRFA.

Many of the state parties and organizations involved in the Generation Challenge Program are bound by the Treaty. Since the SMTA was adopted in 2006, it has become necessary for those states and organizations – and the GCP as a whole - to examine the relationship between the GCP Consortium Agreement and the Treaty; to ensure that that GCP members are in compliance with their international legal obligations; and that the GCP is taking maximum advantage of the flexibilities of the Treaty (and in the legal spaces around the Treaty) to advance the GCP's objectives.

Bioversity International is the convening Centre for the System-wide Genetic Resources Programme (SGRP) of the Consultative Group on International Agricultural Research (CGIAR). The SGRP has a mandate, among other things, to promote harmonized genetic resources policies and practices across the Centres. Partly as a result of its experience and responsibilities as the convening Centre for the SGRP, and partly as a Centre involved in the GCP, Bioversity proposed a series of activities, with a technical workshop as the Centre-piece of those activities, to address the issue of the relationship between the GCP and the Treaty. The leader of GCP Sub-Programme 5 encouraged Bioversity to develop a proposal for support for those activities.

Products

1. The outputs of the project as of the writing of this report (December 2007), include:
2. Twenty-four GCP members/partners trained on the implications of the Treaty vis-à-vis the GCP.
3. A summary workshop report, including recommendations from the workshop's participants to the GCP management team, was developed.

4. A survey of practices within the GCP, concerning the movement of PGRFA and uncertainties by GCP researchers concerning compliance with the Treaty, MLS and the SMTA in particular, was carried out.
5. An informal network, through which participants at the workshop were invited to seek Treaty-related advice and technical backstopping, on an as-needs basis, from the resource people, was established.
6.

Outputs still to be delivered include:

1. A short GCP policy guideline, possibly to be adopted as a supplement to the GCP Consortium agreement, addressing the recommendations set out in the summary workshop report and recommendations included in Appendix 1. GCP management will decide whether or not such a guideline is desirable, and if so, what form and content is most appropriate.
2. Legal instruments in conformance with the policy guideline, for example, a template to be used GCP-wide, for PGRFA under Development, with options for additional legal terms. Again, development of such instruments will be informed by feedback from GCP management.
3. Training materials regarding the Treaty, with special emphasis on the MLS
4. Reference material package for participants (and other GCP members/participants), and message (if deemed appropriate by GCP management) that technical assistance regarding Treaty related matters can be provided ad hoc to the GCP members by the resource people, with Bioversity playing a coordinating role if necessary/appropriate.

G4008.35/G4010.03: Toolbox of available molecular markers useful for marker assisted selection in GCP crops/GCP Molecular Marker Toolkit

G4008.35: March 2008–March 2010
G4010.03: July–October 2010; NCE: December 2010

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Collaborating institutions and scientists:

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The increase in genomics research has lead to a massive amount of publications and databases. With this increase, the search for available user-ready molecular markers cannot only become a real odyssey, as the latest discoveries are often scattered in numerous, expensive peer-reviewed journals or databases, but the information offered through digital resources is not always reliable and does not provide guidance for its appropriate use.

Driven by the demand of agricultural researchers and plant breeders, particularly those in developing countries who face difficulties in accessing up-to-date scientific information on useful molecular markers, GCP designed a rapid-access tool for currently available and validated markers for 19 food security crops, including GCP's 18 mandate crops. The 19 crops covered are: *Musa* spp., barley, beans, cassava, chickpeas, coconuts, cowpeas, faba beans, groundnuts, lentils, maize, millet, pigeonpeas, potatoes, rice, sorghum, sweet potatoes, wheat and yams. GCP's

Molecular Marker Toolkit (MM Toolkit) is online since July 2009 and is accessible on http://www.generationcp.org/sp5/MM-Toolkit. It consists of supporting texts and the toolkit itself, containing information on 273 effectively used markers. The information generated for each marker includes general information (trait identified by this marker, marker code, marker type, ….), the lab protocol, the validation process, the most relevant references, and the name of corresponding experts.

An early phase of the Molecular Marker Toolkit's development saw the compilation of information accessible via internet sources, public databases and papers on the available markers useful in MAS for each of the 19 crops. At a subsequent stage, the findings were analyzed and published in the form of a preliminary list of markers, which was then presented to several crop experts who checked if the markers were effectively used in MAS. In light of both breeders' access to the most up-to-date information and their essential hands-on experience in the use of markers, the collaboration of the breeder community was vital to the analysis. After this revision by experts, the updated information was entered into the toolkit.

For *Musa* spp., coconut, lentil, pigeonpea, sweet potato and yam MAS is not applied yet or the used markers do not comply with the conditions to be included in the toolkit. For these crops the provided information is limited to the actual status of use of molecular markers in these crops. An overview of the available markers useful for MAS in the remaining 13 crops is shown in table 1.

Table 1. Number of each marker type per crop available in the MM Toolkit.

	Total	Type of marker						
		SSR	STS	SNP	SCAR	STMS	CAPS	Unknown type
Barley	**42**	21	17	0	2	0	2	0
Bean	**24**	3	0	0	21	0	0	0
Cassava	**6**	5	0	0	1	0	0	0
Chickpea	**12**	2	0	0	2	7	1	0
Cowpea	**2**	0	0	0	2	0	0	0
Faba bean	**5**	0	0	0	2	0	3	0
Peanut	**4**	2	0	0	1	0	1	0
Maize	**7**	6	0	1	0	0	0	0
Millet	**1**	0	0	0	0	0	1	0
Potatoes	**16**	2	5	1	1	0	3	4
Rice	**61**	29	10	12	2	0	1	7
Sorghum	**16**	13	2	0	1	0	0	0
Wheat	**77**	32	37	0	6	1	1	0
TOTAL	**273**	**115**	**71**	**14**	**41**	**8**	**13**	**11**

The MM Toolkit is complementary to the Genotyping Support Service (GSS) of the Generation Challenge Programme – subprogramme 5, launched in 2006, which aims at facilitating the access of national agricultural research systems (NARS) to genotyping technologies. With this GCP Molecular Marker Toolkit plant breeders in the public sector and small private enterprises, particularly in developing countries, will have access to succinct but complete and validated marker information. By sharing the latest advances in molecular plant breeding, the toolkit will be an important contribution to support modern agricultural science for the benefit of the poor in developing countries.

Crop information

104. G4008.14: Breeding for drought tolerance with known gene information

January 2008–December 2009; NCE: June 2010

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- CSIRO, Australia: Scott Chapman (co-PI), Nick Hansen
- University of Queensland, Australia: Mark Dieters
- CAAS and CIMMYT-China, China: Jiankang Wang, Huihui Li, Changbin Yin
- GCP, Mexico: Jean-Marcel Ribaut
- CIMMYT, Mexico: Matthew Reynolds, Ravi Singh, Hans-Joachim Braun, Gary Atlin
- INRA, France: Francois Tardieu, Claude Welcker
- ICRISAT, India: Dave Hoisington, Shyam Nigam, Vincent Vadez

Summary
The objective of this research was to develop simulation and analysis tools that allow breeders to efficiently estimate and use information about the effects of different genomic regions (genes) on the expression of crop traits such as disease resistance, stress tolerance and yield. The major outputs from the project are these new tools and research publications from the work.

To work with hybrid crops such as maize, a new breeding simulation module (QuHybrid) was developed, starting from QuLine. The major development was the implementation of test crossing and hybrid performance prediction. To make the testcrosses, an additional population defining all the testers was added. When the "testcross" functionality is activated, testcrosses are made between all families and testers. Among-family selection is conducted based on the mean performance of all testcrosses in each family, and within-family selection is conducted based on the mean performance of each individual across all testers. QuLine and QuHybrid are the first two computer tools in the world that can be used to simulate the actual breeding programs for inbred lines development and hybrid development, respectively. These tools allow almost any diploid breeding strategies to be compared and optimized, and are extremely useful research and teaching tools in plant genetics and breeding.

The QuGeneUI (user interface) has been extended to allow it to write and read data in the form of Excel sheets to connect to the iMAS product for GCP. The current interface allows a user to capture information from their molecular map, their QTL map and their list of additive (or epistatic) effects associated with QTL. In addition to the connections to iMAS, the QuGeneUI is able to write dataset to JoinMap formats

and to GGT (Graphical Genotype) formats, and also to Flapjack format (SCRI). The latter is our preferred format as it allows for high-speed data exchange and provides a 'test' for the development of interactions with plant breeding databases. Using the new capabilities of QuGeneUI, the project produced a study of the genetic control of a complex physiological network of QTL affecting leaf and silk elongation of maize (Chenu et al 2009).

ICIM (Inclusive Composite Interval Mapping) was extended to epistasis by simultaneously considering marker variables and marker-pair multiplications in a linear model. Epistatic QTL can be identified by ICIM no matter whether the two interacting QTL have any additive effects. Simulated populations and one barley doubled haploids (DH) population were used to demonstrate the efficiency of ICIM in mapping both additive QTL and digenic interactions. Many improvements have been made in the integrated software package QTL IciMapping, including the interface and functionalities. Mapping methods implemented in QTL IciMapping are single marker analysis, simple interval mapping, inclusive composite interval mapping for additive QTL, inclusive composite interval mapping for digenic interacting QTL, and selective genotyping. The simulation functionality has also been enhanced for power analysis. Version 3.0 of the software was released in 7 September 2010, and is freely available from http://www.isbreeding.net.

Additive and di-genic epistatic effects were estimated in a recombinant maize line population evaluated under different years, locations and water regimes, for a total of 10 environments. For each of the trials large di-genic epistasis effects were identified for male and female flowering traits, respectively up to 48 and 51% in a single trial and to a lesser extent for plant height. The segregation of plant height was regulated mainly by a single QTL identified under 9 environments and explaining an average of 19% of the phenotypic variance (bin 1.06). Several loci with significant additive affects were identified across trials and in general a low correlation was observed between loci expressing additive and epistatic effects. Stable epistatic effects were identified across experiments, as the result of the same di-genic interaction between two loci across trials (plant height), or di-genic interaction between few regulatory genes, but with different loci depending on environment (flowering traits). Major regulatory genes for flowering epitasis were identified on bins 2.08 and 10.05 for female flowering, bins 1.06, 8.04 and 10.04 for male flowering. Those genes might play a key role in plant adaptation, interacting with different genes involved in physiological pathways depending on environmental changes. The identification of large epistatic effects, some of them identified across trials, open new opportunities to achieve genetic gain and improve plant adaptability in maize.

Single backcrossing combined with selected bulk breeding strategy has been successfully used in wheat breeding at CIMMYT to introduce the rust resistance genes from donor parents to elite adapted cultivars. The breeding efficiency of this strategy compared with other crossing and selection strategies was extensively investigated through computer simulation. Results indicated this breeding strategy has advantages in retaining or improving on the adaptation of the recurrent parents and at the same time transferring most of the desired donor genes for a wide range of scenarios. Two times of backcrossing have advantages when the adaptation of donor parents is much lower than that of the adapted parents, and the advantage of three times of backcrossing over two times of backcrossing is minimal. We therefore

recommend the use of single backcrossing breeding strategy based on three assumptions: (1) multiple genes governing the phenotypic traits to be transferred from donor parents to adapted parents, (2) donor parents still have some favorable genes that may contribute to the improvement of adaptation in the recipient parents even under low adaptation, and (3) the conventional phenotypic selection is applied or the individual genotypes cannot be precisely indentified.

Plant breeders select simultaneously for qualitative traits controlled by one or a small number of major genes and polygenic traits controlled by multiple genes, which may be detected as quantitative trait loci (QTL). Simultaneous selection for alleles at both major and minor gene (as QTL) loci was investigated in this project. Given the breeding objective of developing the maximum number of inbred lines combining the six desired major genes and long CL QTL, simulation indicated that a single biparental cross F_1 produced the highest frequency of target genotypes compared with backcross populations. On average, 2.4 individuals with target genotype were present in unselected F_1-derived DH or RIL populations of size 200. A selection scheme for the six major genes increased the number to 19.1, and additional marker-assisted selection (MAS) for CL increased the number of target individuals to 23.0. Phenotypic selection (PS) of CL outperformed MAS in this study due to the high heritability of CL, incompletely linked markers for known QTL, and the existence of unidentified QTL.

Conclusion
Although activities of Objective 3 were delayed and merged into the new Integrated Breeding Platform project, we believe that we have made good advances in applying this research since the start of the project. Below is a list of publications that were directly or partly supported by this project.

Quantifiable Outputs:

1. Characterization of contrasting breeding questions, together with the costs of the components of the system in either a MAS or phenotypic selection context
2. Desktop tools for constructing multiple breeding strategies from existing data
3. Desktop tools for comparing and reviewing breeding strategies
4. Hybrid simulation tool QuHybrid and technical report and Users' manual of QuHybrid
5. Efficient QTL mapping method and software
6. Identification of the QTL distribution mode in maize genome
7. Feasible and cost-efficient MAS strategies for pyramiding multiple favorable alleles in wheat breeding
8. Completion of the workshop/training course
9. Simulation tool QuMARS developed

105. G4008.21: Large scale phylogenomic analyses to gene function prediction for GCP crops

January 2008–December 2009; NCE: February 2010 (not documented)

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- CIRAD: Périn, Christophe

1. Research activities and progresses

1.1. Genome Clustering and update procedure

Following the 2008 activities, we continued with the insertion of new plants' genomes to enrich the previously-computed clustering on the 16 plant genomes currently featured in GreenPhylDB. In order to facilitate this time consuming task, we developed a semi-automatic procedure to handle genome release updates. Updates are definitely a critical step to keep the database attractive for end users and sustain the final product.

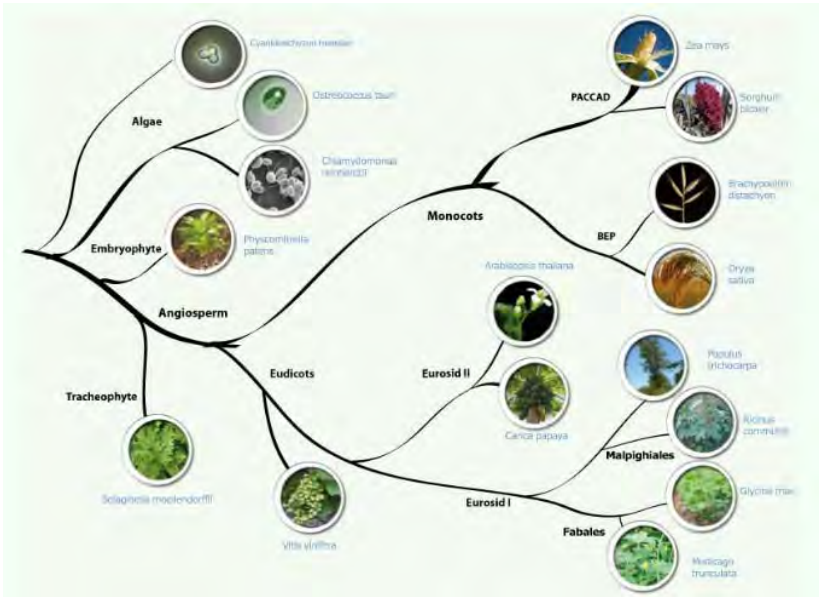

Figure 1: full genomes clustered in GreenPhylDB

1.2. Annotation of gene families

GreenPhylDB stores in first place the results of an automatic clustering of protein sequences. Then, overall consistency of those clusters is manually checked using our own annotation tool that summerizes selected information (statistics on protein domains, enzymatic activities, publications etc.) and guidelines were produced to help with manual annotation of gene families. Annotation has been started and more than 2000 genes families (over 10000) were annotated with emphasis on gene families related to agronomic traits and evolutionary processes. Using Gene Ontology terms (GO), we can identify lists of gene families involved in plant stress response such as drought and propose candidates genes for functional genomics studies.

1.3. Pipeline of analyses

In order to cope with the increase of sequences contained in the clusters, a more efficient pipeline for phylogenomic analyses has been designed and set up on the cluster of computers. Annotated gene families were processed and ortholog predictions (infered via gene tree reconcilation and reciprocal blast hits) are now available on line.

1.4. Website and analyses tools

Version 2 of the the website has been published (http://greenphyl.cirad.fr/). Although the number of data has increased drastically, we have also optmized the database structure and the script code to speed up loading of pages. A tool to localize genes belonging to same gene family along the chromosomes was added to the website.
Note: A version 3 will be released in 2011 with 22 genomes including cassava, cacao, date palm and musa.

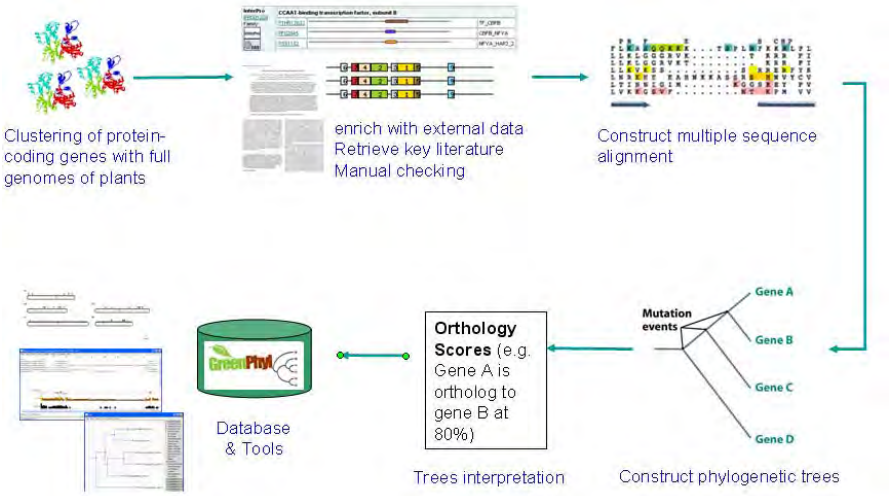

Figure2: main steps of the phylogenomic analyses in GreenPhylDB

References:

Rouard, M., Guignon, V., Aluome, C., Laporte, M.-A., Droc, G., Walde, C., Zmasek, C.M., Perin, C., et Conte, M.G. 2010. GreenPhylDB v2.0: comparative and functional genomics in plants. Nucleic Acids Research 39: D1095-D1102.

Conte, M, Laporte, M-A, Périn, C, and Rouard, M . GreenPhylDB: A Gene Family Database for plant functional Genomics. Available from Nature Precedings <http://dx.doi.org/10.1038/npre.2009.3136.1> (2009)

Conte MG, Gaillard S, Droc G, Périn C (2008). Phylogenomics of plant genomes: a methodology for genome-wide searches for orthologs in plants. BMC Genomics, 2008, 183

Conte MG, Gaillard S, Lanau N, Rouard M, Périn C (2008). GreenPhylDB: a database for plant comparative genomics. Nucleic Acids Research. 2008 January; 36 D991–D998

Wanchana S, Thongjuea S, Ulat VJ, Anacleto M, Mauleon R, Conte M, Rouard M, Ruiz M, Krishnamurthy N, Sjolander K, van Hintum T and Bruskiewich RM (2008). The Generation Challenge Programme comparative plant stress-responsive gene catalogue. Nucleic Acids Research; 2008 January; 36: D943–D946

106. G4008.31: Upgrading the quality and utility of GCP phenotyping data through the development of a database

template to facilitate the storage of data in crop-specific databases

February 2008−February 2009; NCE: January 2010

Principal Investigator and Lead Institute

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The goals of this project were to: (1) create a wizard-driven template ("first generation template") able to store phenotypic observations and all associated data to make them interpretable, whilst assuring compatibility with the GCP domain models; (2) extend to a "second generation template" which is more crop-specific and prescriptive, via the incorporation of mandatory traits and fields (including drought tolerance indicator traits, experimental designs, environmental indicators etc.), both to facilitate future meta-analyses of the phenotypic data and to improve the homogeneity of experimental protocols across GCP projects; (3) document the use of this template in a user manual; (4) export, as far as possible, the data presently lodged in the GCP Central Registry into the 'first generation' template; (5) monitor the use of the templates and the compliance thereof; and (6) explore the possibility of establishing electronic field data capture technology for the GCP community, as a tool to improve the accuracy of phenotyping. The project reached its conclusion in early 2010 with the beta release of the wizard program, the preparation of a user's manual in pdf form, and a set of training videos mounted on the ICIS Wiki website. The program is designed to operate in either a Microsoft Office 2003 or 2007 environment, and instructions are given in both the manual and the video how to download and install the program from the internet.

107. G4009.03: Data standards and best practice for capture and publication of quality GCP and IBP data developed and used

January 2009–December 2009; NCE: December 2010

Principal Investigator and Lead Institute

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- CIP: Simon Reinhardt
- IRRI : Mauleon Ramil, Jeffrey Detras
- IITA: Moshood Bakare, Peter Kulakow with the Cassava breeders' group:
- ICRISAT – B. Jayashree
- Wageningen – Theo Van Hintum, Elizabeth van Strien

Self funded collaborators

- Oregon University : Pankaj Jawal
- Manchester University : Norman Morrison, David Hancock

Summary
In 2009, this project was initially composed of five elements, namely: (1) Ontology, (2) Data Templates, (3) Central Registry, (4) Quality Data Sets and (5) Helpdesk. All components support the delivery of quality data to the scientific community. In 2010, the project refocused on the development of the Crop ontology, data annotation, trait dictionaries and promotion of the CO in breeders' communities.

1. The Crop Ontology

The Crop Ontology is accessible through the online Lookup service developed by the team (http://cropontology.org/). Ontology offers validated terms and concepts to annotate data, and facilitate queries and the retrieval of data/metadata etc. The Crop Ontology describes traits of several economically important crops such as cassava, chickpea, maize, *Musa*, potato, rice, sorghum and wheat. It also describes scales for measuring each trait and the methods for measuring the trait are being gathered for integration into the ontology. Crop specific ontologies were developed by Bioversity, CIMMYT, CIP, ICRISAT, IITA, IRRI for their mandate crops. The main source of breeders' trait names used in developing the Crop Ontology was the International Crop Information System (ICIS), with the addition of the Bioversity key crop descriptors. An additional "crop_ experiment_ ontology" was added for experimental design, environment and treatment factors, based on the ICIS model. To include methods of measurement with units or scales, we need to continue working closely with breeders, or/and researchers trying to harmonize several variables for one trait. Trait dictionaries are being developed by the GCP crop communities and will be linked to the Crop Ontology. The Crop Ontology is embedded into the version 6 of ICIS and data sets were annotated. Training on the methodology for developing the Crop ontology was provided to the new ontology teams. The CO is available in the Bioportal developed by NCBO (http://bioportal.bioontology.org/).. The collaboration with Manchester University leaded to the production of a Crop ontology based tagging prototype called Crop Terminizer search page (http://crops.terminizer.org) able to tag any text with ontology terms. Validated Crop ontology terms have been published in the Plant Trait Ontology (TO) (http://www.gramene.org) and Plant Ontology (PO) (http://www.plantontology.org).

An online tool is needed for crop communities to annotate their phenotyping data with the Crop Ontology terminologies. The integration of the CO within the genebank databases, crop registers and collaboration with GENESYS, GRIN GLOBAL and SINGER will be an important objective. Partners and donors need to be identified to secure the long-term enhancement and use of the Crop Ontology.

2. The GCP data templates version 2.0

The data submission templates were produced and made available with their dictionaries on the GCP central registry for the GCP Passport, SSR genotyping, Phenotyping, QTL, Mapping, DArT genotyping, SNP genotyping, EST data, Gene Expression data, Ecotilling data

3. The Central Registry (http://gcpcr.grinfo.net/) and 5. Helpdesk

259 GCP data sets were uploaded in total with a peak performed in 2009 thanks to a proactive helpdesk performed by Bioversity, CRIL and Wageningen. The Central

Registry is maintained online. The software migration was finally cancelled in agreement with the SP leader.

4. **Quality data**

Quality reports produced by Genomedium and those produced by Wageningen were never validated by the project PIs and therefore are not published.

Peer-reviewed Paper

Shrestha, R., Arnaud, E., Mauleon, R., Senger, M., Davenport, G., Hancock, D., Morrison, N., Bruskiewich, R. and McLaren, G. 2010. Multifunctional crop trait ontology for breeders' data: field book, annotation, data discovery and semantic enrichment of the literature. Submitted to *AoB PLANTS*. plq008 (http://aobpla.oxfordjournals.org/content/2010/plq008.abstract).

The Trait template is available at http://ibp.generationcp.org/confluence/display/MBP/Trait+Dictionaries+for+Fieldbook+Development

More information on the project at http://ontology.generationcp.org

108. G4009.04: Data analysis support for existing projects in SP2 with emphasis on analysis of next generation sequencing data

January 2009–December 2009; NCE: June 2010

Principal Investigator and Lead Institute

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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

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- Greg May and Andrew Farmer, National Centre for Genome Resources (NCGR), Santa Fe, USA.
- David Studholme and Jonathan Jones, The Sainsbury Laboratory (TSL), Norwich, UK.
- Ramil P. Mauleon and Richard Bruskiewich, International Rice Research Institute (IRRI), Las Banos, The Philippines.

Summary

Next generation sequencing has made possible the generation of genomic resources for even orphan crops due to high-throughput sequencing and cost-effectiveness. The data obtained is huge and requires efficient computational set-up for rapid and accurate analysis. Since one of the objectives of the project is to obtain variants or polymorphisms between genotypes, a number of sequential analyses are required (such as mapping, assembly, SNP calling) in processing the raw data. This could be

practically achieved by creating a pipeline that integrates available open-source tools for NGS data analysis.

This project was started with three main objectives: 1) Benchmark available open source short reads assembly and downstream analysis programs/software, 2) Data analysis support, and 3) Data integration, availability and visualization. The proposed NGS data analysis pipeline was integrated with open-source tools like Maq, *NovoAlign* as they have the complete repertoire of analysis functions required and in-house Perl scripts for the identification of SNPs between parental genotypes. The above mentioned tools were benchmarked and the identified SNPs were experimentally validated. GBrowse was configured to visualize the mapped short reads on to the reference sequence, and also the variants. The results are maintained as session based output files to avoid any discrepancy generated by the simultaneous use of the pipeline by different users. Various server side in-house Perl scripts were integrated into the pipeline which automates the process of generating gff3 files, which contains the mapping results and updating the configuration file of database which are required for GBrowse. As an alternate Tablet- alight weight client side tool can also be used.

The proposed activities under objective 1 are completely achieved. All the activities/quantifiable outputs under objective 2 and 3 are achieved except, the implementation of digital gene expression under objective 2 and implementation of the NGS pipeline in Taverna workflow of objective 3 are not achieved. The pipeline can be accessed at http://hpc.icrisat.cgiar.org/ngs/ .

In addition to the proposed objectives, SOAP (Li et al. 2009) has also been benchmarked as it uses Burrows Wheeler Transformation (BWT) algorithm which helps in faster alignment of reads to the reference with reduced memory usage, it will be integrated soon, in the phase II of the project, into the pipeline along with other in-house Perl scripts.

Regarding the data availability, documentation of various procedural/methodological aspects of the pipeline is under progress and several of the datasets have been available either in the public domain or locally, appropriate ones will be posted very soon on Cropforge (http://cropforge.org/)

Conclusions

Benchmarking of tools for the purpose of variant detection showed the mapping tools to be better than *de novo* ones. Among mapping tools, we found MAQ to be one of the most useful ones. We found several tool-specific parameters, which affect the quality of results; the SNP calling was done at variable level of stringency of these parameters. In order to identify highly specific SNPs, the existing approach for depth based consensus calling was modified to include the high quality bases. Several Perl scripts have been written to extract the desired information from the output of the above mentioned tools. Finally, a list of SNPs has been generated for two chickpea genotypes using the three approaches/tools and comparison has been made. A sample of predicted SNPs is experimentally validated to access the accuracy of prediction by said tools/approaches. The pipeline was developed and can be accessed at http://hpc.icrisat.cgiar.org/ngs/ .

Quantifiable Outputs

1. Reference-based mapping/assembly was found more suited for identification of variants between parental genotypes. Among the reference-based mapping/assembly tools, MAQ and *NovoAlign* were the tools of choice based on the desired analysis as well as performance. SOAP was also benchmarked.
2. Parameters having sizeable effect on the output (of mapping, assembly and SNP calling) were identified. A new approach for consensus calling based on high-quality depth was examined to increase the specificity of predicted SNPs.
3. Comparison of *Alpheus* with the selected open source tools showed differences in mapping reads, which in turn was largely due to differences in underlying algorithm and method for mapping.
4. A number of in-house scripts were written for development of NGS data analysis pipeline.
5. SNPs calling was done using above mentioned tools/approaches and were categorized based on variable levels of stringency of following parameters: 1) read depth and 2) frequency of consensus base. Comparison of these sets of SNPs indicated some extent of inconsistency in the prediction. A sample from these sets is being experimentally validated.
6. Though the protocol is formalised it is not implemented as many open source tools are already available. Because of emphasis of the 2nd project (phase II) on SNP discovery for molecular breeding, efforts have been shifted from gene expression analysis to SNP discovery.
7. A genome browser (GBrowse) has been configured which displays reads mapped against reference(s). The servers 'RAM has been upgraded, to cope with the resource hungry operations. As an alternate a client side tool Tablet was used.
8. Several of the (primary or processed) datasets have been posted either at public domain or at ICRISAT intranet.
9. A pipeline has been developed which carries out various analysis using *MAQ*, *NovoAlign* and SOAP discussed above. The pipeline can be accessed at http://hpc.icrisat.cgiar.org/ngs/.
10. Although some efforts were initiated to implement the NGS data analysis pipeline in Taverna workflows, these efforts were discontinued based on discussions with several colleagues. The basic assumption to implement the pipeline in Taverna workflow was to provide a user friendly environment, where a user can upload his data, select the approach, and get the results. However we recognize that this can not be achieved by the Taverna workflow environment.

Diversity

109. G4008.42: Developing DArT markers for several crops in the GCP

January 2008–December 2009; NCE: December 2010

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NO UPDATE SUBMITTED; PROJECT REPORT PENDING UPLOAD

Genomics

110. G4007.02: Validation of drought-response/resistance pathway genes by phenotypic analysis of mutants

August 2007–July 2009; NCE: January 2010

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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- HAU
- IRRI
- VPI

Summary
Research within the GCP and other ongoing research on abiotic stress biology, has provided researchers a number of candidate genes with a potential role in drought response and resistance. These genes have been identified in a number of crops, in response to a variety of environmental stresses and by data derived from breeding, genetics, physiology and genomics, but their exact role in drought response/resistance is unknown. The analysis of mutants is one of the most reliable and time-proven ways of correlating the genotype to a phenotype. The international research community has generated significant mutant resources in the two sequenced plants Arabidopsis and rice (Krishnan et al., 2009). In this project the aim has been to provide drought response phenotypes for an extensive list of candidate orthologous genes in the two plants selected for their potential role in drought responses and resistance mechanisms. The comparative analysis of gene functions between the dicot and monocot plants will be applicable across a wide number of crop plants.

Conclusion
This project was a very good exercise on testing the use of knockout mutants for drought response studies. This has **never** been done at a large scale and so is the first

experience of the participants to make this practical. Many of our assumptions were off the mark, since it was not enough to get a phenotype, but to validate them using other screens, which took a lot of time. However, the genes and mutants characterized in the project are all very significant additions to our knowledge of drought tolerant genes and mechanisms. The experience gained by the PI enabled him to get an NSF project on drought tolerance of rice funded, in which the Co-PIs are collaborators.

Final products to be used by plant scientists outside the project:

1. Establish drought response phenotypes using rice mutants for 100 drought related candidate (SAG) genes
2. Detailed drought physiological analysis of 2 rice transcription factors
3. Established the rice ERECTA (OsER) functions in drought response/resistance and WUE, using mutant analysis
4. Characterized drought response phenotypes for 200 rice orthologous Arabidopsis drought related candidate genes
5. Analyzed 10 Arabidopsis regulatory genes with mutants showing altered drought response phenotypes

Product delivery

111. G4006.13: Targeting and impact analysis of Generation Challenge Programme technologies

November 2006–October 2007; NCE: March 2010

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PROJECT UPDATE EXTRACTED FROM ACCEPTED FINAL TECHNICAL REPORT

Collaborating institutes and scientists

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- International Center for Maize and Wheat Improvement (CIMMYT), Mexico: John Dixon (dixon@aciar.gov.au, now with Australian Center For International Agricultural Research)
- International Food Policy Research Institute (IFPRI), Washington DC: Stanley Wood (s.wood@cgiar.org)

Summary
The international agricultural research and development community is paying more attention to poor and vulnerable populations hindered by agricultural production constraints, especially drought. Past efforts have placed greater emphasis on increasing overall production at the national scale, with emphasis on environments and farmers with the highest potential for improvement. This study uses global crop, climate and poverty data to identify agricultural regions of high priority for research and development in molecular biology and crop genetic resources. Spatial overlay, drought modeling and descriptive statistics are used to make a global assessment of agricultural regions. Our initial analysis showed that drought coincides with high levels of poverty in 15 agricultural regions, especially in South Asia, the Sahel, and eastern and southern Africa. Fourteen crops make up the bulk of food production in these high priority areas. A database was developed for use in priority setting for agricultural research and development.

This report describes additional work to further refine the ex-ante impact targeting approach of the Generation Challenge Program. It describes poverty according to farming system and country for 63 farming systems in the developing countries of Latin America, Africa and Asia. Information on the number of people earning below $1.25 and $2.00 a day, the number of stunted and underweight children and the infant mortality rate was compiled.

The project developed a method for analyzing crop-specific drought throughout the growing season. The seasonal drought index (SDI) estimates the probability of drought for 20-day periods throughout the year. It utilizes a weather generator and the water balance method to simulate rainfall and temperature for every day of the year, running 100 simulations. The ratio of actual evapotranspiration to potential evapotranspiration provides an indicator of water requirements for each crop. The

index can be viewed through an online map server that allows crop improvement specialists to explore drought patterns throughout the world and throughout the growing season.

The project refines descriptions of farming systems in the context of their importance to the GCP. These include an assessment of the likelihood of technology adoption, information on possible poverty exit pathways and other characteristics of the farming system.

The data for this project has all been put online in the context of the "Generation Atlas." The Atlas includes the four sections directly related to this project. The first section shows a website that holds information from the project. The second section is an interactive map that includes the GCP crops, the target constraints to production and socioeconomic and demographic characteristics of the farming systems. A third section includes a site that stores tabular data on the farming systems. Finally, the seasonal drought index is available as an interactive map for exploring drought probability throughout the growing season.

Conclusion This project brought together impact assessment methods, poverty mapping, drought probability analysis and geographic information science and technology to examine priorities for the Generation Challenge Program. In conjunction with partners, it produced the first developing country map of sub national poverty at standard poverty lines. The project developed a crop specific drought probability map and put it in an online interface for the GCP community. It dev will eloped an extensive database of tables and maps for further use by scientists and analysts working in crop improvement.

112. G4008.50: Delivery Plan remote learning modules

August 2008–July 2010; NCE: August 2010

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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- Cornell University, Ithaca, NY, USA
- Stefan Einarson

Summary
The objective of this project is to develop a series of interactive tools to assist scientists involved in GC programs to develop high quality “Delivery Plans,” based on GCP’s current DPKits. The resulting “DP Remote Learning Modules” will allow GCP grantees to successfully complete their required delivery plans to the satisfaction of GCP (and its various stakeholders and funders) without the need of dedicated, in-person, technical assistance seminars.

GCP grantees will be able to follow, from their home country offices, a pre-established sequence of tasks that result in a completed plan that:

- Clearly identifies and articulates expected program outputs;
- Explains how the program outputs will translate into improved conditions for resource-poor farmers in the target areas and crops of the GCP defines the path by which their innovations will travel through "downstream" organizations to reach the organizations that provide direct support to farmers (normally National Agricultural Research Systems [NARS]);
- Requires validation of the objectives and products by the end-user;
- Provides detailed commitments and milestones (timing, description, and type of product) by the research team to ensure synchronized efforts and clear expectations;.
- Can serve as a reference point for GCP management in follow-up and tracking, and to communicate goals and achievements to its various stakeholders.

The development of a remote learning strategy will permit better plans because it will allow more time and opportunity to internalize the concept and its objectives, and allow for pacing of the DPKit development based on the specific conditions of the grantee teams. It will also be considerably more cost-effective than the principal alternatives (seminars, or travelling training teams).

The online, interactive version of the DPKit has been put into trial use since October 2009. Considerable adjustments were made to reflect the comments of GCP and its users, until the final version was presented in August 2010, incorporating all the suggestions and recommendations from GCP and the users.

Conclusion
The online DPKit has been completed and delivered, in close collaboration with GCP. It includes two videos and a manual that incorporate the lessons about the importance of the information and collaboration with the end-user. The comments, criticisms, and requests for changes from GCP and the trial users have been fully incorporated into the DPKit and manual.

Final products to be used by plant scientists outside the project:
End-users will be able to verify the validity and usefulness of the information provided by PIs about the products, constraints and capacity needs, as well as product distribution for each project.

Crosscutting activities

113. G4008.24: From attractiveness to feasibility: A strategic assessment of the capacity to develop and adopt GCP technologies

January 2008–December 2009; NCE: April 2010

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED; PROJECT REPORT PENDING UPLOAD

PROJECTS COMPLETED IN 2009

Research Initiative crops

Cassava

114. G4005.70.02 (CB20b/RF−FS090)/G5005.90 (2005-FS090): Tapping crop biodiversity for the resource-poor in East and Central Africa (*Cassava component*)

July 2005–July 2009

Principal Investigator
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

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- James Gichuru Gethi, Kenya Agricultural Research Institute-Katumani, Kenya
- Isabelle Ralimanana, FOFIFA /DRA, Madagascar
- Mauricio Francisco, Agricultural Research Institute (IIAM), Mozambique
- Gashaka Gervis, Institut des Sciences Agronomiques, Rwanda
- Geoffrey Mkamilo, Naliendele Agricultural Research Institute, Tanzania
- Robert Kawuki, Namulonge Agricultural and Animal Production Research Institute, Uganda

Summary
This project aimed to assess diversity and genetic relationships in cassava genotypes immediately available to plant breeding programs in seven countries in southern, eastern and central Africa. Diversity was measured using both morphological and SSR markers. In addition the project aimed to (i) increase the involvement of NARS from eastern, central and southern Africa in GCP (ii) empower NARS to access and use information made available, particularly through SP1, to broaden the spectrum of crop diversity used in national breeding programs, (iii) work through the regional crop networks (i.e., EARRNET and SARRNET for cassava) to promote the sustainable use of standardized methodologies on a regional basis, (iv) empower a broad spectrum of stakeholders to link into regional and international research activities, (v) initiate an active network of molecular breeders, and (vi) expose NARS germplasm scientists to the facilities and support provided by the BECA platform.

This project has contributed significantly both to capacity building and delivering information on the genetic diversity and genetic relationships of cassava germplasm within southern, eastern and central Africa. Through this project participants have been trained in database development and use using MS Access, cassava phenotypic descriptors have been standardised and participants have been taught to apply them. A

revised descriptor list is to be published in collaboration with EMBRAPA. Seven new phenotypic descriptors were introduced to NARS; protocols for measuring cyanogenic potential, physiological post-harvest deterioration, dry matter using specific gravity, starch content and leaf retention were added. In addition, ease of peeling root cortex (important for mechanical peeling) using a calliper and cortex peel thickness were included. One scientist from each NARS was given exposure to the ILRI-BecA platform and trained in SSR genotyping for up to three months. This study represents the first assessment of diversity in cassava germplasm from DRC, Rwanda and Madagascar, and is the first systematic regional diversity assessment.

Twenty-nine morphological traits provided limited discrimination among cassava germplasm from the region. This indicates a lack of diversity, a general lack of strong adaptive gene complexes to different eco-geographical locations. Local cassava genotypes have been identified with reasonably high levels of dry matter content, harvest index and leaf retention. These traits will be important in future cassava breeding efforts. It is recommended that the data presented is used to define national breeding strategies and provide a rational basis for importing exotic germplasm with diversity that is unlikely to be present within the region.

Farmer-varieties and local and exotic breeding germplasm constituting 1401 varieties were genotyped at 26 SSR loci. The analysis of farmer-varieties alone was most informative and is presented. Levels of genetic diversity according to allelic richness and average gene diversity (He) were similar in all countries although DRC had marginally higher values; 139.55 and 0.61 respectively. This was only slightly greater than varieties from Tanzania with allelic richness (128.48) and gene diversity (0.60).

Calculation of euclidean distance revealed the relatively close relationship of Tanzania with Kenya and Tanzania with Madagascar. DRC was most distantly related to Mozambique. Three main groups resulted when cluster analysis was applied and results illustrated on a dendrogram. Madagascar formed a group on it own possibly reflecting geographical isolation, DRC, Rwanda and Uganda form a second central African group, and Kenya, Tanzania and Mozambique formed a third east African group. The central Africa and eastern Africa groups may reflect two independent introductions of cassava to the region, one from West Africa, via Sudan to northern Uganda, and the other through coastal East Africa.

Structure analysis identified its minimum of two groupings, one with DRC and Uganda germplasm, and the other containing all other countries. This indicates that differences among farmer-varieties within the region are relatively small.

It is important to note that the large majority of farmer-varieties studied are only conserved within breeding programs in the NARS or in farmers' fields. They are not currently conserved in any systematic way as part of a genebank. It is important that the information presented here is used to structure country and/or regional conservation programs. The data presented may also be used to identify germplasm to import to the region to systematically broaden the genetic base of cassava for genetic gain. Specifically germplasm with greater phenotypic variation for traits of interest, than currently available in the region, should be identified and imported.

Conclusion

This project has contributed significantly both to capacity building and delivering information on the genetic diversity and genetic relationships of cassava germplasm within southern, eastern and central Africa. Through this project participants have been trained in database development and use using MS Access, cassava phenotypic descriptors have been standardised and participants have been taught to apply them. A revised descriptor list is to be published in collaboration with EMBRAPA. Seven new phenotypic descriptors were introduced to NARS; protocols for measuring cyanogenic potential, physiological post-harvest deterioration, dry matter using specific gravity, starch content and leaf retention were added. In addition, ease of peeling root cortex (important for mechanical peeling) using a calliper and cortex peel thickness were included. One scientist from each NARS was given exposure to the ILRI-BecA platform and trained in SSR genotyping for up to three months. This study represents the first assessment of diversity in cassava germplasm from DRC, Rwanda and Madagascar, and is the first systematic regional diversity assessment.

Twenty-nine morphological traits provided limited discrimination among cassava germplasm from the region. This indicates a lack of diversity, a general lack of strong adaptive gene complexes to different eco-geographical locations and perhaps general homogeneity across the region in farmer-preferences for the characters studied. Local cassava genotypes have been identified with reasonably high levels of DMC, HI and LR. These traits will be important in future cassava breeding efforts. It is recommended that the data presented is used to define national breeding strategies and provide a rational basis for importing exotic germplasm with diversity that is unlikely to be present within the region.

Farmer-varieties and local and exotic breeding germplasm constituting 1401 varieties were genotyped at 26 SSR loci. The analysis of farmer-varieties alone was most informative. Levels of genetic diversity according to allelic richness and average gene diversity (He) were similar in all countries although DRC had marginally higher values; 139.55 and 0.61 respectively. This was only slightly greater than varieties from Tanzania with allelic richness (128.48) and gene diversity (0.60).

Calculation of Euclidean distance revealed the relatively close relationship of Tanzania with Kenya and Tanzania with Madagascar. DRC was most distantly related to Mozambique. Three main groups resulted when cluster analysis was applied and results illustrated on a dendrogram. Madagascar formed a group on it own possibly reflecting geographical isolation, DRC, Rwanda and Uganda for a second central African group, and Kenya, Tanzania and Mozambique formed a third east African group. The central Africa and eastern Africa groups may reflect two independent introductions of cassava to the region, one from West Africa, via Sudan to northern Uganda, and the other through coastal East Africa.
Structure analysis identified just two groupings, one with DRC and Uganda germplasm, and the other containing all other countries. This indicates that differences among farmer-varieties within the region are relatively small.

It is important to note that the large majority of farmer-varieties studied here are only conserved within breeding programs in the NARS or in farmers' fields. They are not currently conserved in any systematic way as part of a genebank. It is important that the information presented here is used to structure country and/or regional

conservation programs. The data presented may also be used to identify germplasm to import to the region to systematically broaden the genetic base of cassava for genetic gain. Specifically germplasm with greater phenotypic variation for traits of interest, than currently available in the region, can be identified and imported.

Quantifiable outputs

1. A descriptor list available for cassava
2. A workshop conducted to test the descriptor list
3. Morphological characterisation data available for NARS cassava germplasm from two sites in seven countries
4. A draft manuscript for publication
5. A set of SSR markers defined
6. Genotyping data available for NARS cassava germplasm
7. A manuscript submitted for publication
8. A NARS scientist from each of seven countries trained in a molecular marker technology
9. A NARS scientist from each of seven countries familiar with accessing the BecA/ILRI
10. Passport data relating to NARS germplasm available
11. A database of passport and pedigree data, morphological and molecular data of selected NARS cassava germplasm constructed
12. A database of passport and pedigree data, morphological and molecular data of selected NARS cassava germplasm available on the GCP Central Registry
13. A draft publication
14. Final report

Capacity building: Cassava

115. G4007.13.02: Capacity-building *à la carte* 2007–Marker-aided development of nutritionally-enhanced cassava for Nigeria

July 2007–July 2009

Principal Investigator and Lead Institute

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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- Emmanuel Okogbenin, National Root Crops Research Institute (NRCRI), Umudike, Nigeria
- Martin Fregene, CIAT, Colombia
- Ada Mbanaso, NRCRI, Nigeria
- Nnamdi Eke-Okoro, NRCRI, Nigeria

Summary
This Capacity Building a la carte project is directed at developing nutritionally - enhanced cassava varieties in Nigeria. The project therefore seeks to increase the nutritional status of cassava consumers especially the rural poor that largely depends on this crop for food and to boost the commercial potential of cassava in the local and export markets. The focus of this project has been principally directed to increasing both protein and beta carotene contents of cassava. High protein cassava has its greatest uses in the animal feed industry, where the high carbohydrate and protein content makes it an ideal replacement in the tropics for maize. In this project useful germplasm for both traits has been successfully built in the last two years with the introduction of protein and beta carotene from CIAT and IITA, respectively. New beta carotene cassava genotypes have also been developed in the breeding programme at NRCRI through hybridization activities involving crosses among the introduced germplasm and elite cassava lines. Molecular markers were used to introgress CMD resistance into germplasm introduced from CIAT to enhance adaptation and possible release in Nigeria. The introduced and developed germplasm have been micropropagated in vitro and through short-stem propagation to generate copious quantities for field evaluation under a multi-ecological study. The germplasm have been planted so far in the transitional forest zone and in the Guinea savannah. Field evaluations are still on-going to select the best lines for recommendation for on-farm trails with farmers. The project was also aimed at building capacity in the institute in the area of manpower development in the application of marker technology in breeding, and development of infrastructures. Three NRCRI scientists visited advanced centers for training in MAS, germplasm management and conservation and effective handling and analysis of field and molecular data. Two NRCRI staff were also trained in postgraduate programme in the university. A workshop was also organized to complement project activities in the area of phenotyping for beta carotene and protein contents. Infrastructures for laboratory activities in MAS and Tissue Culture were also upgraded within the period. The high protein and beta-carotene rich (high-value) varieties being currently evaluated will provide a unique opportunity to increase production and provide highly nutritious food, animal feed, flour, and chips for the local and export markets. The novel products would also give Nigeria a competitive edge in the export market. To ensure high levels of adoption and commercialization of high protein and beta-carotene varieties, this project will also link up with stakeholders among the secondary and tertiary end users in the industry and impact assessment has also been planned. The project has resulted in the development of CMD resistant genotypes with high beta carotene and protein contents. The genotypes are being further tested for genotype x environment interactions in multiple environments.

Quantifiable Outputs

1. At least 5-10 copies of 507 genotypes of cassava having high protein and beta carotene contents and combining resistance to CMD established from embryo axes and shipped to Nigeria.
2. At least 6 plants of each introduced genotype established in the field and evaluated.
3. At least 1500 planting materials generated from 2-node cuttings for each genotype and further evaluations.
4. At least 2000 genotypes evaluated in clonal evaluation trials
5. At least 150 genotypes evaluated in participatory variety testing

6. At least 20 genotypes developed for high protein and beta carotene contents with high resistance to CMD for crosses and further evaluation
7. At least 50 genotypes combining high protein and beta carotene contents with resistance to CMD deployed in NRCRI crossing block.
8. At least one marker validated for high protein content.
9. At least one marker validated for high beta carotene content.
10. M.Sc. degree training for 2 research staff from NRCRI
11. Hands-on training on conservation of plant genetic resources and marker-assisted selection for 3 scientific staff from NRCRI at CIAT.
12. Value-added cassava genotypes evaluated with flour millers, livestock industry and other cassava food processors
13. Potential impact of value –added cassava varieties on livelihoods of resource poor farmers and Nigerian economy assessed

Groundnuts

Groundnuts: Capacity building

Capacity building a la carte

116. G4007.13.03: Capacity-building *à la carte* 2007–Application of molecular tools for controlled wild introgression into cultivated germplasm in Senegal

July 2007–July 2009

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- Agropolis–CIRAD: Jean-François Rami
- UCB: David Bertioli
- EMBRAPA: Marcio Moretzsohn

Summary
Groundnut is an important crop of the Sahel Zone of Africa. It is a cash crop as well as a major source of dietary proteins and oil, and also a source for animal feeding. Groundnut cultivation in this area faces important constraints, particularly drought stress and diseases, but the narrow genetic basis of the cultivated peanut Arachis hypogaea L. hampers the development of improved varieties through conventional breeding.

During a previous project supported by the GCP, two amphidiploid varieties (A.ipaensis x A. duranensis and TxAG6) have been transferred to ISRA / CERAAS and each of them have been crossed to four different A. hypogaea cultivars from Senegal to produce backcross populations. Populations derived from crosses of this type segregate strongly for many traits. However, considering the nature of the parental cultivars and breeder's priorities in Senegal, investigation of components of drought tolerance, resistance to leaf spot and seed dormancy have been given top priority.

The main objective of this project is to allow the best use of the molecular tools developed earlier in order to optimize the development of breeding material for these priority traits, from the populations. Introgression lines were then developed from the available material using MAS. This requires the use of integrated genotyping at each step of the breeding process. To achieve this goal, we propose to build on the ISRA/CIRAD/EMBRAPA collaboration to ensure capacity building to PhD students and scientists involved in peanut breeding at ISRA and provide technical backstopping at the key steps of the breeding process for all activities related to MAS.

Thus, a PhD student has been trained at the CNRA at Bambey, in the peanut breeding program. Various techniques have been demonstrated to him who now is using them

routinely in the crossings. He attended also two courses on molecular breeding techniques, the first one in Gent, Belgium (August 14-23, 2007) and the second one in Zaragoza, Spain (June 29 to July 3, 2009), hence he get acquainted with some concepts of modern breeding. Moreover, he spent three months in CIRAD at Montpellier for training on molecular breeding techniques.

The genotyping work allowed identifying 206 SSR polymorphic markers. A total of 230 loci were mapped into 21 linkage groups. However, 26 markers revealed segregation distortions.

Conclusions

The implementation of the project has proven to be very helpful for the collaboration between ISRA, CIRAD and EMBRAPA. This has allowed the improvement of the skill for crossing of groundnut with its wild relatives. The DNA extraction protocol for groundnut was also refined. In this regard DNA can now be extracted successfully by our student from our laboratory in CERAAS. This is a very big breakthrough because it will allow us to not send any seed materiel or tissue samples for genotyping, only very clean DNA samples will be sent for genotyping.

Through the project, the level of polymorphism between the different parents was revealed using microsatellite markers.

For the first time a groundnut SRR-based genetic map was constructed, resulting from crosses between cultivated groundnut and an amphidiploid.

Last but not least, the PhD student has gathered even valuable data from its stay in CIRAD at Montpellier that will allow him, along with field data that he already has, to be able to write his dissertation. We hope that by the end of this year he will be able to defend his dissertation.

Quantifiable Outputs

1. One Ph D student trained in various techniques and methods for achieving successful hybridization between amphidiploids and local cultivars
2. One PhD student from ISRA will be hosted in CIRAD for genotyping of BC1 populations.
3. One scientist/PhD student from ISRA will be hosted in UCB/EMBRAPA to get experience on cuttings and bioassays.
4. One PhD student from ISRA will participate to a course in genetic data analysis.
5. Technical backstopping will be provided at each key step of the breeding process to ensure on-time DNA extraction and genotyping.
6. Genotyping of (BC)n plants will be carried out at CIRAD
7. One course will be organized on groundnut markers, maps, and MAS at ISRA
8. Use of a wide network of research stations and different environments

Maize

117. G4007.24: Seed smoke treatment to favour germination under water stressed conditions

December 2007–November 2009

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating scientists and institutes
- Research Centre for Plant Growth and Development, UKZN: Johannes van Staden, Marnie M Light

Summary
Smoke released from burning vegetation functions as an important environmental signal promoting the germination of many plant species following a fire. It not only promotes the germination of species from fire-prone habitats, but several species from non-fireprone areas also respond, including some crops. The germination stimulatory activity can largely be attributed to the presence of a highly active butenolide compound, 3- methyl-2H-furo[2,3-c]pyran-2-one (referred to as karrikin 1 or KAR1), that has previously been isolated from plant-derived smoke. It is well established that the application of smoke and butenolide breaks seed dormancy and yields earlier testa rupture and overall higher germination rate, although these responses can vary between species. Thus, smoke and butenolide treatments have the potential to improve not only the germination percentage but also the seedling vigor of many species. Regarding maize, this effect is more pronounced as smoke and butenolide treatment results in a massive increase in post-germination growth and seedling vigor. Several hypotheses have arisen regarding the molecular background of smoke and butenolide action.

The aim of this study was to gain a deeper insight into the molecular background of how smoke and butenolide exert their effects on seed germination during imbibition and in the early postgerminative stage.

In this project we investigated the changes in the total transcriptome after smoke and butenolide treatment during imbibition. We demonstrated that although smoke-water and butenolide treatment of maize kernels result in a similar physiological response, the gene expression and the protein ubiquitination patterns are quite different. Treatment with smoke-water enhanced the ubiquitination of proteins and activated protein-degradation-related genes. This effect was completely absent from butenolide-treated kernels, in which a specific aquaporin gene was distinctly upregulated. These findings indicate that the array of bioactive compounds present in smoke-water form an environmental signal and may act together in germination stimulation. To achieve a better understanding of the effect of smoke on seedling vigour, we analyzed gene expression in the early postgermination phase also. Nearly the same genes set was activated as in the imbibition stage and it became obvious, that smoke induces stress-

related patways which lead to the better seedling vigor under stressed conditions. It appears that the 'hardening' effect of smoke is similar to that caused by ABA. These findings are supported by the facts that stress and ABA-related messages and promoter motifs were over-represented following smoke-water treatment. In addition, the joint application of smoke-water and ABA decreased seedling vigour which further suggests a possible interaction between the two compounds. Besides their obvious use in agricultural practices, smoke and butenolide can be used in studies to gain further insight into the transcriptional changes during germination.

Conclusion
In conclusion, accelerated protein degradation or induction of the TIP3.1 aquaporin are key features of smoke and butenolide action during the first hours of imbibition. Considering all the knowledge accumulated to date in terms of smoke action we can assume that these physiological events represent only the 'tip of the iceberg' and these can be regarded as the executers of smoke and butenolide action. Furthermore, the smoke-water and bu8tenolide greatly improved the seedling vigour of maize seedlings and can be used as a growth promoter. The microarray study revealed that smoke-water and the active constituent induce stress-related changes in the global transcriptome of imbibed kernels and young seedlings. It appears that the 'hardening' effect of smoke is similar to that caused by ABA. These findings are supported by the facts that stress and ABA-related messages and promoter motifs were over-represented following smoke-water treatment. In addition, the joint application of smoke-water and ABA decreased seedling vigour which further suggests a possible interaction between the two compounds. As far as the nature of smoke and butenolide perception is concerned, it is highly possible that the smoke 'signal' is perceived by a receptor that is shared with the signal transduction system implied in perceiving environmental cues, or some kind of specialized receptor exists in fire-prone plant species which diverged from a more general one present in a common ancestor, and also found in the non fire-prone plants allowing for a somewhat weaker but still significant response. These major integrators of environmental signals stress and hormone response, could be potential targets for future research.

Final products to be used by plant scientists outside the project:
1. Transcriptome data of smoke and butenolide-induced germinating maize kernels and young seedlings

Capacity building: Maize

118. G4007.13.01: Capacity-building *à la carte* 2007−Capacity building for characterising maize for water stress tolerance at KARI−Katumani

July 2007–July 2009

Principal Investigator and Lead Institute
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NO UPDATE SUBMITTED; PROJECT REPORT PENDING UPLOAD

Rice

119. G3005.10: Exploring natural genetic variation: developing genomic resources and introgression lines for four AA genome rice relatives

January 2002–December 2008; NCE: December 2009

Principal Investigator and Lead Institutes
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- Collaborators (institute[s], researcher[s]):
- Susan R. McCouch, Cornell University, USA
- Claudio Brondani, CNPAF-Embrapa, Brazil
- Baboucarr Manneh & Marie Noelle Ndjiondjop, WARDA, Côte d'Ivoire
- César P. Martinez, CIAT, Colombia
- Miguel Diago Ramirez, Fedearroz, Colombia

Summary
Cereals provide the majority of calories consumed by humans. Cereal production faces growing challenges due to increasing human population, changing nutritional requirements and variable environmental conditions that require new approaches to crop production. Wild relatives of modern crop species have survived for millions of years using natural genetic defenses to endure biotic and abiotic aggressions. These wild relatives represent a valuable source of under-utilized genetic variation that is available to plant breeders and represent an invaluable source of genetic information for modern genomics research initiatives. A systematic approach is required to identify and characterize genes from wild species that can be used to enhance crop productivity in a range of environments and under diverse cultural conditions. Using rice as a model, we propose to (1) develop four libraries of interspecific lines called Chromosome Segment Substitution Lines (CSSLs), targeting chromosomal introgressions from different rice relatives, (2) develop a set of 140 molecular markers (called SNPs) identified in genes associated with tolerance to abiotic stress (drought, acid soils, mineral deficiencies or toxicities), (3) validate the utility of the SNPs by using them in the development of the CSSLs in this project and exploring their value in breeding programs for other cereals (4) analyze a set of advanced CSSLs generated from Asian x African rice crosses for their phenotypic response to drought stress. Generating such resources and knowledge will contribute to the objectives of Subprograms 1 and 3 by (i) utilizing *natural genetic diversity* to develop whole-genome libraries of CSSLs as a permanent genetic resource for both breeding and genomics-based research (ii) producing high-throughput, cost-effective markers to *facilitate access to genetic diversity* in a range of different cereal species (iii) making the CSSLs available to breeders and geneticists so that the intersection of their efforts will continue to generate new knowledge.

Conclusion

Although some deviations have occurred from the initial work plan, the main goals for this project have been achieved. As a summary:

- We developed six populations of CSSLs (Chromosome Segment Substitution Lines) that bear introgressions from the AA-genome rice species O. glaberrima, O. barthii, O. meridionalis, O. rufipogon and O. glumaepatula. These populations will constitute a valuable tool for genetic analyses and will allow us to identify key genomic regions that are associated to agronomically important traits.
- We developed a Universal Core Genetic Map for rice. This map has already been demonstrated as a very useful tool to help at designing introgression populations, particularly in the case of interspecific crosses. It is based on microsatellite markers that we discovered and choose with the help of several bioinformatic packages, including some that we develop at CIAT.
- A database, Paddy Map, was created and is available online (http://mapdisto.free.fr). This database aims to provide means to easily and quickly choose a series of genetic markers to be used to genotype a population derived from a specific cross.
- Five first-generation backcross segregating populations were genotyped and five interspecific genetic maps were developed from these data. These maps will be useful to assess the recombination rates for every wild species we use and will facilitate the localization of important genes or QTLs. All the maps we generated were based on the Universal Core Genetic Map.
- In order to fully exploit the information given by the genetic mapping analyses carried out using crosses that involve the O. glaberrima species, we collaborated with the Arizona Genomics Institute to develop a library of Bacterial Artificial Chromosomes (BAC) for this species. The library is available to the international community of plant genomicists, and it will constitute the basis of positional cloning approaches to identify and characterize important genes for O. glaberrima (this work was also supported by USAID funds).
- In parallel to this project, we designed a computer program that helps geneticists at creating CSSL populations. The program is called CSSL Finder at is available for download as freeware at http://mapdisto.free.fr/.
- A bioinformatic tool to facilitate the discovery of single-nucleotide polymorphisms (SNPs) was set up.
- Seven students and four research assistants were trained.
- Four students from Africa and Latin America do shuttle research between their respective centers and Cornell University.
- The international collaboration between several ARIs, CG centers and NARS was strengthened.
- Several publications are in preparation.

We expect the outputs of this project to provide very useful tools to the scientific community, for gene identification in wild species and pre-breeding purposes.

It is worth to mention that this project is now strongly connected to the OMAP project (Rod Wing, Arizona Genomics Institute). Indeed, BAC libraries for four of the parental wild accessions involved in this project have been or will be constructed soon

at AGI. Also, LGDP (Olivier Panaud's group) have developed a BAC library for the TOG 5681 (*O. glaberrima*) accession. Having this genomic resource available will add a great value to the populations of CSSLs developed here, as rice geneticists will have access to complete kits for gene identification and positional cloning in wild rice species.

Quantifiable Outputs:

1. A CSSL population of interspecific lines bearing introgressed genome segments from the AA genome wild species O. meridionalis
2. A CSSL population of interspecific lines bearing introgressed genome segments from the AA genome wild species O. rufipogon
3. A CSSL population of interspecific lines bearing introgressed genome segments from the AA genome wild species O. glumaepatula
4. A CSSL populations of interspecific lines bearing introgressed genome segments from the AA genome cultivated species O. glaberrima in the japonica cultivated background
5. A CSSL populations of interspecific lines bearing introgressed genome segments from the AA genome cultivated species O. glaberrima in the indica cultivated background
6. A set of 125 anchors represented by three SSR loci each. At least one SSR per anchor is polymorphic for any of the worked crosses of this project
7. A database of polymorphic markers eases the definition of the best set of SSRs to be used for a specific cross
8. Lines with superior behavior under stress (drought) conditions

120. G4005.69.01 (CB19a/RF−FS022): Developing and disseminating resilient and productive rice varieties for drought-prone environments in India

March 2005–February 2008; NCE: February 2009

Principal Investigator and Lead Institute

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EXTRACT FROM FINAL TECHNICAL REPORT

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Dr. Satish Verulkar	Indira Gandhi Agricultural University (IGAU), Raipur, Chhattisgarh

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Dr. R. Chandrababu, Dr. S. Robin	Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu
Dr. Shailaja Hittalmani	University of Agricultural Sciences (UAS), Bangalore, Karnataka
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Collaborators for the Upland Rice shuttle breeding network (URSBN):

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Dr. J.C. Bhatt	VPKAS (ICAR), Alomra, Uttrakhand
Dr. Atul Mehta	RRS, Nawagaon-Anand Agricultural University (AAU), Anand, Gujarat
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Summary

During the four years of the project period, more than 2,000 advanced breeding lines coming from IRRI as well as different partners in the Drought Breeding Network (DBN) and Upland Rainfed Shuttle Breeding Network (URSBN) have been evaluated under severe reproductive-stage drought stress, moderate drought stress, and control irrigated situations at the different partners' sites under the DBN and rainfed upland situations under the URSBN. In the DBN, from the evaluation of more than 1,300 advanced breeding lines, at least several promising breeding lines with high yield potential and good yield under severe drought stress were identified. In 2009, the identified promising lines were evaluated under participatory varietal selection (PVS) trials in farmers' fields at Raipur, Hazaribag, Ranchi, Pusa, and Faizabad, along with popular national and local varieties. Results show that lines IR72667-16-1-B-B, IR70215-70-CPA-2-4-1-3, IR55419-04, R-RF-69, R-RF-23, and IR74371-70-1-1

seem promising under severe drought stress as well as under normal irrigated conditions. Promising drought-tolerant breeding lines IR55419-04, IR74371-54-1-1, IR74371-46-1-1, and IR72667-16-1-B-B-3 were also nominated for testing under the All India Coordinated Rice Improvement Program (AICRIP). Breeding line NDR1045-2 has been released as drought-tolerant variety Shusk Smrat in the state of Uttar Pradesh and one of the other lines, IR74371-70-1-1, has been identified by AICRIP for release as a central variety. A proposal for its release for the states of Jharkhand, Orissa, and Tamil Nadu has been submitted. In the URSBN, over the past four years, more than 700 breeding lines have been evaluated under upland conditions. Promising lines have been identified and are being evaluated under PVS. RR 347-2, an elite line identified by the network, has been released by the Central Variety Release Committee (CVRC) of India as Virendra for the uplands of Gujarat and Orissa. DDR 97 (IET 19258) has been released for cultivation in Gujarat and has been identified for central release in Madhya Pradesh. A release proposal has been submitted for RR 354-1 to be released in Rajasthan and RR 345-2 to be released in Jharkhand. A new source of drought tolerance (VLDT 1, VLDT 2, and Sukhawan) for uplands and IR77298-14-1-2, IR55419-04, Dagad Deshi, Laloo 14 Jonga, Kallurundkar, Baranideep, Lalmati, Kalamkati, Sadabahar, Khiradhan, and Mattaikar for lowlands have been identified. Promising stress-tolerant genotypes VL30242, BAU461-06, BAU404-02, BAU446-06, BAU GVT464-07, BAU GVT465-07, BVD110, CR2340-44, NDR1054-4-1, RR616-1, RR427-21 BL-2, VL3288, RR383-21, and NDR 1091-9 were identified.

Conclusions

The project over the past four years has evaluated more than 2,000 breeding lines contributed largely by different national partners from India and IRRI. The four years of continuous research, evaluation of breeding lines, and dissemination of promising breeding lines from 2005 to 2008 in this project project has resulted in the following:

1. For the medium- and late-duration group, identified breeding lines provide an average yield advantage of 800–1,200 kg/ha over current popular mega-varieties (IR36, IR64, Swarna, and Sambha Mahsuri). Thus, the project has largely contributed toward the development of new, farmer-preferred cultivars combining high yield potential with substantially improved drought tolerance.
2. Breeding line NDR 1045-2 was released as variety Shusk Samrat for eastern Uttar Pradesh.
3. Breeding line IR74371-70-1-1 was identified to be released as a drought-tolerant variety by the Central Variety Release Committee of India in Jharkhand, Orissa, and Tamil Nadu.
4. Breeding lines IR55419-04 and R-RF-69 have been identified for release as varieties in Chhattisgarh.
5. RR 347-2 (IET 17901), an elite line identified by the network, has been released by the CVRC as Virendra for the uplands of Gujarat and Orissa.
6. DDR97 has been released as a variety in Gujarat.
7. New sources of drought tolerance (VLDT 1, VLDT 2, and Sukhawan) for uplands and IR77298-14-1-2, IR55419-04, Dagad Deshi, Laloo 14 Jonga, Kallurundkar, Baranideep, Lalmati, Kalamkati, Sadabahar, Khiradhan, and Mattaikar for lowlands have been identified.

8. There has been policy intervention for initiating systematic trials in rainfed areas under AICRIP and, since 2008, the Directorate of Rice Research (DRR), Hyderabad, principally agreed to initiate such trials. In 2008, such testing was conducted under AICRIP at 8 sites.
9. Around 20 lines found promising in the DBN and URSBN trials were nominated for testing in AICRIP and some of them have been promoted to the next scale of testing.
10. The drought breeding program of national partners involved in the two networks as well as that of IRRI have been strengthened.
11. Learning from each year of drought screening and drought phenotyping has been further standardized and improved.
12. Promising breedeing lines generated in this project are being shared with different institutions and rice breeding community in Africa, South Asia and South East Asia. Further, the breeding lines are available for sharing with any interested institutions. In South Asia, the promising lines are primarily disseminated in India, Bangladesh, Nepal via IRRI to institutions not directly linked to this project and via national partners in areas of institutions linked with this project. In South East Asia, the promising lines are being tested in Laos and Vietnam and in Afirca, these are being tested in western Africa in collaboration with WARDA and in eastern Africa by IRRI's newly established station in Tanzania and Mozambique.

121. G4007.08: Integration of genomic tools with conventional screening for developing NERICA rice cultivars for West Africa

August 2007–July 2009; NCE December 2009

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- Institut de Recherche pour le Développement (IRD)/CIAT: Mathias Lorieux
- Institut D'Economie Rural: Fousseyni Cisse

Summary
The regular droughts observed across West Africa seriously affect rice production resulting in significant economic losses. The objectives of the current study were to select from a pool of genotypes those showing best performance in terms of resistance to drought and to leaf blast disease. A total of 327 rice genotypes were screened on the AfricaRice experimental farm over two consecutive years (2007-08 and 2008-09). Two irrigation treatments were considered: full irrigation from sowing to harvest and

imposed 21-day drought from 34 days after sowing onwards. The study indicated a reduction of plant leaf width, grain yield and plant height in both years with drought tolerance indexes of 4.17, 6.52 and 6.90, respectively. Tillering and plant maturity were not significantly affected by drought, whereas values for canopy temperature and leaf greenness were higher under drought conditions than under fully irrigated conditions. The relationship between each parameter evaluated under fully irrigated conditions and under drought conditions was positive and highly significant with the exception of canopy temperature. Strongest correlations were observed for plant height and leaf width (r =0.53** in both cases). Of the different parameters evaluated, only flowering and grain weight under fully irrigated conditions were significantly associated with rice leaf blast disease assessment date. The rice genotype SIK400-b-56-1-361-18 performed best (in terms of lowest value) for plant recovery after drought treatment, leaf rolling and burning, and blast disease symptoms. Genotypes Kogoni91-1, RAM1, RAM27, RAM47, and SIK400-b-56-1-380-24 exhibited best performance in three of the four parameters. If through additional research the results are confirmed, these genotypes might be considered as drought varieties and the interspecific line as NERICA. New sources of resistance to rice yellow mottle virus (RYMV) and bacterial leaf blight (BLB) were identified in O. glaberrima. A new gene conferring resistance to RYMV (named Rymv 2) was also identified. Several quantitative trait loci (QTLs) of resistance to BLB were mapped. QTL7 is being fine mapped and will be used for marker-assisted selection to improve Mega rice varieties that are susceptible to BLB.

Conclusion

The drought studies confirmed the observations of several studies (Efisue, 2006; Hounkpatin, 2007) that values for canopy temperature, flowering, maturity, and leaf greenness were higher under drought conditions than under fully irrigated conditions. Also confirmed were previous observations that tiller number, plant height, leaf width and grain weight were negatively affected by drought. This report indicates that the rice genotype SIK400-b-56-1-361-18 in particular, along with genotypes Kogoni91-1, RAM1, RAM27, RAM47, and SIK400-b-56-1-380-24, performed best in terms of leaf rolling and burning, plant recovery, and resistance to leaf blast. Further studies are necessary to identify the relevant genes, which could be used subsequently for developing new NERICA varieties.

- Twenty accessions resistant to African BLB were found within African cultivated rice (O. glaberrima) germplasm.
- Five resistance QTLs and one specific resistance gene to African BLB were identified.
- The resistant sources identified in this study may be used in specific regional rice breeding programs to develop BLB-resistant varieties.
- Some of the O. glaberrima accessions showed resistance against particular races that might be useful either in cross combinations with material carrying other genes, or in geographical areas where the races are prevalent.
- The new resistance gene identified will be fine-mapped and used in marker assisted selection to develop varieties resistant to BLB.
- Fine-mapping of the QTL 7 will be performed and all specific resistance genes to African BLB will be pyramided in megavarieties with the ultimate aim of improving rice production in Africa.

Final products to be used by plant scientists outside the project:

1. The drought protocol established.
2. One interspecific line identified with good performance in leaf rolling, leaf burning, recovering potential and resistance to BLB.
3. Five genotypes identified with good performance in at least three of the four parameters indicated above.
4. and 5. Two O. glaberrima accessions resistant to RYMV and BLB identified.
6. Genetic diversity of O. glaberrima determined.
7. Genotyped mapping population with SSR markers.
8. Gene resistant to African strains of BLB identified.
9. One marker for BLB developed.
10. Five new fertile interspecific lines available for geneticists and breeders for testing and distribution regionally.

122. G4008.16: Speeding the development of salt-tolerant rice varieties through marker-assisted selection and their dissemination in salt-affected areas of Bangladesh

January 2008–December 2009

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- BINA: Mirza M Islam

Summary

Salt stress is a major constraint across many rice-producing areas because of the high sensitivity of modern rice varieties to salinity. This forces farmers to grow their traditional landraces with low yield and poor grain quality. In Bangladesh, salt stress affects more than 1 million hectares across the southern parts of the country, and poses a serious problem for resource-poor farmers who depend on rice production for their livelihoods, where other crops can barely grow during the monsoon season. Developing modern high-yielding rice varieties that are adapted to these local saline conditions will provide opportunities for improving the lives of farmers living on these marginal lands. Previously, a major salinity-tolerance QTL on rice chromosome 1, named Saltol, was mapped at IRRI using a recombinant inbred line (RIL) population between tolerant Pokkali and sensitive IR29, explaining 43% of the variation for seedling shoot Na+ uptake (Bonilla et al 2002). This project worked to take advantage of modern breeding tools, such as marker-assisted backcrossing (MABC), to develop high-yielding salt-tolerant rice varieties adapted to southern Bangladesh. We are following a precise MABC strategy employing foreground

markers to select for nearby recombinants on each side of the target QTL, and background markers to select against unwanted introgressions. Scientists at IRRI collaborated with their counterparts at the Bangladesh Rice Research Institute (BRRI), Dhaka University (DU), and the Bangladesh Institute of Nuclear Agriculture (BINA) to refine and use an MABC approach to introgress Saltol into popular varieties adapted to target environments.

During this two-year project, Saltol lines were completed, the background was checked with SSR and SNP markers, and seeds were amplified for subsequent field testing in southern Bangladesh. We employed two RILs from the original IR29/Pokkali QTL population, FL478 and FL378, as donors for Saltol, since these RILs have different Pokkali alleles at the Saltol locus and different levels of tolerance between the seedling and reproductive growth stages. Marker-assisted backcross populations using FL478 (IRRI) and FL378 (Dhaka University and BRRI) were developed and used to introgress Saltol into the Bangladeshi mega-varieties BRRI dhan 28, a popular variety for the dry ("boro") season, and BR11, the major variety for the wet ("aman") season. Initial testing using NILs confirmed the importance of Saltol in improving salinity tolerance in rice. Seeds of the introgression lines of these two varieties are being increased for field evaluation with farmers and for subsequent commercialization

Numerous physiological traits are known to be associated with salinity tolerance in rice and Saltol seems to confer tolerance through restricting sodium uptake. The long-term goal is to identify and combine QTLs controlling different physiological mechanisms to achieve greater salt tolerance in high-yielding rice varieties at different plant stages and across different environments. Toward that end, this project also identified novel salinity-tolerance QTLs from the Bangladeshi landraces Capsule and Boilam, which can be used for future molecular breeding efforts and to further augment the tolerance conferred by Saltol. A molecular marker laboratory was also set up at BRRI and used for training local staff from BRRI, BINA, and DU in the use of MABC. Through this unique collaboration, capacity building through improved human resources and research platforms will enable the use of MABC to introgress agronomically useful QTLs/genes into preferred local varieties and breeding lines even beyond the project time frame.

Conclusions

In Bangladesh, salt-affected areas cover more than 1 million hectares and pose a serious problem for resource-poor farmers who depend on rice production for their livelihoods. Our goal is to precisely transfer QTLs conferring salinity tolerance into modern varieties to enable higher and more stable yields for farmers living off these marginal lands. This project is employing a precise marker-assisted backcrossing (MABC) approach to introgress Saltol, a major QTL for salinity tolerance, into several popular varieties that will subsequently be tested in partnership with farmers. At IRRI, BC3F3 lines with the Pokkali Saltol allele were developed in the background of the popular variety BRRI dhan28, which is an important cultivar for the dry ("boro") rice season in Bangladesh. Subsequently, we tested the level of tolerance of this line in preliminary trails, which showed very promising results regarding the impact of this QTL. We subsequently increased the seeds and shipped them for further field testing and potential commercialization in southern Bangladesh. Additional populations were advanced in collaboration with Dhaka University and the Bangladesh Rice Research Institute (BRRI) to transfer additional tolerance alleles into

BRRI dhan28 and BR11, with the latter also recently completed both at IRRI using FL478 as the donor and also at BRRI, using FL378, and its seeds are being increased for field evaluation. Novel QTLs were identified using new sources of tolerance from landraces being grown by farmers in southern Bangladesh, and these QTLs could be combined with Saltol for higher and more stable tolerance. Through partial support of the GCP, the project built a molecular marker laboratory in the Plant Breeding Division of BRRI to incorporate MABC in its breeding programs for agronomically important traits. Sixteen young scientists were trained in the new laboratory for capacity strengthening in MABC and to ensure proper functioning of the new equipment. A training workshop on participatory varietal selection involving young scientists from several institutions was also completed. A policy dialogue workshop involving the minister of agriculture and numerous senior officials in Bangladesh was conducted where the outputs of this project were highlighted and further needs and support for outscaling were presented and discussed. Progress was made in evaluating farmers' perceptions and the impacts of salt-tolerant varieties in saline coastal Bangladesh, and an economic impact analysis of Saltol in Asian countries was completed. The project made significant progress in institutionalizing MABC in three Bangladeshi institutions, and successfully introgressed Saltol into two popular varieties. However, more efforts and support are needed to combine this locus with other novel QTLs with major effects, to achieve higher tolerance, and to develop more resilient varieties that could cover most of the salt-affected rice areas in Asia and elsewhere. This will contribute considerably to food security and enhance farmers' livelihoods in these unfavorable areas that are, in most cases, highly populated with impoverished communities.

Final products to be used by plant scientists outside the project:

1. Saltol introgressed lines completed for BRRI dhan28 and BR11 using FL478 (IRRI).
2. Saltol introgressed lines completed for BR11 using FL378 (DU and BRRI).
3. Saltol introgressed propopulations for BRRI dhan28 using FL378 in progress (DU and BRRI).
4. Optimized markers for MAB of the Saltol QTL with other recurrent parents (IRRI and DU)
5. Additional tolerance QTLs identified from the Bangladeshi landrace Capsule (IRRI)
6. Additional tolerance QTLs identified from the Bangladeshi landrace Boilam (DU)
7. Molecular breeding laboratory set up at BRRI, Gazipur (BRRI, DU, and IRRI)
8. Capacity building and training for MAS and PVS/impact assessment (BRRI, DU, and IRRI)
9. A total 152 crosses were selected and confirmed as true hybrids (BRRI)
10. A total of 4,543 progenies from F2-F6 generations were selected at saline-prone station, Satkhira (BRRI)
11. In total, 182 genetically fixed lines were isolated from the advanced generations (BRRI)
12. A total of 135 breeding lines from OT, PYT, SYT, PVS, and IRSSTN trials were selected during the project period (BRRI)

13. During the ongoing RLR season at BRRI, Gazipur, a total of 23 single crosses and 6 multiple crosses were made in order to combine salinity-tolerance and high-yield parameters of rice (BRRI)
14. Three breeding lines were evaluated by the National Seed Board to release as a variety: two for T. aman and one for boro (BRRI).
15. Salinity screening at the reproductive stage has been established at BINA
16. Some salt-tolerant F4 and F5 lines have been identified (BINA)
17. MAB has begun and several crosses have been made accordingly (BINA)

123. G4008.41: Application and validation of the major QTL phosphate uptake 1 (*Pup1*)

January 2008–December 2009

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- IRRI: Abdelbagi Ismail

Summary
The major rice QTL Phosphate Uptake 1 (Pup1) was originally identified in a screening in a phosphorus (P) –deficient field under upland conditions in Japan. After fine mapping, the Pup1 locus was sequenced in the tolerant donor variety Kasalath. Based on comparative sequence analyses, gene-specific Pup1 markers have been developed that are diagnostic for Pup1 in a wide range of rice varieties. These markers, in conjunction with SSR and SNP background markers are now being used to develop Pup1 introgression lines of three Indonesian upland varieties at ICABIOGRAD and two irrigated indica varieties at IRRI (Philippines) by a marker-assisted backcrossing approach. Two field experiments were conducted with the Indonesian BC2F2 Pup1 lines and tolerance of Blast infection was assessed in the BC2F3 generation in a green house experiment. The indica Pup1 lines are currently at the BC2F2 generation and the BC2F1 generation has been genotyped using the SNP marker platform at IRRI. Phenotypic data of BC1F1 plants suggest that the few remaining background introgressions and heterozygous loci contribute to the overall better performance of the segregating breeding lines in comparison to the parental lines IR64 and IR74. The lines are now being further purified by selfing and one additional backcross to quantify the effect of Pup1. Contrasting BC2F2 lines will be analyzed under P-deficiency and drought stress during the DS2010 at IRRI. Purified lines will be available later next year.

A Pup1 marker survey of more than 150 diverse rice accessions revealed that Pup1 is largely absent from irrigated varieties but conserved in many upland breeding lines.

Accessions with contrasting Pup1 haplotype were selected and phenotyped in two seasons under drought stress (IRRI upland farm) and P-stress conditions (Pangil, lowland site). Preliminary data analyses suggest that Pup1 confers a significant yield advantage under drought conditions whereas no consistent significant effect of Pup1 was observed under anaerobic, lowland conditions. However, more detailed analyses of the date are still ongoing. Selected accessions with contrasting Pup1 haplotypes were analyzed using the SNP marker platform at IRRI to assess if other chromosomal regions might contribute to the observed positive performance of accessions with the Pup1 locus. The identified regions are now being assessed in detail.

A beneficial effect of Pup1 under upland but not under lowland conditions is well in agreement with our molecular assessment of Pup1 candidate genes and other phenotypic data that suggest that Pup1 is associated with drought tolerance (see project G3008.04: Drought from Different Perspective: Improved Tolerance through Phosphorus Acquisition). Detailed analyses of root traits in Pup1 near isogenic lines (NILs) grown under drought and –P conditions showed that +Pup1 NILs maintained longer root hairs under drought stress but not under well-watered conditions. In addition, a higher degree of root lignification was observed in +Pup1 NILs (see G3008.04 and annual report 2008 this project). The importance of these findings is supported by recent data from a micro array analysis that revealed several differentially expressed cell wall-related genes, with some having been associated with root hair development and elongation (Pariasca-Tanaka et al 2009).

Conclusions

The main objective of the project was the development of Pup1 introgression lines by marker assisted backcrossing. The markers that are now available are diagnostic across a wide range of rice accessions and we have started to contact NARES partners in India and Africa to genotype local rice accessions. Pot experiments and field data derived from diverse accessions with contrasting Pup1 haplotypes so far suggest that Pup1 is most effective in upland/drought prone environments, however, final conclusions on the effect of Pup1 under lowland/irrigated conditions can only be drawn after IR64-Pup1 and IR74-Pup1 lines have been phenotyped under P-deficient irrigated and drought conditions at IRRI next year. The upland sites that were chosen for phenotyping of the three upland Pup1 introgression lines in Indonesia were not ideal since stress (aluminum toxicity) was too severe at one site (Jasinga) and residual P was too high at the other site (Lampung). ICABIOGRAD is now identifying more suitable sites. The field experiment at Lampung showed that the Pup1 lines performed as good as the parental lines. Since the Pup1-recipient parents are already very well adapted to local upland conditions, the yield advantage of Pup1 might be relatively small and therefore difficult to quantify in field experiments given the general variability of field data. More extended evaluations of the Indonesian Pup1 lines will be conducted next season at IRRI and in multi-location trials during the WS2010 after seeds have been increased and additional genotyping of selected lines has been completed.

Key Products Developed by the Project

1. At least five Pup1 introgression lines developed and tested in multilocation trials
2. Target environment for Pup1 identified
3. Effect of Pup1 on drought tolerance quantified
4. At least two students trained
5. At least two publications in peer-reviewed international journals

124. G5005.22: Network of Indian institutions and scientists working on development of drought-tolerant rice varieties

March 2005–February 2008; NCE: February 2009

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FINAL REPORT PENDING UPLOAD

125. G7009.03: Rice Challenge Initiative start-up project

June 2009–December 2009

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Summary
The Generation Challenge Programme launched seven Challenge Initiatives, including one on rice with a focus on drought in Africa, during a meeting held in Montpellier in February 2009. Three projects were written during the course of that meeting and sent to the GCP management team for review, as a result of which the GCP management team and the review and advisory panel recommended both merging the three projects and also actively involving partners from national programs. The coordination and integration of the three projects were subsequently discussed over three days during which common understanding was reached on the content of the projects, the objectives and expected outputs. We also discussed the key sites for drought phenotyping in each participating country.

Conclusion
By meeting in Cotonou, an overall review of the three projects was done. Participants were exposed to each detail of the projects and ensuing discussion on individual activities was intense and constructive to the overall satisfaction of all those involved. The project coordinator was identified and given the responsibility of leading the writing of the combined proposal in a participative manner with contributions and active participation from all NARS and other partners of the project. The final project proposal and budget was approved by the GCP. The sites for drought phenotyping were identified in each NARS country.

Final products to be used by plant scientists outside the project:

1. Rice CI proposal written and approved
2. Rice phenotyping sites evaluated for capacity to undertake work for Rice CI

Wheat

126. G3007.06: Genetic dissection of drought-adaptive mechanisms in bread and durum wheat through large scale phenotyping methodologies

August 2007–August 2009; NCE: December 2009

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- Peter Langridge, ACPFG, Australia
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- B. Mishra, DWR, India
- Ravish Chatrath, DWR, India
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Summary
Declining water resources and unpredictable rainfall are serious threats to crop productivity throughout the world. Although wheat is relatively well adapted to moisture stress, and breeding progress using conventional approaches has resulted in significant improvements in productivity in rain-fed areas, there is considerable scope to improve the scale and pace of progress through exploiting the genetic diversity that exists in wheat genomes. This project has used a collaborative model, combining partners with expertise in genetics, breeding and physiology thus facilitating the design of agronomic and genetically relevant mapping populations, a realistic and rigorous approach to phenotyping, and application of the most appropriate biotechnologies. The collaborators work in three major wheat producing countries (India, Mexico and Australia) where the crop is either rain-fed or grown with restricted irrigation and the research material (bread wheat and durum wheat mapping populations) offers a unique ability to dissect the genomic effects of drought tolerance (particularly for the D genome). The project provides selection tools and methodologies including genetic and physiological markers that can be applied in breeding programs worldwide and well characterized experimental populations that can be used to develop similar tools in other stress prone environments.

This project focuses on large scale phenotyping methodologies in three mapping populations (DH1=Kukri × RAC875, DH2=Excalibur × Kukri, DIC=Atil × *Triticum dicoccon*). Field trials have been conducted at CIMMYT, Mexico, during the past 3 years, with results available for 2 seasons of irrigated, drought and hot irrigated conditions for each of the populations. The DH1 and DH2 populations have also been extensively trialled under rain-fed conditions at approximately 20 sites over 3 years in

southern Australia for the genetic dissection of drought adaptive mechanisms. Additional trial information is also available from sowing under drought and irrigated conditions in India. These trials have enabled the compilation of a comprehensive database of physiological information, for the three populations, that is available for the genetic dissection of drought and/or heat adaptation.

Through a combination of precision phenotyping, on the three populations, in conjunction with deployment of the latest molecular marker technologies, genetic markers associated with drought adaptive traits have been identified. Well characterised genetic maps have been constructed for each of the bread wheat populations, with the DH1 population comprising 455 molecular markers (7.6 cM average distance) and the DH2 population comprising 393 molecular markers (6.3 cM average distance). Primary map construction of the Atil × *T. dicoccon* populations has also been completed. The map consists of 894 markers (853 DArTs and 41 SSRs), with an average marker spacing of 2.5 cM. The genetic analysis of the DH1 and DH2 populations has identified five target regions for detailed mapping (1B, 3B, 6A, 7AS, 7AL). For each region, screening of around 850 lines in the RIL populations has been undertaken to identify lines showing a recombination event within the target interval.

Field evaluation can often involve too much uncontrolled variation to allow the dissection of drought adaptive traits into their physiological components. For this reason the project has also investigated the application of high throughput phenotyping under controlled environments. It is well know that accurate simulation of drought in a controlled environment is difficult to achieve, therefore, a comprehensive range of drought adaptive traits have been evaluated in field environments for comparison to those measured in controlled conditions. From this research we have identified that yield, grain number and grain weight appear to be the most likely candidates for such an approach, however, other traits also offer likely possibilities. Chlorophyll content and flag leaf waxiness were other traits that represented the most likely targets for further work.

To complement this research, the development of new phenotypic screening protocols was also initiated. A technique was designed to measure crop establishment through analysis of ground cover by utilizing digital photography and image analysis software. Protocols were developed to obtain photographs at high speed and to automate the processing, and analysis, of the photographs to increase speed and reduce human interpretation. The Digital Ground Cover analysis tool provided an accurate representation of percentage vegetation coverage, with several advantages being identified over other measurement tools. In addition, the potential for developing a high throughput screening protocol for root growth was investigated. The study established a methodology for the high throughput identification of lines with increased root depth using relatively inexpensive gypsum blocks. Subsequent research has also investigated the dynamics of root response to moisture stress and availability.

Finally, one of the aims of this project was also to generate mapping populations for drought adaptation that are not confounded by phenology. Twelve populations consisting of approximately 1000 RILs have been developed. Preliminary studies indicate that 80% of the individuals in the populations have a 9–12 day difference in the number of days to anthesis in an irrigated environment. This is a positive indication of the usefulness of the populations, as under drought conditions, and after

a reduction in the size of the populations, this range is expected to be further reduced, ensuring the development of a valuable set of mapping populations.

Conclusion
The key aim of this project was to identify candidate loci for drought adaptive traits for potential application in MAS, positional cloning and screening of genetic resources for new drought adaptive alleles. To complement this objective, research also aimed to validate high throughput drought screening protocols that could be realistically extrapolated to performance in field environments, and to generate new mapping populations that will not be confounded by major phenological genes. A comprehensive database of physiological information has been compiled, and has lead to the identification of potentially valuable candidate loci in both of the bread wheat populations. Activities with the populations have also focused on fine mapping of the loci in the bread wheat populations, and combining phenotypic and genotypic data for QTL discovery in the durum wheat population. Furthermore, glasshouse experiments were designed to assess drought adaptive traits in a controlled environment and identified some likely targets for further study. The generation of mapping populations for drought adaptation that are not confounded by phenology is also well advanced.

Final products to be used by plant scientists outside the project:

1. Data base for 800 genotypes of their phenotypic expression for ground cover (GC) canopy temperature (CT), carbon isotope discrimination (CID), and soluble stem carbohydrates (SSC), yield and agronomic traits
2. Report identifying QTLs associated with yield, drought adaptive (GC, CT, CID and SSC) and agronomic traits
3. Novel large-scale phenotyping methodologies
4. Analysis and report identifying the degree to which a range of drought-adaptive traits can be reliably estimated in controlled environments
5. Crossing plan among complementary lines to develop populations with negligible diversity for flowering across a range of environments

127. G7009.08: Improving drought tolerance in wheat for Asia

November 2009–December 2009
Startup grant for wheat RI in China and India

Principal Investigator
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Cross-crop projects

128. G3005.01: Identifying genes responsible for failure of grain formation in rice and wheat under drought

January 2005–December 2007; NCE: June 2009

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutions and scientists

- K. McNally, R. Bruskiewich, R. Mauleon (IRRI, Philippines)
- R. Dolferus, L. McIntyre, R. Richards, T. Condon (CSIRO Plant Industry, Australia)
- Z.-Q. Ma (Nanjing Agricultural University, Nanjing, China),
- S. Kikuchi, K. Satoh (NIAS, Tsukuba, Japan),
- R. Chandra Babu (TNAU, Coimbatore, India).

Summary
Drought is the main cause of yield loss in rainfed rice and wheat, and losses are most severe when drought occurs at the flowering stage. A drought phenotyping technique was used to standardize the drought stress protocol, using the fraction of transpirable soil water as the covariable against which physiological and molecular responses can be measured. Microarray data from experiments on rice and wheat panicles, anthers, and peduncles were collected, subjected to statistical and cluster analysis, and (where possible) related to promoter structure, genome location, and role in metabolism. Transcriptional responses to stress of specific genes or subfamilies of genes were assessed using RT-PCR. Included in this group were invertase genes of wheat and rice, ABA response element binding factor (ABF) genes of rice, and vacuolar H+-PPase genes of wheat. RNA in situ hybridization was used to establish whether genes within a network were indeed coexpressed in specific cell types. In the case of transcription factors OsABF1, OsABF5, OsVP1 and their putative target genes, coexpression was shown in the primary and secondary vascular systems. In wheat, CSIRO has focused on ESTs that were identified in cDNA libraries from the reproductive parts of the plant and revealed four wheat cell wall invertase, three NCED, and three ABA8OH genes. RT-PCR confirmed that these genes are expressed in wheat anthers and ovules. The RT-PCR fragments of these genes were cloned and will be used to screen the anther- and ovule-specific cDNA libraries for additional candidate and full-length copies of these genes. Segregating populations of rice RILs were characterized to identify lines with divergent responses to stress during flowering. Three mapping populations including a large F_5 IR64/Moroberekan population were phenotyped at IRRI under field-managed drought stress during reproductive stage. Data were used for QTL analysis of peduncle elongation and spikelet sterility traits. In India, lines showing extremes of drought response were identified in four F_2 populations derived from crosses of drought-tolerant Indian

landraces (PL and PMK) and three improved varieties (IR20, Co 43, and IWP), and the populations were used in QTL analysis of reproductive-stage processes under drought. Stable QTLs were identified for reproductive-stage drought resistance and some were found to be consistent across genetic backgrounds. Overall, this project delivers new insights and products that could be exploited for dissecting the complexity of reproductive-stage drought and opens new opportunities for gene discovery and efficient breeding of rice and wheat under water-scarce environments.

Conclusions

The project focused on two major processes involved in reproductive-stage drought and spikelet sterility: peduncle elongation and anther dehiscence. Because cell-wall invertases play an important role in carbon allocation to developing organs, we examined the tissue-specific expression and drought sensitivity of the corresponding genes (OsCIN1 to 9) at heading in the widely grown drought-sensitive cultivar IR64. OsCIN1-5,8 were expressed to varying degrees in the flag leaf, panicle, anthers, and peduncle at 1 day before heading. Our data showed clearly that cell-wall invertase genes, as a class, respond rapidly to water deficit in anthers and peduncles and, through a reduction in sink strength, help to coordinate a delay in anthesis and heading. By contrast, vacuolar invertase OsVIN2 was up-regulated by drought stress in flag leaves, panicles, anthers, and peduncles. Although OsCIN1-3,5,8 were active in the peduncle, only OsCIN2 was expressed strongly and preferentially at the base, where cell division and cell elongation occur. Drought stress halted peduncle elongation and reduced tremendously OsCIN2 transcript level, on rewatering, peduncle elongation was restored and OsCIN2 transcript level recovered partially. The ABA level of peduncles increased significantly under drought stress and returned to the control level on rewatering. Detached peduncles floated on water elongated little and lost all OsCIN2 transcripts, but on addition of GA_3, they elongated rapidly and maintained high OsCIN2 transcript levels. ABA antagonized both peduncle elongation and maintenance of OsCIN2 transcript levels. We conclude that this antagonism is a potential intervention point for breeding strategies directed at enhancing panicle exsertion and spikelet fertility during or after drought stress at heading.

The development and standardization of the dry-down approach using FTSW as stress covariable allows careful comparison of degree of stress with degree of yield reduction through gradual stress development and simultaneous analysis of gene expression profiles. Our findings confirm that the GA-ABA antagonism is a major feature of drought responsiveness in the anther and the peduncle. Therefore, a first potential intervention point for enhancing spikelet fertility would be to maintain plant water status in the reproductive structures, through dehydration avoidance mechanisms, to reverse the drought-induced rise in ABA content in the anthers. Two potential additional intervention points for carbon supply to the growing peduncle under drought stress are the overexpression of the cell-wall invertase OsCIN2 and the conversion of rice to fructan accumulation.

Microarray analysis reveals that the number of genes responding to drought stress in rice and wheat is very large, with numerous differences seen between drought-tolerant and drought-sensitive lines. At present, the number of genes is too large to be conveniently used as a source of candidate genes for drought tolerance. Complementary approaches should facilitate the analysis and identification of the key

pathways through the use of mutants, RNAi lines, RILs, and advanced backcross lines. Microarray analysis also showed repeatedly that different members of a gene (sub)family are expressed in a given tissue, with some members being up-regulated by stress, some down-regulated, and some unaffected. RNA in situ hybridization showed that some of these genes are expressed in the same cell types, raising questions about the specific roles of the proteins encoded by the different transcripts. This situation is seen not only for transcription factors where the DNA binding site is conserved, implying competition for the same promoter sites (e.g., OsABF1 and OsABF5) but also for protein kinases and phosphoprotein phosphatases. The potential for cooperation and antagonism between members of the same protein (sub)family should be further investigated in rice and wheat under drought to select the key genes to be used in transformation.

The combination of transcript profiling, DEG, and QTL analyses allowed the mapping of many drought QTLs specific for reproductive-stage processes and the discovery of several regions of coordinated expression in rice. These data should be further tested and validated in wheat. This approach opens promising avenues for fine mapping of yield-related QTLs and gene discovery for marker-assisted selection in rice and wheat.

Final products to be used by plant scientists outside the project:

1. Bioinformatic analysis to identify four genes differing between accumulators (wheat) and non-accumulators (rice) of fructans
2. Bioinformatic analysis conducted on replicated rice microarray data to identify key cis elements in drought-responsive promoters
3. Five genes analyzed by TILLING
4. Four candidate genes linked to drought-induced arrest of peduncle growth identified for RT-PCR
5. Eight mega varieties screened for differences in drought stress on peduncle elongation, anther dehiscence, and grain filling

Crosscutting activities

Breeding

129. G4008.30: Development of a GCP phenotyping network

February 2008–February 2009

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Participating consultants
- John C O'Toole, OK, USA

Summary
This report is a first step towards the establishment of a strategic network of field drought phenotyping sites for GCP target crops in order to provide the necessary genetic resources for breeders working towards water-limited environments.

A list of potential candidate field phenotyping sites was developed for South America, Africa and Asia with respect to their expected capacity to serve as phenotyping sites. These sites were visited by three consultants using a standard form of minimum data entry. Sites were visited in Africa, South America, China, India and Thailand. Visited sites were assessed according to data on climate, crops, soils, biotic factors, human resources, expertise in plant water relations, management effectiveness, infrastructure, evidence of current research quality, transportation, security, and more.

Recommendations are made with respect to site potential as FPP, LPP or TPP. Additional inputs from GIS information, crop models and crop homology maps as developed under a stand alone component 2 of the project are reported by that project.

The conclusions and recommendations of this report are submitted directly to GCP Management Team as a project output document entitled: "Recommendations to the GCP Management Team on how to define and implement the GCP phenotyping network".

Conclusion
Submitted as Output #3 entitled "Recommendations to the GCP Management Team on how to define and implement the GCP phenotyping network".

Key Products Developed by the Project
1. List of candidate LPP and FPP sites based on site visits.
2. Detailed reports for the visited sites submitted to GCP management Team as computer files.
3. Recommendations to the GCP Management Team on how to define and implement the GCP phenotyping network.

Capacity building

130. G4007.21: Genotyping Support Service

August 2007–February 2009

Principal Investigator and Lead Institute
Humberto Gómez Paniagua, GCP

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- Xavier Delannay, GCP
- M Carmen de Vicente, GCP

Summary
The GSS started with a pilot phase in 2006. The pilot phase offered the opportunity to development of administrative and legal procedures, which were later applied with the applicants to the first open call (August-October 2007). The management of this first call allowed the implementation of further improvements to the procedures.

In its first call, GSS received 33 applications, out of which 25 were selected (then 6 ceased). The 19 requests in process are as follows: Potato 5, Cassava 4, Rice 3, Sweetpotato 2, Maize 1, Chickpea 1, Coconut 1, Yam 1, Musa Ensete 1. These applications were received from Tanzania 4, Bolivia 1, Kenya 3, India 1, Ghana 3, Brazil 2, Iran 1, Sri Lanka 1, Chile 1, Ethiopia 1 and China 1. Fourteen of these applications aim to conduct molecular characterization of germplasm and five address plant breeding issues such as marker assisted selection, gene tagging or genetic map development. The workshop for this call is planned on January 2009.

Activities covered in the current reporting period are as follows:
1) Refinement of accepted proposals
2) Development of an improved procedure to contract service providers
3) Fine-tuning of the applications: “Genotyping Support Service Request Form”, applicant’s DNA quality requirements and standards to control the quality of the genotyping labs results
4) Preparations for the 2nd GSS workshop: workshop venue and enhancement of content
5) In close collaboration with Cropster, development of a module to manage new rounds of call for applications to the GSS
6) Monitoring implementation of results of finished services as well as progress in ongoing services, 7) Opening of 2nd call for GSS applications.

Conclusion
General conclusions can be summarized by referring to the increasing demand of services, as evidenced through the new call for applications. This indicates that the GSS fills indeed a real need of developing country partners in an efficient manner. Several contrasting situations were found in this call and innovative solutions devised to cope with them and a much larger workload. These actions allowed the GSS to deliver the expected services and outputs in an effective manner.

Human resources (support to teams, students, travel grants, workshops)

131. G4005.71.01.03: CB21: Fellowships/travel grants (2005)

January 2005–December 2009

Principal Investigator and Lead Institute
Carmen de Vicente, GCP.

PROJECT REPORT PENDING UPLOAD

132. G4009.05: Training workshop on marker-assisted breeding

April 2009–September 2009

Principal Investigator and Lead Institute
Carmen de Vicente, GCP

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes

- IAMZ
- WUR

Summary
The course was conducted during the week starting June 29th to July 3rd, 2009 at IAMZ in Zaragoza Spain. It was addressed to NARS participants involved with the Tropical Legumes I and the Genotyping Support Service GCP Projects. The objective of the course was to introduce the participants to the analysis of single and MET trials using mixed models, and demonstrate how these models can be extended to detect QTL and QEI. The methods illustrated both in the context of conventional QTL mapping (i.e. using designed segregating populations) and in the context of LD mapping (i.e. using diverse populations). The course also touched on the crucial steps of molecular map construction as well as on the analysis of population structure in diverse populations.

Conclusions
Nineteen researchers involved in TLI or GSS received high quality instruction regarding the application of advanced statistical procedures and the use of software for the analysis of molecular markers in the context of breeding. The use of molecular markers will help researchers improving the accuracy of their assessment of phenotypic and genotypic performances.

Quantifiable Outputs

1. 5 days of theoretical and practical sessions of advanced statistical analysis using mixed models.
2. 19 researchers from NARS engaged in either TLI or GSS trained.

Learning materials and resources

133. G4005.63: Interactive Resource Centre & Helpdesk

August 2007–July 2009

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Summary
The Interactive Resource Center & Helpdesk continues to grow its user base, increasing by about 30% per year. Over the last year over 6,400 'unique users' (as identified by StatCounter) have hit the site, from over the world but with a high percentage from Africa (see map figure, Appendix F).

The newest addition this year is a Facebook page, linked and complementary to the IRC site. Facebook is a free social networking site where users can post news and have discussions. I am hopeful this may facilitate discussions, but it is too early to say how well this will work. So far there are about 12 people signed up, from Africa, India, and South America.

This year I have also worked more closely with the GCP Bioinformatics Helpdesk, in cases where I received questions that were more appropriate for that helpdesk (in particular, we have had questions on QTL analysis).

A number of new items were added last year, in response to user requests and suggestions from the GCP Management Team. A Frequently Asked Questions (FAQ) site was added and will be updated regularly. A Helpdesk Team page was added to give faces to some of the people that help answer questions. Due to the high number of questions about DNA extractions, a DNA Extractions Troubleshooting page was added, along with a new protocol for removing RNA from DNA Extractions. "What's New" was added to the main page so users can immediately see the latest additions.

In addition, I now keep an email list of people who have requested to be alerted to announcements and new items. This is over 130 people now, mostly from Africa; I send out an update every few months.

Two of the most well-received items have been the Sorghum Contact list and the Millet contact list. These were compiled by looking online for people working on sorghum, compiling a list, then sending them an email asking them to reply if they did NOT want to be posted on the page. No one requested to be taken off the list, indeed many people replied to suggest "descriptor words" to go with their name and to suggest other people to be on the list. In addition, I am still receiving requests from people to be added to the lists. Currently the sorghum list contains approximately 100 scientists and the millet list over 85. Both lists are available sorted by country or by last name. The lists have already been used by a number of people to announce news

of interest to the community, so I feel this has been a highly useful resource. The Sorghum list is available here http://irc.igd.cornell.edu/SorghumContacts.html, the millet list here http://irc.igd.cornell.edu/MilletContacts.html. People can write to me to request a file with the list (I did not make this downloadable to avoid it being used for spam). A list for cassava, the next most highly requested crop, is in progress and contacts have been made to begin compiling more to the list, before sending it out.

Towards the goal of increasing awareness and usership, a news item was posted to the Sorghum e-News newsletter, and reciprocal links have been exchanged with a number of other groups, including the GIPB (Global Partnership Initiative for Plant Breeding). A poster was presented and fliers made available at the GCP ARM 09 as well.

As usual, 2 or more news items have been posted each month, prioritizing journal articles that may be relevant to plant breeders, and news of possible funding sources. New links to several learning modules, as well as new protocols, have been added.

A number of users have requested a posted list of Genotyping Services (where samples can be sent for genotyping) as well as where primers can be ordered. I have begun discussions with colleagues on how to do this (including the GCP GSS director) but it has been a little more complicated than I expected, given that they change frequently, and I would want to post a list but not recommend any in particular. I will continue to work on this.

Conclusions

In summary, I am very pleased with the progress of the IRC this year. Nearly all goals were met, and the reception of the Sorghum and millet lists was very positive. Number of users has increased each year, and the new map shows a clear impact has been made in Africa in particular.

Quantifiable Outputs

1. Questions are answered on a same-day basis
2. FAQ will be posted
3. Photos and bios of the scientists behind the Helpdesk will be featured on the site
4. A Comparative Genomics learning module will be posted
5. Links to 2-4 other learning modules posted
6. Additional protocols will be posted (minimum 3)
7. List of available genotyping services posted
8. 1-2 news articles featured each month
9. survey sent to known users and posted online
10. Responses compiled
11. Report of the results published
12. Lists of researchers and contact info for scientists working on sorghum will be made available, including contact info and areas of interest
13. Contact lists for millet researchers will be made available
14. Contact lists for cassava researchers
15. 2-4 News articles describing the IRC & Helpdesk will be sent to relevant newsletters (ie. AMMANET, Plant breeding newsletter, etc)
16. A «world map» of users will be drawn

Crop information

134. G4005.22: Development of GenerationCP domain models and ontology

January 2005–December 2008; NCE: December 2009

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists
- IRRI, Thomas Metz, Martin Senger, Graham McLaren
- Bioversity, Elizabeth Arnaud, Tom Hazekamp, Adriana Alercia
- CIMMYT, Rosemary Shrestha, Guy Davenport
- CIP, Reinhard Simon
- ICRISAT, Jayashree Balaji

Summary

The GCP domain model and ontology form the backbone of semantic integration standards for GCP data and tools in the GCP platform and network.

Previous years of effort in this task established a mature scientific domain model documented on the Pantheon project web site (http://pantheon.generationcp.org). Since 2007, the focus of the task has shifted to the development of ontology to parameterize the model for specific semantics, in particular, ontology for plant and trait characteristics across GCP mandated crops. This work is also documented on the Pantheon web site and also, for end users, on the GCP McClintock web site (http://mcclintock.generationcp.org).

Conclusions
- Production-quality of the GCP domain model is now routinely applied, using formalized GCP use cases, to platform and network systems integration of GCP databases and tools.
- Full potential of semantic integration of GCP data, in particular, phenotyping data sets, awaits further elaboration of GCP ontology dictionaries and elaboration of ontology management support in the GCP platform.

Key Products Developed by the Project:
1. GCP domain models published at http://pantheon.generationcp.org applied to GCP platform and network implementations to manage GCP data.
2. Data template and GCP platform tools cross-linked to ontology browser/selector tool connected to GCP ontology database.
3. GCP domain model ontology published online with full documentation and populated with priority sets of target ontology, for application to GCP data annotation

135. G4006.08 Data analysis support for existing projects in SP2 with emphasis on integrating results across gene expression and QTL mapping experiments

January 2006–December 2008; NCE: June 2009

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- IRRI, Ramil Mauleon, Richard Bruskiewich, Hei Leung
- NIAS Shoshi Kikuchi
- JIC Andreas Magusin
- CIMMYT Trushar Shah, Jose Crossa, Yunbi Xu
- CIP, Reinhard Simon

Summary

- Maxd GCP is currently being used to store and manage microarray expression data, QTL data from maize and rice studies are currently being managed in a GCP implementation of Generic Genome Browser (Gbrowse), CMap (Comparative Map viewer), and Ensembl.
- Improvements and extensions of the analysis tools were made for MAANOVA, EASE and Mapman.
- Open source EST analysis software and in house scripts and Perl modules written for EST analysis are available on the Paracel machine.
- The GCP pipeline and analysis tools are being used in GCP projects and the results were presented at the GCP ARM.
- Various consultations/collaborations with GCP & non-GCP partners using the analysis pipelines were done during the year.
- 15 and 22 million Solexa reads from the two chickpea genotypes (ICC1882 and ICC4859) were mapped (with default parameters) against a reference dataset

Conclusions
The project has supported a number of projects for which there was little bioinformatics support. The ultimate goal of adding value through comparing datasets is now starting to be achieved.

Final products to be used by plant scientists outside the project:

1. List of orthologue candidate genes or syntenic regions for rice, maize and wheat (IRRI-CIMMYT)
2. Candidate genes from chickpea associated with drought traits identified. (ICRISAT)
3. Two day workshop for up to 20 scientists prior to the 2008 ARM (All)

136. G4006.16: Development of an integrated GCP Informatics Platform

January 2006–December 2009

Principal Investigator and Lead Institute
Martin Senger, IRRI; m.senger@cgiar.org

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institututes and scientists

2006

- Guy Davenport - CIMMYT
- Reinhard Simon – CIP
- Richard Bruskiewich – IRRI
- Martin Senger – Consultant with IRRI
- Akinnola Akintunde - ICARDA
- Manuel Ruiz - CIRAD
- Jayashree Balaji – ICRISAT
- Andrew Farmer – NCGR, Consultant with CIMMYT
- Maseru Takeya - NIAS
- Jane Morris – African Centre for Gene Technologies (ACGT)
- Natalia Martins – EMBRAPA

2007

- Sub-Task I – SP1 Use-Case (lead from CIRAD)
- Manuel Ruiz – CIRAD
- Brigitte Courtois - CIRAD
- Sub-Task II – SP2 Use-Case (lead from NIAS)
- Shoshi Kikuchi, Maseru Takeya - NIAS
- Richard Bruskiewich, Ramil Mauleon – IRRI
- Marcos Costa, Georgios Pappas – EMBRAPA
- Sub-Task III SP3 Use-case (Lead from CIMMYT)
- Guy Davenport – CIMMYT
- Kyle Braak –CIMMYT
- Manilal William – CIMMYT
- Key Collaborators from related projectsTemplate GCP Final Technical Report Updated October 2010
- Martin Senger – Consultant with EBI
- Mathieu Rouard - Bioversity

2008

- Manuel Ruiz (CIRAD)
- Pierre Larmande (CIRAD)
- Xavier Argout (CIRAD)
- Guy Davenport (CIMMYT)
- Marcos Costa (EMBRAPA)
- Jayashree B (ICRISAT)
- Senthilvel S (ICRISAT)
- Richard Bruskiewich (IRRI)

- Martin Senger (EBI/IRRI)
- Andrew Farmer (NCGR)

2009

- Milko Skovic, Bioversity
- Guy Davenport, CIMMYT
- Reinhard Simon, CIP
- Anthony Collins, CIP
- Manuel Ruiz, CIRAD
- Jayashree Balaji, ICRISAT
- Richard Bruskiewich, IRRI

Summary
The global distribution of GCP partnerships, the complexity of the GCP crop research agenda, and the existence of many legacy and 3rd party technologies, have driven an ambitious GCP SP4 - Crop Bioinformatics development agenda since May 2004. Since that time, members of the SP4 team have collaborated to develop elements of a GCP platform – a semantic "domain model", Java software application programming interface ("API") and GCP use case specifications - for the capture, storage, access, integration, analysis, and publication of data from local and distributed data sources wrapping the results of GCP SP1, 2, and 3 research, and related public data. Previous years' efforts on platform development was partitioned across several commissioned research tasks and later consolidated into one comprehensive task (led by M. Senger) in 2009.

This work has led to the deployment of several information systems sharing a common approach to data integration. This model-driven architecture is based on the GCP domain model instantiated through a middleware layer and extended by GCP ontologies. User documentation for the GCP data standards (domain model and ontologies) is on the GCP McClintock site.(http://mcclintock.generationcp.org). Examples of these systems are the International Rice Information System (IRIS),(http://koios.generationcp.org/germplasm/) the International Wheat Information System (IWIS), (http://cril2.cimmyt.org/germplasm/index.jsp?crop=wheat#) and the International Maize Information System (IMIS). (http://cril2.cimmyt.org/germplasm/index.jsp?crop=maize#) In addition, some genetic analysis databases are also accessible, such as TropgeneDB, (http://tropgenedb.cirad.fr/) OrygenesDB (http://orygenesdb.cirad.fr/) and GenDiversity, (http://gendiversity.cirad.fr/Home) as well as genetic resource information systems like SINGER and EURISCO. Since these systems use the GCP middleware, applications accessing data through the platform will be able to retrieve data from any or all of the databases and integrate them through analysis and visualization tools.

Conclusion
It is still the firm opinion of the GCP platform development team that the "Model Driven Architecture" and "Service Oriented Architecture" concepts underlying the GCP platform effort are feasible and productive concepts. However, the progress and impact of the project to meeting the global challenges of crop informatics, has fallen somewhat short of original vision and expectations.

The GCP domain model and platform API is being reviewed and updated to meet MBP project needs by the GCP MBP team, with possible external (public/private) partner feedback. The project has been presented to other major bioinformatics research initiatives such as the iPlant Collaborative in the USA. The standards have also been presented to private crop industry partners (Bayer, Syngenta). However, to date, wider adoption of the project standards globally outside the GCP is not observed. For this reason, the future impact of the GCP data standards and platform products globally is, unfortunately, highly uncertain.

The GCP semantic data ("domain model") standards underlying the GCP platform do represent significant crop informatics expertise. It could be productive to revisit the documentation of the standards and enhance their completeness and professional quality.

The GCP "DataSource" implementations (i.e. Java libraries) for specific systems such as ICIS and BioMoby web services do need to be simplified, generalized and better documented, to allow wider code sharing with other teams globally who may wish to implement GCP DataSources.

Steadily increasing global internet access and integration suggest that semantic and software integration standards like those developed within the GCP will likely become more rather less important in the future. However, those standards need to keep pace with emerging technological changes and opportunities, for example, "Cloud" computing. Survival of the GCP semantic and platform standards may require new partnerships with public and private sector partners. The GCP MBP project represents one good avenue within which to nurture the required partnerships.

137. G4006.17: GCP quality management and data quality improvement

January 2006–December 2008; NCE: June 2009

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Collaborating institutes and scientists

- CGN–WUR: Theo Van Hintum
- ICRISAT: B Jayashree
- CIP: Reinhard Simon
- Agropolis–CIRAD: Claire Billot

Summary
In 2008, this project incorporated the project GCP Software Engineering and Collaboration Platforms as an objective. The project addressed the following issues

that have strong implications on data quality and/or quality management in the GenerationCP.

- The Laboratory Information Management System (LIMS) designed and implemented at ICRISAT meets the requirements of a moderately high throughput molecular genotyping facility. It is being presently used for the capture of high throughput simple-sequence repeat (SSR) genotyping data and information management in parental screening and genotyping of mapping populations or germplasm collections. It interfaces with laboratory instruments like the ABI genetic analyzers and robotic liquid handlers. Some efforts have been made to help customize this system for use at other centers that generate high-throughput genotyping data such as IITA and BeCA (Kenya). The objective of support through the GCP project 2006-17 was to provide for the setting up of a LIMS help desk that could provide help with the evaluation, customization, and adaptation of the LIMS by interested groups/institutes.
- The collaboration systems CropForge and CGPWiki were maintained and supported. This activity was a continuation of the former project GCP Software Engineering and Collaboration Platforms. User training was provided and training materials were published.
- The data quality of the SSR marker data sets submitted to the GCP Central Repository was analysed and reported in a GCP meeting and the detailed results submitted to GCP management.
- A white paper on the Requirements for GCP Projects Producing Primary Data was written as part of a CG-wide effort on improved research data management. The paper was provided to GCP management and allows GCP management to better specify service level agreements for data-producing projects.
- CIP has failed to submit a report for the reporting period. Although work was carried out to the best of the knowledge of the PI, no final assessment of the actual outputs can be made. The PI was advised to prepare the final technical report without the input from CIP.

Conclusion

Data quality assurance and quality management are essentially governed by policies and procedures in every center/institute. The GCP can contribute to these, but not build them or replace them. Tools and methods can be developed and documented through GCP funding, but their uptake and application at individual centers/institutes cannot be ascertained as they often require an institutional commitment that goes beyond the GCP-funded projects. A prime example is the implementation of a LIMS.

Final products to be used by plant scientists outside the project:

1. ICRISAT LIMS.
2. Data quality resolution methodology.
3. Collaboration platform for software development and quality management.
4. White paper on Research Data Management.

138. G4006.35: Statistical support for the design and data analysis of GCP projects

January 2009–December 2009

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Collaborating institutes and scientists

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- UNAM (presently UC–D): Joost van Heerwaarden

Summary
The increasing capability to generate information at the phenotypic, and specially at the molecular level has highlighted the need of adequate statistical methods to maximize the benefits out of the valuable data produced. This is not an exception within the GCP, where an increase in demand of statistical support has been notorious since the start of the program. Whereas other GCP projects focus on the development and deployment of appropriate methodologies for the type of data produced within the GCP, this project aims at facilitating the use of those methods by capacity building and by consultancy. The strategy followed in this project in order to achieve this objective consisted of three main activities: activity 1 consisted in developing material where statistical issues related to design and analysis of experiments are presented in a didactical way, and that can be accessed by researchers when confronted with their own experiment planning or data analysis. This material has been made accessible online. The second activity consisted in delivering courses or workshops where the material developed in activity 1 was presented in detail and where researchers had the opportunity to interact directly with statisticians. Two courses were organized, one at CIAT, Colombia and the other one at IAMZ, Zaragoza, where GCP researchers were confronted with an overview of methods that are useful to address their specific questions, including issues in relation to experimental design, molecular map construction, genetic diversity analysis, population genetics, QTL mapping (and its extensions to multi-environment QTL mapping), association mapping, etc. This courses were an excellent opportunity to test the developed training material by activity 1. Finally, activity 3 consisted in providing statistical advice to GCP researchers to address specific questions related to their breeding or research problems. Examples of the topics that required our advice includes experimental design for molecular map construction and for diversity studies, and for analysis questions focus on methods for QTL mapping and association mapping.

Final products to be used by plant scientists outside the project:

1. Training material available on line.
2. Capacity building via intensive training.
3. Statistical support to GCP projects.

139. G4007.09: Design and analysis of marker–trait associations studies with special attention for genetically challenging crops

August 2007–December 2008; NCE: October 2009

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- Imperial College London, UK: David Balding
- NIAB, UK: Ian Mackay; Wayne Powell
- SCRI / BIOSS, UK: Christine Hackett; Dave Marshall
- Leiden University Medical Center, The Netherlands: Hans van Houwelingen; Jeanine Houwing-Duistermaat
- Wageningen UR, the Netherlands: Marcos Malosetti; João Paulo; Marco Bink; Hans Jansen

Summary/Conclusion
General mixed model methodology was developed for LD analysis. Furthermore, protocols were defined for data quality and investigation of genetic diversity. The mixed model LD methodology includes facilities to model intra-trial genetic and error variation and inter-trial genetic correlation, as well as genetic relatedness and population substructure. The methodology is documented in several papers and at GCP and Biometris websites. Corresponding training material was developed and has been applied in various GCP courses.

Final products to be used by plant scientists outside the project:

1. Training material available on line (presentations, website)
2. Procedures to perform all steps of LD mapping in the freely available software GenStat Discovery
3. Literature in the form of papers and book chapters

140. G4007.10: Support to GCP scientists regarding issues related to bioinformatics and data handling

August 2007–July 2009; NCE: December 2009

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Elisabeth van Strien - WUR

Summary
The objective of the project is to make the expertise, software tools and methodology developed on the basis of GCP-SP4 funds better accessible to the GCP scientists and the rest of the world. This is achieved by inventorying these resources, establishing a help desk, and re-organising the bioinformatics portal.

By the time the project started, the range of SP4 products had become very wide but their accessibility was low. To improve this situation, an inventory of expertise was made by approaching all (former) SP4-PIs asking them about their field of expertise and creating an expert database for internal use. In 2009 this expert database was updated. The SP4 helpdesk has functioned and is functioning as foreseen, with a maximal 48hour response time (sp4helpdesk@generationcp.org). The helpdesk is promoted to the GCP community by a webpage, outlining goal, intended audience and contact details. ('The SP4 helpdesk on bioinformatics and biometrics', http://www.generationcp.org/bioinformatics.php?da=08106902). The helpdesk was actively promoted at the 2009 ARM in Mali via a poster presentation, bilateral interactions, and via dispersal of handouts on data analysis courses organized by GCP SP4.

The SP4 products and services produced in the past years were inventoried on the basis of the project reports. These products and services were displayed on the GCP bioinformatics portal that had to be completely reorganised. (http://www.generationcp.org/bioinformatics.php) The current portal gives easy access to the most useable products. In addition, the portal pages were greatly extended in 2009 to include also less tangible products.
Promotion of the GCP SP4 products and services was achieved by posters at the 2008 and 2009 ARMs, and at the TDWG 2009 meeting in Montpellier.

Conclusions
- The project has created better access to the SP4 products and expertise by inventorying the expertise and products and reorganising the GCP Bioinformatics portal.
- The SP4 helpdesk has had few requests for support. This can be a result of scientists not knowing about it or not needing it, or may be due to a basal knowledge gap.
- Very many SP4 products are now accessible, where before they were hidden on local disks and in a shape that could not be used (interpreted) by others. Value to these products was added by prioritizing and unearthing these.

Quantifiable 2009 Outputs
1. Regular updates of the website
   Status: SP4 Helpdesk website is maintained as planned
2. Test reports and plans for improvements when required
   Status: the GCP SP4 bioinformatics portal pages were updated and completely re-arranged
3. Visits of the SP4 bioinformatics pages is monitored

4. New releases of database made available for internal use
   Status: Expert database is updated
5. List of transactions
   Status: List of transactions was created.
6. List of products
   Status: products are displayed on the SP4 bioinformatics portal pages.
7. Publication of products on bioinformatics portal pages
   Status: the updated, re-arranged GCP SP4 bioinformatics portal page display identified SP4 products and services.

141. G4007.11: Further development and support for use of iMAS by NARS and the other user communities

January 2008–December 2008; NCE: December 2009

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Richard Bruskiewich, IRRI
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Summary
The iMAS system provides a single unified computing and decision support platform to facilitate marker-aided selection and breeding through integration of a number of freely available open-source quality computing tools. The system frees the user from the painful, time-consuming and error-prone manual preparation of input data files required by a host of computing software involved in the computational process for marker-assisted selection and breeding. The provision of simple-to-use online decision guidelines allows the user to correctly and confidently use the different computing tools and to interpret and use their outputs to facilitate making decisions for marker-aided selection and breeding.

The system comprises of six modules: Data Validation, Phenotyping, Linkage Map Building, QTL Analysis, Genome Display, and MABC Sample Size. The Data Validation module helps the user to check whether the required initial input data files have been prepared in accordance with the rules required by iMAS. The Phenotyping module generates experimental design and undertakes biometric analyses. The Linkage Map Building module builds linkage maps. The QTL Analysis module undertakes QTL analyses. The Genome Display module helps pictorially visualize the genomic content to select genetic material of desired genomic composition. The MABC Sample Size module helps determine the optimal sample size for marker-aided backcrossing. Salient features of the system are a seamless integration of different computing tools into one single platform, extensive simple-to-use online decision guidelines and manual, and the provision of a windows interface to all DOS-based programs, the last one making it easier for a user to correctly, comfortably and confidently use these programs. The first beta version of iMAS (iMAS 1.0) was released at the ARM in South Africa in September 2007. The final version of iMAS is V 2.0 and was released in December 2009.

Conclusion

The idea behind iMAS was to give users an easy to use integrated tool / platform to facilitate user to carry out all kind of biometrical analysis at one place, without bothering about various formats requirements of various software. In addition to this bottleneck with many software to most molecular breeders is non availability of clear guidelines to take correct decision for various biometrical analyses. As a solution to this iMAS provides an online decision guide which can assist users to select correct options and making correct move. With the newest version of iMAS v 2.0, one can start from genotypic and phenotypic data and can end up with putative QTLs. In addition to this iMAS also incorporates graphical software which can display genome content with high resolution graphics and also depict location of putative QTLs with linkage map. This display in also extended to multi location / environment QTLs, overall iMAS project has been very successful and received appreciation from a large user base.

Diversity

142. G4006.02: A dataset on allele diversity at orthologous candidate genes in GCP crops (ADOC)

January 2006–December 2007; NCE: December 2009

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- OL2: Merideth Bonierbale, Reinhart Simon, Roland Schaftleitner, CIP (Peru)
- OL3 : Dave Hoisington, Rajeev Varshney, Spurthi Nayak, Jayashree B, ICRISAT (India)
- OL4 : Dominique Brunel, Pierre Mournet, INRA-CNG (France)
- CrP1: Rice: Ken McNally, IRRI (Philippines)
- CrP2: Barley: Michael Baum, Wafaa Choumane, ICARDA (Syria)
- CrP3: Sorghum: Tom Hash, Rajeev Varshney, Dave Hoisington, ICRISAT (India)
- CrP4: Bean: Matthew Blair, CIAT (Colombia)
- CrP5:Chickpea: Rajeev Varshney , Hari Upadhyaya, Dave Hoisington, ICRISAT (India)
- CrP6: Cassava: Martin Fregene, CIAT (Colombia)
- CrP7: Potato: Merideth Bonierbale, Reinhart Simon, Roland Schaftleitner, CIP (Peru)

Summary
The ADOC project (GCP 2006-02) aimed to characterize allelic diversity at orthologous loci of candidate genes for drought tolerance in seven GCP crops (rice, barley, sorghum, bean, chickpea, cassava and potato), working on reference collections of around 300 accessions for each crop. The overall objective was to infer from sequence data potential adaptive selection pressures acting on the most important genes, and compare diversity of orthologous sequences between model species (on which precise physiological data were undertaken, leading to potential candidate genes) and GCP target species.

Six gene families (ERECTA, DREB, SS, SPS, ASR and VIN) were selected as the initial subset of target genes. Except the DREB gene family, for which a specific focus has been given to DREB2A, and the SPS gene family in cereals, for which only the Os01g69030 orthology group was studied, they represent a set of relatively small gene families acting at different levels of the drought stress response (transcriptional regulation, carbohydrate metabolism…) for which a comparative analysis of gene families was undertaken.

After an initial bibliographic overview and database survey, consensus primers were used to amplify and identify a few new sequences in some species from the project. After aligning all available sequences for every crop studied, a list of specific primers was designed by the different partners in order to amplify candidate genes. Specific primers were tested on a few representatives of the reference collections. Phylogenetic trees could be drawn. In cereals, a model was proposed for Asr gene number evolution, based on the general model from Salse et al (2008). It suggests an initial number of six Asr genes in the poaceae diploid ancestor, and involves different gene losses after the whole genome duplication, some of them specific to several taxa.

Crop partners defined a reference collection of around 300 accessions for each crop, based on neutral polymorphism and passport information, from global germplasm collections. Those reference sets could be available for further drought phenotyping. DNA was extracted from reference collections and used for large scale Sanger sequencing of allelic series of candidate genes sequences.

Obtaining complete gene families was easier in whole sequenced genomes like rice and sorghum. Polyploidy and heterozygosis induced difficulties in analyzing data for cassava and potato; however sequences for a few genes were obtained and analyzed for SNP diversity across all species. A total of 10Mbp was sequenced and allelic variation could be assessed on a minimum of 60 and a maximum of 347 accessions, for 70 candidate gene's sequences.

Different patterns and intensity of sequence diversity have been found within gene families and between species. The lowest diversity was found for DREB2A sequences, in all crops considered. Some outliers were found compared to the overall distribution of diversity indices, like some Asr genes, sucrose synthase genes, and vacuolar invertase-related sequences in barley. Different patterns and range of sequence diversity were found within gene families and between species for orthologous genes, suggesting different constraints acting on those genes.

Population structure influenced partially haplotype patterns. A large range of haplotype diversity was found and the degrees of this differed between species. For a few genes like OsAsr3, computation of a sequence-based neutrality test suggested selection events acting at the species and/or subgroup level. SNP data were collected and are integrated into the GCP central repository.

Conclusion

The ADOC project has provided a set of data for allelic diversity on potential candidate genes families for drought tolerance. Unfortunately, completion of gene families cannot be guarantied for genomes not yet fully sequenced. Orthology between monocots and dicots was also difficult to assess for families encountering many gene duplications after speciation's events.

A large range of diversity was observed on the candidate sequences analyzed, depending on gene families and species. The presence of co-orthologs for some specific genes changed the diversity pattern observed between different plant species. Therefore, functional inference based on solely sequence homology between two species must be taken with caution, because of potential subfunctionalization acting on paralogs.

When computing selection indices, a few candidate genes presented a significant departure from neutrality, suggesting selection events acting on them. Population structure was very different among our seven species. It influenced greatly the diversity pattern observed. Therefore it must be taken into account before any attempt to identify specific haplotypes that would represent adaptive potential in stressed environments.

More functional information is required now on reference collections in order to identify potential candidate genes for drought adaptation and their best alleles within reference collections. Our SNP data should facilitate the use of those candidate sequences in association mapping with drought tolerance traits. In comparison with high throughput SNP data scattered along the chromosomes, our data will allow to use haplotypes instead of unique SNPs.

Final products to be used by plant scientists outside the project:

1. Reference samples of 283 to 300 accessions for seven GCP crops (rice, barley, sorghum, chickpea, bean, potato, cassava)
2. Consensus and specific primers for some candidate genes
3. SNP data for genetic mapping in some GCP crops (COS markers) and association mapping with phenotypic traits

143. G4008.40: Workshop on 'Reference sets of food crop germplasm for international collaboration'

January 2008–December 2008; NCE: December 2009

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NO UPDATE SUBMITTED; PROJECT REPORT PENDING UPLOAD

Collaborating institutions

- System wide Genetic Resource Programme (SGRP), Rome, Italy
- Global Crop Diversity Trust (GCDT), Rome, Italy
- Global Partnership Initiative for Plant Breeding Capacity Building (GIPB), Rome, Italy

Product delivery

144. G4006.14: *Ex ante* impact analysis of marker-assisted selection technologies supported by the Generation Challenge Programme

December 2006–December 2008; NCE: April 2009

Principal Investigator and Lead Institute
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EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- VPI: Jeffrey Alwang
- IRRI: Abdelbagi Ismail
- CIAT: Martin Fregene

Summary
Ex ante impact analysis was used to estimate benefits of GCP investments and to validate an approach to impact assessment that might be used broadly in the GCP to document progress to donors and others. Two GCP projects: "Revitalizing marginal lands: Discovery of Genes for Tolerance of Saline and Phosphorus Deficient Soils to Enhance and Sustain Productivity," and "Development of Low-Cost Technologies for Pyramiding Useful Genes From Wild Relatives of Cassava into Elite Progenitors" were examined. Economic surplus analysis was used, and total economic benefits of the projects were projected based on the situation with and without the new technologies (traits). Benefits were calculated taking into account (a) area planted to crops currently affected by target stresses, projected changes in area under cultivation, and production of the crops in specific countries, (b) the nature of the markets for the crops, (c) projected yield and cost changes due to the new technologies, (d) estimated time for discovery, development, and deployment of the DNA marker technologies and associated germplasm, (e) estimated time required to breed, test and disseminate superior new cultivars, including rates of adoption by farmers, and (f) the discount rate for benefits and costs that occur in the future. Results indicate that marker-assisted breeding (MAB) in rice will save at least 3-6 years compared to conventional breeding (CB) and result in significant incremental benefits in the range of $50 to $500 million depending on the country, abiotic stress, and lag for CB under base assumptions. For cassava, benefits for MAB to incorporate resistance to cassava mosaic disease, green mites, white flies, and post harvest deterioration vary from $34 to $817 million depending on the country. Three graduate students were engaged in the project. One completed her MS thesis in June 2008, a second completed his thesis in August 2008, and a third completed her thesis in June 2009. The third student assessed gender impacts of improved cassava varieties in Nigeria, with a focus on labour use by gender in cassava production, harvesting, processing, and marketing and on decision-making by gender within cassava households. She surveyed 200 households in Nigeria with assistance from the National Root Crops Research

Institute and used Probit analysis to analyze the data. She found that households that adopt cassava varieties with improved insect and disease resistance allocate more female labour to cassava production, processing, and marketing than do non-adopting households. One reason appears to be the expansion of female labour for planting, fertilizer application, weeding, harvesting, processing and marketing of cassava that occurs as profits rise. One implication is that improved varieties may increase pressures for mechanization of certain tasks completed by women in cassava. There is little change in male labour use, but women in adopting households relinquish some control over decision making to men with respect to input purchases, labour allocation, and borrowing when improved varieties are adopted.

Conclusion

The economic surplus analysis for the rice and cassava examples has been completed, and two MS theses contain the results. A third MS thesis on gender impacts of improved cassava varieties in Nigeria is also completed. Results indicate that marker-assisted breeding (MAB) in rice will save at least 3-6 years compared to conventional breeding (CB) and result in significant incremental benefits in the range of $50 to $500 million depending on the country, abiotic stress, and lag for CB under base assumptions. For cassava, benefits for MAB to incorporate resistance to cassava mosaic disease, green mites, white flies, and post harvest deterioration vary from $34 to $817 million depending on the country. The costs of MAB exceed those of conventional breeding alone, but the difference in benefits of MAB as compared to conventional breeding so far exceeds the difference in costs, that MAB is clearly the more cost effective approach to obtaining the new technologies. Gender impacts are felt as well through changes in labour use and household decision making.

Quantifiable Outputs:

1. Map of technology-impact pathway for MAS technologies for rice
2. Summary of current status of development of the various products in the MAS rice project
3. Data file with production, area, price, and trade data for rice in the affected countries
4. Summary of published data on rice crop losses due to saline and phosphorous deficient soils and to submergence
5. Per hectare budgets for rice in each affected area with and without the improved technologies
6. Economic surplus analysis results available for the rice technologies
7. Net present values and internal rate of return estimates obtained.
8. A roughly 30 page report prepared and in review that summarizes the rice impact analysis.
9. The final report completed for the rice impact study
10. MS student from Asia completes MS thesis and returns home with training for future impact studies
11. Map of technology-impact pathway for MAS technologies for cassava
12. Summary of current status of development of the products in the gene pyramiding cassava project
13. Data file with production, area, price, and trade data for cassava in the affected countries
14. Summary of published data on cassava crop losses due to insects, diseases, and PPD

15. Per hectare budgets for cassava in each affected area with and without the improved technologies
16. Economic surplus analysis results available for the cassava technologies
17. Net present values and internal rate of return estimates obtained.
18. A roughly 30 page report prepared and in review for the cassava impact analysis.
19. The final report completed for the cassava impact study.
20. MS student from Africa completes MS thesis and returns home with training for future impact studies
21. Report describing the impact assessment protocols and methods to facilitate other impact studies
22. Results of both the rice and cassava impact studies will be summarized in 3-4 page "impact briefs"
23. Publishable journal article manuscript prepared out of each MS thesis
24. Report summarizing, with examples, how the impact methods could be applied with less detailed data to provide rough estimates of benefits for a larger set of projects.
25. MS thesis completed for gender impact analysis (new output)

145. G4008.36: Getting the focus right: Food crops and smallholder constraints

April 2008–December 2009

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Collaborating institutes and scientists

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- CIAT: Glenn Hyman
- Food crop breeders, crop management, socio-economics and GIS specialists in CG centers including IRRI, CIMMYT, ICRISAT, CIAT, WARDA and IITA
- Numerous NARS and NGO institutions and staff in South and East Asia, Sub-Saharan Africa, Latin America

Summary
To determine the most important production constraints and possible solutions to the constraints for six major food crops in 15 farming systems with high poverty in Sub-Saharan Africa, South Asia and East Asia, surveys were conducted with 672 experts representing a diversity of backgrounds and experience. Respondents reported large gaps between highest achieved crop yield on smallholder farms and average yield on farm. Yield gaps were smallest for rice (about 60% of current average smallholder farm grain yields), mid size for wheat and cassava, and larger (sometimes double current farm yields) for sorghum, cowpea and chickpea. Gaps were also smaller in the high input and yield farming systems of East Asia and largest in the marginal, dryer systems, particularly in Sub-Saharan Africa. Four categories of production constraint

(abiotic, biotic, management and socio-economic) were considered important contributors to yield gaps. Abiotic and management constraints were more important for wheat, socio-economic and management issues for rice and cassava, and abiotic constraints for sorghum. Biotic constraints dominated the legumes. A diversity of specific constraints was reported for the crops in the systems. The most severe widespread constraints for wheat involved the deficiency, high cost and poor management of N fertilizer, and problems associated with grain filling drought stress, mid season drought and irrigation management. Those for rice also included N fertilizer problems, soil fertility depletion, various leaf, stem and head pests and diseases, weed competition and inadequate water management. Striga and weed competition, soil resource degradation, soil fertility management, and drought were the most severe overall for sorghum. Pod, leaf, stem and flower insect pests and the high cost of their control dominated cowpea. Helicoverpa pod borer, Botrytis grey mould and control costs were the most severe for chickpea. Unsuitable varieties/poor seed, soil infertility and fertilizer constraints were also widespread with the legumes. Marketing problems and lack of finance were concerns for cassava along with weed competition, African cassava mosaic virus and poor varieties/planting materials. Many solutions were proposed to alleviate the most important constraints. The findings can inform priority setting for the Generation Challenge Programme on important food crops in major farming systems with high poverty.

Conclusions

In conclusion, large smallholder farm yield gaps were reported for most crops in most farming systems, implying significant scope for improvement of farm yields if the most serious constraints can be identified and alleviated. Abiotic, biotic, management and socio-economic constraints were all important contributors to yield gaps. Many serious specific constraints were reported for the crops in the systems. Most serious constraints were considered to be getting worse. Respondents proposed many variety/germplasm interventions to address the most important constraints, and suggested policy/socio-economic and crop management solutions. Proposed solutions to biotic and abiotic constraints often involved the development of improved germplasm with tolerance or resistance to various pest, disease, nutrient and water stresses.

Overall, this comprehensive report of findings on constraints and opportunities for a range of major food crops will provide an important set of knowledge to guide GCP priority setting. The GCP should find many of the suggestions on varietal, germplasm or genetic solutions helpful to support their current investments in particular thrusts with the crops surveyed and to guide the deployment of their resources into the future. Some of the ideas on management and policy related interventions will also be helpful to guide the wider agricultural research and development community.

Quantifiable Outputs:

1. Preliminary constraints results for 3 crops, as tables and graphs (wheat, rice, sorghum)
2. Draft report for 6 food crops (wheat, rice, sorghum, cassava, cowpea, groundnut) including methodology, identification of constraints and estimated local and aggregate losses for each constraint.
3. Final report and journal article submitted
4. Information on potential solutions to important constraints in draft report.

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