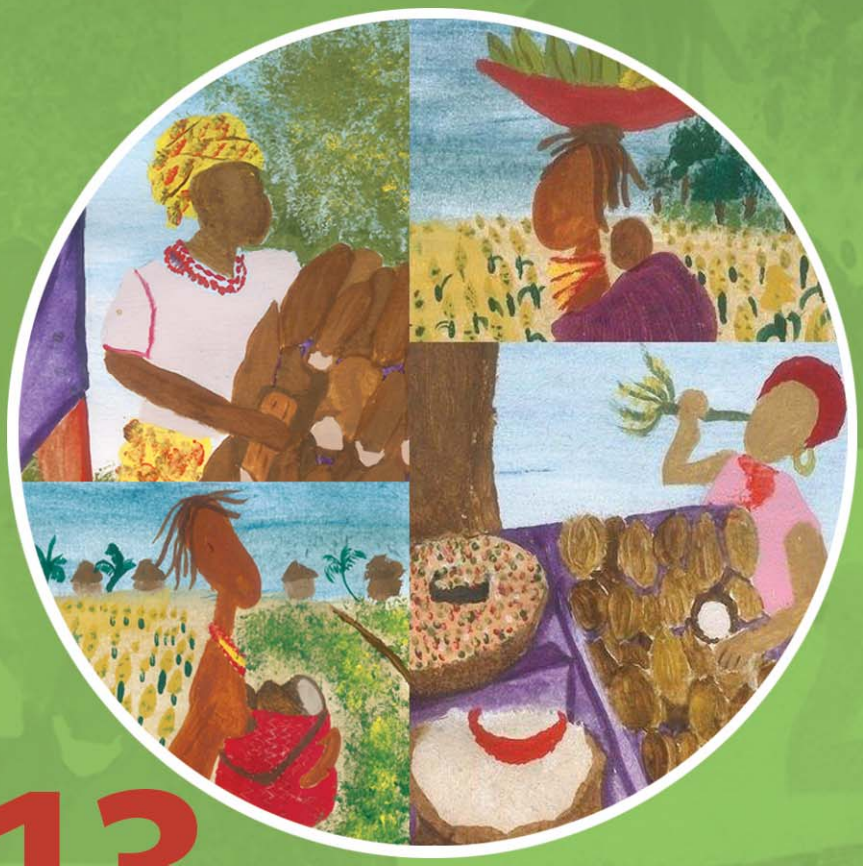


2013 Project Updates

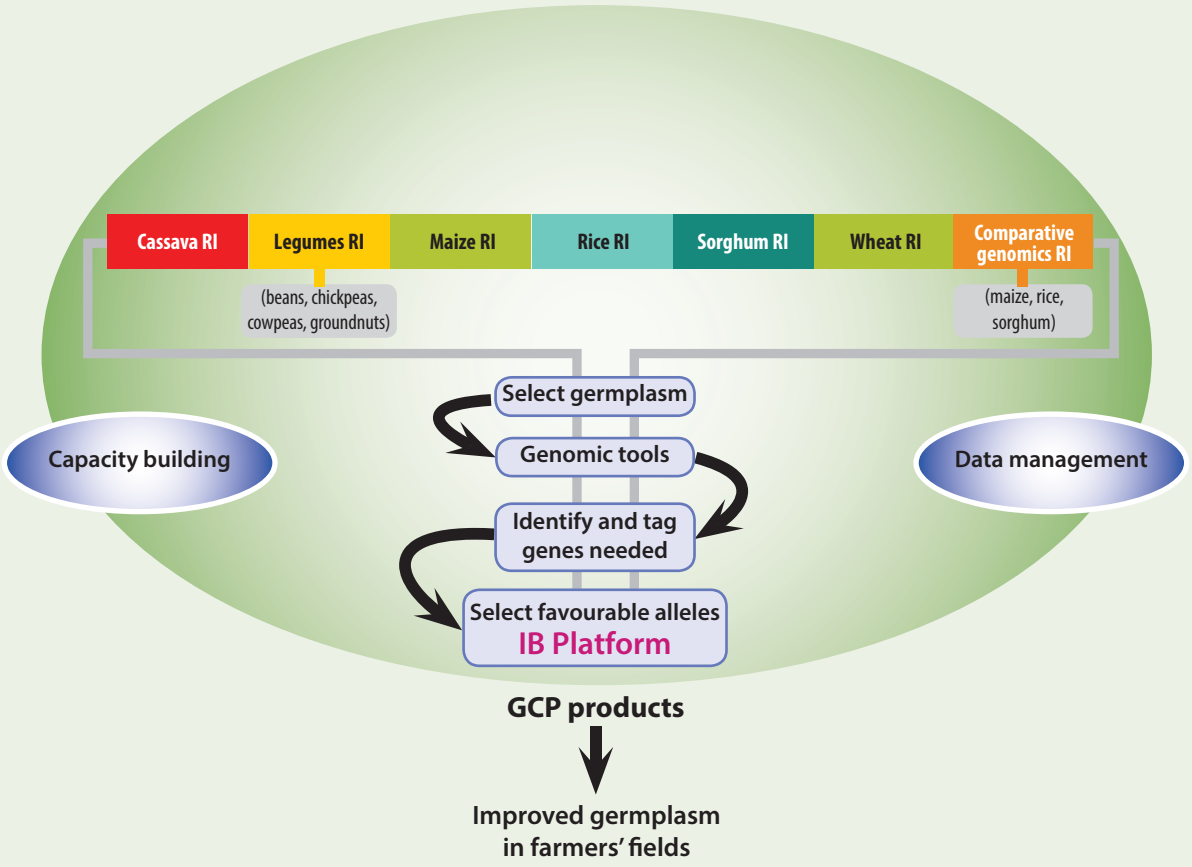
Generation Challenge Programme

Partnerships in modern crop breeding for food security

Our activities and structure

In Phase II, GCP (2009–2014), GCP primarily focuses on seven Research Initiatives (RI) organised by crop and crop clusters (see diagram) as well as a service component – the Integrated Breeding Platform (IBP): a web-based one-stop shop for information, analytical tools and related services to design and carry out integrated breeding projects. The RIs are trait- and country-specifi c. As selected user cases of IBP, the RIs aim to demonstrate that modern and integrated breeding approaches can have a significant impact on crop productivity in developing countries.

GCP's Research Initiatives (RIs) and research support activities



Generation Challenge Programme

Partnerships in modern crop breeding for food security

2013 Project Updates

(incorporating projects completed in 2012 and 2011)

June 2013

Generation Challenge Programme (GCP)
Hosted by CIMMYT
(Centro Internacional de Mejoramiento de Maíz y Trigo;
the International Maize and Wheat Improvement Center)

Mailing address:
c/o CIMMYT, Apdo Postal 6–641
06600 Mexico, DF Mexico

Physical address:
c/o CIMMYT Km 45 Carretera México–Veracruz
El Batán, Texcoco, México, CP 56130
Tel: +52 55 5804 2004 **Fax:** +52 55 5804 7558

Email: generationcp@cgiar.org or info@generationcp.org

www.generationcp.org /GCProgramme

Correct Citation: CGIAR Generation Challenge Programme, 2013. 2013 Project Updates (incorporating projects completed in 2012 and 2011). Texcoco, Mexico: Generation Challenge Programme.

Compiled by: Gillian Summers
Cover illustration: Recomposition by Gillian Summers, based on original artworks by Rhoda Okono entitled *Homeward bound* and *Market place at down*
Art direction: Miguel Mellado E. and Eliot Sánchez P.

Editorial note: Project updates are published as presented by the authors, except for updates that extended beyond the set word limit where text beyond this point has been omitted, indicated by continuation dots/suspension points (...)

This publication has been printed in black-and-white. For colour images, please contact the appropriate Principal Investigator.

Contents

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
AltSB	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
Δ13C	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
F1 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

2013 PROJECTS
I. Research Initiatives
Cassava

1. G7010.01.03: Implement MARS Project for drought tolerance in Africa

- *G7010.01.03: March 2010–February 2014 (successor of G7009.09: December 2009–February 2010)*

Principal Investigator and lead institute

Emmanuel Okogbenin, National Root Crops Research Institute (NRCRI), Umudike, Nigeria. Tel: 234-8063905548

Collaborating institutes and scientists

- National Root and Tuber Crop Improvement Institute (NRCRI), Umudike, Nigeria: Chiedozie Egesi
- Crop Research Institute (CRI), Kumasi, Ghana: Elizabeth Parkes Parkes
- Savannah Agricultural Research Institute (SARI), Ghana: Joseph Adjerbeng Adjerbeng
- Cornell University, Ithaca, NY USA: Tim Setter
- Donald Danforth Plant Science Center, St. Louis, MO, USA: Martin Fregene

Cassava (*Manihot esculenta*) is an important staple in sub-Saharan Africa and holds great potential for improving agricultural productivity in dry marginal environments where expansive cultivable land exists. Molecular breeding through marker-assisted recurrent selection (MARS) offers an efficient strategy for improving cassava productivity in the dry ecologies of Africa

Mapping population

The MARS project in under the GCP Research Initiatives has successfully developed three mapping populations. The populations which are F_1 crosses developed using non inbred parents include TMS98/0505 x TMS 98/0510 denoted as family A; TMS98/0505 x TMS98/0581 denoted as Family B; and TMS98/0505 x TMS 97/02324 denoted as Family C.

Genotyping of Mapping population

Although an existing cassava map published in 2012 (Rabbi et al., 2012) was available, the identification of additional polymorphic SNP markers in our mapping populations which hadn't been mapped necessitated genotyping of over 800 SNP markers selected in 2011 during polymorphism survey of parents used in developing the mapping populations.

Construction of Linkage Map

In 2012, mapping population B with the biggest progeny size was gentyped at KBiosciences. Genotyping of this population has been completed and from which subset of markers (selected based on wide genome coverage) would be used for genotyping families A and C. A total of 856 SNP markers were used to genotype Family A population. The markers were tested for segregation ratio following Chi square test (test for deviation from expected Mendelian segregation for each marker) and the best segregating and informative markers were used in mapping. The Genetic linkage map was constructed using JoinMap. The grouping of linked markers was done using LOD. Regression mapping was used for map construction. Map distances were calculated as Kosambi mapping function. Linkage group analysis resulted in a genetic map of 1582.8 cM containing 505 SNP markers distributed in 21 linkage groups. The number of markers mapped is 71 SNP markers more than the recently SNP/SSR published by Rabbi et al (2012). The number of marker per linkage group ranged from 6 - 48 markers while the length of the linkage of linkage ranged from 25.6 cM - 143.7 cM. The average distance between adjacent markers for the map is 3.69 cM. The inter-marker distance ranged from 1.92 cM to 6.69 cM which is less than 10 cM targeted in this project for the implementation of MARS.

Establishment of Phenotyping trial

In 2012, the mapping populations were established for phenotyping studies for key traits linked to drought tolerance and high productivity in the dry ecologies. Two mapping populations (Family B and C were planted in Minjibir, Kano State, Nigeria) while the third mapping population (Family A) was planted at SARI, in Tamale, Ghana. Having successfully generated some planting materials in 2011, clonal evaluation trials to evaluate for productivity and drought tolerance traits were initiated. The trial was established using augmented design (which is an incomplete block design). The design is best suited for cassava and vegetatively propragated crops were planting materials are highly limited (due to low multiplication ratio) at initial stages of development and also high advantageous for high plant population size as with the mapping populations. The plants were subjected to stress for a period of six months without rainfall.

Phenotyping for drought tolerance and productivity

Phenotypic data for pests and disease, growth and morphological parameters (plant height, number of leaves formed, branch height, branching levels, and number of scars), productivity parameters (yield, harvest index, foliage e.t.c) as well as physiological data (stomatal conductance abscisic acid) were evaluated. Cassava mosaic disease (CMD) was very low indicating high CMD resistance in the populations generally with over 95% of the genotypes in the mapping populations showing good CMD resistance and confirms the suitability of the population for QTL mapping. It also shows the appropriateness of the parents (of the mapping populations) which among other considerations were selected for CMD resistance. Phenotypic data shown wide genetic variation in the mapping populations with significant difference for highly key productivity traits. Preliminary QTL mapping identified seven QTLs for dry yield and one QTL for harvest index of moderate effects which is now been used to slow to select the next parents for the next recombination cycle.

Infrastructural development of phenotyping sites

To efficiently improve phenotyping capacity in the NARs for the successful execution of this project the GCP has facilitated infrastructural development of experimental sites through topographic survey, provision of weather station, setting up of irrigation systems, purchase of physiology equipment and associated facilities to enhance quality data both in Ghana and Nigeria. Infrastructural development of drought phenotyping trial sites was completed at Tamale Ghana while that of Minjibir station in Nigeria is nearing completion.

Reference

Rabbi, IY, Kulembeka HP, Masumba, E, Marri PR, Ferguson M. 2012. An EST-derived SNP and SSR genetic linkage map of cassava (Manihot esculenta Crantz). Theor Appl Genet. 125(2):329-42. doi: 10.1007/s00122-012-1836-4.

2. G7010.01.02: Improving and deploying markers for biotic stresses in cassava

- *G7010.02: March 2010–February 2014 (successor of: G7009.10: December 2009–February 2010)*

Principal Investigator and lead institute

Chiedozie Egesi, National Root Crops Research Institute (NRCRI), Umudike, Km 8 Ikot Ekpene Road, Umuahia 440001, Nigeria; (234) 703 496 2100 cegesi@yahoo.com

Collaborating institutes and scientists

- National Root Crops Research Institute (NRCRI), Umudike, Nigeria: Emmanuel Okogbenin, Joseph Onyeka, Adeyemi Olojede
- Crop Research Institute (CRI), Kumasi, Ghana: Elizabeth Parkes, Adelaide Agyeman
- Agricultural Research Institute (ARI) Naliendele, Tanzania: Geoffrey Mkamilo
- Donald Danforth Plant Science Centre, St. Louis, MO, USA: Martin Fregene

1. Research activities and progresses at NRCRI, Nigeria

1.1 Phenotyping for cassava mosaic disease (CMD) in two mapping populations in clonal evaluation trial

Phenotyping of cloned individuals from two biparental F_1 populations segregating for cassava mosaic disease (CMD) were done for the second season. The two mapping populations were established in clonal evaluation trials at two sites in Nigeria (Umudike and Otobi). The trial consisted of 340 individuals from 97/2205 x TMS 30555 and 141 individuals from 96/1089A x TMS 30555. The genotypes were scored for CMD on a severity scale of 1 to 5 where 1 represents no symptoms and 5 very severe symptoms.

1.2 SNP genotyping for mapping in CMD segregating populations

Genomic DNAs from 340 progenies of 97/2205 x 30555 were genotyped on KASPar SNP platform of KBiosciences. Out of more than 900 polymorphic SNP markers identified from parental screenings 577 SNPs were polymorphic in the progenies of family 97/2205 x 30555. A genetic linkage map for this family was constructed with JoinMap software and it has the 577 markers distributed among 18 linkage groups. The total length of the linkage map is 1708.3 centi Morgans (cM) with average marker intervals ranging from 1.28 to 5.43 cM. QTL analysis is ongoing.

1.3 Evaluation for resistance to cassava bacteria blight (CBB) and cassava green mite (CGM)

1.3.1 Mining for resistant gene donors in African landraces

Two hundred and fifty six (256) genotypes were evaluated for CBB and CGM resistance under field conditions at NRCRI sub-station at Otobi, Nigeria. For the CBB evaluation, of the 256 landraces evaluated, a total of 59 (23%) were resistant to CBB. Majority of the accessions (74%) were moderately susceptible while approximately 3% were highly susceptible. Considering resistance to CGM, 21% of the landraces were resistant, 52% moderately susceptible and 25% highly susceptible.

1.3.2. Evaluation of elite breeders' lines for resistance to cassava bacterial blight (CBB) and cassava green mite (CGM)

One hundred and eighty three (183) elite lines mostly from IITA breeding stock were evaluated at Otobi, Nigeria. Fifty five (30%) were resistant to CBB, 85 genotypes (46%) were moderately susceptible while 23.5% were highly susceptible. With respect to reaction to CGM, 13% were resistant, 59% moderately susceptible and 27% highly susceptible. The results from this study identified new sources of resistance to both CBB and CGM (Table 1). These resistant genotypes have been established in a hybridization block at NRCRI main station Umudike for development of mapping population towards genetic studies for resistance to these two important biotic stresses.

Table 1. Clones identified as resistant to cassava bacterial blight (CBB) and cassava green mite (CGM).

NRCRI germplasm	CBB score	CGM score	IITA germplasm	CBB score	CGM score
NR/8010	1	1	188/023543	1	1
74/2	1	2	189/00748	1	1
74/640	1	2	TME-226	1	1
73/30	1	2	T983/00016	1	1
73/192	1	2	188/01226	1	1
73/404	1	2	088/00623	1	1
75/647	1	2	TME-236	1	2
75/726	1	2	TME-478	1	2
77/169	1	2	088/00378	1	2
TMX4009	1	2	087/00183	1	2

Disease and pest severity scores on the basis of 1 to 5 where 1 is no symptom and 5 is very severe symptoms.

2. Research activities and progresses at ARI, Tanzania

2.1 Development of 4 bi-parental populations for validation of cassava brown streak disease (CBSD) SNP markers

Four bi-parental CBSD populations were developed for validation of CBSD SNP markers. The families are Namikonga x AR 37-80, Kiroba x 3C83-13, Muzege x Cheupe and B2C20-65 x AR 37-80. Phenotyping for foliar symptoms of CBSD has commenced with data collection already conducted in 3, 6 and 9 months after planting. The skewing of the progenies tended to the resistant phenotype, an indication that we may be dealing with a few oligogenic genes. The symptoms in the roots including necrosis will be assessed at harvest.

Tangible outputs delivered

The project has successfully conducted multi-environment disease response assessment of the 2 mapping populations giving a distinct classification of response types in the two segregating populations. Genotyping of the populations for CMD resistance with SNP markers have been completed and gene tagging and QTL analyses is ongoing. Also, new CBSD segregating populations for SNP marker validation have been developed, field-established and are being phenotyped. The evaluation of new germplasm resources for the identification of new sources of resistance to CBB and CGM has identified germplasm with combined resistance to the two biotic stresses.

3. G7010.01.01: Improvement and eva luation of the existing cassava reference set for Africa

- *April 2010–March 2013; NCE: March 2014*

Principal Investigator and lead institute

Morag Ferguson, International Institute of Tropical Agriculture (IITA); m.ferguson@cgiar.org; c/o International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi 00100, Kenya; (+254) 20 422 3000; Fax: (+254) 20 422 3001

Collaborating institutes and scientists

- IITA: Dominique Dumet, Ismail Rabbi, Peter Kulakow, Jorge Franco, Edward Kanju, Melaku Gedil
- CIAT: Luis Augusto Becerra Lopez-Lavalle, Hernan Ceballos, Clair Hershey, Daniel Debouck
- Agriculture Research Institute, Tanzania: Geoffrey Mkamilo
- Crops Research Institute, Ghana: Elizabeth Parkes
- National Root Crops Research Institute: Chiedozie Egesie

Abstract

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.

To date 564 cassava varieties have been genotyped for 1,536 SNPs using an Illumina GoldenGate assay (Illumina Inc., San Diego, CA). This consisted of approximately:

- One hundred and fourteen 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.
- 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'.
- 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'.

Average SNP call rate was 0.98, with an average minor allele frequency of 0.49. Genotyping results will be used to 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.

Cassava: Capacity-building activities:

Community of practice project

4. G7010.01.05: A Cassava breeding community of practice in Africa for accelerated production and dissemination of farmer-preferred cassava varieties resistant to pests and diseases

- *January 2011–December 2013 (Successor of: G4008.26, January 2008–December 2010)*

Principal Investigator and lead institute

Emmanuel Okogbenin, National Root Crops Research Institute (NRCRI), Umudike, Nigeria. Tel: 234-8063905548

Collaborating institutes and scientists

- National Root and Tuber Crop Improvement Institute (NRCRI), Umudike, Nigeria: Chiedozie Egesi
- Crop Research Institute (CRI), Kumasi, Ghana: Elizabeth Parkes
- NAARI Namulonge, Uganda: Anthony Pariyo
- International Institute for Tropical Agriculture, Ibadan, Nigeria: G. Melaku
- International Center for Tropical Agriculture (CIAT), Cali, Colombia: Luis Augusto Becerra Lopez-Lavalle

The cassava breeding community of practice (CoP) in Africa project seeks to facilitate the uptake and strengthen modern breeding strategies in cassava at NARS.

1. A practice of sharing experiences and information

Breeder to Breeder Information exchange visit is a strategy deployed in the CoP for sharing practical experience from on field situations among countries. The visits have primarily focussed, among countries, on molecular breeding in cassava improvement, germplasm exchange, resistance breeding for cassava brown streak disease (CBSD) and plant response/ mechanisms to CBSD infection. In close association with the IBP, the CoP webpage supporting information sharing was further strengthened by populating its database. Given the need for wider impact in molecular

breeding, the CoP membership expanded in 2012 from four to thirteen African countries covering the different sub-regions of the continent.

2. Routine use of markers and field based breeding

A total of 64 genotypes developed via MAS for *CMD2* resistance have been selected and integrated into the cassava breeding scheme. The 64 genotypes evaluated showed good resistance to the disease although with differential response. Cassava mosaic disease (CMD) evaluation results also indicate that resistance profile of these genotypes were influenced by their different genetic backgrounds. MAS for *CMD2* gene is currently been undertaken using SSRs. Attempt to map the CMD2 gene with SNP in a segregating mapping population is at advanced stage. Thirteen SSR markers in the genomic region of the CMD2 gene have been selected and this is being used to identify SNPs within the CMD 2 region to facilitate the use of SNPs for MAS in cassava breeding. To improve stable and durable resistance pyramiding CMD resistance has been done. In addition to the M. *glaziovii* and TME3 sources, the breeding programmes has increasingly used TMS98/1089A and TMS97/2205 as new sources of CMD resistance in crosses. Selection have revealed that the number of the families selected in breeding programme were dominated by progenies from TME3, TMS96/1089A and TMS2205. Source population improvement is also being accomplished for CBSD in East Africa to improve breeding for the disease.

Official release of MAB developed cassava varieties in Tanzania

Through collaborative activities involving other partners (ARI, CIAT, IITA) the CoP supported breeding initiatives leading to the release of four cassava varieties Latin American genetic background combining CBSD and CMD resistance developed via MAS on in Tanzania. Similarly in Nigeria, CoP supported the release of MAS developed genotype of LA genetic background for CMD resistance, high starch content and good yield.

Marker-aided development of partial inbred lines

Partial inbred lines have the advantage of reducing heterozygosity and reducing genetic load in cassava. The scheme combined both selfing and marker analysis to estimate heterozygosity. Parent lines used for this activity were six in Ghana, five in Nigeria and seventeen in Tanzania. Inbreeding depression analysis for the Nigeria populations indicate that harvest index, dry matter content, and vigour were not severely affected in performance at S_1. Hetrozygosity at index estimated at S1 using SNPs ranged from -213 to -43.13 following Scotti *et al.,* (2000) procedure. Advanced selfed generations up to S_2 and S_3 have been made.

Improvement of elite gene pools for multiple disease resistance

Gene pool improvement combining several key breeding traits such as dry matter, disease and pest resistance, drought tolerance and high yield were initiated by selecting top performing F_1 lines developed from crosses between Latin American and African elite lines. The F_1 lines were selfed to develop new progenies fixed for CMD and CGM alleles and improved agronomic traits Hybridization activities in the countries used no less than 20 F_1 parent lines for selfing to generate F_1S_1 which are currently under field evaluation and would be selected with aid of markers. The next stage is to do intercross among the F1S1 to combine multiple resistance genes for disease and pest and other traits and take advantage of heterosis for enhanced genetic performance.

Strengthening the capacity of National program breeders

A major highlight in the CoP is capacity building to strengthen the knowledge base and technical abilities of breeding staff in modern breeding strategies. The CoP workshop on molecular breeding was held in Tanzania in 2012 covering training on diagnostic tools for virus detection and use of Galaxy tablet and IB fieldbook by breeders. The next workshop of the CoP is scheduled for Ethiopia in 2013.

Reference

Scotti, C., F. Pupilli, S. Salvi and S. Arcioni. (2000). Variation in vigor and in RFLP-estimated heterozygosity by selfing tetraploid alfalfa: new perspectives for the use of selfing in alfalfa breeding. Theor. Appl. Genetics 101: 120-125.

Legumes

Beans

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

- *G6010.03: May 2010–May 2014 (TLI Phase II)*

Principal Investigator and lead institute
Bodo Raatz, CIAT, International Center of Tropical Agriculture
A.A. 6713, Cali, Colombia, Tel: +57 2 4450000 b.raatz@cgiar.org

Collaborating institutes and scientists

- SARI, Ethiopia: Daniel Ambachew
- SARI Selian, Tanzania: Sosthenes Kweka
- DARS Malawi, Virginia Chisale
- DR&SS, Zimbabwe, Bruce Mutari
- KARI, Kenya, David Karanja

Improving common bean productivity for marginal environments in sub-Saharan Africa

Common bean is the major food legume for direct human consumption and of particular importance for nutrition in developing countries in Africa and around the globe. Production is challenged by various biotic and abiotic constraints. TL1 aims to improve production by breeding for improved varieties and development of breeding tools.

1. SNP based markers for MAS

Various previously known markers were converted to SNP based assays.

SNP based markers are used to tag the important Bean Common Mosaic Virus (BCMV) resistance gene *bc-3*. First a CAPS marker was developed, for use in simple agarose gel system, used in evaluation of >6000 plants in 2012. Secondly, a Tm-shift assay was developed (Wang et al. 2005, Fig 1) as a gel free system. This marker was also established at the Kbio/LGC platform.

Tm shift markers were also developed for the bruchid resistance locus Areclin, two Common Bacterial Blight (CBB) resistance loci and two Angular Leaf Spot (ALS) loci. In an ongoing finemapping project the ALS resistance gene from G5686 was mapped to a 1.4 MB region on chromosome 4.

All markers are now available for MAS by CIAT and partners.

To develop new markers for Bean Stem Maggot (BSM) resistance, phenotyping data was collected in Ethiopia and ~100 additional SNPs were evaluated, QTL analysis is in progress.

2. Development of drought tolerant germplasm

36 elite Andean drought lines evaluated under irrigated and rainfed conditions at CIAT HQ, in 2012 enjoying the first excellent drought season in years. As in previous years, breeding lines proved to have superior productivity compared to commercial checks under stress (Fig 2). Best correlating traits for drought yield were found to be biomass (0.41) and pod harvest index (0.22).

Multi locational trials are now carried out in 4 partner countries. Data will be used to select best lines across environments for varietal tests and physiological analyses will discover yield-related traits to be used in breeding selection.

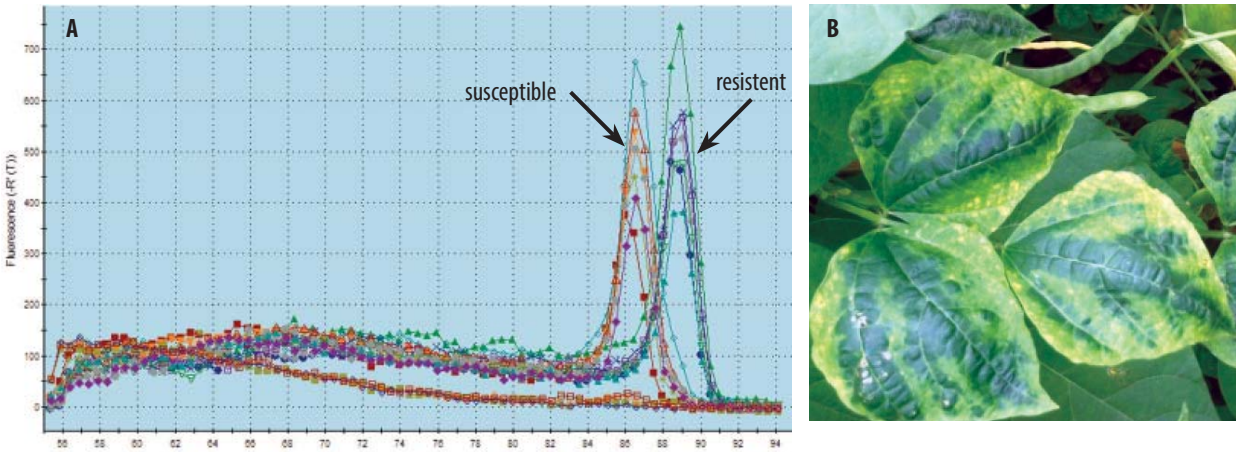


Fig 1: A: Marker for the *bc-3* resistance gene in Tm shift assay read in real-time PCR machine. B: damage caused by BCMV.

3. MARS and MAGIC populations

Additional phenotypic data of the MARS population of ~200 lines was collected at CIAT HQ, Colombia. SNP genotyping yielded additional 14.000 data points using the fluidigm platform. QTL analysis is now in progress to determine markers to be used in recurrent selection.

An 8-parental MAGIC mapping population was developed, using parents that excel for various traits (drought, nutrition, acidic soils, Al tolerance, virus resistance). Population is currently growing in F4:5 generation and first phenotyping will commence in July. Leaf sampling for DNA extraction initiated in May, genotyping will follow soon.

4. Training

30+ regional breeders joined a workshop held at CIAT Kawanda, Uganda. PhD student visited CIAT HQ for 3 months. A PhD and a Master's student finished their degrees

5. Data management

Data base is being populated, use of trait dictionary implemented, partners were trained to use defined data formats

Literature:

Jun Wang, Karen Chuang, Mandeep Ahluwalia, Sarika Patel, Nanette Umblas, Daniel Mirel, Russell Higuchi, Soren Germer,2005, High-throughput SNP genotyping by single-tube PCR with Tm-shift primers. BioTechniques 39:885-893

Chickpeas

6. G4011.08: Harnessing the potential of MAGIC population for gene discovery and breeding applications in chickpea

- *Duration: August 2011–July 2014*

Principal Investigators and lead institute

Pooran Gaur & Rajeev Varshney, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, India Tel: 040-3071 3305, Fax: 040 3071 3074 Email: p.gaur@cgiar.org & r.k.varshney@cgiar.org

Collaborating institutes and scientists

- ICRISAT, India: Mahendar Thudi, Srinivasan Samineni and VS Arun Kumar Sama
- Egerton University, Kenya: Paul Kimurto
- Ethiopian Institute of Agricultural Research (EIAR), Ethiopia: Asnake Fikre

Context: For harnessing the genetic diversity present in the elite chickpea germplasm, a multi-parent advanced generation intercross (MAGIC) population is being developed using eight cultivars/elite lines (ICC 4958, ICCV 10, JAKI 9218, JG 11, JG 130, JG 16, ICCV 97105 and ICCV 00108) from South Asia and sub-Saharan Africa. Higher recombination frequencies and greater genetic diversity in MAGIC lines will provide greater opportunities and precision in QTL detection. In addition, these lines will be used directly in breeding programs for development of cultivars adapted to different environments in Asia and sub-Saharan Africa.

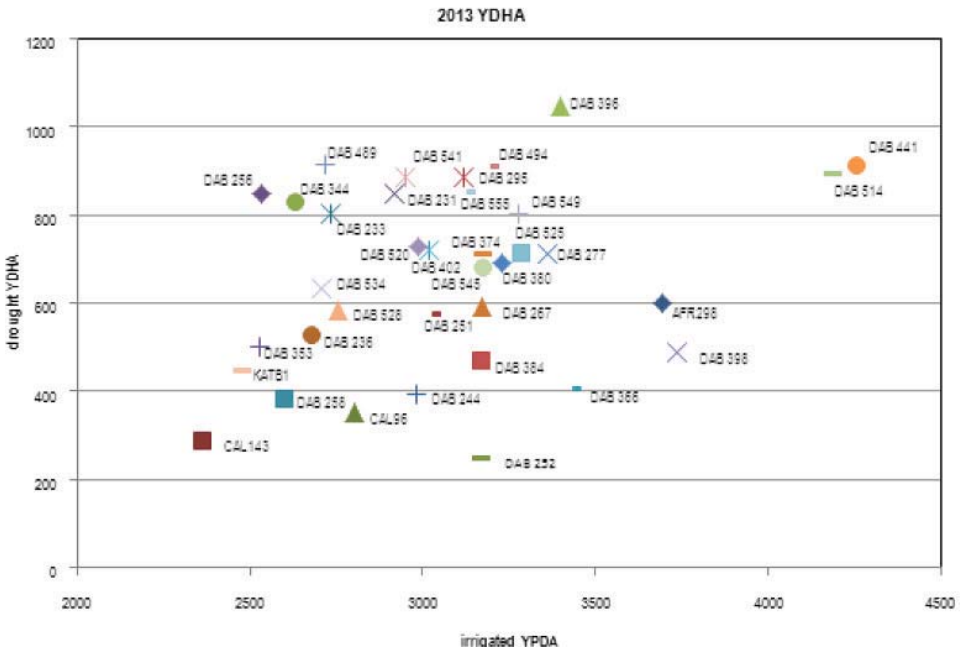


Fig 2: Yield under irrigated and rainfed conditions in Palmira, Colombia. Checks shown in grey.

Objective 1: Development of MAGIC population
Eight well-adapted and drought tolerant desi chickpea cultivars/elite lines (ICC 4958, ICCV 10, JAKI 9218, JG 11, JG 130, JG 16, ICCV 97105 and ICCV 00108) from Ethiopia, Tanzania, Kenya and India were identified in Phase I of TL-I and TL-II projects for use as parents in development of MAGIC lines. These 8 parents were intercrossed in all possible combinations, excluding reciprocals, by generating 28 two-way (Oct 09-Feb 10 in field), 14 four-way (Jun 10-Sep 10 in the greenhouse) and 7 eight-way crosses (Oct 10-Feb 11 in field). F_1s were advanced to F_2s in the greenhouse (Mar 11-Jun 11). A total of 1200 F_2 plants were grown during Jun 11-Sep 11 in the greenhouse. These were advanced to F_3 (Oct 11-Feb 12 in field), F_4 (Feb 12-May 12 in field), F_5 (Jun 12 - Sep 12 in greenhouse), and F_6 (Oct 12-Feb 13 in field) using single seed descent (SSD) method. DNA was isolated from all 1200 F_6 plants and these plants were used for development of F_7 progenies. A total of 1200 F_6 derived F_7 MAGIC lines are being grown (May-Sep 2013) for seed multiplication in an off-season nursery at Hiriyur in Karnataka state of India.

F_4 populations from 2-way, 4-way and 8-way crosses are being used directly in breeding programs. F_4 bulk seed of eight-way crosses was supplied to 15 NARS partners in India for their evaluation and selection of single plants during 2012/13 crop season. The partners have selected desirable plants for development of progenies. A total of 377 heat tolerant progenies have been developed at ICRISAT-Patancheru from heat tolerant plants selected from the F_4 population of 8-way crosses.

Objective 2: Analysis of genetic diversity in MAGIC population
The eight parents used in the development of MAGIC lines were genotyped using 70 highly polymorphic SSR markers earlier reported by Nayak et al. 2010 (Theor Appl Genet 120:1415–41) and Thudi et al. 2011 (PLoS ONE 6(11): e27275) and 747 SNP markers (651 SNPs through KASPar assays and 96 SNPs on BeadXpress platform). In addition, large scale sequence data was generated on these lines through restriction site associated DNA (RAD) sequencing approach and whole genome re-sequencing (WGRS). The sequence data generated through RAD sequencing on five genotypes ICC 4958, ICCV 10, JG 130, JG 16 and ICCV 108) ranged from 6.51 million bases to 11.68 million bases and the cleaned data ranged from 6.47 to 11.34 million bases. Similarly, the sequence data generated through WGRS on JAKI 9218, JG 11 and ICCV 97105 ranged from 27.95 – 38.24 million bases. The sequence data generated will serve as reference for identification of the haplotype groups among the MAGIC lines developed. Further for the development of additional SNP markers the parental lines of the population was sequenced using Illumina MiSeq and detailed analysis is in progress for identification of candidate SNP markers using data from RAD sequencing, WGRS and Illumina MiSeq.

Objective 3: Selection of candidate lines possessing maximum diversity
About 1000 F_6 MAGIC lines are being genotyped using genotyping-by-sequencing approach (GBS) and candidate lines possessing maximum diversity will be selected.

Objective 4: Phenotypic evaluation of selected MAGIC lines for drought tolerance related traits
This activity will be carried out during Year 3. About 200-300 F_8 lines will be selected based on genotypic information and evaluated for yield and other agronomic traits under moisture stress (rainfed) and non-stress (irrigated) conditions at three locations (ICRISAT-Patancheru, Egerton University-Kenya and DZARC-Ethiopia).

Objective 5: Marker-trait association analysis for drought tolerance related trait
This activity will be carried out during Year 3. Phenotypic and genotypic data collected from MAGIC lines will be used for marker-trait association analysis.

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

- *Duration: May 2010–May 2014 (TLI Phase II)*

Principal Investigator and lead institute
Rajeev K. Varshney, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru - 502 324, Greater Hyderabad, India Tel: 040-3071 3305, Fax: 040 3071 3074; Email: r.k.varshney@cgiar.org

Collaborating institutes and scientists

- ICRISAT, India: Pooran Gaur, Mahendar Thudi, L Krishnamurthy, Trushar Shah, Rachit Saxena
- ICRISAT, Kenya: NVPR Ganga Rao
- Ethiopian Institute of Agricultural Research (EIAR), Ethiopia: Asnake Fikre
- Egerton University, Kenya: Paul Kimurto

Chickpea (*Cicer arietinum* L.) is the world's second largest grown food legume with developing countries accounting for over 95% of production and consumption. TLI Phase 2 aims at further harnessing the resources developed during Phase 1 for chickpea crop improvement. Based on phenotyping data of the

reference set generated in Phase 1, 38 pre-breeding populations developed from the superior lines are in different generations in sub-Saharan Africa and India. Further, 28 two-way crosses, 14 four-way crosses and seven eight-way crosses were made for developing MAGIC populations. DNA was isolated from 1200 F_6 progenies and F_7 seeds harvested in February 2013 (Activity 1). A genome-wide Physical map was developed spanning 574 Mb that contributed to sequencing of the chickpea genome. Using a GBS approach 49 SNP markers were integrated in the QTL region, as a result, the QTL interval is narrowed down from 35 cM to 14 cM (Activity 2). In continuation of Phase I activities on molecular breeding for drought tolerance traits, 20 BC_3F_5 lines have been evaluated at ICRISAT-Patancheru and by TL-II partners at Nandyal, Dharwad, and Gulbarga in India. Several lines with more than 10% increase in yield under rainfed and irrigated conditions have been identified. NARS partners as leaders initiated MABC activities in Kenya and Ethiopia and completed three backcrosses for enhancing drought tolerance in elite cultivars at their location. Using OptiMAS, eight superior lines were selected from the cross JG 130 × ICCV05107 and true F_1s from first recombination were chosen and second recombination cycle is in progress (Activity 3). Sixteen scientists from sub-Saharan Africa and Asia were trained by organizing a workshop on "Modern breeding technologies for chickpea improvement". One PhD and three MSc students from NARS obtained their degree. In addition, two PhD students from NARS have been working in the area of molecular breeding at ICRISAT-India as well as in Kenya and Ethiopia (Activity 4). Data generated during TLI Phase 1 were curated into the Integrated Breeding System workflow (Activity 5).

Activity 1: Utilize genetic diversity to develop breeding and MAGIC populations

Genetic diversity present in chickpea germplasm is being utilized in two ways. In the first approach, 38 populations are being developed based on the genotypes identified in TLI Phase 1 or TLII, which includes five crosses at EIAR, two at EU, 20 at ICRISAT-Kenya and 11 at ICRISAT-India. In the second approach, a MAGIC population is being developed by using a set of 8 well-adapted and drought tolerant lines (ICC 4958, ICCV 10, JAKI 9218, JG 11, JG 130, JG 16, ICCV 97105 and ICCV 00108) from Ethiopia, Kenya and India identified in TLI and TLII. The following 28 two-way, 14 four-way and 7 eight-way crosses and F_1s were advanced to F_6 using the single seed descent method. DNA was isolated from all 1200 F_6 progenies, F_7 seeds were harvested in February 13. Sequencing of 8 parental lines of MAGIC population using Illumina MiSeq is underway.

Activity 2: Develop genomic resources for enhancing MABC and MARS activities

In collaboration with Marker Services of Integrated Breeding Platform, successful KASPar assays were developed for a total of 2,005 SNPs. These KASPar assays were validated and screened for polymorphism on 94 genotypes. Agenome-wide physicalmap spanning 574 Mb was developed in collaboration with the National Institute of Plant Genetic Research (NIPGR), New Delhi (S Bhatia and AK Tyagi) and UC-Davis, USA (Ming Cheng Luo). Genetic map positions for 245 BES-SSR markers permit an initial integration of BAC contigs with the chickpea genetic map. Efforts towards fine mapping of the "QTL hotspot" identified on CaLG04 of RIL population ICC 4958 × ICC 1882 during TLI Phase I, employing available chickpea genome sequence information genotyping by sequencing (GBS) approach resulted in the integration of 49 SNP markers in the QTL region. As a result, the QTL interval is narrowed down from 35 cM to 14 cM.

Activity 3: Employ MABC and MARS activities to improve superior lines

Evaluation of 20 BC_3F_4 (JG11 × ICC 4958) and their parents for root traits in the ROS as well as under field conditions, indicated that most of the BC_3F_4s had an RLD greater than JG 11and an increase in yield (11.2% under rainfed and 24.3% under irrigated conditions). Further, multi-location evaluation of 20 BC_3F_5 MABC lines lead to identification of several lines with at least 10% higher yield than the recurrent parent JG 11 and 22-47% increase in seed size. Under TLI Phase 2, NARS in SSA and India (as a part of complementary grant from DBT) are leading the MABC activities. Three cycles of MABC have been completed in both the crosses at Ethiopia and Kenya. In the case of MARS population development, a set of 8 lines were selected for recombination cycles using OptiMAS. The first recombination cycles were made at Patancheru and second recombination cycle is in progress.

Activity 4: Strengthen capacity of NARS partners

A workshop on "Modern breeding technologies for chickpea improvement" trained sixteen chickpea scientists from TLI, TLII and other projects – twelve from Africa (Ethiopia, Kenya, Tanzania, Malawi, Algeria) and four from Asia (India, Nepal, Bangladesh, Myanmar). One PhD student (Mr Kebede Teshome) and three Master students (Mr Abebe Sori, Mr Moses Oyier, Getachew Tilahun and Mr Abebe Sori) obtained their degree. Three PhD students (Ms Serah Songok, Mr Musa Jarso and Ms Alice Koskie) are currently working on MABC and MARS activities.

Activity 5: Management and storage of data
Data generated from TL I Phase 1 and Phase 2 are being curated and stored in the Integrated Breeding Workflow System (https://www.integratedbreeding.net/integrated-breeding-workflow-system-be-released-june-2013).

Cowpeas

8. G6010.02/G7010.07.01: Improving cowpea productivity for marginal environments in Africa

- *G6010.02: May 2010–May 2014*
- *G7010.07.01: May 2010–May 2014 (TLI Phase II)*

Principal Investigator and lead institute
Philip Roberts, University of California, Riverside; Department of Nematology, Riverside, CA 92521, USA; 951-827-7332; FAX 951-827-3719, Philip.Roberts@ucr.edu

Collaborating institutes and scientists

- University of California, Riverside: Timothy Close, Shizhong Xu
- Institut Senegalais de la Recherches Agricole, Senegal: Ndiaga Cisse
- Institut de l'Environment et des Récherches Agricole, Burkina Faso: Issa Drabo
- Eduardo Mondlane University, Maputo, Mozambique: Rogerio Chiulele
- International Institute of Tropical Agriculture, Nigeria: Ousmane Boukar

Context: This project has twenty six Quantifiable Outputs for cowpea (Vigna unguiculata) over five Project Activities: 1) Develop MAGIC population, 2) Develop genomic resources in support of marker-assisted breeding, 3) Employ marker-assisted recurrent selection (MARS) and marker-assisted backcrossing (MABC) to develop improved breeding lines and varieties, 4) Capacity building for modern breeding in Africa, and 5) Curation and storage of data. The genomics revolution has enabled rapid advances in genotyping capabilities and construction of high-density genetic maps that enable breeding strategies with the potential to accelerate delivery of improved crop varieties. Cowpea has a suite of powerful new tools and trait-linked markers that promise to enhance and expedite genetic gain. With this increased potential, however, comes greater data volume and complexity including knowledge about genotyping, marker-trait associations, marker platforms and genotypic and phenotypic data. Training in modern breeding, including sample tracking and data management, is a key activity so that cowpea breeders can effectively use the new tools.

Findings and implications
An eight-parent MAGIC population of > 300 lines has progressed through two-way, four-way and eight-way intercrosses, and is presently at the F5 stage of inbreeding. Inbred lines clearly segregate novel combinations of morphological traits from the parents such as leaf shape and seed coat patterns, implying that the wide range of abiotic and biotic stress traits present in the parents is also present in novel combinations among the inbred lines. MARS and MABC progress varies among locations. Following phenotyping and SNP genotyping at the F2:3 stage and ICI mapping, the most advanced MARS populations have completed the third round of intercrossing and entered the selfing phase. MARS crossing and progeny selection decisions have been guided by OPTIMAS, integrating favorable QTLs for drought tolerance, yield, seed size and Striga resistance. MABC is underway to introgress resistance to Striga, insects, fungal pathogens and nematodes into elite backgrounds with drought tolerance. Progress toward simple introgression has clearly been accelerated by background selection, reducing the necessary number of generations to just two or three.

Links to previous work
This is phase II of the Tropical Legumes 1 project. All work in the present project is linked to work accomplished in phase I, which included development of SNP genotyping and construction of an initial consensus genetic map, and drought trials of biparental RIL populations and several hundred germplasm accessions in African and US locations.

Next steps and challenges
The MAGIC population will be advanced by single seed descent to F7, and complete genotyping in four-way and eight-way progenies for line validation. This population will be a powerful breeding and genetics resource for the cowpea community. The initial performance evaluations of the MARS populations to be conducted in the next year will provide assessment of the breeding value gained from applying the MARS breeding scheme to elite biparental cowpea populations. The MABC populations will result in improved preferred African cowpea varieties with key donated traits and also a framework for analyzing the breeding value gained from genotyping for foreground and background selection. We will

continue to format the phase II datasets for curation in the data management system. A primary challenge will be to ensure the project outputs are used or advanced in other programs beyond the Tropical Legumes 1 and II projects.

References

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

Lucas, M.R., Diop, N., Wanamaker, S., Ehlers, J.D., Roberts, P.A., Close, T.J. 2011. SNP consensus mapping and comparative genomics of cowpea [*Vigna unguiculata* (L. Walp)]. The Plant Genome. 4:218-225.

Pottorff, M., Wanamaker, S., Ma, Y.Q., Ehlers, J.D., Roberts, P.A., Close, T.J. 2012. Genetic and physical mapping of candidate genes for resistance to *Fusarium oxysporum* f.sp. *tracheiphilum* race 3 in cowpea [*Vigna unguiculata* (L.) Walp]. PLoS ONE 7:e41600.

Lucas, M.R., Huynh, B., Diop, N., Roberts, P.A., Close, T.J. 2013. Markers for breeding heat tolerant cowpea. Molecular Breeding 31: 529-536.

Huynh, B-L., Close, T.J., Roberts, P.A., Hu, Z., Wanamaker, S., Lucas, M.R., Chiulele, R., Cisse, N., David, A., Hearne, S., Fatokun, C., Diop, N.N., Ehlers, J.D. 2013. Genepools and the genetic architecture of domesticated cowpea [*Vigna unguiculata* (L.) Walp.]. The Plant Genome doi 10.3835/plantgenome2013.03.0005.

Groundnuts

9. G6010.01: Improve groundnut productivity for marginal environments in sub-Saharan Africa

- *May 2010–May 2014 (TLI Phase II)*

Principal Investigator and lead institute

Vincent Vadez, ICRISAT, Patancheru 502324, AP, India; v.vadez@cgiar.org; +91 4030713463; Fax: +91 4030713074

Collaborating institutes and scientists

- Nalendiele Research Station, Tanzania: Omari Mponda
- Chitedze Research Station, Malawi: Albert Chamango
- ISRA – CERAAS, Senegal: Issa Faye, Daniel Fonceka
- University of Georgia Athens, USA: Andrew Paterson
- CIRAD: Dr Jean-Francois Rami / Daniel Fonceka
- EMBRAPA, Brasil: David Bertioli and Soraya Leal-Bertioli

The project tackles two major constraints of peanut production in sub-saharan Africa: foliar diseases (early leaf spots, rust and rosette) and drought. The main focuses are on bringing in new genetic diversity from cultivated germplasm or wild relatives, developing genomic tools for their use in breeding, generating relevant genetic material to map QTLs for these constraints, breeding tolerance and resistance traits in farmer preferred lines, training partners and adding information generated to the IBP for further use.

New diversity for drought and disease resistance - 60 genotypes with large yield contrast under intermittent drought across years and locations, all with good agronomic traits, have been re-tested in Niger and India. ICG12697, ICG15287, ICG3140, ICG434, ICG8751, ICGV02266, ICGV02290, ICGV88145 and ICGV97182 in Niger and ICG12879, 55-437, ICG4729, ICG3584, ICGV97183, ICG11088 in India were confirmed drought tolerant. Some are tested in PVS trials for direct release in TLII.

Three rosette resistant lines (ICG 13099, ICG 14705 and ICG 15405), with pod yield of ICG 14705 equivalent to the best rosette resistant ICGV-SM 90704, five ELS resistant lines with ICG 12509 as good as the best ELS elite variety ICGV-SM 95741, and two rust resistance line with ICGV 02446 with same yield as the best rust resistant elite check ICGV 94114, were confirmed. On-farm PVS trials were started in Malawi and Tanzania. Detached leaf assays have also been put in place in Malawi.

Chromosome substitution lines (CSSL) of synthetic AixAd in the background of Fleur11 have been distributed to India, Brasil, Malawi and Niger. Several entries show a higher yield under drought than drought tolerant 55-437, while certain CSSL show resistance to ELS in Senegal. Two new AB-QTL populations are being developed in Senegal using synthetics developed at ICRISAT. Five new synthetics produced in Brazil have been crossed to cultivated lines and tested for rust resistance, all of them being immune except one. Overall, evidences are accumulating that wild relative, through synthetic allotetraploids, contribute outstanding new sources of resistance to diseases.

Develop genomics tools for cultivated groundnut - 96 informative SNPs were used to develop KASPar assays (GKAMs, Groundnut KASPar Assay Markers). From the screening of 96 GKAMs on 94 genotypes,

90 GKAMs were validated and 73 GKAMs showed polymorphism. These were screened on 280 diverse genotypes of the reference set and 72 GKAMs were found polymorphic. The average polymorphism information content (PIC) value for polymorphic GKAMs was 0.31 in the reference set.

Map disease resistance QTL and introgress existing disease resistance QTLs

One population for ELS in Mali, two for rust in Malawi and two for rosette in Malawi have been advanced to F6 and phenotyped. Most DNA polymorphic populations for rust (ICGV 93437 × ICGVSM 95342, 61 SSRs), ELS (ROBUT 33-1 × ICGV 95714, 111 SSRs) and rosette (CG7 × ICGVSM 90704, 119 SSRs) will be genotyped. As a part of TLI and National Fund project from India, 43 QTLs were identified for foliar disease resistance based on two populations (TAG 24 × GPBD 4 and TAG 26 × GPBD 4). For rust resistance, in addition to IPAHM103, four new markers (GM2009, GM1536, GM2301 and GM2079) were associated with the major QTL. One major QTL was identified for late leaf spot (LLS) namely $QTL_{LLS}01$ (GM1573/GM1009-pPGPseq8D09) showing PVE up to 62.34%. The major QTL for rust has been introgressed in leading varieties in India through marker-assisted backcrossing (MABC).

Develop new populations for drought tolerance mapping and breed for disease resistance

From 22 new mapping populations developed earlier for drought, two are being advanced to RILs, based on further confirmation of parental lines in the field. In Mali, 3 new sources of resistance to ELS are used in 13 populations involving early maturing rosette tolerant lines that are highly susceptible to foliar diseases and advanced to F4.

BC3F1 introgression of disease resistance into farmer accepted and adapted varieties is on-going namely: rosette resistance from ICGV-SM 90704, ICGV-SM 01731 and ICG 12991 into CG7, Chalimbana, JL24, Pendo, Nyanda, ELS resistance from elite resistant lines ICGV-SM 95714 and ICGV-SM 93555into Pendo, JL 24 and Nyanda, and rust resistance from ICGV 94114 and ICGV 95342 into Pendo and Nyanda.

Strengthen capacity of NARS partners
10 young scientists from different participating countries have taken part in training in India and Niger or Mali, and were trained in both domains of drought adaptation and disease resistance, on activities dealing with phenotyping, breeding or genetics.

Management and storage of data

Pedigree and phenotyping data are captured: (i) in Agrobase at ICRISAT India/Mali; (ii) in IBFB at ISRA, Senegal; (iii) in Excel sheet in ICRISAT Niger. Genotyping data are captured in GDMS at ICRISAT Patancheru, while those for CSSL construction from BC1 to BC3 sent to ICRISAT for upload in GDMS.

Legumes: Cross-cutting activities

10. G6010.05: Cross-cutting crop activities (drought phenotyping, data management and capacity building) (TLI project, Objective 5)

- *May 2010–May 2014 (TLI Phase II)*

Principal Investigators and lead institutes

- Drought phenotyping: Vincent Vadez, ICRISAT
- Data Management: Trushar Shah, ICRISAT
- Capacity building: Ndeye Ndack Diop, GCP

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

Activity Leader and lead institute:

Vincent Vadez, ICRISAT Patancheru 502324, AP, India; v.vadez@cgiar.org; +91 4030713463; Fax: +91 4030713074

Collaborating institutes and scientists

- ISRA, Senegal: Issa Faye – Nouhoun Belko
- NCSU (Raleigh, USA): TR Sinclair
- CIAT, Colombia: IM Rao

The Tropical Legume I project is targeting drought as the major constraint limiting crop production of 4 legumes (groundnut, bean, cowpea, chickpea) in sub-Saharan Africa. In the first phase of TLI, each crop had a different approach to drought. In this second phase, we introduced some cross-learning from experience in different crops, by comparing possible adaptive traits across crops and by using common protocols and methods. Adapting to water limitation falls into three critical domains: (i) managing water to ensure water is available for grain filling; (ii) water capture; (iii) efficient remobilization to grain. In addition, drought patterns vary across time scales and locations leading to large genotype-by-environment interactions that hamper breeding progress. Therefore, the last but most important component of that objective is the use of crop models for different legumes, constructed on the same model structure, to pre-empt possible trait effects on yield and then to better guide best breeding targets.

Managing water - From the results gathered over the past few years, it is now clear that species share a number of traits of adaptation to water limited conditions, including the capacity to restrict transpiration under high VPD or to modulate the leaf area (groundnut, cowpea, and chickpea). We made progress towards the mapping of some of these traits. While this was not originally planned, prior progress were sufficient to bring us to that stage. QTLs for the transpiration rates under high vapor pressure deficit and for the leaf area were identified in cowpea, with each parent controlling some of the alleles for these traits (which is exciting because it opens the opportunity to choose recombinant lines based on the "content" in these QTLs, and the knowledge of their effects on water use, that would lead to different water usage and then specific capacities to adapt to a range of water limitations). Similar work has been done in a RIL of chickpea. A RIL population of groundnut was also screened in the field, in lysimeters and in pots for water saving traits, to map both agronomic and trait QTLs.

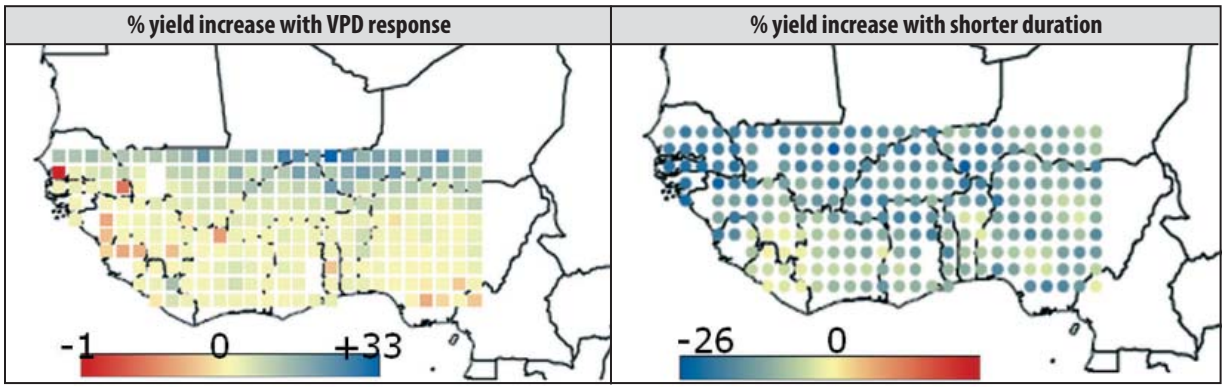
Lysimetric assessment of water capture – Here also tolerant lines of chickpea and cowpea have the capacity extract more water during the post-anthesis period, which is putatively related to water saving traits. In bean, higher yielding lines appear to have deeper and more profuse rooting, although this relationship was not strong. We have tested cowpea, bean and groundnut in lysimeters with low and high N soil content. Groundnut performs equally well under low and high N soil, while cowpea shows yield decreases under low N nutrition, especially under water limited conditions, and bean suffers the N deficiency more than the water limitation. These data suggest that, depending on legume crops, care needs to be taken about the role of symbiotic N fixation (SNF). It also shows large variation in all species for the SNF-dependent yield.

Partitioning traits – It was confirmed in bean that superior adaptation to drought was associated with higher pod partitioning and pod harvest index values, and in the case of bean, it was not related to the capacity to extract water during the grain filling period.

Crop model simulation - The crop simulation model has been validated for target regions of chickpea and groundnut. The model has been upgraded to run multiple locations simultaneously, which now gives the potential of rapidly testing the effect of traits (where genotypic variation exist) on yield across large geographic areas and across years, with output given in the form of GIS maps for easy interpretation. Key to this progress was the finding that Marksim-generated weather data are equally suitable to observed weather data to assess these trait effects. Several PhD students are being trained to take full responsibility in the modeling efforts in their respective crops.

Several leaf area development coefficients have been assessed in field trials in groundnut and in chickpea and few more coefficients still need parameterization in representative cultivars of different crops.

Crop simulations have continued in groundnut, chickpea and even soybean. In groundnut it shows that: (i) transpiration sensitivity to high vapor pressure deficit would increase groundnut yield above 10 degree latitude in WCA; (ii) that drought has mostly a negative effect on yield above 10 degrees latitude; (iii) that shortening the cropping cycle of groundnut by about 10 days would decrease yields by about 20-25% across the entire block of locations in WCA (see graph below). In chickpea, it shows that faster rooting would have a small (5%) negative effect on yield, except on short duration cultivars. The genetic trait with most effect would be the capacity to extract water from deeper layer (10% yield benefit), while irrigating with 30 mm at the beginning of seed growth would bring about 30-40% yield benefit. The latter illustrates that crop modeling can test either genetic or agronomic effects, or any combination.



Activity 2: Data curation of the Tropical Legumes I project

Activity Leader and lead institute
Trushar Shah, ICRISAT; tm.shah@cgiar.org
Patancheru, Hyderabad, 502324

Collaborating institutes and scientists

- ICRISAT: Vincent Vadez, Rajeev Varshney, Pooran Gaur, Prasad Peteti
- IITA: Sam Ofodile, Boukar Ousmane
- CIAT: Alberto Guerrero, Steve Beebe, Bodo Raatz
- UC-Riverside: Jeff Ehlers, Tim Close

Most of the data from Phase I of the TLI project has been curated and is available in the respective central databases. The data from the reference sets as well as the marker, map and QTL information available would be of particular interest to the crop breeders. The tools to query this information are through the Integrated Breeding Workflow System. This data has been migrated to the latest version (version 2) of the IBDB database that uses the Chado schema.

A data catalogue has been developed for the datasets arising from TLI Phase II from the reports submitted and this has been verified by the activity leaders. Germplasm information, Marker information, Evaluation data, Genotyping data, Mapping data, QTL data generated from different breeding activities/ schemes such as MARS, MABC and Magic populations are being curated and stored in the Integrated Breeding Databases. Trait dictionaries were developed along with ontologies and were uploaded to crop ontology website (http://www.cropontology.org/). Sequence data has been uploaded to public databases such as NCBI and on the ICGGC website.

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

Activity Leader and lead institute
Ndeye Ndack Diop, GCP; nn.diop@cgiar.org

This cross activity is part of Tropical legumes 1 project and covers 3 areas that have been set to provide support towards an effective implementation of the different objectives of the project 1) Crop comparative drought phenotyping, 2) Data management, and 3) *Building the capacity of sub-Saharan African scientists, and project management* that will be reported here.

One important element of the capacity building under the TL1 project is the training of the next generation of scientists (MSc and PhD level) that is undertaken in each crop objective of the project (for details refer to the corresponding project). Their research is an integral part of the project and addresses one or several milestones of the project. In addition, a more general training is undertaken, taking advantage of the annual meetings of both TL1 and TL2. For TL1 partners, the focus has been on Fieldbook and GDMS, both of which are tools developed under the IBP initiative. For TL2 partners, the focus has been given to the introduction to MB concepts, Fieldbook and QA/QC applications. The 2013 training following the TLs joint annual meeting

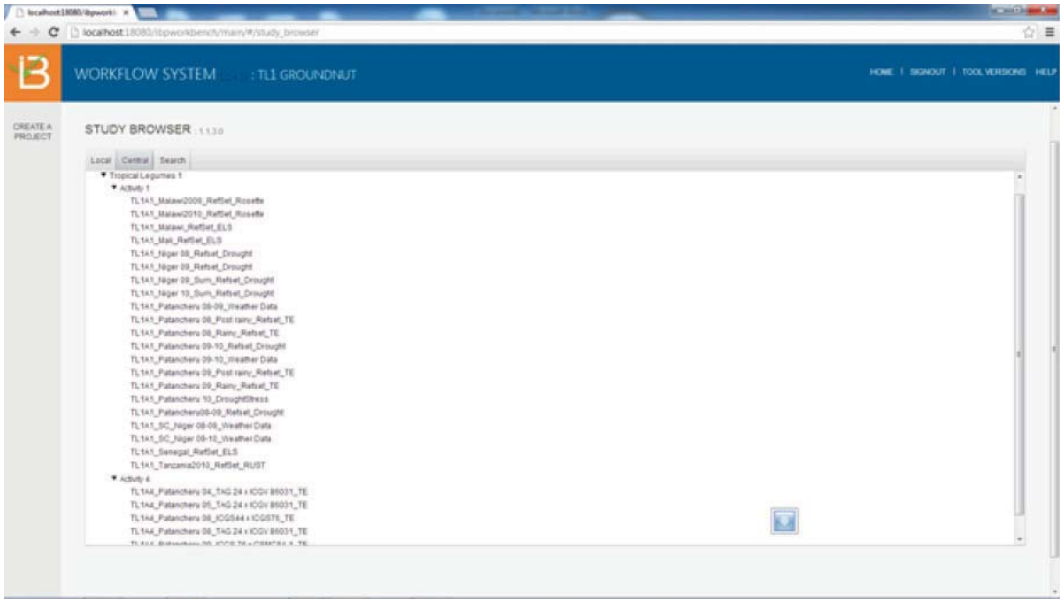


Fig 1: A screenshot of datasets in the Groundnut Central Database under the Tropical Legumes Project by activity.

has focussed on analysing the fingerprinting data that was completed earlier in 2013, designing nurseries and advancing them to the next generation, and querying the database for genotypic and phenotypic data. These training workshops tend to be 1 to 2 days per year and might not be the most efficient to ensure that trainees have a full understanding of the concepts and tools. Still, they are important to introduce the new developments to senior scientists that usually attend the meeting, as they are the leaders of the breeding programmes and will be defining the direction to be taken.

More training courses have been implemented for the people that are likely to implement the experiments and analyse the data. Training courses were offered to developing-country partners (junior scientists, MSc and PhD students whose theses focused on populations generated by TLI), on phenotyping for drought, on the analysis of phenotypic and genotypic data, and on the application of modern breeding techniques. One of those training courses is the Integrated Breeding Multi-Year Course (IB-MYC), organised in conjunction with IBP, which aims to provide the trainees with the necessary knowledge on molecular breeding and to use the tools to analyse their data in the most efficient way. Partners of both TLI and TLII have been invited to join the IB-MYC programme. They represent 32.3% of the 170 trainees over the 3 regions (West and Central Africa, East and Southern Africa and South and South-East Asia). TLII partners have been given the opportunity to initiate a small MB project and to introduce MB into their breeding programmes by providing access to genotyping support through commissioned GSS projects.

In addition to the training of scientists, it is also important to train the technicians who work with them, given the need to assure quality data. Technician training was conducted on a regional basis to overcome language barriers and to include as many technicians as possible. The training covered improved the management of agricultural research plots and trials, including field preparation and homogeneity, trial installation and care during the season, planning and budgeting of research work, as well as methods of data collection, focusing on the use of electronic tablets and data-management software.
Most of the infrastructure support was implemented earlier in the project, between 2010 and 2011. The latest support was a greenhouse for the chickpea team in EIAR in Ethiopia.

TL1 and TL2 scientists have initiated a joint meeting, first for 1 day in 2012, and in 2013 by joining the annual meetings for TL1 and TL2, for East and Southern Africa to discuss integrative activities between the 2 projects. TL1 partners defined the products that are ready for transfer in TL2 breeding programmes. TL2 partners defined the important traits that are missing from TL1 activities and how to integrate them into the discovery process undertaken by TL1 or by initiating relevant crosses. The low uptake of MB techniques by the NARS partners, despite the effort undertaken by GCP to train the partners during TL meetings and through IB-MYC, is one of the main challenges we face with the national partners. One area to be explored would be partners from CGIAR centres and ARIs to collaborate closely with the NARS on their MB programme in a mentorship type of joint programme. This has been a success in the collaboration between Agropolis-CIRAD and ISRA (Senegal) in the development of the CSSL population. This model could be extended to include the TL2 national partners and TL1 partners.

11. G6010.06: Forensic marker service activities at GCP (including 6 tropical legume crops)

- *February 2012–May 2014*

Principal Investigator and lead Institute

Chunlin He, Generation Challenge Programme, c/o CIMMYT, Km 45 Carretera México-Veracruz, El Batán, Texcoco, Estado de México, CP 56130, MEXICO; c.he@cgiar.org
Tel: +52 (55) 5804 2004; Fax: +52 (55) 5804 7691

Collaborating institutes and scientists

- Generation Challenge Programme, c/o CIMMYT, Texcoco, Mexico: Xavier Delannay, Ndeye Ndack Diop, Mae Christine Maghirang
- International Institute of Tropical Agriculture (IITA), Nigeria: Ousmane Boukar and Hesham Agrama
- International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India: Pooran Gaur
- International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Kenya: Ganga Rao
- International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Malawi: Emmanuel Monyo
- International Center for Tropical Agriculture (CIAT), Colombia: Bodo Raatz and Steve Beebe

The forensic project (for Quality Assurance and Quality Control or QA/QC) was proposed to enhance the breeding process with higher standard by using DNA markers for the improvement of key tropical legume (TL) crops including cowpea, chickpeas, pigeonpeas,

common bean, soybean and groundnut. The project aims to increase the breeding efficiency and thus the legume productivity and profitability in target regions of sub-Saharan Africa and India.

1. KASP SNPs developed at GCP for forensic marker services

A set of KASP SNP markers has been developed for each of the TL-crops through collaboration with the partners and service provider.

2. Fingerprinting data generated from the elite germplasm of TL-crops

A set of fingerprinting data has been generated for each of the tropical legume crops through SNP genotyping at KBioscience/LGC. These data are important resources for molecular breeding programs.

3. Utilization of the KASP SNPs and fingerprinting data to QA/QC activities

Some of the immediate uses of the KASP SNPs and fingerprinting data for QA/QC include, but are not limited to: (1) identity confirmation of breeding lines; (2) identity confirmation of F1 and BC1 materials; (3) line-finishing assistance (verification of parentage, residual heterozygosity, duplication, etc.); (4) Other uses: analysis of diversity and similarity in selected material of the breeding programmes, choice of polymorphic markers to use in genotyping of biparental progenies, QTL detection and mapping, marker-assisted breeding (MAB). The major uses of (1), (2) and (3) from the above were presented and discussed at the TL1/TL2 meeting in May 2013 in Campala, Uganda, they will be on the agenda of the next genotyping projects. Some of the users indicated strong interest in using them.

Challenges

Although many breeders demonstrated interest in the forensic projects, there may be challenges in their implementation: (1) these data sets may be limited for fast germplasm recycling in breeding programs due to small number of lines used; (2) some breeders may need further training and understanding the importance of QA/QC projects, especially in considering the preparation and shipment of actual samples for the genotyping.

Legumes: Capacity-building activities:

Capacity-building projects: training components for TLI scientists and students

12. G4009.07.01: Capacity-building à la carte 2009 – TL1 students for analysis of drought tolerance in common beans

- *October 2009–October 2010; NCE: May 2014*

Principal Investigator and lead institute

Bodo Raatz, b.raatz@cgiar.org
CIAT, International Center of Tropical Agriculture
A.A. 6713, Cali, Colombia, Tel +57 2 4450000

Table 1. The number of KASP SNPs developed at GCP for the 6 TL-crops from the forensic project

Crops	Partners	No. of SNPs or SSRs	Status
Cowpea	University of California Riverside - Jeff Ehlers	1122 SNPs	Available for genotyping
Chickpeas	ICRISAT - Rajeev Varshney	2005 SNPs	Available for genotyping
Pigeonpeas	ICRISAT - Rajeev Varshney	1616 SNPs	Available for genotyping
common bean	USDA - Perry Cregan, CIAT – Stephen Beebe	1497 SNPs	Available for genotyping
Soybean	USDA - Perry Cregan; IITA - Melaku Gedil	1082 SNPs	Available for genotyping
Groundnut	ICRISAT - Rajeev Varshney	91 SNPs and 90 SSRs	Available for genotyping

Table 2. Fingerprinting data of 6 TL-crops from the forensic project

	KASP SNP development				Fingerprinting		
Name of crop	#Samples	#SNPs	#Data points	Name of crop	#Samples	#SNPs	#Data points
Chickpeas (1)	95	2,005	190,475	Chickpeas (1)	30	1,144	34,320
Chickpeas (2)	225	63	14,175	Chickpeas (2)	170	1,128	191,760
common bean	95	1,497	142,215	common bean	94	770	72,380
Cowpeas	23	1,122	25,806	Cowpeas	177	1,059	187,443
Groundnut	94	90	8,460	Groundnut	200	91	18,200
Pigeonpea	93	1,616	150,288	Pigeonpea	200	1,200	240,000
Soybean	40	1,082	43,280	Soybean	206	1,029	211,974
Total			1,091,579				956,077

Note: the numbers in shaded cells are expected data as the lab work of the projects are in progress.

Collaborating institutes and scientists

- SARI, Ethiopia: Fitsum Alemayehu
- DR&SS, Zimbabwe: Godwill Makunde

TLI students for analysis of drought tolerance in common beans

Common bean is the major food legume for direct human consumption and of particular importance for nutrition in developing countries in Africa and around the globe. Production is challenged by various biotic and abiotic constraints. This project is carried out under the Tropical Legumes- I (TL-1) project as a complementary training component. It aims to improve understanding of the molecular basis of drought tolerance.

Objective 1: MARS (Fitsum Alemayehu, PhD student)

F. Alemayehu was visiting CIAT for three months for training and drought yield evaluations. During his stay the MARS population was evaluated and he received trainings in statistical analysis of field data and QTL analysis linking phenotypic and SNP data using TASSEL software.

i): Development of crosses for marker assisted recurrent selection

A population of ~200 lines is used that was developed at CIAT HQ, Colombia. Elite drought resistant germplasm was combined with high yielding varieties. It is a somewhat complex population descending from five crosses, to capture more genetic variation.

ii): Generation advance under drought pressure of these recurrent selection populations at SARI (F. Alemayehu, D. Ambachew)

Existing crosses from lines of the drought population were shipped to Ethiopia to be advanced under conventional drought selection. This will serve as a control to the MARS approach and may deliver locally adapted breeding lines for variety testing.

iii): Phenotyping under drought

Additional phenotypic data of the MARS population of ~200 lines was collected at CIAT HQ, Colombia. Drought conditions this season were excellent and these data will be combined with phenotypic evaluations of previous seasons to allow a solid initial QLT analysis.

iv): Genotyping of drought population for QTL analysis

Additional SNP genotyping yielded further 14.000 data points evaluating ~ 170 markers. Genotyping was carried out using the fluidigm platform at CIAT HQ. QTL analysis is now in progress to determine markers to be used in recurrent selection.

Objective 2: Association Mapping for drought traits (Godwill Makunde)

In this project the reference collection was evaluated for drought related traits and genome wide association studies were performed using SNP markers to identify marker-trait associations. This work was part of G. Makunde's PhD thesis which is now completed. G. Makunde has since left his institute to work for some university.

13. G4009.07.02: Capacity-building à la carte 2009 – Capacity-building in modern cowpea breeding

- *October 2009–October 2010; NCE: May 2014*

Principal Investigator and lead institute

Philip Roberts, University of California, Riverside; Philip.Roberts@ucr.edu Department of Nematology, Riverside, CA 92521, USA; 951-827-7332; FAX 951-827-3719

Collaborating institutes and scientists

- University of California, Riverside: Timothy Close
- Institut Senegalais de la Recherches Agricole, Senegal: Ndiaga Cisse
- Eduardo Mondlane University, Maputo, Mozambique: 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 subsequently retook the TOFL test. The student from Mozambique was accepted into the PhD program at UC Riverside and started at UC Riverside in Jan. 2012. He has made good progress on his dissertation research, focused on genetic analysis of nematode and pathogen resistance and breeding potential of a core collection of Mozambique cowpea germplasm accessions and land races. 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. The focus of her dissertation research is genetics and breeding for drought tolerance and Macrophomina resistance in cowpeas adapted to Senegal.

Tangible outputs delivered in Phase I, first year Phase II

Two African PhD students with improved English skills, mentored in PhD programs in Senegal and at UC Riverside focused on cowpea genetics, pathology and modern breeding.

14. G4009.07.03: Capacity-building à la carte 2009 – 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: May 2014*

Principal Investigator and lead institute

Rajeev K Varshney, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, India Tel: 040-3071 3305, Fax: 040 3071 3074 Email: r.k.varshney@cgiar.org

Collaborating institutes and scientists

- Egerton University, Kenya: Paul Kimurto Richard Mulwa
- ICRISAT, India: Pooran Gaur, Mahendar Thudi

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, of 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. After two generations of selfing ten BC_3F_3 lines with high background recovery were chosen for seed multiplication in main crop season 2013. After seed multiplication these lines will be evaluated under rainfed and irrigated environments for yield and harvest index related traits.

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 Feb 2011. After foreground selection, 6 plants were found to be homozygous at the donor allele QTL region. Background selection with 40 SSR markers that are equally distributed throughout the chickpea genome indicated >90% genome recovery of the recurrent parent. These six lines were selfed during off-season (Jul-Sep 2011) in green house to obtain BC_3F_2. The BC_3F_2 were further advanced to BC_3F_3 during main crop season Oct 2011- Feb 2012. Seed multiplication these ten lines will be taken up during main crop season in 2013 and subsequently these lines will be evaluated for yield and harvest index related traits under rainfed and irrigated environments.

15. 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: May 2014*

Principal Investigator and lead institute

Vincent Vadez, ICRISAT, Patancheru 502324, AP, India;
v.vadez@cgiar.org;
+91 4030713463; Fax: +91 4030713074

Collaborating institutes and scientists

- CERAAS, Senegal: Ndiaga Cisse
- Egerton University : Paul Kimurto
- ICRISAT-Niger : Falalou Hamidou
- North Carolina State University, Raleigh: Tom Sinclair

The Tropical Legume I project is targeting drought tolerance as one of the major traits limiting crop production of 4 legumes (groundnut, bean, cowpea, chickpea) in sub-saharian Africa. Unfortunately, drought pattern vary across time scales and locations leading to large genotype-by-environment interactions that hamper breeding progress. An approach to tackle these limitations is to better understand what traits contribute to crop adaptation across these scenarios. This is the object of a transversal Objective 5 in the TLI project. The present grant aims at training young scientists to that approach because skills to precisely phenotype these traits in a marker-assisted breeding approach are indeed critical for the success of breeding in sub-Saharan Africa. Our current object is to train a critical mass of plant breeders and technicians to the techniques and protocols that they will need to master to generate relevant phenotyping data. These training activities have been going on since the beginning of the TLI project. While a lot of training usually takes place in form of short workshops, we have acquired the conviction that workshops cannot replace long-term, hands-on training, especially on complex constraints such as drought. Therefore, the principle of these training activities is to identify, in collaboration with the partners, where the training needs are, and then to tailor specific training to individuals over periods of 4-6 months.

This year we had six trainees under that project: Ruth Wangari from Egerton University in Kenya, Omar Halilou and M Boulama from Niger, Nouhoun Belko, Mareme Niang and Tossim Habalo from CERAAS senegal. The areas of training follow by and large the areas of the cross-specie objective of the TLI project:

Water use traits

Trainees are routinely doing dry-down experiments, which are a standard protocol to impose controlled water stress conditions, and which can be adapted to different conditions and purposes. They are also trained to measure the transpiration response to high vapor pressure deficit, which has become one of the salient traits for drought adaptation. The protocols used depend on the number of entries to test, i.e. growth chambers for entry numbers below 30-40, or outside natural conditions during key periods of the year for numbers above 40. An alternative to measuring the VPD response is to measure the canopy temperature and training in using infrared camera and in treating images has been provided. Examples of training activities: Tossim Abalo on testing peanut, Ruth Wangari on chickpea, Nouhoun Belko on cowpea.

Rooting and water extraction

In the scope of TLI-Objective 5 we use lysimeters, i.e. long and large PVC tubes that are used to monitor water extraction by regular cylinder weighing. Similar system is now set up at ICRISAT-Niger and CIAT. Training was then about monitoring of weight data, TDR assessment of profile of water extraction, and generating result output. Comparison of cowpea and peanut in that system has been done in Niger (papers in progress). Measurements of root traits (with WinRhizo) have also been undertaken at a small scale to compare root length density data to water extraction.

Large scale phenotyping
This is a must if good physiological knowledge is to be expanded to a breeding use. Several mapping populations, segregating for some key traits, have been phenotyped (e.g. the transpiration response to low and high VPD in a cowpea RIL and a chickpea RIL), by two trainees (Nouhoun Belko and Ruth Wangari). The main purpose here was to tackle the unavoidable "logistical" issues around large scale phenotyping (timing of measurement, staggering of replication, team work, task sharing, quality, etc.)

Field phenotyping
This is of course the end point. Trait and field phenotyping are both needed. Several students (eg Omar Halilou, Filipo Machamba) have been trained to monitor field trials, from the experimental design, seed packeting, layouting, monitoring of irrigations, harvest.

Crop simulation modelling
This is the last but not the least. Several students have been trained to use a family of crop models for legumes (i-Legumes) developed by Afshin Soltani and Tom Sinclair. Each student has acquired the skills for a crop he/she knows. So far one student has been trained for peanut (Omar Halilou) and bean (Jaumer Ricaurte) and another one is planned for cowpea (Nouhoun Belko).

Links to previous work
This followed a training course on phenotyping at ICRISAT in March 2008, and then a continuous stream of long term (3-6 months) trainees every year ever since (4-8/year)

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 do good, precise, high throughput phenotyping. So, beyond training of people it also involves future development in infrastructure.

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

- *May 2010–June 2014*

Principal Investigator and lead institute
Rajeev K Varshney, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, India Tel: 040-3071 3305, Fax: 040 3071 3074
Email: r.k.varshney@cgiar.org

Collaborating institutes and scientists

- ICRISAT, India: M Thudi, C Siva Kumar, RK Saxena and PM Gaur
- Egerton University (EU), Kenya: P Kimurto
- Ethiopian Institute of Agricultural Research (EIAR), Ethiopia: A Fikre,

Marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS) - two most important molecular breeding approaches are gaining importance in the recent past for more precise selection and introgression of desired traits into elite cultivars. Chickpea is most important food legume in the arid and semi-arid regions especially in Sub-Saharan Africa (SSA) and South Asia (SA) challenged by drought. Significant genomic tools like SSRs (simple sequence repeats), SNPs (single nucleotide polymorphisms) and DArT (Diversity Array Technologies) arrays and a hotspot containing QTLs for several drought related traits has been identified in Phase I of Tropical Legumes –I (TL-I). Cost-effective KASPar genotyping assays were developed and validated for a set of 2005 high confidence SNPs in collaboration with Integrated Breeding Platform. Comprehensive transcript map with 138 loci (including 568 novel SNP markers using KASPar assays; Hiremath et al. 2012, Plant Biotechnol J 10: 716-732) and a genetic map with 1291 loci (BES-SSRs and DArT markers; Thudiet al. 2011, PLoS One 6(11)27275) were developed. Further large number of SNP identified using Genotyping-by-sequencing approach (GBS), of which setoff 49 novel SNPs were mapped in the "QTL hotspot" region indentified during TLI Phase I based on ICC 4958 × ICC 1882 recombinant inbred line population which narrowed down the interval of this region from 35cM to 14 cM. With the infusion of additional funds from other projects, two BAC libraries were constructed, 46,112 clones were assembled and a physical map was developed spanning 574 Mb that contributed to sequencing of the chickpea genome. One PhD and 4 MSc students from NARS obtained their degree. In addition, two PhD students from NARS have been working in the area of molecular breeding at ICRISAT-India as well as in Kenya and Ethiopia. Sixteen scientists from sub-Saharan Africa and Asia were trained by organizing a workshop on "Modern breeding technologies for chickpea improvement".

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, successful KASPar assays were developed for a total of 2,005 SNPs from 2,486 genes containing high confidence SNPs. A total of 1,484 SNPs were determined to be polymorphic. A total of 568 SNPs were integrated in the transcript map of chickpea (ICC 4958 × PI 489777) (Plant Biotechnol J 10:716-732). In addition BES-SSRs and DArT loci developed during TLI Phase I were used for constructing a comprehensive interspecific genetic map for ICC 4958 × PI 489777 recombinant inbred line (RIL) population (Thudi et al. 2011, PLoS One 6(11):27275). Further, using a GBS approach high-quality genotyping data have been assembled for 828 SNPs on the ICC 4958 × ICC 1882 RIL population and a comprehensive genetic map comprising a total of 1,013 marker loci, spanning a distance of 723.64 cM was constructed. A set of 49 SNP markers were integrated into the "QTL hotspot" region. As a result, the QTL interval is narrowed down from 35 cM to 14 cM.

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***

1.3 ***Next generation sequencing of pooled DNA of BACs identified***

Although construction of local physical map for selected genomic regions was proposed in the proposal and two sub-activities namely 1.2 and 1.3 as mentioned, a genome-wide physical map was developed. In brief, two new BAC libraries were constructed in collaboration with the National Institute of Plant Genetic Research (NIPGR), New Delhi (S Bhatia and AK Tyagi) and UC-Davis, USA (Ming Cheng Luo). High information contig fingerprinting (HICF) of 69,984 BAC clones gave high-quality fingerprinting data for 67,164 clones. Fingerprinting of an additional 1,144 BAC clones, 773 of which correspond to NB-LRR disease resistance gene clusters (UC-Davis) and 371 of which have been genetically mapped via BAC-end derived SSRs (BES-SSR) (Thudi et al. 2011, PLoS ONE 6(11):27275), was also completed. A total of 46,112 clones were assembled into 1,174 contigs, with 3,256 singleton clones. The physical map developed spans an estimated 574 Mb. Genetic map positions for 245 BES-SSR markers permit an initial integration of BAC contigs with the chickpea genetic 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" held during Oct 25 – Nov 19, 2010 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).

2.2 ***Training of at least 4 MSc and 2 PhD students from NARS institutes in chickpea genomics and breeding.***

Mr Kebede Teshome, a PhD student registered at Haramaya University (HU), Ethiopia, has now graduated. Ms Serah Songok, PhD student registered at EU, Kenya is currently summarizing the results of research for her PhD dissertation. Mr Musa Jarso, a breeder from EIAR, has registered at Addis Ababa University (AAU), Ethiopia has taken up three backcrosses towards enhancing drought tolerance through MABC approach in elite Ethiopian cultivars. Ms Alice Koskie, a PhD student has registered at WACCI, Ghana, is currently working on MARS and MABC activities. Mr Abebe Sori, registered at HU, Ethiopia; Mr Moses Oyier, registered at EU, Kenya; and Mr Getachew Tilahun, registered at AAU, Ethiopia have all obtained their Masters degrees.

Maize

17. G4008.56: AMDROUT: Asian maize drought-tolerance project

- *November 2008–October 2013; NCE: July 2014*

Principal Investigator and lead institute
B. S. Vivek, CIMMYT, bvivek@cgiar.org
c/o ICRISAT, Patancheru-502324, Hyderabad, INDIA.
Telefax: +91-40-30713779/80

Collaborating institutes and scientists

- Krishidhan Seeds, India: I.S. Singh
- Syngenta India: R.P. Singh
- Indonesian Center for Food Crops Research and Development, Indonesia: M. Azrai
- National Maize Research Institute, Vietnam: Le Quy Kha
- Institute of Plant Breeding, Philippines: Eureka M. Ocampo
- Department of Agriculture, Thailand: P. Grudloyma
- Yunnan Academy of Agricultural Sciences, China: F. Xingming

Background

Over 80% of the 19 million hectares of maize in South and South-East Asia is grown under rainfed conditions and is prone to drought. Addressing the problem of drought can provide excellent 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 elite CIMMYT inbreds are available whose resultant hybrids out yield commercial checks. Most of the donor investment has been directed at Africa with little 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. 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 (with SNP markers) within bi-parental (or pedigree) breeding populations generated using African white drought tolerant donor inbreds and elite Asian adapted yellow inbreds. MARS projects from African populations have not shown major QTLs for yield under drought. 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).

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 were CML538, CML440, CML505, CZL0719, and CZL00009. Recipient lines identified to be of value to this project include: CML470, VL1012767, VL108733, VL108729, VL1012764, and CML472.

Genetic Gains

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

Population Name	CIMMYT-Asia recipient line	DT donor line	F3 families	Polymorphic SNPs
AMDROUT1	CML470	CML444	294	353
AMDROUT2	VL1012767	CML444	189	391

For both populations: Cycle 1 (c1) was formed by recombining lines that showed good performance in test crosses. c1F2 was formed by recombining c1 plants that were visually appealing. In the same season but on a second set of c1 plants, cycle 2 (c2) was formed by recombining individual c1 plants that showed high GEBVs (top 5-10%) based on genotyping.
AMDROUT1: Per se performance of c1F2 was 40% better while that of c2 was 80% better than the F2. Per se performance of c2 was 25% better than c1F2 (Fig 1).
AMDROUT2: Per se performance of c1F2 was 40% better and that of c2 60% better than the F2. Per se performance of c2 was 16.5% better than c1F2 (Fig 2).

Discussion

C1F2s were derived by two recombinations, each based on phenotypic-only selection. C2 was derived by one recombination based on phenotypic selection and a subsequent recombination based on marker effects (i.e. genotype only). C2s of both AMDROUT1 and 2 showed superiority over c1F2s of respective populations. Thus, a combination of phenotypic with genotypic selection gives higher gains compared to phenotypic-only selection.

Tangible outputs delivered

- AMDROUT1c3, AMDROUT2c3
- AMDROUT1c2, AMDROUT2c2, AMDROUT(5x6)c2
- AMDROUT1c1F2, AMDROUT1c2F2, AMDROUT2c1F2, AMDROUT2c2F2, AMDROUT(5x6)c1F2.
- S1 bulks derived from AMDROUT1c2, AMDROUT2c2, AMDROUT1c1F2

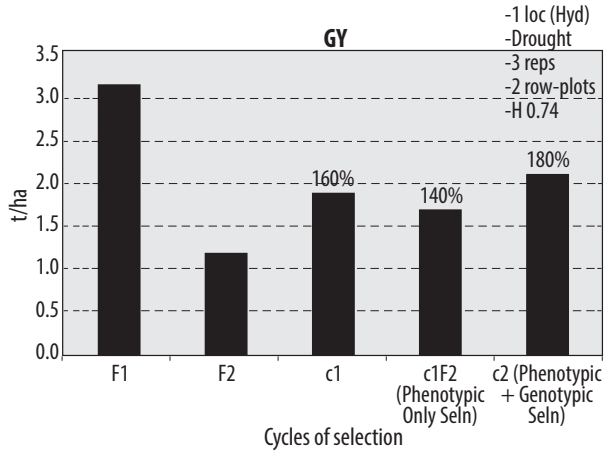


Fig 1: Comparison of genetic gains of Population AMDROUT1 for phenotypic-only vs phenotypic+genotypic selection

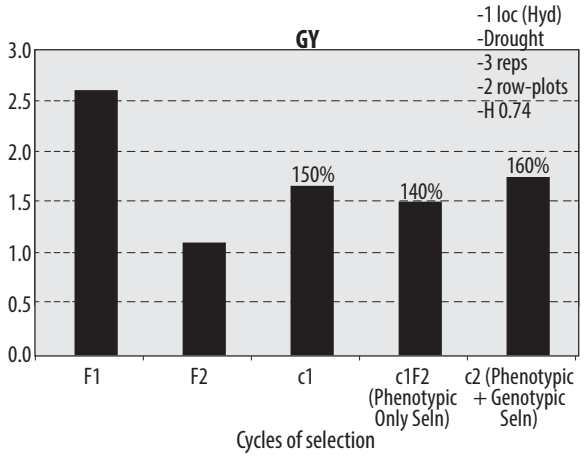


Fig 2: Comparison of genetic gains of Population AMDROUT2 for phenotypic-only vs phenotypic+genotypic selection

Rice

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

- *November 2008–October 2011; NCE: April 2013*

Principal Investigator and lead institute
Kenneth L. McNally, International Rice Research Institute; K.McNally@cgiar.org
DAPO Box 7777, Metro Manila, Philippines; +63 (2) 856-6133; Fax: +63 (2) 580-5699

Collaborating institutes and scientists

- IRRI, Philippines: A. Kumar, A. Henry
- AfricaRice, Nigeria: S. Mande
- Tamil Nadu Agricultural Univ., India: R. Chandra Babu
- Barwale Foundation, Hyderabad, India: D. Joshi, V. Shenoy
- Univ. Aberdeen, UK: A. Price
- Charles Sturt Univ., Australia: L.J. Wade
- Nagoya Univ., Japan: A. Yamauchi
- CRURRS, Hazaribag, India: M. Variar, N. Mandal, S. Anantha
- IGKV, Raipur, India: S.B. Verulkar
- NDUAT, Faizabad, India: J. Dwivedi
- UAS, Bangalore, India: H.E. Shashidhar, S. Hittalmani
- CRRI, Cuttack, India: O.N. Singh, P. Swain

Although there is a strong case for the role of roots in plant performance under drought stress, appropriate methods for evaluating them in relation to performance under drought (particularly in rice) are less clear-cut. There is also a strong need for advances in phenotyping to match the rapid progress in genotyping of rice. A better understanding of the genetic variation in rice for drought response and root traits - as well as practical methods for studying them - is needed by the research community. This project focused on rice root phenotyping of genetic diversity in response to drought.

Outputs from the rice root phenotyping network
The major aspect of this project was to conduct root phenotyping on the OryzaSNP panel, which is a set of 20 diverse genotypes that has been mapped for over 170,000 SNP markers. This phenotyping has been achieved through root studies from eight different sites or screening methods conducted in Australia, India, Japan, Nigeria, the UK, and the Philippines. The data from the OryzaSNP root phenotyping work is available on the public database IRIS and is now undergoing collective analysis for GxE effects and phenotype/genotype association studies. Genotypes from the aus subgroup have stood out for deep root growth and yield under drought (Henry et al., 2011; Gowda et al., 2012). In continuation with the efforts to generate phenotyping data for association studies, root/drought phenotyping of the aus panel (250 genotypes) that will subsequently be used for association mapping has been completed in three field seasons (drought and well-watered treatments), one greenhouse lysimeter study, and is ongoing using the herbicide placement at depth method. Preliminary genotyping data is now available for use in genome wide association. This and additional work with NILs and CSSLs (Kano-Nakata et al, 2013; Swamy et a. 2013) is helping us identify genomic regions associated with drought response and root traits.

A root methods manual was published that describes all of the screening protocols that have been optimized for rice drought studies as part of this project. The focus of this manual is the description of relatively high-throughput, low-cost, and precise root phenotyping techniques that have been developed for drought studies on rice. Field phenotyping protocols for root studies and a range of root phenotyping container systems are described.

Activities characterizing breeding lines in target drought-prone environments
Another large component of this project was the evaluation of 10 advanced breeding lines at partner sites in the India Drought Breeding Network (CRURRS Hazaribag, IGKV Raipur, NDUAT Faizabad, and CRRI Cuttack). This evaluation included capacity building and supplying of equipment for root sampling and scanning at two sites (Hazaribag and Raipur) and additional measurements (such as canopy temperature) were conducted in India and at IRRI to contribute to the physiological characterization of these breeding lines. Interestingly, drought-tolerant variety Sabahghi dhan showed multiple traits that may confer its high yield under drought, but several other genotypes stood out as promising since they performed even better than Sabahghi dhan.

This project was conducted with the viewpoint that characterizing rice root responses to drought can have a major contribution to improvement of rice productivity under drought stress. Two PhD and 2 MS students from India, the Philippines, and Tanzania (Kijoji et al., 2012) were fully supported by this project, and several others from Iraq, Japan, and Sri Lanka were also trained by contributing to this research. In addition to the root phenotype/genotype data generated by this project, we hope that our work will help empower these students and other rice researchers in partner countries to screen

for root traits in local germplasm, and to realize the huge genetic potential of rice for root traits that can be effective for drought resistance.

References

Gowda VRP, Henry A, Vadez V, Shashidhar HE, Serraj R. 2012. Water uptake dynamics under progressive drought stress in OryzaSNP panel rice accessions. Funct. Plant Biol. Functional Plant Biology 39: 402-411.

Henry A, Gowda VRP, Torres R, McNally K, Serraj R. 2011. Genetic variation in root architecture and drought response in *Oryza sativa*: rainfed lowland field studies of the Oryza SNP panel. Field Crops Res. 120:205-214.

Kano-Nakata M, Inukai Y, Wade LJ, Siopongco JDLC, Yamauchi A. 2011. Root development, water uptake, and shoot dry matter production under water deficit conditions in two CSSLs of rice: functional roles of root plasticity. Plant Prod. Sci. 14:307-317.

Kijoji AA, Nchimbi-Msolla S, Kanyeka ZL, Klassen SP, Serraj R, Henry A. 2012. Water extraction and root traits in *Oryza sativa* x *Oryza glaberrima* introgression lines under different soil moisture regimes. Functional Plant Biology 40: 54-66.

Swamy B. P. M, Ahmed HU, Henry A, Mauleon R, Dixit S, et al. (2013) Genetic, Physiological, and Gene Expression Analyses Reveal That Multiple QTL Enhance Yield of Rice Mega-Variety IR64 under Drought. PLoS ONE 8(5): e62795. doi:10.1371/journal.pone.0062795

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

- *November 2008–October 2011; NCE: September 2013*

Principal Investigator and lead institute

Boonrat Jongdee, The Bureau of Rice Research and Development: boonrat@brrd.in.th; boonrat@brrd.mail.go.th
Ubon Ratchathani Rice Research Center, Ubon Ratchathani 34000, Thailand,
Tel: 0066-45-344-103; Fax: 0066-45-344-090

Collaborating institutes and scientists

- Bureau of Rice Research and Development (BRRD), Rice Department, Thailand: Grienggrai Pantuwan
- The University of Queensland, Australia: Shu Fukai
- National Agricultural and Forestry Research Institute, Laos:
- Phoumi Inthapanya
- Cambodia Agricultural Research and Development Institute, Cambodia:
- Ouk Makara

The rainfed lowland rice ecosystem is the major food production system in the Mekong region, covering North and Northeast Thailand, Laos and Cambodia. In this ecosystem, drought is considered to be the main constraint for rice production, and development of drought resistant varieties will stabilize yield. The current drought screening method utilizes a late planting to impose a prolonged terminal drought in wet season. There are 3 main growing conditions that rice plants experience in drought-prone rainfed lowlands; 1) flooded condition where soil is anaerobic, 2) aerobic soil conditions after disappearance of standing water before drought develops which is often repeated resulting in intermittent drought, and 3) terminal drought conditions where rice plants are severely effected by lack of water . The second condition can be important particularly where frequent rainfall events prevent development of severe terminal drought conditions, and thus the project tested the importance of this condition for selection of widely adapted varieties.

To assess the usefulness of intermittent drought screening method the relative performance of genotypes was also tested under traditional flooded and terminal drought conditions for two years in both the wet and dry seasons (water condition experiments). The same genotypes were also tested in 5-7 multi-location trials under farmers field conditions in two years. These sets of experiments were conducted in Thailand, Cambodia and Laos. We show here the results from Laos where 25 common genotypes were tested in a total of 10 water condition experiments on research stations and 12 multi-location on-farm experiments.

Mean yield reduction in intermittent and terminal drought was 19 and 37% respectively in wet season and 43 and 64% respectively in dry season, indicating that the proposed intermittent drought screening in dry season, and the current terminal drought screening in wet season would provide the magnitude of drought severity that would be appropriate for screening. Genotypes shown to be drought tolerant, based on the drought response index (DRI), were consistent in intermittent and terminal drought screening. There was no positive relationship between potential grain yield obtained under flooded conditions and DRI, indicating that some drought tolerant lines can have high potential yield, thus adaptation to a wide range of rainfed lowland conditions are expected. In low yielding environmen**ts**, genotypes that had high DRI produced higher yield. The study did not show strong evidence for the use of delay in flowering because rainfall events occurred during the screening cycle.

Grain yield from on-farm multi-location experiments was highly correlated with that of flood and aerobic conditions but not with terminal drought conditions. The genotypic variation in grain yield in multi-location trials was significantly correlated with variation in DRI. Thus, on-farm genotypic variation can be explained by the variations in potential yield and drought tolerance. Drought tolerance can be identified from intermittent and mild drought condition or prolonged terminal drought conditions, but the intermittent drought condition can be more readily created without the need for inducing prolonged terminal drought conditions.

With the imposition of slightly more severe stress in intermittent conditions than that generated in the drought stress of the present experiment, genotypes with drought tolerance can be readily obtained from wet season screenings. This method can be used for both photoperiod sensitive and insensitive varieties, and thus promote development of widely adapted varieties for rainfed lowland rice ecosystem. The next step would be to use this method for screening a large number of genotypes. Maps identifying where early and late season drought are likely to have serious effects on rice yield have been developed and can be utilised to enhance breeding efforts for target environments in Laos, NE Thailand and Cambodia.

20. G4008.45: A Nested Association Mapping (NAM) population of rice: laying the bases for highly efficient QTL characterisation

- *August 2008–July 2010; NCE: July 2013*

Principal Investigator and lead institute

Mathias Lorieux, IRD/CIAT, Colombia –
mathias.lorieux@ird.fr, m.lorieux@cgiar.org
CIAT - AA6713 – Cali – Colombia. Tel +57 2 445 01 43

Collaborating institutes and scientists

- AfricaRice, Benin: Marie Noelle Ndjiondjop – m.ndjiondjop@cgiar.org
- CIAT, Colombia: César P. Martinez – c.p.martinez@cgiar.org
- CIAT, Colombia: Edgar Torres – e.torres@cgiar.org

Introduction/Background

Modern breeding strategies often fail to include precise genetic information. Marker-Aided Selection (MAS) strategies have proven to be more efficient than conventional selection in several cases, but still suffers of (1) lack of precision in the localization of the genes of agronomical importance (the so-called QTLs, for Quantitative Trait Loci) and (2) are often limited to the alleles available in the crossing scheme used for QTL detection, i.e., the genetic information obtained from a particular cross between two genotypes (or lines) will not be useful when working with other genotypes. We propose to develop a new genetic resource, called a Nested Association Mapping (NAM) population (Yu et al 2008), that would (1) help in linking the genomic tools available for rice, (2) give access to a much higher allelic diversity at the important QTLs than "conventional" mapping approaches do, (3) allow fine mapping of QTLs (i.e., localise them with high precision on the rice genome), thus increasing significantly the efficiency of MAS strategies, and (4) provide interesting and promising genetic materials (advanced lines) for direct introduction in breeding schemes.

Selection of parental lines and F1 production

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.

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. The study of 37 out of the 41 parents gave 53 alleles with an average of 2.5 alleles per locus.

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

Production of SSD 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 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.

We now have produced about 200 F_7 lines for 20 combinations (10 at CIAT and 10 at AfricaRice). We are now in the process of importing seeds from AfricaRice to CIAT.
Implications

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.

Future plans
We plan to genotype the 4,000 NAM lines using low-resolution whole-genome sequencing in the frame of the France Génomique-IRIGIN project. We expect to obtain a first release of the data by mid 2014.

Our wish is to distribute the NAM resource to all interested partners who can score the population for their favourite traits. The data would be centralized in a common database with the aim to make the data available to the scientific community.

References
Yu, J., Holland, J. B., Mcmullen, M. D., & Buckler, E. S. (2008). Genetic Design and Statistical Power of Nested Association Mapping in Maize. Genetics 178:539–551

21. G4011.04: Dissemination and community of practice for newly developed drought-tolerant QTLs pyramided breeding lines

- *July 2011–June 2014*

Principal Investigator and lead institute
Arvind Kumar, International Rice Research Institute, IRRI; akumar@irri.org
DAPO BOX 7777, Metro Manila, Philippines; (63) 2-580-5600; Fax: (63) 2-580-5699

Collaborating institutes and scientists
- Indira Gandhi Krishi Vishwavidyalaya, Raipur, India: S.B. Verulkar
- Central Rice Research Institute, Cuttack, India: O.N. Singh
- Central Rainfed Upland Rice Research Station, Hazaribag, India: N.P. Mandal
- Directorate of Rice Research, Hyderabad, India: T. Ram

Research activities and progress
The present project aim to evaluate a set of improved drought tolerant Vandna NILs and IR64 NILs under irrigated control and reproductive stage drought stress in India identify the promising lines for different drought-prone regions, multiply and generate large amount of seeds of promising NILs for further dissemination and genotype and phenotype marker-assisted recurrent selection (MARS) populations under irrigated control, reproductive stage drought stress, blast, and bacterial leaf blight; identify the QTLs, lines with QTLs and attempt to combine QTLs for grain yield under drought, blast tolerance and bacterial blight using IR55419-04/Sambha Mahsuri mapping population.

Findings and implications
Four Vandna NILs along with Vandna were screened under irrigated control and reproductive stage drought at Hazaribagh and Hyderabad. Vandna NILs showed yield advantage of 0.5 tha^{-1} under drought over Vandna without any yield penalty under irrigated situation. Four IR64 NILs along with IR64 were evaluated at more than sixteen locations in India under All India coordinated Rice improvement program. Combined over all the locations, two NILs IR 87707-445-B-B and IR87707-446-B-B showed yield advantage of 30 to 130% and 4% over IR64 under drought and irrigated control situation respectively.

The two parents IR55419-04 and Sambha Mahsuri involved in the development of MARS population were surveyed for parental polymorphism using 778 SNPs. 123 SNPs were polymorphic. Four hundred lines were genotyped using polymorphic SNPs and additional SSR markers in regions with no SNPs available. The population was screened for blast, BLB and for grain yield under drought stress as well as non-stress situations at IRRI. Seven hundred plants were selected under stress. One major effect QTL $qDTY_{11.1}$ was identified with an additive effect of 117 kg under drought. Epistatic interaction between two loci, one on chromosome 3 and other on chromosome 6 was detected to show increased yield under drought. For blast resistance one locus was detected on

chromosome 3 (Table 1). Plants were confirmed for the presence of donor specific alleles and first cycle of recurrent selection was carried out. From the first generation recurrent selection, many lines with 1.0 tha^{-1} or more yield advantage over Sambha Mahsuri under drought and phenotypically similar to Sambha Mahsuri were identified (Table 2). Progenies obtained from the first cycle were genotyped and second cycle of recurrent selection carried out. Currently genotyping is being carried out to select introgression lines with QTLs fixed and having grain quality similar to Sambha Mahsuri and having high grain weight.

Next Step

Efforts will be made to get released through national / state the identified promising Vandna Nils as well as IR64 NILs and disseminate them to large number of farmers through large scale seed production. For the MARS lines, the promising lines identified in recurrent cycle one will be evaluated at two locations in India in 2014DS and the lines selected after the second cycle of recurrent selection will be advanced in 2013WS and evaluated under drought and irrigated control in 2014DS season at IRRI.

Table 1 QTLs for grain yield under drought and blast identified

Chromosome	Interval	Additive Effect	Trait	Donor
11	Kid2746 – Kid 287	117	Yield -drought	IR55419-04
3 and 6	Kid3806 –RM520			
	Kid1613-Kid3434	350	Yield -drought	IR55419-04
3	RM16-RM520	-	Blast	IR55419-04

Table 2: Promising lines identified to show yield advantage over Sambha Mahsuri under drought

ENTRY	DTF (days) Non-stress	HT (cm) Non-stress	DTF (days) Drought	HT (cm) drought	Grain yield kgha-1 Drought
IR 99742:1-39-4-63	82	90	77	66	2063
IR 99742:2-23-7-17	79	79	88	72	1979
IR 99742:1-33-3-229	96	93	85	67	1814
IR 99742:1-14-2-87	76	78	82	78	1753
IR 99742:1-39-4-147	84	78	82	69	1649
IR 99742:1-14-2-53	79	88	82	72	1582
IR 99742:1-33-3-228	90	80	83	70	1559
IR 99742:2-23-7-135	80	72	83	62	1447
IR 99742:1-61-6-10	81	84	83	70	1368
IR 99742:1-39-4-83	80	91	84	67	1352
IR 99742:1-33-3-158	79	86	84	72	1340
IR 99742:1-33-3-69	78	79	86	77	1299
IR 99742:1-33-3-317	79	82	82	71	1288
IR 99742:1-33-3-304	81	82	83	79	1279
IR 99742:1-14-2-124	82	86	84	72	1270
IR 99742:1-33-3-152	81	75	88	69	1228
IR 99742:1-39-4-47	79	83	85	64	1209
IR 99742:1-33-3-328	78	74	86	75	1167
IR 99742:1-33-3-28	79	79	83	74	1066
Sambha Mahsuri	84	88			0
$LSD_{0.05}$	NA	NA	4	7	969

22. G4011.06: Field phenotyping for drought resistance of the MARS population developed under the GCP Rice RI

- *November 2011–October 2012; NCE: October 2013*

Principal Investigator and lead institute

Cécile Grenier, CIRAD, UMR AGAP, F-34398 Montpellier, France; CIAT, Cali, Colombia; (57) 2-445-0000 c.grenier@cgiar.org; cecile.grenier@cirad.fr

Collaborating institutes and scientists

- CIAT: Manabu Ishitani, Michael Selvaraj, Santiago Jaramillo
- CIRAD: Alain Audebert

1. Research activities and progress at CIAT

The MARS population composed of 230 lines derived from the cross IR64 x B6144-F-MR-6-0-0 was evaluated under upland rainfed condition at the CIAT experimental station in Santa Rosa (Meta) Colombia. Drought was imposed in the stress plot by withholding irrigation and applying the stress for two weeks after panicle primordial stage was reached (50 days after sowing). In the control experiment irrigation was supposed to be applied during the whole duration of the crop cycle.

Various agronomic traits were scored and average plant yield per plant derived from the plot yield divided by number of plants in the plot. Transpiration was estimated with canopy temperature. Measurements were performed after three weeks of stress using a thermographic camera, only on the stress treatment. Environmental conditions were monitored to measure soil moisture as well as climatic conditions during the course of the experiment.

2. Research activities at CIRAD

Thermographic infra red pictures taken at the CIAT research station were then studied at CIRAD. The digital data was analyzed to calculate the ratio between canopy temperature (Tc) and atmospheric temperature (Ta), the two being simultaneously recoded in

the field. To normalize canopy temperatures against micro-meteorological weather fluctuations, results were improved by using CWSI (Crop Water Stress Index) obtained with the vapor pressure deficit and air temperature.

During the process of image and data analysis a student from Africa Rice will follow training in CIRAD on new technologies for drought phenotyping; their concept and use for plant breeding.

3. Outputs delivered

A large variation for response to drought was recorded among the entries, but above all, a severe sterility syndrom seems to have affected both treatments (Figure 1).

All the thermographic pictures taken on the drought treatment were analyzed and revealed a great variability among entries. The histograms for transpiration (Tc-Ta) and CWSI showed high variability (Figure 2), where tolerant material has cooler canopy temperature and thus a low CWSI and negative Tc-Ta.

4. Comments

The drought season started approximatedly one month after the sowing date. From Januray 10th the irrigation was suspended in one treatment for a period of three weeks. This year suffered from an intense drought period which lasted well above a month, and the scarity of water stored in the reservoir did not permit irrigation to be provided to the experiment according to the plans. This may have caused a stronger stress in the drought treatment and even a certain pressure in the controlled treatment which visibly suffered stress with symptom of sterility. Natural rainfall pattern returned by mid February but the impact of several weeks of drought had already done ireversible damages in many lines. These limitations do not allow us to consider the control treatment as a condition to measure the crop yield potential.

Further analysis combining soil and atmospheric humidity should allow better understanding of the genotype response to the drought stress. Although this trial will not give a true evaluation of potential under favourable condition and tolerance under drought condition, it should represent a screen to identify lines with tolerance to extremely severe drought conditions.

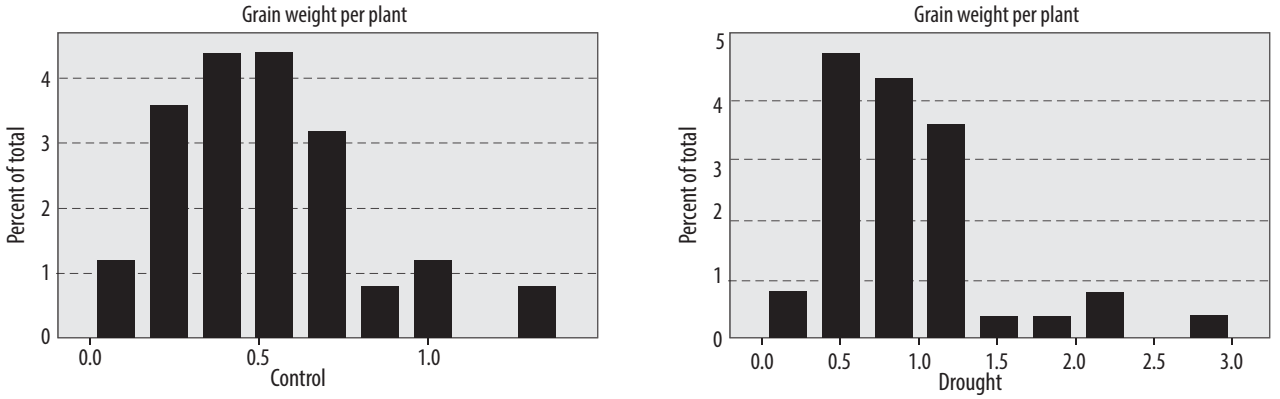


Figure 1: Distribution (%) of lines for various ranges of grain weight per plant, under control and drought conditions. Only entries where grain could be harvested are plotted.

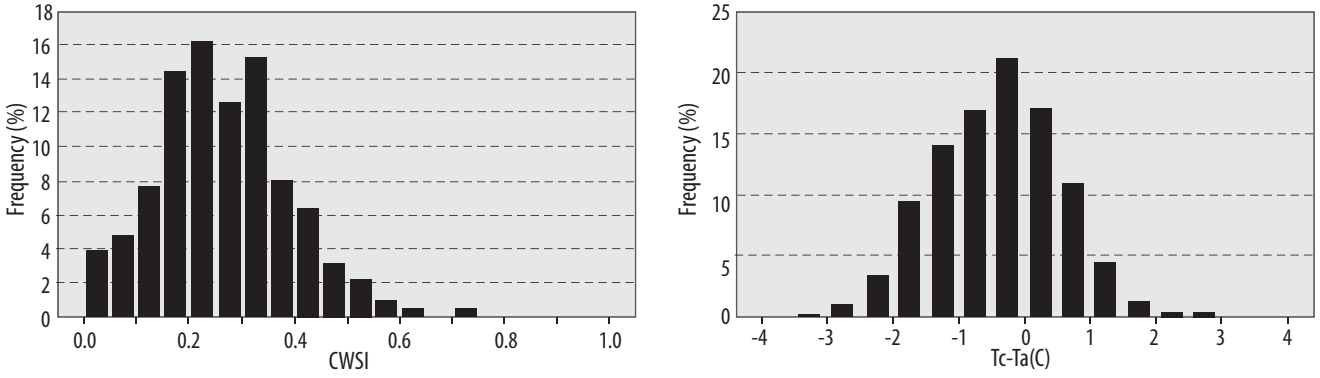


Figure 2: CWSI and (Tc-Ta) histogram for 250 lines phenotyped under drought condition.

5. References

Audebert A, Chatel M, Grenier C, Ospina Y, Rodriguez F, 2009. Breeding for water use efficient rice: toward large scale phenotyping under field conditions for Marker Assisted Recurrent Selection (MARS). Poster presented during the InterDrought III Meeting: Shanghai-China 11 – 16 October 2009

Kumar R, Venuprasad R, Atlin GN, 2007. Genetic analysis of rainfed lowland rice drought tolerance under naturally-occurring stress in eastern India: Heritability and QTL effects. *Field Crops Research*, 103:42-52

Stam P, 1995. Marker-assisted breeding: *In*. JW Van Ooijen and J Jansen (ed) Biometrics in plant breeding: Application of molecular markers. Proceedings of the ninth meeting of the EUCARPIA section Biometrics in plant breeding. CPRO-DLO, Wageningen, Netherlands, pp. 32-44

23. G4011.07: Project Name: Rice Multi-parent Advanced Generation Inter-Crosses (MAGIC) Phase II

- *November 2011–October 2013*

Principal Investigator and lead institute

Hei Leung, International Rice Research Institute, Los Banos, Laguna, Philippines

Collaborating institutes and scientists

- IRRI: Glenn Gregorio, Ed Redona, RK Singh, Chitra Raghavan, Nonoy Bandillo, Ramil Mauleon, Mike Thomson
- IRRI – East South Africa: Rosemary Murori
- CSIRO, Australia: Emma Huang
- Cornell University: Susan McCouch
- National Taiwan University, Taiwan: Chih Wei Tung

Context

MAGIC *(***M**ulti-parent **A**dvanced **G**eneration **I**nter-**C**rosses*)* is an experimental method that increases the precision with which genetic markers are linked to quantitative trait loci (QTL). MAGIC populations have uses beyond trait mapping. The highly recombined germplasm can be used directly as source materials for the extraction and development of breeding lines and varieties adapted to different environments. The increased recombination in MAGIC populations can lead to novel rearrangements of alleles and greater genetic diversity. Thus, MAGIC populations provide an underpinning genetic resource that can facilitate the discovery, identification and manipulation of new forms of allelic variability.

We have developed 4 multi-parent populations: *indica* MAGIC (8 *indica* parents); MAGIC plus (8 *indica* parents with two additional rounds of 8-way F1 inter-crossing); *japonica* MAGIC (8 *japonica* parents); and Global MAGIC (16 parents – 8 *indica* and 8 *japonica*). All the 16 parents are improved varieties with desirable traits for biotic and abiotic stress tolerance, yield, and grain quality. The *indica* MAGIC population is the most advanced of the MAGIC populations developed thus far and comprises 1328 lines produced by single seed descent (SSD). The purpose is to fine map QTLs for multiple traits and to directly and indirectly use the highly recombined lines in breeding programs.

Findings and Implications

Products: The MAGIC populations have served as a source of new germplasm for breeders. In each growing season, breeders have selected lines from different populations for their respective breeding objectives. As an illustration, selection for early maturing lines with good phenotypic acceptability were made in the last 2013 DS. These included: a) MAGIC Global - 280 breeding lines with 5 panicles per line, b) *Indica* MAGIC S6:8 - 80 bulked lines and c) MAGIC Plus - 120 breeding lines *via* panicle to row selection. A total of 480 lines were selected.

New results and concepts*:*

- Pilot experiments of applying genotyping-by-sequencing (GBS) and phenotyping to 200 lines demonstrated that the dataset can be used for Genome-wide association GWA mapping. At the S4 stage of SSD a subset (200 lines) of this population was genotyped using the GBS approach and was phenotyped for multiple traits, including: blast and bacterial blight resistance, salinity and submergence tolerance, and grain quality. After filtering GBS data, approximately 17,000 SNP markers were used for GWAS.
- Genome-wide association mapping identified several known major genes and QTLs including *Sub1* associated with submergence tolerance and *Xa4* and *xa5* associated with resistance to bacterial blight. Moreover, the genome-wide association study (GWAS) results also identified potentially novel loci associated with essential traits for rice improvement. Table 1 shows the approximate intervals defined by the SNP significantly associated with the trait. We expect resolution to increase with higher SNP density through better sequencing technologies. For details of GWAS results, see **Bandillo et al 2013**.
- Recently we also obtained GBS data on a set 190 MAGIC PLUS lines also at the S4 stage (SSD). Preliminary GWAS for bacterial blight, brown spot

and salt tolerance confirm the associations detected in the *indica* MAGIC population. We are currently using the mpMAP package for interval mapping to determine the ability to fine map (Huang et al. 2011).

GBS data from, recombination fractions and linkage disequilibrium (LD) for *indica* MAGIC and MAGIC plus populations were compared. Results showed that two extra rounds of recombination in MAGIC Plus (5 meioses) *vs* indica MAGIC (3 meioses) yielded smaller LD, as expected from increased recombination. This will potentially increase the mapping resolution for multiple traits.

Next steps

- Provide MAGIC lines to breeding programs
- Genotype and phenotype 1,200 indica MAGIC lines (S8 generation)
- Determine recombination breakpoints in the genomes, and whether there are hot spots (more recombination).
- Advance remaining populations (*japonica* MAGIC, Global MAGIC)
- Apply genotyping and phenotyping to a set of Global MAGIC to determine representation of genomes of the 16 founder parents.
- Apply GWAS to identify complex traits, such as yield, drought tolerance, quantitative disease resistance and other quantitative traits.

References

Bandillo N, Raghavan C, Muyco PA, Sevilla MA, Lobina IT, Dilla-Ermit CJ, Tung CW, McCouch S, Thomson M, Mauleon R, Singh RK, Gregorio G, Redona E, Leung H. 2013. Multi-parent Advanced Generation Inter-Cross (MAGIC) populations in rice: progress and potential for genetics research and breeding. Rice 6:11 doi:10.1186/1939-8433-6-11

Huang BE, George AW (2011) R/mpMap: a computational platform for the genetic analysis of multiparent recombinant inbred lines. Bioinformatics 27:727-9.

24. G7010.04.01: Improving rice productivity in lowland ecosystems of Burkina Faso, Mali and Nigeria through marker-assisted recurrent selection (MARS) for drought tolerance and yield potential

- *May 2010–April 2014*

Principal Investigator and lead institute

Marie-Noëlle Ndjiondjop–AfricaRice Center (AfricaRice), 01 BP 2031 Cotonou, Benin
m.ndjiondjop@cgiar.org; Tel: +229 21 35 01 88; Fax: +229 21 35 05 56

Collaboratoring institutes and scientists

- Africa Rice Center, Benin: Sow Mounirou, Ramaiah Venuprasad, Ibnou Dieng, Koichi Futakuchi, Sander Zwart
- Institut de l'environnement et des recherches agricoles, Burkina Faso: Drissa Hema
- Institut d'économie rurale, Mali: Fousseyni Cisse
- National Cereals Research Institute, Nigeria: Alhassan Maji
- International Rice Research Institute, Philippines: Arvind Kumar, Amelia Henry
- Institut de recherche pour le développement, Montpellier, France: Alain Ghesquière
- Centre de coopération internationale en recherche agronomique pour le développement, Montpellier, France: Tangui Lafarge, Audebert Alain and Nour Ahmadi
- International Center of Tropical Agriculture (CIAT), Cali-Palmira, Colombia: Cecile Grenier

Work packages (WPs) of Project G7010.04.01:

- WP 1: Characterisation of the IV-TPE, establishment of drought evaluation sites and description of ideotypes fitting major sub-classes of TPE
- WP 2: Phenotyping for yield potential and drought tolerance

Table 1. SNP markers associated with traits and the approximate sizes of interval defined by the markers.

Trait	Locus	Chrom	No. of significant makers associated with trait	p-value cut-off	Physical distance between the end markers
Submergence tolerance	Sub1	9	16	5.43E-08	0.6 Mb
Amylose content	waxy	6	14	7.48E-09	0.58 Mb
Grain length	GS3	3	25	8.17E-05	6.7 Mb
Grain width	GW8	8	4	4.20E-05	0.8 Mb
Gelatinization temperature	SSIIa	6	19	6.07E-15	1.8 Mb
Salt tolerance	qSKC1	1	20	9.18E-04	3.0 Mb
Resistance to BLB strain PX086	xa5	5	3	7.21E-08	0.13 Mb
Resistance to BLB strain PX061	Xa4	11	8	9.62E-08	1.7 Mb

- WP 3: Developing improved lines combining favourable QTL alleles for drought adaptation and productivity for target environments in Burkina Faso, Mali and Nigeria
- WP 4: Rice drought molecular biology and breeding community of practice for West Africa
- WP 5: Project and information management

Context
The project focuses on the rainfed lowland ecosystems in Burkina Faso, Mali and Nigeria. Within four years it is expected to establish (i) the drought profiles of the target population of environments (TPE) in inland valley lowlands; (ii) the identification of traits of interest for targeted environments, using novel phenotyping methodologies enabling an efficient separation of genetic (G) and environmental (E) effects; and (iii) the integration of the information on drought profiles with novel phenotyping methodologies in a marker-assisted recurrent selection (MARS) scheme to develop better-adapted germplasm for each major target environment.

Major research activities and results (product and impact)

Work Package 1. Characterisation of the Inland Valley (IV)-TPE and establishment of drought evaluation sites and designs, and description of ideotypes fitting major sub-classes of TPE.

Principal Investigator and lead institute
Sander Zwart, AfricaRice Center (AfricaRice), S.Zwart@cgiar.org

Activity 1: Identification of the extent of inland valley lowlands in the target Countries
Digital elevation models have been downloaded and mosaics have been created. Field validation has been performed and algorithms are developed and started to perform mapping across the three target areas/states.

Activity 2: Characterization of inland valleys through farmer surveys
Approximately 200 surveys have been conducted in Mali and two states in Nigeria, whereas in Burkina Faso the work has been delayed but expected to commence in June-July 2013. The conducted surveys are currently being digitized in a data base system. Data quality will be checked and the data base will be cleaned up where necessary. When data for Burkina Faso become available it will be added.

A work meeting is planned for October to work on the analysis of the surveys.

Activity 3: Identification and characterization of drought evaluation sites
Finished

Activity 4: Adaptation of SARRAH model to the project objectives
Finished

Activity 5: Validation of crop simulation modeling tools
The TPE activity started in 2010 with the design and implementation of an on-station experiment to generate calibration/validation data for crop modeling. Drought was induced in two of the seasons to assure valid calibration inputs for water-limited rice yields. Early 2013 the experiment ended and the last measurements are being analyzed and the quality of the data base is being checked. The ORYZA2000 model will be used for the TPE characterization.

Activity 6: Characterization of drought profile of the TPE and definition of rice ideotypes using modeling and GIS
The input data for the spatial data analysis in GIS are being prepared. These include remote sensing maps of precipitation and evapotranspiration. The maps are being validated using ground measurements from meteorological stations. These stations data have been ordered, downloaded and imported in a MS Access database. Finally, a Digital Elevation Model (DEM) for the three target countries have also been downloaded and imported. The DEMs will be used to delineate the boundaries of the sub-catchments that will be the smallest unit of analysis.
The AfricaRice crop modeler received training in the SAMARA model developed by CIRAD. Potential rice-yields under water-limited (drought) conditions will be modeled for at least 10 stations in each country depending on data availability. A data base with meteorological data from numerous stations in the three target countries is being prepared and will be available within 3 weeks.

Work Package 2. Phenotyping the MARS populations for yield potential and drought tolerance, as well as promising lines in various environments.

Principal Investigator and lead institute
Koichi Futakuchi, AfricaRice Center (AfricaRice), k.futakuchi@cgiar.org

The project uses two phenotyping methods. Firstly, yield and yield components are evaluated under two water regime conditions (irrigated and stressed) and, secondly, during the drought period, the plant transpiration capacity is estimated through measurement of the canopy temperature.

Activity 1: Development of facilities for field evaluation of yield potential and drought tolerance
In order to sustain molecular breeding projects with focus on drought, infrastructures were developed in AfricaRice and NARS countries. A rainout shelter was constructed at AfricaRice for controlled-drought evaluation. This rainout shelter is being used to screen the MARS populations. Meanwhile, field drought evaluation facilities (irrigation systems, soil moisture content monitoring equipment such as diviner, and weather station) were installed in Burkina Faso, Mali and Nigeria to support our partners' phenotyping efforts. Besides contribution to a more accurate phenotyping of the MARS populations, these facilities are also being used to train young research on drought research, in order to strengthen West African drought screening capacities.

Activity 2: Facility for evaluation of yield potential and drought tolerance related to secondary traits under controlled conditions (rain-out shelter)
In order to sustain molecular breeding projects with focus on drought, infrastructures were developed in AfricaRice. A rainout shelter was constructed for controlled-drought evaluation. This rainout shelter is being used to screen the MARS populations.

Activity 3: Field evaluation for yield potential and general adaptability
The general adaptability trials were already completed, and the first MARS population of 230 $F_{3:5}$ lines derived from a cross between IR64 and B6144F and 10 check varieties, have undergone 12 field evaluations, for yield potential under favorable conditions (2 years) during the wet season, in four locations (Banfora-Burkina Faso; Longorola-Mali; Ibadan and Badeggi-Nigeria) following a topo-sequence (Valley fringe and valley bottom). A wide level of variability for target traits, irrespective of the ecology, was observed among the progenies in all locations. The population average yield was 5.06 $t.ha^{-1}$ in Banfora, 4.19 $t.ha^{-1}$ in Badeggi, and 4.08 $t.ha^{-1}$ in Ibadan. Three progenies out-yielded all the checks in Badeggi, and several yielded more than the two parents. The second population of 230 $F_{3:5}$ lines derived from a cross between IR64 and ITA212 and 10 check varieties will be evaluated in the same locations this coming raining season.

Activity 4: Field evaluation of yield under drought
The first MARS population of 230 $F_{3:5}$ lines derived from a cross between IR64 and B6144F and 10 check varieties, have undergone 8 field evaluations, for yield under cyclical drought during the dry season (one year) in five locations (Banfora-Burkina Faso; Longorola-Mali; Ibadan and Badeggi-Nigeria Los Banos-Philippines and, Santa Rosa-Colombia). A wide level of variability for target traits, irrespective of the water regime, was observed among the progenies in all locations. Yield reduction due to drought averaged respectively 72%, 33% and 47%, in Ibadan, Longorola and Banfora (Figure 1). Stability estimated from environmental variance revealed that some lines (16 in Ibadan, 24 in Burkina, 9 in Mali) had more stable yield than the best check. The second population of 230 $F_{3:5}$ lines derived from a cross between IR64 and ITA212 and 10 check varieties are currently under evaluation for yield under drought in four locations, along with 44 best entries from previous experiments.

Activity 5: Methodological pre-studies for indirect (secondary traits) phenotyping for drought tolerance
A bi-parental *indica* population of 230 $F_{3:5}$ lines derived from a cross between IR64 and B6144F, and 10 control varieties, were phenotyped under drought condition at the Villavicencio CIAT experimental station (Colombia) during the dry season 2012/2013. Irrigation was suspended for two weeks at reproductive stage (45-60 days after sowing). Drought response was evaluated based on canopy temperature obtained with a numerical infra-red thermographic camera. The methodology was improved by introducing the crop water stress index (CWSI) calculated with climatic data collected from in situ weather station to normalize canopy temperatures against micro-meteorological weather fluctuations. Leaf canopy temperature at reproductive stage exhibited strong and significant genotypic differences that were negatively correlated with soil moisture content. This

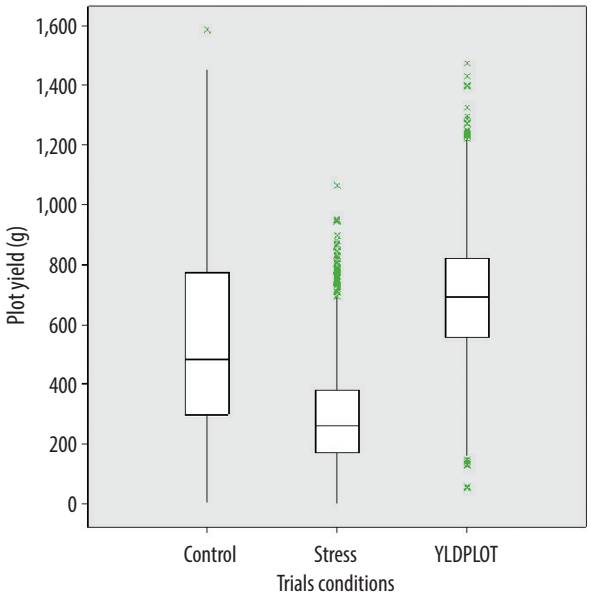


Figure 1. Plot yield of the first MARS population (IR64 x B6144F-MR-6-0-0) determined from 13 experiments data related to yield potential under favorable conditions and drought stress. Trial conditions (Stress= cyclical drought from 45 days after sowing; Control= well watered repetitions during dry seasons; YLDPOT = yield potential trials during the wet seasons).

phenotyping approach allowed identifying genotypes with good maintenance of transpiration capacity (and thus sustained growth) under drought stress; this ability can possibly be related to two main causes; either a lesser volume of water necessary for plant functioning (thus reducing the need for water extraction from the soil) and/or a greater root system depth, rendering available a larger amount of water. Independently the two variables (canopy temperature during stress and yield) showed a wide diversity: we observed that canopy temperature during the drought period displayed values differing by more than 5 °C between sensitive and tolerant lines. Similarly, yield values differed strongly between genotypes. A negative correlation was observed between canopy temperature and yield, thus highlighting the importance of water availability during the reproductive period. This phenotyping approach permitted identifying genotypes able to sustain adequate growth under drought stress. The integration of phenotypic data obtained through the high throughput phenotyping with molecular data (generated by AfricaRice with 484 single nucleotide polymorphism (SNP) markers) will constitutes a significant advance for the identification of genomic regions associated to this trait. If these results are confirmed, they will allow developing efficient marker-assisted recurrent selection (MARS) approaches for breeding rice for drought resistance.

Activity 6: Phenotyping under controlled drought (rain-out shelter, AfricaRice, Cotonou)

The population was evaluated for drought tolerance under controlled-environment in a rainout shelter (ROS). A wide level of variability for target traits, irrespective of the water regime, was observed among the progenies. The population average yield was 3.9 $t.ha^{-1}$ under the ROS. Yield reduction due to drought averaged 72.6% under the ROS. Stability estimated from environmental variance revealed that 5 lines under ROS had more stable yield than the best check. The set of 44 best entries from previous experiments are also under evaluation for yield under drought in the ROS and soon the second MARS population will also be evaluated.

Activity 7: Conventional breeding of MARS population and MARS end-products

To document the advantage or gain of MARS approach over conventional breeding, a parallel conventional breeding program using the same population evaluated using MARS, is being carried out at AfricaRice, Ibadan. From a panicle-row nursery comprising 1172 $F_{3:6}$ entries (IR64 x B6144-MR-6-0-0 cross) established during the 2011 wet season, 167 lines ($F_{3:7}$) were evaluated in replicated trials under two conditions (stress and control in the 2012 dry season. Due to repeated rains, the stress level was low. The mean yields of stress and control trials were 3.6 and 4.0 t ha-1, respectively. Combined analysis over stress and control trials was performed to select good yielding lines. The mean yield of entries ranged between 2.2 and 5.4 t ha-1. Based on the combined analysis, the best 100 lines (yielding > 3.7 t ha-1) were chosen and evaluated under rainfed conditions in the wet season. They are being evaluated under drought conditions this year.

Work Package 3. Developing improved lines combining favorable QTL alleles for drought tolerance and productivity for target environments in Burkina Faso, Mali and Nigeria

Principal Investigator and lead institute
Marie-Noëlle Ndjiondjop–AfricaRice Center (AfricaRice), m.ndjiondjop@cgiar.org

Activity 1: Development of populations of F3:5 segregating lines from bi-parental crosses

One thousand eighty hundred eighty two F_3 lines as well as progenies at F_1 and F_2 generations were firstly developed from bi-parental cross, involving four cross combinations ARC1 (IR64 x BW348-1), ARC2 (IR64 x B6144F-MR-6-0-0), ARC3 (IR64 x ITA212) and ARC4 (from IR64 x WAB 638-1). These F_3 progenies have undergone evaluation in NARS partners' fields for general adaptability. Two new populations ARC5 (IR64xIRAT104) and ARC6 (IR64xKogoni 91-1), respectively at $F_{3:4}$ generations were added. The six populations were multiplied for seed and generation increase ($F_{3:5}$) during the wet season at AfricaRice Ibadan. In addition seeds were back up at AfricaRice-Cotonou for safety. During a monitoring tour, the breeders decided to choose the ARC2=IR64 x B6144F-MR-6-0-0 (refer below as first MARS population) and ARC3=IR64 x ITA212 (refer below as second MARS population), populations for phenotyping and further QTLs detection because they were well adapted to their environments showed wide variability in term of morphological traits, whereas the flowering dates between lines were below 15 days. The $F_{3:5}$ seeds of the first population were shipped to all partners for yield potential screening in wet season and for drought tolerance in the dry season in NARS countries, IRRI, CIAT and AfricaRice. Moreover, $F_{3:5}$ seeds of the second MARS population were distributed to NARS partners and AfricaRice for phenotyping purpose.

Activity 2: QTL mapping for drought tolerance and yield potential

At the molecular level, leaf samples were collected at $F_{3:4}$ generations for the two populations. DNA were extracted and shipped to Kbioscience Lab for genotyping with 502 and 440 SNP markers respectively. Five QTLs related to yield were detected on chromosomes 1, 3, 10 and 11 in Badeggi, Banfora and Longorola (Table 1). The QTL on

Table 1. Example of 7 QTLs detected for yield, days to 50% flowering and plant height in the first MARS population (IR64 x B6144F-MR-6-0-0). The location and field conditions are provided in the first column.

Location	Environment	Trait	Chr.	Position	-log10(P)	%Expl. Var	Add.eff	High value allele	s.e.
Burkina, (also in Ibadan, IRRI, Mali, Nigeria)	Drought, (Yield potential and Drought trials)	Days to 50% flowering	3	6.63	21.408	57.57	4.223	B6144F-MR-6-0-0	0.389
Ibadan	Drought treatment	Yield	3	160.88	7.395	20.832	0.167	IR64	0.029
Ibadan	Drought treatment	Days to 50% flowering	8	34.35	3.844	9.208	1.439	B6144F-MR-6-0-0	0.372
Ibadan	Irrigated	Yield	10	83.77	3.289	7.464	0.216	B6144F-MR-6-0-0	0.061
Mali	Irrigated	Yield	11	12.74	4.658	12.774	35.181	IR64	8.103
ROS, (also in Burkina, Mali, Nigeria, IRRI)	Drought, (Yield potential and drought trials)	Yield and plant height	1	151.04	3.83	9.996	8.497	B6144F-MR-6-0-0	2.198
ROS	Irrigated	Yield	6	61.46	3.76	8.935	9.616	B6144F-MR-6-0-0	2.515

Chromosome 3 explained 36.38 and 10.83% of the yield phenotypic variation in IRRI and Badeggi, respectively. This QTL is also related to plant height and days to flowering. In Ibadan and under the ROS, two QTLs for grain yield under drought stress, explaining 20.83% and 9.99% of the phenotypic variation, were detected. These results underline the successful development of a reliable phenotyping network**.** A three-step optimization process of the MARS approach is being undertaken using OptiMAS, to combine favorable alleles of those QTLs into a single line. As a result, a simulating of the breeding value of the population using OptiMAS with 6 QTLs, revealed 0.541 and 0.026 for mean molecular score and variance respectively. Moreover, two complementary subsets of 5 lines (mean molecular score = 0.929, variance = 0.001) for the QTLs of interests were made for recombination

Work Package 4. Work package 4: Rice drought molecular biology and breeding community of practice for West Africa

Principal Investigator and lead institute

Venuprasad Ramaiah–AfricaRice Center (AfricaRice), r.venuprasad@cgiar.org

Activity 2: Information and technology exchange between and within NARS

A monitoring tour was organized from 03 to 08 June 2013 (see picture1), where all the NARS partners have visited ongoing drought experiments in Burkina Faso and Mali. Participants discussed current trials, coming activities related to remaining work packages mainly completing WP1, and next trials in WP2.

Activity 3: Group training by AfricaRice through the Molecular Breeding Platform

During the monitoring tour group training for NARS partners, on statistical analysis; QTL detection and simulation for Rice CI data was also discuss, so that each partners participate to make decisions about recombination cycles. This training will be held later this year.

Activity 4: Facilitation of linkages with related projects and existing varietal evaluation and release systems

At this level of the project, the Rice CI project is linked to:

- The AfricaRice Breeding Task Force for participatory varietal selection, as the same people (Maji for Nigeria, Hema for Burkina Faso and Cissé for Mali) are also focal points of the Rice CI in their countries.

Activity 1: Training and capacity-building: PhD and MSc students working under the Rice CI project

Name	Nationality	University	Date of selection	Thesis tittle
Mr Abraham Shaibu	Nigerian	University of Nigeria, Nsukka,	2010	Assessment of the Utility of Oryza glaberrima in Drought Tolerance Rice Breeding
Mr Jean Sangare	Malien	Unversity of Abomey Calavi	2011	Drought response of bi-parental MARS population derived from crosses between IR64xKogoni
Mr Abdourhamanne Konate	Burkina Faso	University of Abomey Calavi	2011	Identifying morphological features and physiological processes as an integrated overall strategy for tolerance to water deficit in rainfed lowland rice
Aboubacar Dairra	Malien	University of Abomey Calavi	2010	MSc courses are ongoing

Monitoring tour at IER Sikasso (Mali)

Monitoring tour at IER Sikasso (Mali)

Monitoring tour at INERA Banfora (Burkina)

Monitoring tour at INERA Banfora (Burkina)

Picture 1: Monitoring team inspecting performance of the MARS populations under drought conditions as well as the performance of the lines under conventional breeding process selected by Rice CI breeders from 2012 Yield Potential Trials

- GRiSP phenotyping task force - the Product Leader and AfricaRice's physiologist (also coordinator of controlled-drought phenotyping) participated in the GRiSP phenotyping workshop held on 28-30 March 2011 at CIRAD, Montpellier. The GRiSP R&D Product Line 2.3: Rice varieties tolerant of abiotic stresses, milestones M2.3.1.8 and 9.

Work Package 5. Project and information management

Principal Investigator and lead institute
Ibnou Dieng, AfricaRice Center (AfricaRice), i.dieng@cgiar.org

Activity 1: Data gathering and information management
The Rice CI Databases comprising molecular and phenotypic data was set up at AfricaRice (Central database) and in NARS institutes (local database). So far, nomenclature and standards have been established for F_1, F_2 and 1882 F_3 lines derived from the four cross combinations ARC1, ARC2, ARC3, and ARC4. The ARC2 and ARC3 populations were chosen by the breeders for MARS and their nomenclatures and standards were established for $F_{3:4}$, $F_{3:5}$, $F_{3:6}$. Workbooks including layouts were created for each of the 19 trials implemented in the project for the first MARS population. The data collected from these trials at partners' sites were received on excel spreadsheets and entered on the Rice CI database. The Rice CI database is being managed using the GCP IBP Platform: the IRIS system and more recently the IBWorkbench.

Activity 2: Project management
Best efforts are being made to improvement communication and transparency in the project information management. Last year monthly online meetings were done between the project coordinator, the GCP manager of the project and the Product Delivery Coordinator. In addition to that, meetings with workpackage leaders are being organized on a quarterly basis.

Rice: Capacity building activities

Community of practice

25. G4009.09: A Community for strengthening rice breeding programmes using genotyping building strategy and improving phenotyping capacity for biotic and abiotic stresses in Mekong region

November 2009–October 2012; NCE: July 2013

Principal Investigator and lead institute
Jonaliza L. Siangliw, BIOTEC; jsiangliw@gmail.com; jonaliza.sia@biotec.or.th Rice Gene Discovery Unit, Kasetsart University, Kamphangsaen, Nakhon Pathom 73140 Thailand; (66) 34-355192; Fax: (66) 34-355195

Collaborating institutes and scientists

- BIOTEC, Thailand; Theerayut Toojinda
- Department of Agricultural Research, Myanmar: Khin Soe
- National Agriculture and Forestry Research Institute, Laos: Bounthong Bouahom
- Cambodian Agricultural Research and Development Institute, Cambodia: Ouk Makara

1. Development and conversion of backcross introgression lines using MAS

Most of the activities were devoted in developing introgression lines by students and staff from Department of Agricultural Research, National Agriculture and Forestry Research Institute, Cambodian Agricultural Research and Development Institute and Ubon Ratchatani University. Most of the line conversion activities used backcrossing or selfing techniques.

Table 1. Status of line development of Mekong rice varieties included in the project

Institute	Population	Target	Status
DAR	Aromatic Manawthukha with bacterial blight resistance	BC3F3	Finished
DAR	IR53936 with salinity and submergence tolerance	BC2F4	BC2F4
DAR	Gene mapping of cooked rice elongation using Paw Sam Mhwe	F4	Finished
NAFRI	Glutinous rice with submergence tolerance and blast resistance	F4	Finished
CARDI	Aromatic CAR3 with submergence tolerance and brown planthopper resistance	F2	Finished
CARDI	Phka Rumdol with brown planthopper resistance	F2	Finished

2. Building of facilities for phenotyping capacity

Submergence ponds to validate submergence tolerance were built in DAR, NAFRI and CARDI. Greenhouses to screen bacterial leaf blight, blast and brown planthopper were built in DAR, NAFRI and CARDI, respectively.

3. Trait evaluation

The facilities built in each institute were used to screen materials (germplasm and breeding materials) in order to assess the efficiency of the facilities.

Table 2. Screening of traits introgressed into Mekong rice varieties

Institute	Trait	Population tested	Results
DAR	Submergence tolerance	200 Myanmar germplasm	7 lines had 20 or more percentage of plant survival
	Salinity tolerance	65 Myanmar germplasm	7 lines showed tolerance to salinity stress
	BLB resistance germplasm	34 Myanmar and 27 breeding lines	1 variety showed resistance to 4 Myanmar isolates
NAFRI	Submergence tolerance	30 Breeding lines and 10 TDK1 with sub-mergence tolerance	All lines showed higher level of survival than TDK1
	Blast resistance	120 breeding lines	1 susceptible; 126 moderate resistant to resistant reaction
CARDI	Submergence tolerance	46 breeding lines of aromatic CAR3 with submergence tolerance and 4 aromatic CAR3	11 breeding lines tolerant to submergence
	Bph resistance	34 IRRI lines to select donor of Bph resistance	5 lines moderately resistant

4. Workshop on trait validation

Phenotyping workshop was conducted in DAR, NAFRI and CARDI in 2011.

Institute	Trait	No. of participants
DAR	Submergence tolerance and bacterial blight resistance	15
NAFRI	Submergence tolerance and blast resistance	15
CARDI	Submergence tolerance and brown planthopper resistance	15

5. Field trials

The materials that were developed in the previous phase were evaluated for their adaptation and field performance in the station and farmer's field. Many of those materials will be released as new varieties.

Institute	Population	No. of lines	Number of locations	Remarks
DAR	Aromatic Manawthukha	1	9	To be released as new variety
	Salinity tolerant Sin Thwe Latt	1	3	To be released as new variety
NAFRI	Aromatic TDK1	3	5	To be released as new variety
	Improved IR57514	2	5	To be released as new variety
CARDI	Aromatic CAR3	1	10	Under field trial

26. 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*

Fellow and lead institute
Armin Bhuiya, International Rice Research Institute; a.bhuiya@irri.org

Collaborating institutes and scientists
Scientific supervisors are –

- International Rice Research Institute (IRRI), Abdelbagi M. Ismail. Postal address: DAPO Box 777, Metro Manila, Philippines. Email: abdelbagi.ismail@cgiar.org
- Bangladesh Agricultural University (BAU), M. Wazuddin. Postal address: Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. Email: m.wazuddingpb@yahoo.com

Rice (*Oryza sativa L.*) cultivation is the most important economic activity on earth as it is the second largest use of land for food production. Salinity is one of the most common abiotic stresses in rice growing areas worldwide. As modern rice varieties are highly sensitive to salinity, 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 (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 to 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. F_2 population from a cross between the Kutipatnai and Azucena was developed to identify QTLs associated with salinity tolerance in rice. Another mapping population was developed by a cross between Ashfoll and IR64. DNA extraction has been done from the F_2 population of Ashafoll/ IR64 for genotyping and F_2:F_3 has been used for phenoyping. Parental survey has been done among Kutipatnai, Azucena, Asfoll and IR64 with 313 SSR and Indel markers. 150 SSR and Indel Markers has been selected as polymorphic between parental lines for each population but 106 of these markers were good and subsequently used for genotyping the mapping populations of Kutipatnai / Azucena. Genotyping of 288 F_2 progenies of Kutipatnai / Azucena and phenotyping of the $F_{2:3}$ families were carried out for salinity tolerance at seedling stage. Two weeks after salinization at an EC of 12 dS m^1 ,the $F_{2:3}$ lines has been characterized for several physiological traits associated with salinity tolerance including *Standard evaluation system* (SES) score, survival%, shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, Na uptake, K uptake, Na/K ratio and leaf chlorophyll content. A genetic linkage map has been constructed with the 106 polymorphic markers which cover 1396.6 cM with an average distance of 13.18 cM between loci. A total of 14 QTLs have been identified using Composite Interval Mapping with the software QGene 4.3.10 for the phenotypic traits observed in the present study. A major QTL on chromosome 1, *qSKC1.1*, was identified spanning S01038 to RM7075 marker interval explaining a phenotypic variance of 11 % for Shoot K^+ concentration with LOD significance of 6.1. Another QTL, *qSL1.1* on chromosome 1 was mapped for shoot length which also explained a phenotypic variance of 11 %. Other QTLs explained phenotypic variances in range of 5.1 – 11 %. The positive QTL allele for all these QTLs were contributed by tolerant parent Kutipatnai rendering them fit ones for marker assisted improvement of salt susceptible varieties.

Flash flooding or submergence is also a major problem in rainfed ecosystems in the coastal region of Bangladesh. More than 15 million ha in South and South East Asia- two million ha in coastal Bangladesh- are affected by flash floods. Coastal saline areas invariably experience frequent submergence for short periods with saline or freshwater during the 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 a high yielding variety (HYV) will be more effective for rice cultivation in coastal regions. 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 were made between BR11-*Saltol* and BR11-*Sub1* and 350 F_2 seeds of BR11-*Saltol* /BR11-*Sub1* were genotyped for q*SALTOL* using the SSR markers RM493, RM3412, and AP3206, and for q*SUB1* using closely linked markers SC3 and ART5. Eighteen selected $F_{2:}$ F_3 plants with homozygous alleles for both the QTLs were screened separately for tolerance of salinity (10 dS m^{-1}) and under complete submergence for 14 days. Finally 9 lines have been selected, which were tolerant for both salinity (EC 10) and Submergence. These selected lines were again screened in submergence demo tank with saline water (EC 8) and also in demo field with EC6. These lines were also characterized for morphological and agronomical traits such as days to flowering, plant height, no. of tiller, no. of panicle, panicle length, filled grain, unfilled grain, weight of filled grain, weight of unfilled grain etc. ; physiological traits such as SES for salinity and submergence, Plant vigor, Chlorophyll content, Na & K uptake in root and shoot , Na-K ratio in root and shoot etc. and several grain quality traits.

Sorghum

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

- *November 2008–October 2011; NCE: December 2013*

Principal Investigator and lead institute

Andrew H. Paterson (PI), Plant Genome Mapping Laboratory, Univ. Georgia (paterson@uga.edu)

Collaboratoring institutes and scientists

- SARI, Tamale, Ghana: I.D.K. Atokple
- ICRISAT, Patancheru, India: C. Tom Hash
- Marathwada Agricultural University, Parbhani, Maharashtra, India: S.P. Mehtre
- National Research Center for Sorghum, Hyderabad, India: Ragimasalawada Madhusudhana

Objective 1. Fine-scale characterization of qualitative factors related to drought tolerance.

Ninety-one (91) double-QTL introgression homozygotes, and their single-QTL introgression parents, as well as donor parent, B35 and recurrent parent, S35 and a local check, Kapaala were evaluated in field trials conducted under irrigated dry season and rain-fed conditions for their agronomic performance. The dry season trial was planted in February 2012 using furrow irrigation at the Golinga Irrigation Scheme in the Tolon District, Northern Region of Ghana. Each line was sowed on two ridges spaced 75 cm apart, using a spacing of 25 cm between hills and one plant per hill. The ridges were 3 m long and the experiment was replicated 3 times. A similar setup was established in July 2012 for the rainy season, except for 2 plants per hill. Fertilizer was applied at the rate of 60 kg N/ha and 30 kg/ha for each of phosphorus and potassium. For the dry season, water was applied as frequently as possible to avoid drought stress until the fourth week, and watering was withheld for a cycle of 12 days before re-watering. For the rainy season the crop was totally rain-fed without any irrigation.

Data were collected on plant height (PLHT), days to 50% flowering (DFF), panicle weight (PANWT), grain weight (GRNWT), stover weight (STOVWT), and leaf area index (LAI) using standardized procedure in each case. For selection based on trait performance, genotypic values for PLHT and DFF were ranked from lowest to highest (because they mostly have negative correlation with grain yield in sorghum) while those of the other traits were ranked from highest to lowest. Respective ranges were 127- 275 cm, 70-96 days, 116.2-611.8 g/m^2, 54.2-367.7 g/m^2, 2833-8833 g/m^2, and 1.61-4.55 for PLHT, DFF, PANWT, GRNWT, STOVWT and LAI during the dry season. Mean value ranges of 133-397 cm, 60-101 days, 189-619 g/m^2, 89-432 g/m^2, 3333-10333 g/m^2 and 0.69-4.06 were recorded for PLHT, DFF, PANWT, GRNWT, STOVWT and LAI respectively during the rainy season. For both dry and rainy season, significant genotypic differences were observed for all the traits except LAI under the dry season (Appendix 2). For plant height, no genotype was ranked among the top 10 for both the dry and rainy season; one genotype (41) only was ranked among the top 10 regarding DFF for both the dry and rainy season; three genotypes were ranked among the top ten during both seasons for each of PANWT (23, 31, 39) GRNWT (31, 49, 60) STOVWT (54, 63, 40) and LAI (36, 57, 60). The top ten lines selected for each of the traits will be evaluated in replicated trials across various districts of northern Ghana during the 2013 cropping season for further selections.

Objective 2. Build a framework for dissecting quantitative mechanisms of drought tolerance.

Total RNA from thirteen diverse accessions [IS 27587, IS 14216, IS 18868, IS 21691, IS 22632, IS 2205, E 36-1, IS 9830, N13, IS 15401, M35-1 (IS 1054), S35 (ICSV 111) and CSM63E] was extracted at nine phenological growth stages in chronological order (*viz*., germinating seeds; seedling growth stage roots and shoots (ca. 10 das); juvenile growth stage roots and shoots (ca. 25 das); pre-boot stem elongation stage roots, stems, developing inflorescence and leaves; boot stage roots, stems, developing inflorescence and boot leaf; anthesis stage roots, boot leaf and developing inflorescence; mid-grain fill stage boot leaf and developing grains; soft-dough stage boot leaf and developing grains; and mature dry grain), and shipped to UGA during December 2011 for sequencing and transcriptome profiling for SNP identification and development. Transcript profiling yielded reads ranging from 29-57 million shotgun . shotgun reads were mapped to the reference sorghum genome by bwa v0.5.9. Variations were discovered using samtools package v0.1.18, using high mapping score reads (>=30), high Base score (>=30) and read depth of 4-60. A variant is called when 90% of the reads differ from reference (P(ref|D)<0.1). The functional effect of non-synonymous substitutions has been predicted by plant-specific evolution conservation profiles. Detailed analysis is in progress.

Activity 2. Empirical testing of relationships between drought tolerance (and other phenotypes) and specific genes, gene families and functional SNPs.
A $F_{3:8}$ sorghum RIL population derived from cross-(N13 × E 36-1) segregating for staygreen trait, a well characterized post-flowering drought tolerance mechanism, was field evaluated for three years during postrainy season of 2008, 2009 and 2010. A set of 240 entries including 220 individual RILs along with repeated parental genotypes and checks was evaluated with 2 rows/replication x 3-reps each for an irrigated control and stress treatment in an alpha-lattice design. Data on days to flowering, plant height (cm), weekly senescent/staygreen scores (from one week before flowering till maturity), no. of tillers, no. of harvestable panicles, grain numbers per plot, grain number per panicle, test weight (g/100 seed), panicle harvest index (%), fresh stover weight/plot (g/plot), dry stover weight/plot (g/plot), grain harvest index(%) were recorded. This data is being assembled for further analysis. In addition, T. Hash is growing a diversity panel previously identified for the staygreen character itself, that we plan also to explore.

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

- *July 2008–July 2013; NCE: October 2014*

Principal Investigator and lead institute

Jean-Francois Rami, UMR AGAP CIRAD; rami@cirad.fr
CIRAD - UMR AGAP TA 108/03, Av Agropolis, 34398 Montpellier CEDEX 5, FRANCE; Tel: +33 4 67 61 44 65; Fax: +33 4 67 61 56 05

Collaborating institutes and scientists

- Institut d'Economie rurale (IER), Mali: Niaba Teme, Sidi Bekaye Coulibaly, Mamoutou Kouressy
- CIRAD, France: Michel Vaksmann
- Syngenta seeds, France: Denis Lespinasse, Michel Ragot.

This project aims at improving sorghum productivity in semi-arid environments of Mali through integrated marker assisted recurrent selection (MARS). In this project, the MARS methodology was used as a proof of concept of using molecular markers to accelerate and monitor genetic progress in the breeding process. This is conducted through recombination cycles that aim at cumulating QTLs' favorable alleles identified as part of the breeding process for many traits and in many environments. This approach aims at getting the most of breeding populations, and finally of resources allocated to the breeding program. MARS can be seen as a pipeline of genetic material improvement through enrichment in good alleles for target traits and corresponding identified QTLs.

The breeding phase of the project is still in progress and will be mostly completed by the end of 2014. In short, two populations developed from the crosses of Tiandougou with Keninkeni (P114) and Tiandougou with Lata3 (P118) have been used in this project. Populations (about 400 individuals) have been advanced up to F_3 generation and seed increased to $F_{3:5}$ bulks for field testing. The two populations have been evaluated in 2010 and 2011 respectively in 6 environments (3 locations x 2 sowing dates). QTLs have been detected for both populations for a large number of traits (grain productivity, flowering time, plant morphology, grain quality, etc.) in the 6 environments (figure 1). QTL ideotypes have been designed for each population through working meetings with breeders. Two MARS cycles (C1 and C2) have been conducted for the first population (P114) from 2011 to 2013. The C3 cycle is ongoing. The first cycle (C1) for the second population (P118) will be conducted during 2013 cropping season. The families obtained through the first cycle of recurrent selection (C1) for the P114 population have been phenotypically evaluated during the 2012 cropping season together with parental lines and elite checks. Several (about 15) MARS breeding lines have showed superior and stable (over 4 environments) performance as compared to the elite checks evaluated in the same experiments. More breeding lines will be evaluated over the coming seasons, as part and beyond the project, from subsequent MARS cycles of both populations and will provide elite material to IER breeding program for on station and on farm testing and variety release.

In terms of capacity building two PhD students are completing their thesis as part of this project, on sorghum grain quality and panicle architecture respectively. One MSc student from Mali is also studying in Montpellier, working on flowering time and photoperiod sensitivity. Near Infrared Reflectance Spectroscopy (NIRS) is a key technology for sorghum grain quality evaluation and for incorporating these important traits in breeding programs. NIRS is rapid and non-destructive and has therefore the potential for predicting several grain quality parameters for thousands of sample in a cropping season. We have initiated as part of the project the transfer to IER of NIRS grain quality calibration that have been developed by CIRAD as well as training of IER staff member on NIRS maintenance and calibration development methodologies.

Figure 1: Graphical representation of the QTLs detected for population 118 on chromosome 1 for all traits and environments. The color represents the sign of the effect (Blue: positive allele from Tiandougou parent, Red: positive allele from Lata-3 parent) and the intensity of the color represents the Lod value.

Globally, the implementation of MARS in the sorghum breeding program at IER is a success, and more breeding lines will be available for evaluation and variety development over the coming year. The skills developed by the team in conducting such integrated breeding approach will allow development of new MARS projects using new parental lines including genetic material being currently developed as part of BCNAM companion project.

29. G4012.01/G7009.04: Development and evaluation of drought-adapted sorghum germplasm for Africa and Australia (G4012.01: Phase II)

- *January 2009–June 2014*

Principal Investigators and lead institutes

- Andrew Borrell, University of Queensland (UQ/QAAFI); a.borrell@uq.edu.au. Hermitage Research Facility, 604 Yangan Road, Warwick, QLD 4370, Australia; (61) 7-46603640; Fax: (61) 7-46603600
- David Jordan, University of Queensland (UQ/QAAFI); david.jordan@daff.qld.gov.au Hermitage Research Facility, 604 Yangan Road, Warwick, QLD 4370, Australia; (61) 7-46603622; Fax: (61) 7-46603600

Collaborating institutes and scientists

- Institut d'Economie Rurale, Mali: Sidi Coulibaly and Niaba Teme
- Institut de l'Environnement et de Researches Agricoles, Burkina Faso: Clarisse Pulcherie Barro/Kondombo
- Institut National de Recherche Agronomique du Niger: Soumana Souley
- Kenya Agricultural Research Institute: Clement Kamau
- Ethiopian Institute of Agricultural Research: Alemu Tirfessa and Asfaw Adugna
- Agricultural Research Corporation, Sudan: Mohamad Abdalla

1. Research activities and progress in Australia

1.1 Increase seed of introgression lines

Seed of the introgression lines was increased during 2012 in a glasshouse at Hermitage during Jan-Mar (since daylength can be regulated in this facility, there was no problem inducing flowering in photoperiod sensitive lines) The aim was to have sufficient seed available for the wet season planting in Africa in 2012 to further increase seed and for preliminary phenotyping in Mali and Ethiopia.

1.2 Develop and finalise contracts for project
Andrew Borrell is currently finalising sub-contracts between the University of Queensland and the six African countries. A contract is already in place between UQ and IER in Mali.

1.3 Send seed of RIL populations to Mali
Seed of four RIL populations totaling 917 individuals will be sent to Mali in June 2013. Sidi is currently organizing import permits and the seed has already been packaged in Australia.

1.4 Develop evaluation protocol for experiments in Africa
In May 2013, Andrew Borrell and Sidi Coulibaly developed evaluation protocols for standardised experiments in all six African countries. All partners provided input into this document.

2. Research activities and progress in Africa
2.1 Increase seed of introgression lines
Seed of 126 introgression lines was increased during 2012 at Cinzana Research Station in Mali by Sidi Coulibaly. Sufficient seed was produced in 64 of these lines for evaluation at two sites in six countries (Mali, Burkina Faso, Niger, Ethiopia, Sudan and Kenya) during 2013. Seed of the introgression lines was also produced by Alemu Tirfessa and Asfaw Adugna at Melkassa Research Station in Ethiopia during 2012.

2.2 Evaluate populations based on adapted material from Mali that has been enriched for stay-green QTL using marker assisted selection for QTL (introgression lines) for which there is strong evidence of effectiveness
64 stay-green enriched lines will be evaluated along with two locally adapted lines (2 x 2 reps = 4), making a total of 68 lines. These lines will be assessed in 12 trials during 2013 (6 countries x 2 sites per country). Sidi organised and distributed phytosanitary certificates to all partners on 14 June 2013 and will send seed of all test lines shortly. The aim is to plant experiments in late June or early July in all countries. The experimental design will consist of 68 lines x 2 reps x 2 locations (272 plots per country). There will only be enough seed to evaluate lines under water-limited conditions i.e. no irrigation control. Data will be collected on phenology (anthesis & black layer), visual stay-green rating, tillers/plant, height to flag leaf/base of panicle/top of panicle, green leaf area at maturity, grain yield, grain size, plus any relevant grain quality assessment.

2.3 Assist in the establishment and implementation of a molecular breeding community of practice for sorghum in Africa
An effort has been made to establish a network of sorghum scientists in Africa (a community of practice). This effort was initiated at a CoP workshop in Hyderabad during 2011 as part of the GCP Research Meeting. As a follow-up, African partners were invited by Andrew Borrell and Vincent Vadez to attend a review of an ACIAR stay-green project in Hyderabad in February 2013. This meeting was attended by four of our partners: Sidi Coulibaly (Mali), Clarisse Pulcherie Barro/Kondombo (Burkina Faso), Clement Kamau (Kenya) and Asfaw Adugna (Ethiopia). On-going training in Australia and Africa will continue to strengthen this network.

2.4 Training in Australia for visiting African scientists on sorghum crop improvement
Four people will be trained in February 2014 for a three-week period at Hermitage Research Facility in north-eastern Australia. One scientist has been selected from each of the following four NARS in the Sorghum CoP (Burkina Faso, Niger, Sudan and Kenya). Scientists from Mali (Drs Coulibally and Teme, IER) and Ethiopia (Taye Tadesse, EIAR) have already been trained at Hermitage in 2012. Participants will work with Australian scientists in the sorghum improvement program (conventional breeding, molecular breeding and physiology). They will also receive training in the wider aspects of the program.

2.5 Training in Africa by visiting Australian scientists on sorghum crop improvement
It is planned that some training will be undertaken in Africa by Andrew Borrell and Barbara George-Jaeggli when they visit trial sites in 2013 or 2014.

2.6 Evaluate mapping populations (RILs) in Mali
It is planned that one of four RIL populations will be evaluated in Mali during 2013. Sidi is currently organizing import permits and the seed has already been packaged in Australia.

2.7 Evaluate F1 hybrids in Mali
Six F1 hybrids will be evaluated based on two Malian males (PI585749 & PI609278) crossed with three elite female parent lines from DAFF (A1*9_B010054, A1*F_B963676 & F2_ms3*3_R931945-2-2) that contrast in the level of stay-green.

30. 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
Niaba Témé, IER BP 258, Rue Mohamed V, Bamako, Mali
Tel (+223) 20 22 26 06/20 23 19 05, niabateme@gmail.com

Collaborating institutes and scientists
- IER: Sidi B. Coulibaly, Mamoutou Kouressy
- CIRAD: M. Vaksmann, J. F. Rami
- ICRISAT, Mali: E. Weltzien, F. Rattunde
- ICRISAT, India: T. Shah

High yielding sorghum variety development in Africa targets a wide range of harsh and volatile environments where local landraces show higher grain quality, adaptation with lower grain yield potential. Backcross Nested Association Mapping (BCNAM) approach is tested by two institutions, IER and ICRISAT, to enhance grain yield and quality and to identify QTLs linked to various traits of interests. The main objectives are to develop new breeding populations, to phenotype, to identify key genomic regions, to initiate efficient molecular based breeding approaches using experimental sorghum populations and to boost capacity of national breeding programs for wider use of germplasm diversity using molecular tools. Fifty seven (57) BC1 populations, 20 from ICRISAT and 37 from IER were developed using two techniques: hand emasculation and genetic male sterility methods from 2009 to 2011. There were three recurrent parents, ten common and 20 specific donor parents used for the development of genetically enriched populations. Off-season and main cropping seasons were used to accelerate population development. Selection pressure was applied to eliminate lines with extreme levels of tall plant height and early or late maturity during the BC1F2 generation.

Number of families per population varies from 70 to 200. Twenty two (22) populations at IER and eleven (11) at ICRISAT were phenotyped in 2012/2013 cropping season for more than 50 agronomic traits measured. There were two dates of sowing in each two contrasting locations at IER and two sowings, low phosphorus (LP) and high phosphorus (HP) conditions at IRCISAT. At IER, 3077 families including checks were phenotyped for the first date of sowing at Sotuba and 2492 for the second dates at Sotuba and for both dates of sowing at Cinzana. An augmented design was used for field layout for each population to collect data and NIRS was used for parental lines grain quality data. DNA samples were taken from BC1F3 families and conformity test of progeny to parental lines was run using 50 SNP markers.

First sets of phenotypic data are collected from IER (22 populations) and ICRISAT (11) and are being analyzed. There are varying and interesting agronomic results within and across populations from the different date of sowings. Early phenotypic data show that early sowing has grain yield advantage over late sowing at both Sotuba and Cinzana sites. Populations having Grinkan as recurrent parent show higher yield potential than Kenikeni. Donor parents CSM388, CSM417, Konotene, IS23540, E36-1 and SC566-14 combine well with Grinkan and CSM 417 combines well with Kenikeni.

Photoperiod sensitivity of recurrent and donor parental lines was evaluated at two sites (Sotuba and Sikasso) with two different sowing dates. Parental lines and recurrent parents vary from non-sensitive (few) to medium and highly sensitive to day length. The recurrent parents and lines were generally earlier maturing at the lower latitude (Sikasso). Genetic male sterile (ms3) populations of Kenikeni and Grinkan are accessible. Grain and fodder qualities of the parental lines are also available. Adaptation of parental lines to post flowering drought stress was evaluated during the cool offseason of 2012/2013 and results are under analysis. Genetic distances among the parental lines (34) are known using 1320 SNPs markers.

Second year of populations phenotyping is planned for this coming cropping season at both IER (two sites, two sowing dates per site) and at ICRISAT (high and low phosphorus conditions). Grain quality (grain vitrosity, pericarpe thickness, 1000 seed weight) are being evaluated. Parental responses to post flowering water stress will be re-evaluated in late sowing time in august 2013. Two master and four bachelor students completed their trainings on population development and phenotyping since 2009 while two PhD and one master students are pursuing their studies. A scientific officer is being hired at present. There is an urgent need to hire a postdoctoral scientist for analyzing the BCNAM population data for both phenotypic and genotypic data and QTL detection. Varying and interesting lines will be available targeting dual purposes sorghum for food, feed and fodder if variety development and release are achieved. Population development is completed, seed is being multiplied and made available to partners, and road should be paved for variety development and release once the GCP will have ended in 2014.

Acknowledgements
IER, GCP, CIRAD, ICRISAT-Mali, Sorghum Programme, LSEP of Sotuba, Cinzana Station research and Sotuba accounting teams.

Wheat

31. G3008.01: Generating new wheat germplasm with enhanced drought/heat tolerance using AB genomes genetic diversity

- *November 2008–October 2011; October 2013*

Principal Investigator and lead institute
Satish Misra, Agharkar Research Institute, India; satishmisra@yahoo.com.
Genetics Department, Agharkar Research Institute, G.G. Agharkar Road, Pune 411 004, India; Tel: +91-20-25654357 (Ext. 267)
Fax : +91-20-25651542

Collaborating institutes and scientists

- Agharkar Institute, Pune, Maharashtra, India: S. Tetali, S. Tamhankar, A.J. Hingane.
- CIMMYT, Mexico: D. Bonnett, M. Zaharieva, S. Dreisigacker, J. Crossa and T. Payne
- Plant Breeding Institute, Sydney University, Australia: R.Trethowan and P.Sharp
- University of Agriculture Sciences, Dharwad, Karnataka, India: R.R. Hanchinal, A. Shreenivas Desai, I. Kalappanaar, K.K. Math and B. Nirmala Yenagi

1. Research activities and progress at CIMMYT, Mexico:

At CIMMYT, diverse Dicoccon lines originating from 38 countries, provided by CIMMYT and ICARDA gene banks were pulled together and molecular diversity analysis was performed using a set of 35 highly polymorphic SSRs. The molecular diversity groups largely reflected 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 hexaploid wheats by crossing the subset of 108 dicoccum with three different *Ae. tauschii* accessions. Molecular diversity analysis indicated that the subset of 108 reasonably well covers the diversity of full set of 308. Seed was distributed to project collaborators or further multiplication.

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

At Plant Breeding Institute, Sydney, emmer wheats were crossed with hexaploid bread wheats. Seeds were obtained from almost 50 crossing combinations. The hexaploids used for crosses involved Indian cultivars (PBW502, PBW550, DBW16, DBW17), the drought tolerant CIMMYT lines (Berkut and Waxwing*2/Kiritati), the durum based synthetic (Sokoll) and the emmer based synthetic (*T. dicoccon* P194625/*Ae. tauschii* 372). Backcrosses were made to the hexaploid wheat parent and more than 1000 double haploids were produced from the derived BC_1F_1. The F_2 was sown in large populations and F_2 derived F_5 materials were planted in field in Australia for stress assessment. The seed of superior F_5 lines was sent one year ago but lost at DWR. A complete set is now with Australian quarantine and will arrive in ARI Pune for further evaluation.

The DH materials were increased in the field in 2012 and are now being planted in replicated trials under moisture stress to assess the impact of the *T. dicoccum* introgression. DNA has been extracted and all materials will be genotyped in 2013 to identify tetraploid introgressions in the best performing materials under stress.

3. Research activities and progress at Agharkar Research Institute, Pune, India.

At Agharkar Research Institute, durum based and emmer based synthetic wheats were inter crossed in the research farm situated at Hol. One hundred seventy six lines were developed out of around 30 cross combinations. They are stable lines ready for further usage. 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. Sixty seven F_3's and 48 F_2's were planted in this season 2012-13.

New Dicoccum based synthetics arrived from CIMMYT were planted for seed multiplication and sharing with UAS Dharwad.

Indian Dicoccum germplasm consisting of 48 genotypes was studied for molecular diversity using total 47 polymorphic SSR markers distributed over all 14 chromosomes. Number of alleles per locus ranged from 2 to 9 with average 3.87 per locus. A total of 201 alleles were detected at 52 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 (UAS), Dharawad

At UAS, Dharwad, total of 50 BC_1's, 53 F_2's, 55 F_3's, 50 F_4's, 9 BC_1F_3's, 44 F_5's (SSD method) and 48 F_5's (Bulk method) were generated by utilizing genetically diverse popular and existing bread wheat varieties, durum and dicoccum based synthetic hexaploid wheat obtained from CIMMYT. The developed materials were evaluated at Dharwad, Wellington and Ugar for physiological, morphological and pathological parameters. Among the breeding materials generated F_4's progenies were showed the maximum chlorophyll content (43.5 to 57.5) and F_5's (SSD method) progenies were showed narrow range of canopy temperature (28.1 to 33 °C). These F_4's and F_5's breeding materials can be effectively utilized for further breeding programme. Overall breeding materials generated under the GCP project were showed resistant to ever evolving leaf rust pathogen as well as emerging spot blotch disease and these resistant sources can be utilized for accelerating the disease resistance breeding programme in wheat.

Next steps and challenges

1. Seeds of direct crosses and synthetics generated at PBI, Sydney which will be reaching very soon to ARI, Pune will be shared between project collaborators.
2. SBL generation by crossing drought tolerant bread wheat varieties with newly prepared Synthetic Hybrid Wheats.

32. G3008.08: Breeder-friendly high-throughput phenotyping tools to select for wheat adaptive traits in drought environments

- *November 2008–October 2011; NCE: October 2013*

Principal Investigators and lead institutes

Francis Ogbonnaya (till early 2012), ICARDA; current email: Francis.Ogbonnaya@grdc.com.au
Wuletaw Tadesse (2013), ICARDA; W.Tadesse@cgiar.org
Fernanda Dreccer, CSIRO; Fernanda.dreccer@csiro.au
CSIRO Plant Industry, Cooper Laboratory, University of Queensland Campus, Warrego Highway, Gatton, 4343 Queensland, Australia

Collaborating institutes and scientists

- INRA-CRRA, Centre Aridoculture, Morocco: Hassan Ouabbou
- ICARDA-INRA Cooperative Research Program, Morocco: Sripada M. Udupa
- CSIRO Plant Industry, Australia: Tony Condon
- Ethiopian Institute for Agricultural Research (EIAR), Ethiopia: Firdissa Eticha
- Kulumsa Agricultural Research Centre (KAR), Ethiopia: Solomon Gelacha
- International Centre for Maize and Wheat Improvement (CIMMYT), Mexico: Matthew Reynolds

1. Research activities and progresses at CSIRO, ICARDA and collaborators

Up to ca. 200 wheat lines, comprising a set of 72 lines for trait comparison and 200 ICARDA lines, were grown in 32 environments representing contrasting mega-environments for drought. Locations were in Syria, Lebanon, Morocco, Ethiopia, Mexico and Australia over three years. Sites were mostly rainfed, but irrigated treatments were also run in selected locations for benchmarking. Information from all sites has been collated together with a pedigree matrix. In the first instance a MET analysis will be done assuming the lines are independent, followed by another MET analysis including the relationships matrix to reflect the parentage. The purpose is to investigate if the difference in adaptation patterns observed in the GxE can be explained by the pedigree structure. In addition, the role of environmental attributes and their impact on yield will also be investigated as part of the GxE analysis.

Two courses have been held as part of capacity building first in Aleppo, Syria in 2010- attended by 8 from 5 countries, the second on breeding targets for water limited conditions and a phenotyping workshop was help in Rabat in May 2013. This was attended by 9 people- from 6 countries. In both cases, people were mainly involved in breeding activities in their countries of origin.

2.2 Content

2.2.1 Ongoing projects initiated in 2012, or earlier

a. Context:

The objectives of the project were to assess the relative impact of putative key traits on drought adaptation by comparing related lines contrasting in trait value in different drought mega-environments and develop and validate field-based, high throughput phenotyping tools to assess traits putatively relevant to water limited conditions. Then apply this knowledge to characterize elite ICARDA lines and build capability in non-invasive phenotyping methods and the estimated value of different traits, particularly within the Central and West Asia and North Africa (CWANA).

b. Findings and implications: Present the latest findings from your research.

The analysis of data on overall performance of lines and analysis of trait value has not finished. However, based on performance of ICARDA lines in Ethiopia and Morocco, crosses have already been implemented by local breeders. For instance, in Moroco, 23 lines were handed to breeders to be included in preliminary trials and crossing blocks. Similarly, with phenotyping methods, the course, resources (protocols, presentations, reading material) and a support network have been put in place as a mechanism to support the new practices. Genome wide association analysis will also be carried out to identify QTLs contributing to various adaptive traits that could be targets for marker-assisted selection of desirable traits for highly desirable traits in breeding programs amongst the national agricultural systems including Ethiopia, Morocco and the target region.

d. Next steps and/or challenges

Next steps are to continue with data analysis, paper writing and looking for funding to do a thorough characterisation of the environment in the target regions and analysis of more favourable trait combinations using simulation modelling.

33. G7010.02.01: Breeding and selection strategies to combine and validate quantitative trait loci for water-use efficiency and heat tolerance of wheat in China

- *April 2010–March 2014*

Principal Investigator and lead institute

Ruilian Jing, Institute of Crop Science, CAAS; jingrl@caas.net.cn
No. 12 Zhongguancun South Street, Haidian District, Beijing 100081, China; Tel./Fax: +86 10 82105829

Collaborating institutes and scientists

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

1. Background of the project

QTLs have been identified for traits related to drought and heat stress adaptation in a range of different wheat mapping populations. The expectation of the project is that the frequency of favorable alleles for tolerance to moisture stress and heat will be improved.

2. Findings and implications

2.1 Confirmation and selection of QTL for physiological traits associated with drought and heat tolerance

Using joint linkage-association mapping, 70 validated QTL markers for physiological traits related to drought and heat tolerance were selected as candidate markers for marker assisted recurrent selection (MARS). Among them, 11 markers linked with two QTL. These candidate QTL are for parameters of chlorophyll fluorescence kinetics (Fv/Fm, 9), canopy temperature Depression (CTD, 12), water soluble carbohydrate content (WSC, 31) and chlorophyll content (CC, 29).

2.2 Populations selected for molecular marker assisted selection based on the diversities of genotyping and genotyping

To select the available populations for MARS, we detected the SNP polymorphisms between parent pairs used in the candidate crosses by KBioscience UK. The genetic relationship of 30 parent accessions was revealed by 1864 SNPs (Fig. 1).

Based on the diversities of phenotyping and genotyping, 8-19 top lines were screened from the original crosses to make new crosses for pyramiding

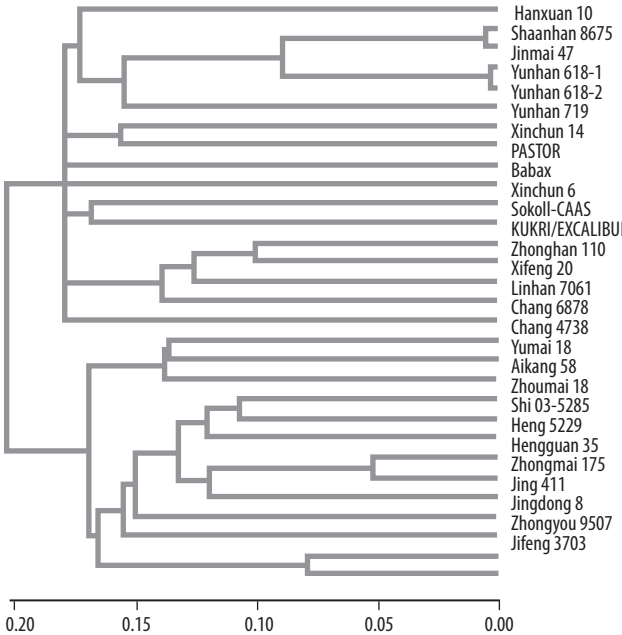


Fig. 1 Genetic relationship of 30 wheat accessions based on 1864 SNPs

the target alleles. The range of cross numbers are 16-46F_1 and 10-15F_2 for the original crosses, respectively (Table 1). The polymorphic SSR markers for QTL between individual parent pairs ranged from 12 to 36.

3. Next step

Because of the spring character of wheat in Xinjiang, the candidate QTL and their elite alleles need to be further confirmed. We are going to screen and validate the QTL for the spring wheat synchronously in 2013.

Table 2 Populations selected for the molecular marker assisted recurrent selection

No.	Original cross	Genera-tion	No. of lines	Polymorphic marker	Top lines	Cross
1	Jingdong 8 × Aikang 58	$F_{2:4}$	207	36	8	$16F_1$
2	Yannong 19 × Yunhan 618-2	F_6	395	13	9	$15F_2$; $18F_1$
3	Hengguan 35 × Jifeng 3703	$F_{3:4}$	320	27	17	$46F_1$
4	Chang 6878 × Chang 4738	$F_{2:4}$	220	12	13	$37F_1$
5	Xinchun 6 × PASTOR	F_5	224	19	19	$10F_2$; $30F_1$

References

Weiyu Li, Bin Zhang, Jianan Zhang, Xiaoping Chang, Runzhi Li, Ruilian Jing. Exploring elite alleles for chlorophyll content of flag leaf in natural population of wheat by association analysis. *Acta Agronomica Sinica*. 2012, 38: 962–970.

Delong Yang, Ruilian Jing, Xiaoping Chang, Wei Li. Quantitative trait Loci mapping for chlorophyll fluorescence and associated traits in wheat (*Triticum aestivum*). *Journal of Integrative Plant Biology*. 2007, 49: 646–654.

Delong Yang, Ruilian Jing, Xiaoping Chang, Wei Li. Identification of quantitative trait loci and environmental interactions for accumulation and remobilization of water-soluble carbohydrates in wheat (*Triticum aestivum* L.) stems. *Genetics*. 2007, 176: 571–584.

34. G7010.02.02: Molecular breeding and selection strategies to combine and validate QTLs for improving WUE and heat tolerance in India

- *July 2010–June 2014*

Principal Investigator and lead institute

K Vinod Prabhu, Indian Agricultural Research Institute; kvinodprabhu@rediffmail.com
Indian Agricultural Research Institute; New Delhi, India. Tel: (91)11-25841481; Fax: (91) 11-25841481

Collaboratoring institutes and scientists

- Indian Agricultural Research Institute, New Delhi: G P Singh
- Punjab Agricultural University, Ludhiana: Praveen Chhuneja
- National Research Centre on Plant Biotechnology: N K Singh
- Jawaharlal Nehru Krishi Vishwa Vidyalaya, Regional Station, Pawarkheda: P C Mishra
- Agharkar research Institute, Pune: S C Mishra

Research activities and progress of lead and collaborating institutes

Physiological evaluation of genetic populations and germplasm resources has been carried out in India to implement standard drought and heat phenotyping protocols and new breeding and selection strategies

Table 1: Multi-location phenotyping and SSR markers based genotyping for inter-family intermating among best identified progenies in F_5 generations

MARS Populations	Number of progenies in Multi-location trial	Number of Best identified progenies	Polymorphic SSR markers for genotyping	Crosses attempted among bestprogenies for first round of recurrent selection
DBW43XHI1500	160	10	36	100
HUW510XHI1500	160	12	20	40
BW7203XBW9149	180	12	17	64
PBW442XBWL0056	180	10	11	27

Table 2: Marker- assisted backcross breeding for transfer of QTLs for moisture and heat tolerance traits.

Backcross populations	Population size in BC_1F_1	Number of best progenies selected	Traits targeted for foreground selection
HD2733/WH730	**725**	**66**	**Yield, CT**
HD 2733/C306	**645**	**62**	**Yield, CT**
HD2733/HD2888	**454**	**20**	**Yield, above-ground biomass**
GW322/HI 1500	**540**	**68**	**Stay-green, Chl content, CT**
GW322/HD2987	**780**	**38**	**Stay-green, NDVI, Yield**

using MARS and MABB is being carried out to improve water use efficiency and heat tolerance of wheat in India.

1. Physiological trait based phenotyping among the international core-set and Indian set

An International core set consisting of 145 lines of genetic resources and an Indian set comprising 17 lines was evaluated under restricted irrigation and rainfed conditions at four locations for three years. Data was recorded for canopy temperature, chlorophyll content, NDVI, flag leaf area, stomatal conductance, yield and its component traits.

2. Combining QTLs through marker assisted recurrent selection and first round of designed intermating among selected F_5 families

Multi-location phenotyping has been done at four target locations under rainfed and irrigated conditions for two years. The best progenies had been selected for combining favorable QTLs for stress tolerant traits. Genotyping was conducted with 40-50 polymorphic microsatellite markers and first cycle of intermating was carried out in two populations.

Development of backcross populations for introgression of known QTLs into elite backgrounds Genotyping of the parents of backcross populations was carried out with 400-500 microsatellite markers. Five backcross populations were advanced to $BC_2 F_1$ generation after the identification of target regions with donor QTLs for stress tolerant traits in the foreground selection. Background selection with polymorphic SSR markers is in progress.

References

G. P. Singh, Neelu Jain, Ramya Kurien, Vinod, J.B.Sharma and K. V. Prabhu Molecular breeding and selection strategies to combine QTLs for improving drought/ heat tolerance along with rust resistance genes in wheat

Comparative Genomics

35. G7009.07: Cloning, characterisation and validation of *Alt$_{SB}$*/Al tolerance in rice

- *October 2009–October 2012; NCE: March 2013*

Principal Investigator and lead institute
Leon Kochian, Robert Holley Center for Agriculture and Health, USDA-ARS, Cornell University, Ithaca, NY 14853; Email: LVK1@cornell.edu; Phone: 1-607-255-5480; Fax: 1-607-255-2459.

Collaborating institutes and scientists

- Susan McCouch, Department of Plant Breeding, Cornell University, Ithaca, NY 14853

Context: Rice is significantly more Al tolerant than other cereal crop, yet mechanisms of rice Al tolerance are largely unknown and until this project began, no genes underlying natural variation in tolerance had been reported. The goal of this project was to use comparative genomics to identify rice homologs to our sorghum Al tolerance gene, *SbMATE*, and verify if these genes are involved in rice Al tolerance. We also proposed to use joint linkage-genome wide association (GWA) mapping to identify novel rice Al tolerance genes as well as verify candidate *SbMATE* homologs with sufficient diversity to use for breeding for enhanced rice acid soil tolerance.

Findings, implications, and next steps: Early in this project, we discovered that rice *SbMATE* homologs play at most a small role in rice Al tolerance. SbMATE-mediated Al tolerance in sorghum involves Al-activated citrate exudation from the root tip. The Al chelates toxic Al in the rhizosphere and prevents it from entering the root. We found this physiological mechanism is not prevalent in rice. Rice must employ true tolerance mechanisms to tolerate the high levels of Al in the root tip cell wall and symplasm. Recently, Ma's lab identified *OsFRDL4 as the SbMATE homolog and a root tip citrate transporter which plays a minor role in rice Al tolerance. We conducted joint linkage-GWA analysis of rice Al tolerance using an Azucena* (tolerant) x IR64 (sensitive) RIL population and 383 diverse rice accessions that were genotyped using a 700K rice Illumina SNP chip. The linkage mapping identified 3 Al tolerance QTL on chr 1, 2, and 12. The QTL on chromosome 12 was quite significant, explaining 20% of the variation in Al tolerance. We subsequently map-based cloned the chr 12 QTL and found the gene responsible is *ART1* (*Al resistance transcription factor 1*), a zinc-finger transcription factor and a master regulator of the rice Al tolerance response. ART controls the Al-induced expression of a number of other candidate Al tolerance genes. Our findings are significant, as we are the first to show genetic variation in a potentially important candidate rice Al tolerance gene that now will be an important breeding target.

To develop plant material appropriate for field testing and breeding applications, we have generated three sets of reciprocal NILs, each containing one, two or three of the Al tolerance QTL (including the *ART1* QTL) in either the Azucena (Al-tolerant) or IR64 (Al-sensitive) background. Because favorable alleles were distributed in both parents, this approach will evaluate the effect of favorable and unfavorable alleles in the two divergent backgrounds, providing material of interest for both lowland and upland breeding programs. These NILs are an excellent resource as donors of Al tolerance allele(s) for our future use in applied breeding programs.

From the GWAS analysis of Al tolerance phenotyping of the rice diversity panel, we identified 48 genomic regions associated with Al tolerance. The majority of these regions were subpopulation-specific, highlighting the subpopulation-specific genetic architecture of complex rice traits. A number of regions co-localized with *a priori* candidate genes and previously identified QTLs. Three regions corresponding to induced Al sensitive mutants (ART1, STAR2, Nrat1) were also identified to be involved in natural variation for Al tolerance. We have also found novel GWAS QTL and are focusing on 1 very significant peak identified across all subpopulations on chr 7. Different loci in this region may contribute to Al tolerance in different subpopulations. When analyzed together, the SNPs across the region show significant association with Al tolerance. This has interesting implications for plant breeding, where introgression of a large region containing multiple favorable alleles at related loci may be more effective than trying to target individual alleles. To begin to dissect the region to identify candidate genes, we scanned the region using the MSU Rice Genome browser and identified over 120 rice gene models in the region, a large portion being transposons. We also identified a number of cell wall-related genes in the region and because it is clear rice must have cell wall Al tolerance mechanisms, this region is of great interest to us. We will conduct haplotype and gene expression analyses in these candidate cell wall genes, to investigate their function and determine whether they play a role in rice Al tolerance.

Finally, we investigated the functional significance of natural allelic variation in the Al transporter gene *Nrat1* on chromosome 2, which we found underlying a GWA peak explaining 40% of the variation in *aus* Al tolerance. We then verified a controversial hypothesis that Nrat1 was a root tip Al uptake transporter that helped protect the cell wall by moving cell wall Al into the cell, where it is stored in the vacuole. Expression of rice *Nrat1l* in Arabidopsis, which like sorghum uses root organic acid efflux and not true Al tolerance mechanisms, greatly increased Al tolerance. Hence we may have a new target in Nrat1 for improving Al tolerance in rice and other cereals. We have evidence in maize that a functional homolog of rice Nrat1 exists, and we will investigate its possible role in improving maize Al tolerance.

36. G7010.03.01 Cloning, characterisation and validation of *Pup1*/P efficiency in maize

- *April 2010–March 2014*

Principal Investigator and lead institute

Leon Kochian, Robert Holley Center for Agriculture and Health, USDA-ARS, Cornell University, Ithaca, NY 14853; Email: LVK1@cornell.edu; Phone: 1-607-255-5480; Fax: 1-607-255-2459.

Collaborating institutes and scientists:

- Embrapa Maize and Sorghum, Sete Lagoas, Brazil: Claudia Guimarães, Sidney Parentoni, Jurandir Magalhães, Vera Alves, Maria José Vasconcelos, Sylvia Sousa, Roberto Noda
- USDA-ARS/Cornell: Lyza Maron, Miguel Pineros, Randy Clark, Ed Buckler, Jon Shaff,
- Moi University/KARI, Eldoret, Kenya: Sam Gudu
- JIRCAS, Tsukuba, Ibaraki, Japan: Mathias Wissuwa

Project Abstract

Context: 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. Yet resource-poor farmers in drought-prone upland and rain fed lowland environments typically apply little fertilizer. Hence there is an important need to use modern plant breeding to improve the P acquisition efficiency of major food crops on these types of soils. Members of our Challenge Initiative recently cloned via high resolution mapping of the Pup1 P efficiency QTL in rice along with candidate gene analysis the first major P efficiency gene in plants. This rice gene, *OSPSTOL1*, encodes a root protein kinase that appears to improve root growth and change root architecture to allow the rice plant to more effectively mine P from low-P soils. In this project, we are using comparative genomics to identify and test maize *OSPSTOL1* genes as candidate maize P efficiency genes. Verification involves joint linkage-association mapping of P efficiency in both maize bi-parental RIL populations and a diversity panel combining Brazilian maize breeding lines adapted to acid, low P soils with the Buckler association panel. The same populations will be used to conduct linkage-association analysis of 3D root system architecture (RSA) to see if any RSA traits co-localize to the same genomic region as *ZmPSTOL1* homologs and/or P efficiency QTL.

Findings, implications, and next steps: Our initial research involved bioinformatics-based search of maize homologs using OsPSTOL1 as the query. This analysis identified four predicted genes in the maize genome sharing more than 65% of sequence identity with *OsPSTOL1*, which are our initial candidate genes for analysis. These genes are located on chromosomes 3, 4 and 8. SNPs for these 4 genes between the parents of the maize RIL population were identified and converted to CAPS markers and used to map their physical position on the maize genome.

The members of the Embrapa elite maize diversity panel were each crossed with two maize lines that are members of the two major maize heterotic groups generating 321 testcross hybrids. These testcross hybrids were evaluated in the field under low and high P over two growing seasons for traits related to P efficiency. The traits evaluated were: grain yield; anther silk interval; plant height; ear height; and phenotypic indices for P accumulation and partitioning. These datasets are currently being analyzed. Also, a genetic linkage map was constructed in our maize RIL population using SNP, SSR and the *ZmPSTOL1* candidate genes as markers. The RILs were backcrossed to both parental lines generating 280 progeny, and the backcross populations were evaluated on low P soil in the field for grain yield; shoot dry weight; phosphorus efficiency acquisition; phosphorus internal utilization efficiency and phosphorus use efficiency. QTLs for phosphorus use efficiency (PUE), phosphorus acquisition efficiency (PAE) and phosphorus internal utilization efficiency (PUE) were identified from the field phenotyping. Six QTLs were identified for PUE, six for PAE and five for PUTIL. Most of the QTLs mapped for PUE were coincident with the genomic regions mapped for PAE, in agreement with the high correlation (0.89) between these traits, which were also highly correlated with grain yield under low P (0.96 and 0.85, respectively). The RIL population itself was also mapped for 2D RSA traits. The maize seedlings were grown in

paper pouches with low P nutrient solution, digital photography was used to capture 2D digital images of each root system, and root system traits were analyzed using RootReader 2D software. QTLs for root traits, total plant dry weight, root:shoot ratio, P content and P utilization efficiency evaluated in nutrient solution under low P were also mapped in the RIL population. Three to four QTLs were mapped for each trait, explaining from 23 to 32% of the total genetic variance. One genomic region looks very intriguing on chr 8, flanked by the maize candidate genes *ZmPSTOL1* and *Zm1PSTOL1*, in a region spanning from 92 to 97 cM. In this region, QTLs were identified for volume of fine roots, total root dry weight and P acquisition efficiency. The co-localization of QTLs for these traits with the *ZmPSTOL1* suggest that this gene may be involved with the development of fine roots that positively affects plant growth and P acquisition efficiency under low P availability in nutrient solution. These same RILs will in the near future be mapped for 3D RSA traits using the RootReader 3D platform.

37. G7010.03.02: Cloning, characterisation and validation of Alt_{SB}/Al tolerance in maize

- *April 2010–March 2012; NCE: March 2013*

Principal Investigator and lead institute

Claudia Guimarães, Embrapa Maize and Sorghum;
claudia@cnpms.embrapa.br
Rd MG 424, Km 65, Sete Lagoas - MG 35701-970, Brazil;
+55 31 30271300; Fax: +55 31 30271279

Collaborating institutes and scientists

- Embrapa Maize and Sorghum, Jurandir Magalhaes, Sidney Parentoni, Lauro Guimarães, Andrea Carneiro, Newton Carneiro, Robert Schaffert, Vera Alves
- Robert W. Holley Center for Agriculture and Health, USDA-ARS / Cornell University, Leon Kochian, Lyza Maron, Jiping Liu, Miguel Pineros, Ed Buckler
- Moi University, Sam Gudu

1. Validation of functional ZmMATE *or Al tolerance QTLs in Brazilian maize crosses*

1.1 Validation of *ZmMATE1* as a gene underlying a major Al tolerance QTL in maize

A segregating population composed by 118 RILs derived from Cateto Al237 x L53 cross was saturated with 54,455 GBS-based SNPs, as well as candidate genes such as, *ZmMATE1*, *ZmMATE2*, *ZmALS* and *ZmNrat1*. Aluminum tolerance was evaluated as relative net root growth (RNRG) after five days treatment in {39} µM Al^{3+} activity, compared to the root growth without Al in nutrient solution. Five genomic regions were significantly associated with Al tolerance on maize chromosomes 2, 3, 5, 6, and 8 jointly explaining 65% of the total phenotypic variance for RNRG. A major Al tolerance QTL was detected on chromosome 6 (*qALT6*), which was co-localized with *ZmMATE1*, and with a single expression QTL (eQTL) explaining 70.9% of *ZmMATE1* expression. Recently, we presented that three copies of *ZmMATE1* was highly associated to Al tolerance in this cross (Maron et al. 2013).

Three near-isogenic lines (NILs) for *qALT6* were developed by marker-assisted backcross using an STS marker constructed *ZmMATE1* and the SSR umc1018. These NILs showed a two-fold increase in Al tolerance when compared to the recurrent parent and maintained the Al-induced *ZmMATE1* expression as high as in the donor line, Cateto Al237. NILs and their parental lines were evaluated on acid soils with three levels of Al saturation (0, 20 and 40%) in the first 20 cm. The field experiment showed that NILs introgressed with *qALT6* presented grain yield similar to their recurrent line L53 in corrected soil and statistically superior on acid soils, whereas the yield performance of was significantly reduced under 20 and 40% of Al saturation (Figure 1). The effect of *qALT6* on maize yield was evaluated in near-isogenic hybrids (NIHs) derived from crosses of L53 and NILs with maize elite lines currently used in maize commercial hybrids. The results indicated a significant yield improvement of NIHs in 40% of Al saturation compared to the single-cross hybrids with L53. In general, no differences were detected under corrected soil, except for L3xNIL13 that presented an increased yield performance in comparison to L3xL53 single-cross hybrid.

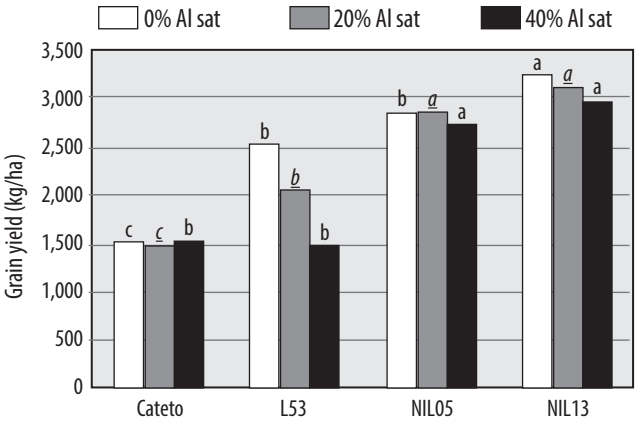


Figure 1. Grain yield of maize NILs introgressed with the *qALT6* and their parental lines obtained under three Al saturation levels in a typical Brazilian Cerrado soil. Same letters with the same pattern indicate similar mean based on Scott-Knott test at $p < 0.05$, for each soil condition.

Thus, we present strong evidences that *ZmMATE1* is a functional MATE member underling the major Al tolerance *qALT6*, which is likely to modulated Al tolerance by Al-induced *ZmMATE1* expression. Additionally, *qALT6* was able to improve yield stability on acid soils in maize NILs as well as in crosses with elite line. Therefore, marker-assisted introgression of *qALT6* is expected to improve maize adaptation to acidic soils that possibly can be expanded to other tropical regions.

1.2. Other candidate genes on Al tolerance QTL regions

ZmMATE2 was another candidate gene putatively involved in a novel Al tolerance mechanism in maize, as proposed by Maron et al. (2010). As *ZmMATE2* was physically predicted at 20.60 Mbp on chromosome 5, this gene was not included within our *qALT5*. Additionally, maize NILs for *ZmMATE2* were as Al-sensitive as their recurrent line L53, indicating that this candidate gene was not controlling Al tolerance in our population. Another suggestive probability peak was detected close to *qALT5*, at 69.30 Mbp, but without reaching the significance level determined by the complete model. Interestingly, in this neighborhood we identified the predicted gene GRMZM2G168747 at 74.61 Mbp, sharing 83% of sequence identity to rice *Nrat1*. *ZmNrat1* was specifically expressed in maize roots, showing a differential expression profile along the first three centimeters of the seminal root tips from 1 to 72 hours of Al exposure. The expression pattern of ZmNrat1 was compatible to the results from a microarray experiment in maize (Maron et al., 2008) and to its putative homologue from rice (Xia et al. 2010). Our results suggest that further experiments are required to validate the involvement of this candidate gene in maize Al tolerance.

2. Validation of* ZmMATE *genes or Al tolerance QTLs in a panel of Kenyan and Brazilian maize lines

We characterized 353 Kenyan and 79 Brazilian maize lines for Al tolerance, which were partially genotyped with 1085 SNPs. A group of maize lines selected based on Al tolerance and genetic diversity was evaluated for *ZmMATE1* expression. Besides Cateto Al237, only seven Brazilian and three Kenyan maize lines showed the Al-induced *ZmMATE1* expression. All Brazilian lines were derived from L228-3, a dent elite line developed by Embrapa breeding program. However, both lines developed in Kenya were derived from crosses using Cateto Al237. Thus, *ZmMATE1* was not expressed in two major Kenyan sources of Al tolerance, 203B and CON5. Additionally, no QTL for Al tolerance was mapped on chromosome 6 using 203B as Al tolerant line, confirming that ZmMATE1 was not functional in this germplasm.

3. Tools for marker-assisted selection to improve Al tolerance in Kenyan lines

Three adapted Kenyan lines were identified as donor sources of *qALT6*, CatetoAl237xL3-5, ATPS4SintOPVxR12C10-5 and SynthAlxR12C10-8. Even without specific markers for *qALT6*, two SNPs were identified flanking the *qALT6*, PZA00606_4 and PHM15961_13, among this group of maize lines. However, the recurrent lines have to be selected based on the interest for Kenyan breeding program. Additionally, molecular data of 1085 SNPs is available for this group of Kenyan lines that can be applied in a marker-assisted backcross program to speed up the development of maize cultivars well adapted to acid soils. These useful tools were transferred to Kenyan breeds in the scope of GCP maize MAS project (G7010.03.05).

Tangible outputs delivered

a) Functional *ZmMATE* genes and/or Al tolerance QTLs in maize;
b) Identification of sources of functional *ZmMATE1* in Brazilian and Kenyan maize germplasm;
c) Molecular markers flanking *ZmMATE1* polymorphic in a group of Brazilian and Kenyan lines
d) 1085 SNP markers evenly distributed along maize genome in a group of Brazilian and Kenyan lines that can be used for MABC

References

Maron LG, Guimarães CT, Kirst M, Albert PS, Birchler JA, Bradbury PJ, Buckler EF, Coluccio AE, Danilova TV, Kudrna D, Magalhaes JV, Piñeros MA, Schatz MC, Wing RA, Kochian LV (2013) Aluminum tolerance in maize is associated with higher MATE1 gene copy number. *Proc Ntl Acad Sci USA*, March 11.

Maron LG, Kirst M, Mao C, Milner MJ, Menossi M, Kochian LV (2008) Transcriptional profiling of aluminum toxicity and tolerance responses in maize roots. *New Phytol*, 179:116-128.

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

38. G7010.03.03: Establishing a molecular breeding programme based on the aluminium 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 and lead institute
Eva Weltzien, ICRISAT-Mali, E.Weltzien@icrisatml.org; B.P. 320 Bamako, Mali; Tel: (223) 20223375, Fax: (223) 20228683

Collaborating institutes and scientists

- Moi University, Kenya: Sam Gudu
- Kenya Agricultural Research Institute (KARI): Dickson Ligeyi
- Institut National de Recherche Agronomique du Niger (INRAN) Soumana Souley
- EMBRAPA, Maize and Sorghum, Brazil: Robert Schaffert, Jurandir Magalhaes, Alvaro V. Resende, João H. Viana, Claudia T. Guimarães, Sylvia M. Souza and Vera Alves
- Cornell University, Institute for Genomic Diversity, Theresa Fulton, Sharon Mitchell
- USDA-ARS Robert Holley Center for Agriculture and Health, USA: Leon Kochian
- ICRISAT–Niger: C. Tom Hash (replacing Bettina Haussmann)
- ICRISAT–Mali: Fred Rattunde

Summary
188 sorghum genotypes from West and Central Africa were evaluated for grain yield under low and high P soil conditions in Mali, Niger and Senegal from 2006-2012. From all 188 genotypes crown root angle, mycorrhiza infestation and P uptake rates were measured in 2011 in a pot trial with low P soils. All 188 genotypes were genotyped with Genotyping-by-Sequencing at Cornell University yielding 308 623 SNPs across the whole genome after imputing missing data. SNPs in six different candidate genes for various P adaptation mechanisms were identified after sequencing their PCR products across all 188 genotypes at the University of Hohenheim. Additionally, all genotypes were evaluated for 31 SNPs in eight sorghum candidate genes for Pup1 and 9 SNPs for the aluminum tolerance locus AltSB. Only 9% of the 188 genotypes carried the alleles for aluminum tolerance. Genome-wide-association mapping and genomic selection for grain yield and different adaptation mechanisms are currently carried out.

1. Research Activity: Phenotyping
In 2012 all field trials for phenotypic evaluation under low and high P conditions of 188 sorghum genotypes from West and Central Africa were completed. Due to environmental problems during the cropping seasons only 27 trials (14 –P and 13 +P) showed large enough yield reductions in –P compared to +P thus assuring high quality P stress data. Relative small GxE-interaction and very small GxP-interaction was detected, pointing to a general good adaptation of West African sorghums to low P soils (Table 1). A broad sense heritability of 0.89 (based on Cullis et al., 2006) across all trials (–P=0.79; +P=0.76) confirms the low GxE and the possibility of selecting across environments. Nevertheless, direct selection under –P conditions might still be superior and advisable as shown by Leiser et al. (2012).

Table 1: Variance components (+ s.e.) of a combined REML analysis for grain yield across 14 low P and 13 high P environments.

Random Term	Component	s.e.
Gen	852.5	136.4
Gen x Year	159.1	40.4
Gen x Loc	263.5	52.1
Gen x P_level	6.8	16.4
Gen x Year x Loc	217.9	35.2
Gen x Year x P_level	90.8	20.3
Gen x Loc x P_level	74.2	17.6

Data from our pot trial show that there is significant genotypic variation among genotypes for mycorrhiza infestation and crown root angle. But both traits showed rather low heritability values thus genotypic selection for those traits in such a pot trial is not very promising. Further we could not detect any relationship between mycorrhiza or root angle and grain yield under field conditions. Nevertheless, landrace genotypes, which showed specific –P adaptation, had more shallow roots and higher mycorrhiza infestation rates, thus both traits can be of importance for genotypic adaptation to low P soils. The non existing relationship of both traits to actual grain yield under low P conditions points to the different mechanisms used by the genotypes.

2. Research Activity: Genotyping
All 188 genotypes were genotyped at Cornell University with genotyping-by-sequencing (GBS). After filtering and imputation of missing SNPs with the software NPUTE (Roberts et al., 2007)such as disease association studies. Common remedies for this problem include removing affected markers and/or samples or, otherwise, imputing the missing data. On small marker sets imputation is frequently based on a vote of the K-nearest-neighbor (KNN we could detect 308 623 SNPs across the whole genome. Our imputation accuracy was always above 96% for each separate chromosome.

Across all 188 sorghum genotypes six candidate genes, namely OsPHR2, SIZ1, PHO2, OsSPX1, PHT1, Pht1;6 were amplified using PCR, sequenced, aligned and SNPs were detected with a call rate of 65-80% and 1-10% polymorphism. Generally only a few SNPs per gene could be detected (0-9 SNPs). Additionally, all genotypes were genotyped with the KASPAr system at KBioscience for 31 SNPs in eight sorghum candidate genes for the Pup1 locus. All these SNPs are ready for use for further analysis in a candidate gene approach association study for adaptation of sorghum to low P conditions and validation of the Pup1 gene in West African sorghums. Furthermore all genotypes were evaluated for the aluminum tolerance locus Alt_{SB}. Out of the 188 genotypes 9% carried the alleles for aluminum tolerance, thus showing the need to increase Alt_{SB} in our breeding material.

References

Cullis, B.R., A.B. Smith, and N.E. Coombes. 2006. On the design of early generation variety trials with correlated data. J. Agric. Biol. Environ. Stat. 11: 381–393. doi:10.1198/108571106X154443.

Leiser, W.L., H.F.W. Rattunde, H.-P. Piepho, E. Weltzien, A. Diallo, A.E. Melchinger, H.K. Parzies, and B.I.G. Haussmann. 2012. Selection Strategy for Sorghum Targeting Phosphorus-limited Environments in West Africa: Analysis of Multi-environment Experiments. Crop Sci. 52: 2517–2527. doi:10.2135/cropsci2012.02.0139.

Roberts, A., L. McMillan, W. Wang, J. Parker, I. Rusyn, and D. Threadgill. 2007. Inferring missing genotypes in large SNP panels using fast nearest-neighbor searches over sliding windows. Bioinformatics. 23: i401–i407. doi:10.1093/bioinformatics/btm220.

39. G7010.03.04: Developing rice with dual tolerance of phosphorus deficiency and aluminum toxicity: marker-assisted pyramiding of Pup1 with novel tolerance QTLs.

- *April 2010–March 2014*

Principal Investigator and lead institute

Sigrid Heuer, International Rice Research Institute, replaced 01st April 2013 by
Tobias Kretzschmar, International Rice Research Institute, t.kretzschmar@irri.org DAPO Box 7777, 1301 Metro Manila, Philippines, Tel: +63 (2) 580 5600 ext 2761/2253

Collaborating institutes and scientists

- International Rice Research Institute, Abdelbagi Ismail
- Japan International Research Center for Agricultural Sciences, Matthias Wissuwa
- Institute for Agricultural Biotechnology & Genetic Resources Research and Development, Sugiono Moeljopawiro
- Institute for Agricultural Biotechnology & Genetic Resources Research and Development, Joko Prasetiyono

Phosphorus deficiency and aluminum toxicity are soil-related abiotic stresses that typically coexist on the acid soils that are very common in the humid tropics. Both stresses severely inhibit root growth and it is therefore important to develop rice with tolerance of both, Al toxicity and P deficiency. For P-deficiency tolerance, the major QTL *Phosphorus uptake 1* (*Pup1*) is available and its beneficial effect under P deficiency has been demonstrated in different environments (Japan, the Philippines, Indonesia) and genetic backgrounds (Nipponbare, IR64, IR74, Situ Bagendit, Dodokan) (Ref 1-2). For Al-toxicity tolerance, a tolerant variety from Indonesia (Dupa) has been identified and the underlying QTLs will be mapped within this project.

One of the main objectives of this project is to develop and disseminate molecular markers and tolerant breeding materials to NARES in Asia and Africa for the improvement of yield in stress environments and low-input systems.

Findings and implications:

The major gene in the *Pup1* locus has now been cloned and functionally characterized via transgenic validation approaches. The gene was named *Phosphorus starvation tolerance 1* (*OsPSTOL1*) and codes for a Ser/Thr protein kinase. Our current understanding of the gene function suggests that it acts as an enhancer of root growth and that the *OsPSTOL1*-mediated larger root system provides better access to P and other nutrients. The *OsPSTOL1* data were published in August 2012 in *Nature* (Ref 3) and received considerable international attention by the scientific community as well as public media.

In order to disseminate the *Pup1* molecular marker technology and initiate breeding of tolerant rice varieties across Asia, a training workshop with participants from seven Asian countries was held in August 2012.

The IR64-Pup1 and IR74-Pup1 breeding lines have been further advanced and evaluated in two field experiments at IRRI. Ten Pup1 lines of BC2F5 generation were screened under phosphorus deficient conditions. Agronomic data were collected, and the lines showing the best performance were selected for SNP genotyping for the latest confirmation. Likewise, the Indonesian Pup1 breeding lines have been further

evaluated at ICABIOGRAD and at IRRI. The data are now being compiled for final analyses and selection of the best lines for seed dissemination

Next steps

Mapping of the aluminum (Al) tolerance QTL from Indonesian donor variety Dupa will be continued. The hydroponics-based phenotyping protocol has been optimized and the mapping population was phenotyped. The population will be genotyped within the coming months.

Various physiology studies will be conducted to reveal the mechanism of Aluminum tolerance. Dupa and five other tolerant varieties will be tested. The physiology study will include: elemental analysis, ROS detection, Rhizosphere and pH barrier leakage and root cell wall lignifications. A preliminary study was conducted in May 2013. Forty Indonesian local varieties were screened for tolerance to Aluminum toxicity by using hydroponic Magnavaca solution. The data is being analyzed, and the five most tolerant plants will be included in to the physiological studies.

Dissemination of Pup1 seed material to participants of the Pup1 workshop is in its final stages. Once finalized, it will lead to relevant field trials being conducted in Vietnam, Thailand, India, Nepal and Tanzania as well as further locations within the Philippines.

An additional candidate with likely implications on phosphorous starvation tolerance from the Pup1 locus is currently being investigated in depth via transgenic validation studies.

References

1. Chin JH, Gamuyao R, Dalid C, Bustamam M, Prasetiyono J, Moeljopawiro S, Wissuwa M, Heuer S. 2011. Developing rice with high yield under phosphorus deficiency: *Pup1* sequence to application. Plant Physiol. 156:1202-1216.
2. Chin JH, Lu X, Haefele SM, Gamuyao R, Ismail AM, Wissuwa M, Heuer S. 2010. Development and application of gene-based markers for the major rice QTL *Phosphate uptake 1* (*Pup1*). Theor. Appl. Genet. 120(6):1073-1086.
3. Gamuyao R, Chin JH, Pariasca-Tanaka J, Pesaresi P, Dalid C, Slamet-Loedin I, Tecson-Mendoza EM, Wissuwa M, Heuer S. 2012. The protein kinase pstol1 from traditional rice confers tolerance of phosphorus deficiency. Nature 488:535-541.

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

- *April 2010–March 2014*

Principal Investigator and lead institute

Samuel Gudu, Moi University, samgudu2002@yahoo.com P.O Box 3900-30100 Eldoret Kenya.

Collaborating institutes and scientists:

- Moi University Kenya: E. Ouma;
- University of Eldoret: B. Were
- Kenya Agricultural Research Institute, Kitale: D.O Ligeyo
- Embrapa Maize and Sorghum (Brazil): C.O. Guimarães; S. Parentoni; J. Magalhães; V. Alves; S. Sousa; L. Guimarães
- JIRCAS: M. Wissuwa A. Ismail;
- IRRI: A. Ismail, S. Heuer
- USDA-ARS (Cornell University): L. Kochian; J. Shaff, L. Maron; M. Pineros; J. Liu, Ed Buckler

Background

The goal of the project is to improve maize grain yields in acid soils of Kenya where yields are depressed by low available phosphorous and aluminium toxicity amongst other factors. The summary of results obtained in genetic diversity among maize inbred lines using SNP markers, mapping of Al toxicity tolerance gene(s), solution culture screening and determination of Al induced ZMATE gene expression in these inbred lines that we now use in developing maize hybrids and synthetics is given below.

Genetic Diversity among maize inbred lines being used to develop varieties adapted to acid soils

A total of eighty six (86) diverse inbred lines of maize used for breeding for Al tolerant and Phosphorus use-efficiency maize varieties were genotyped with 1250 SNP markers to determine genetic diversity among them. The SNPs classified the 86 maize lines into 3 major groups that reflect their geographical origin and interbreeding. High genetic distances among and within accessions in the pool reveal high diversity and provides a wealth of variation that are currently being harnessed in our maize breeding program to develop maize cultivars adapted to Al toxic and Phosphorus deficient acid soils that limit maize production in Kenya.

Evaluation of aluminium toxicity tolerance of maize inbred lines in nutrient solution

A total of two hundred (235) maize inbred lines were screened for tolerance to Al in the standard Maganavaca et al. (1987) nutrient solution without or with Al stress at a concentration of 39 µM Al^{3+} activity, respectively. The inbred line 203B-14, a derived of the Kenyan 203 population, was ranked as the most tolerant line in this study. The top 20 positions of most tolerant lines were dominated by derivatives of the Kenyan 203B and CATETO from Brazil. The study confirms the superiority of the 203B population as a new major source of tolerance to Al toxicity and the potential of its derivatives in developing maize cultivars adapted to acid soils. Ten distinct clusters based on Relative Net Root Growth (RNRG) of seedlings subjected to 39 µM Al activity is shown in Table 1 below.

Table 1. Cluster analysis using Scott and Knott's method showing the different clusters based on Al tolerant capacity as measured by the RNRG of the 235 lines. Means were compared at a significance level of $p < 0.05$.

	RNRG			
Group	Lowest limit	Highest limit	accessions	% of Accessions
a	1.05	1.16	04	01.70
b	0.95	1.04	06	02.55
c	0.80	0.94	24	10.21
d	0.68	0.79	24	10.21
e	0.57	0.67	33*	14.04
f	0.49	0.56	30	12.77
g	0.40	0.48	33	14.04
h	0.30	0.39	47	20.00
i	0.20	0.29	28**	11.91
j	0.10	0.19	06	02.55

* and ** Groups where the tolerant and susceptible controls are located respectively

Mapping the aluminium tolerant gene(s) in Kenyan Al tolerant maize line 203B

An F2:3 mapping population derived from a cross between 203B-1 × SCH3 was generated between the highly Al tolerant maize line (203B-1) and a sensitive line (SCH3) from Kenyan. The F2 population was genotyped using 183 polymorphic SNP markers between the two parents. Novel genomic regions associated with tolerance to Al not coincident with earlier reported genomic regions were identified in the 203B-1. This line together with other derivatives of 203B are useful in the development of maize varieties for acid soils of the world.

Determination of Al induced ZmMATE1 gene Expression.

Forty Kenyan maize inbred lines derived either from Brazilian CATETO, the Al tolerant standard, or purely from Kenyan sources were used to determine Al induced ZMATE gene expression using Quantitative real-time PCR to quantify *Zm*MATE1 RNA transcripts. Using the non-responsive L53 Al sensitive reference line, *Zm*MATE1 expression levels were as high as 16 folds in the accession SYN AL × R12C10 – 8 and other lines that had CATETO background, but as low as 0.54 folds in the accession MUL 891 and all the Kenyan inbred lines without CATETO genetic background even when they are highly Al tolerant. Most of the inbred lines from Kenya, including those that exhibited high Al tolerance under nutrient solution culture, however, exhibited exceptionally low levels of the gene (*Zm*MATE1 expression < 2 folds). This suggests a different gene from *Zm*MATE1 could be responsible for their Al tolerance.

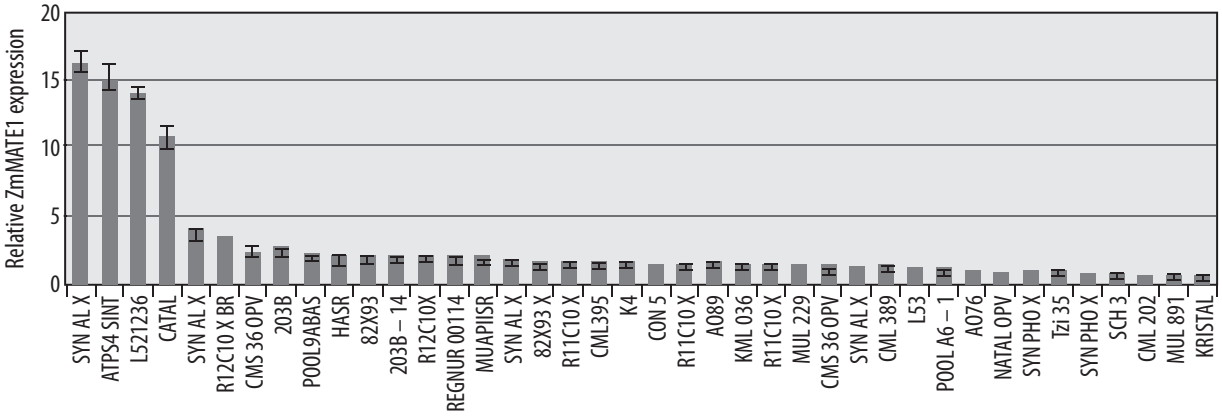


Figure 1. *Zm*MATE1 relative expression levels among 40 accessions after 6 hours of exposure to Al^{3+} ions at an activity of 39 µM Al activity in nutrient solution culture.

41. 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, and identification of other P efficiency QTLs

- ***April 2010–March 2014***

Principal Investigator and lead Institute
Jurandir Magalhães, Embrapa Maize and Sorghum; jurandir@cnpms.embrapa.br
Rd MG 424, Km 65, Sete Lagoas - MG 35701-970, Brazil; +55 31 30271283; Fax: +55 31 30271279

Collaborating institutes and scientists

- Embrapa Maize and Sorghum (Brazil): Robert Schaffert, Claudia Guimarães, Vera Alves, Sylvia Morais de Souza, Alvaro Vilela Resende, João Herbert Moreira Viana USDA-ARS Robert W. Holley Center for Agriculture and Health (US): Leon Kochian, Jiping Liu, Randy Clark, Zhangjun Fei
- Boyce Thompson Institute – Cornell University (US): Zhangjun Fei
- Institute for Genomic Diversity – Cornell University (US): Steve Kresovich, Martha Hamblin, Sharon Mitchell, Theresa Fulton
- INRAN (Niger): Soumana Souley
- 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. *Pstol1* (formerly designated *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 have cloned the gene encoding *Pstol1*, which was found to encode a protein kinase that enhances early root growth and P acquisition (Gamuyao et al., Nature 488:535-539, 2012). This project is establishing a framework based on comparative genomics to identify sorghum *Pstol1* 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 *Pstol1*candidate genes and P efficiency assessed both in the field and under controlled conditions in the laboratory and greenhouse. Our preliminary data generated so far based on association analysis suggests a role for *Pstol1* homologs in controlling changes in root morphology and P acquisition in sorghum. These results are now being validated by means of bi-parental QTL mapping. 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

In the 2012 annual report we presented the following partial results:

1 – A bioinformatics and phylogenetic analysis was undertaken to identify likely homologs in sorghum of the *Pstol1* gene from rice.
2 – Amplicons within *Pstol1* homologs were sequenced in a small discovery panel and SNPs and indels at a frequency compatible with association analysis were identified.
3 – These SNPs were converted into the KASPar system from LGC Genomics (http://www.lgcgenomics.com/) and our sorghum association panel was genotyped with those markers. The panel was also characterized for population structure.
4 – The association panel was phenotyped for root system morphology using 2D imaging. In addition, the panel was phenotyped for phosphorus efficiency traits in the field in a low-P site in Brazil.
5 – Phenotyping for P efficiency traits was also conducted in the panel in a low-P site in Tsukuba - Japan. In addition, selected sorghum accessions from West Africa and from the association panel were phenotyped for root traits using the "shovelomics" approach.
6 – A system to phenotype the sorghum association panel for root architecture (3D analysis) was developed at Cornell University.
7 – We simulated type I error using the unified mixed model developed by Yu et al. (Nature Genetics 38:203-208, 2011) considering population structure (Q) and kinship (K) alone in addition to a Q +K model and defined the best model for association analysis.
8 – Association analysis was conducted to link allelic variation at *Pstol1* homologs and traits related to P acquisition.

The most important result so far is the detection of significant associations for root morphology and traits related to P acquisition. Due to the quantitative nature of those traits, further validation is needed. For that, we phenotyped a large RIL population (400 individuals) for root morphology. In addition, this population was genotyped by GBS (Elshire et al., PLoS One 6:e19379, 2011). Missing data was inputed using NPUTE (http://compgen.unc.edu/wp/?page_id=57) and a QTL scan was conducted using initially a General Linear Model (GLM). Figure 1 below shows initial results for the QTL scan based on root volume, indicating a QTL peak colocalized with *Pstol1* homologs on chromosome 3.

3 – Next steps

(1) Conclude the QTL analysis for validation of the association results, (2) phenotype the sorghum association panel for root architecture and conduct association analysis, (3) select markers for genotyping different sorghum materials.

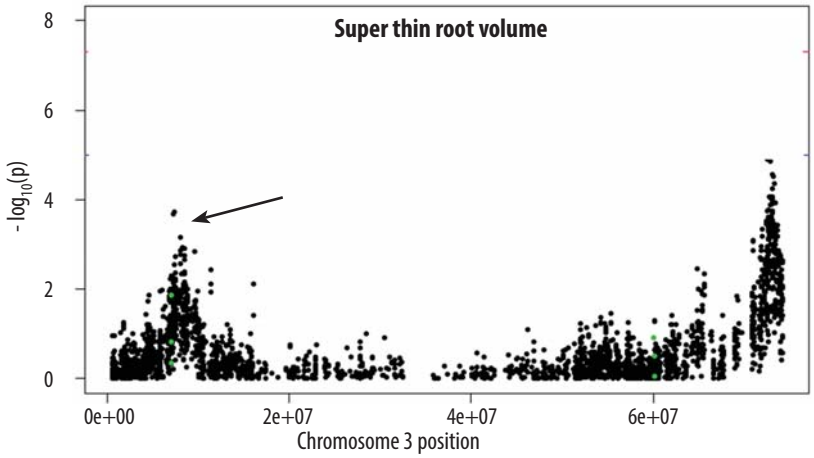


Figure 1: QTL scan for root volume based on GBS data. Points in red are SNPs within *Pstol1* homologs. The arrow indicates colocalizaton between *Pstol1* homologs and a QTL for root volume.

Theme 3 – Crop information systems

Projects for this theme are listed below under Integrated breeding information system

Theme 4 – Capacity building

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

- *March 2008–February 2014*

Principal Investigator and lead institute

Eric Danquah, WACCI, University of Ghana, edanquah@wacci.edu.gh PMB 30, Legon, Ghana; (233) 21-520605-8; 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

Abstracts

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 first phase of the WACCI PhD which was a 5-year programme ended in December 2012. The second phase began in January 2013 and is now a 4-year programme. In this new phase, students undertake one year of coursework and advanced modules on special topics in plant breeding and related areas at the University of Ghana. In the second year, students return to their research institute for two and a half years of field research. They then return to the University of Ghana in the final six months to complete and submit their theses.

Currently, the Programme is in the sixth year and has enrolled a total of fifty-four students from eight West and Central African countries (Burkina Faso, Cameroon, Ghana, Kenya, Mali, Niger, Nigeria and Senegal). Four of the fifty-four students are supported by grants from the Generation Challenge Programme (GCP).

Table 1. 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, Dramane Sako and Ruth Thompson are in the final year and are currently in their home institutes rounding up work on their thesis research. They would return to WACCI to complete and submit their theses from August to November, 2013.

Below is a list of the students, their thesis topics and supervisory committees.

Table 2. List of Students in the final year

No.	Name	Thesis Topic	Supervisory Committee
1	Dramane Sako	Quantitative Trait Loci analysis for yield components and panicle architecture in sorghum	E.Y. Danquah*, V. Gracen, S. K. Offei
2	Thompson Ruth N.A.	Delayed post -harvest physiological deterioration in cassava	S. K. Offei*, K. Asante, E.Y. Danquah

Names with* - Main supervisors

The remaining two students on GCP support are currently in the fourth year and are also conducting their field research in their home institutions. Supervisory visits to these students in their home institutions have been scheduled for June, 2013. Below is a table of the thesis topics and supervisory committees of the students.

Table 3. Thesis topics of the fourth year students

No.	Name	Proposed Thesis Topic	Supervisory Committee
1	Joseph Benoit Teyioue Batieno	Genetic analysis of drought tolerance and resistance to macro-phomina phaseolina in cowpea.	E.Y. Danquah*, M. Yeboah, K. Ofori
2	Joseph Adjebeng-Danquah	Genetic studies of cassava genotypes for improved yield under drought environments of northern Ghana	S. K. Offei*, K. Asante, V. Gracen

Names with* - Main supervisors

Training of the four students at the PhD Level is on-going and all four students are expected to submit their PhD thesis for review and examination.

III. Services

G8009 Integrated Breeding Platform (IBP)

- *July 2009–July 2014*

Project details can be found in the table at the end of the narrative.

Introduction

The Integrated Breeding Platform (IBP) project aims to create a sustainable, web-based, 'one-stop shop' for information, analytical tools and related services for the design and execution of integrated breeding (IB) projects, particularly targeting developing country breeders.

Progress

Management and communication: Pascal Flament of Groupe Limagrain took over as Chairman of the IBP Scientific and Management Advisory Committee. An experienced private-sector breeder, Hamer Paschal, was added to the Project Management Team. Two private companies, Efficio LLC and VSNi, were contracted to fill capacity gaps.

Communication: The IBP web portal had well over 700 registered clients by the end of 2012 and continues to carry varied information and news of interest to our clients. A Chinese-language version of the portal was launched in June 2012.

Business Plan: The IBP Operational/Business Plan remained in abeyance, but discussions on the second phase of the project will include defining a sustainability strategy.

Capacity building: The three-year Integrated Breeding Multiyear Course (IB–MYC) commenced in 2012, focusing CB efforts on a selected group of developing country researchers for maximum impact. Two workshops were organised for field technicians. A learning resources section was established on the IBP portal.

Communities of Practice (CoPs): After the first seven, the establishment of additional CoPs was suspended to allow for a strategy review to further advance the concept. The Cassava CoP was expanded to an additional nine African countries: Côte d'Ivoire, DR Congo, Ethiopia, Kenya, Liberia, Malawi, Mozambique, Sierra Leone and South Sudan, and held its meeting in Dar es Salaam, Tanzania, in August 2012. The Sorghum CoP utilised the facilities of the IBP portal to collaboratively refine the sorghum trait dictionary. The Data Management CoP was involved in the IB–MYC training programme, with a view to identifying and training future trainers.

IBP information system and tools

Existing tools for data management and breeding logistics: IBP continued to support legacy International Crop Information System (ICIS) tools, but this declined significantly during 2012 and is to cease in 2013.

Integrated Breeding WorkBench administration and configuration application: Efficio LLC was contracted to develop the IB WorkBench - a prototype of which was demonstrated during the IBP annual meeting. The WorkBench integrates germplasm and phenotyping data browsing tools, the Breeding Manager application, the IB FieldBook, the Breeding View statistical pipeline and the OptiMAS decision support tool. Other applications will be added as they become available.

Breeding Manager Application: This application was released in June 2012 and, based on initial user feedback, improvements were made to the functionality for managing seed inventories and to the user interface for the germplasm import tool and the crossing manager tool. Prototypes of the pedigree input tool, the pedigree editor and a Germplasm Browser were also produced for testing.

Integrated Breeding Field Trial Management System application: Optimisation of the crop ontology system was completed. A set of 50 key breeding traits was consultatively identified for each supported crop and incorporated into the IB FieldBook. The first version of the Integrated Breeding Database (IBDB-v1) was designed. The redesign of the IB phenotypic database was contracted to a team led by Efficio LLC. Version 2 of the IB FieldBook (IB–FB) was released in June 2012. This version enables users to launch the IB–FB both as a stand-alone application and from the IB WorkBench, and includes enhanced functions for data import, export, exchange and analysis.

Integrated Breeding Genotypic Data Management System (GDMS) application: The GDMS was refined to handle marker and genotyping information as well as maps and quantitative trait loci (QTLs), and to allow for exchange of data with the IBP analytical tools using the Flapjack file format. Fingerprinting/genotyping data from GCP-funded projects were loaded into the system and testing commenced.

Integrated Breeding Analytical Pipeline application: Considerable progress and improvements were made on the following phenotypic data analysis application tools in Genstat and R: single-site analysis tool (SSA), multienvironment analysis tool (G × E), variance component analysis tool and mixed model G × E tool. Good progress was also made on the QTL analysis tool, QTL × E analysis tool and genetic diversity tool, which are the components of the Molecular Genetic Analysis application. Code for the QTL analysis tool (single- and multienvironment) was upgraded and released through the IBP portal. A beta version of the R analytical pipeline (R-AP) was developed, including modules for analysing single- and multisite plant breeding trials, linkage mapping using bi-parental populations, computing selection indices and estimation of genetic parameters using common mating designs. A private sector provider, VSNi, was contracted to develop it. A beta version of Breeding View was released to selected testers in December 2012, extending the current pipelines for single-trait analysis and single-trait QTL linkage analysis to run for multiple traits. A new pipeline was incorporated for G × E analysis. Improvements were made in interoperability with other tools.

Integrated Breeding Decision Support System application: The Marker-assisted Back-crossing tool (MBDT) was enhanced: improvements were made to the GUI; users are now able to import data containing multiple marker types; and, the tool can now read directly from the IB database. The first stable stand-alone version of OptiMAS with a GUI was distributed at the IBP annual meeting for testing and released through the IBP portal in October 2012. The input file was changed to the Flapjack format to allow interoperability, and was reinforced to prevent dataset errors. Faster computation, better visualisation of results and improved selection options were implemented. A new user support website for the tool was completed.

Simulation applications: Functional enhancements were made to the QuGene GUI. All help documents are now located in a simple drop-down menu at the top, and button options are more easily accessible. A new tool, NetCDFViewer, was developed to summarise and display QuGene outputs. Progress was made on the implementation of QuGene online within the iPlant Collaborative infrastructure – additional output control was built to facilitate compilation and summarization of results from very large simulations that are possible on using iPlant. Other enhancements allow the storage and management of massive simulations via NetCDF database tools. The simulation component of the project will, however, be discontinued after June 2013, as the project focuses more on aspects that are a higher priority for IBP's key target groups.

Services

Breeding Plan Development: Prototypes for the planning tool for marker-assisted recurrent selection (PMARS), the planning tool for marker-assisted backcrossing (PMABC) and the planning tool for marker-assisted selection (PMAS) were completed in November 2012. The current versions can help breeders design a breeding scheme by choosing parents and making crosses up to the development of the final line.

Genotyping services: Four service providers were retained in 2012: KBioscience/LGC Genomics (KBLG, for SNPs), and BecA, DNA Landmarks and ICRISAT (all SSR). Since most of the projects involve SNP markers, KBLG has become the dominant service provider. 30 marker service requests were handled. A number of clients also contacted KBLG directly, but used SNP markers developed through GCP. Conversion of 1,000 to 2,000 SNPs to the KBLG KASPar platform is now complete for 10 important crops – maize, cowpeas, chickpeas, pigeonpeas, rice, cassava, sorghum, wheat, soya beans and common beans. The Genotyping Support Service continued to introduce new users to molecular breeding through a system of commissioned projects targeting collaborators in developing countries.

Information management and data curation services: These services were enhanced by training and support functions provided by four expert consultants. Inventories of datasets from the nine supported crops were established as part of these efforts to assist partners to curate and appropriately format the data from their projects. The nine central crop databases were made available for download from the IBP portal. A web application to query these databases was also installed for beans,

cassava, cowpeas, maize, rice and wheat. All nine crop databases were updated to work with the IB FieldBook. Additional tablets were distributed for data collection in the field, bringing the total number to 216 (20 in 2011 and 196 in 2012). Information management and data curation were covered in depth during the Year 1 IB–MYC workshops. A participatory process to establish and document 50 important breeding traits was completed for GCP's nine target crops.

Design and analysis service: This service is well established. Training was delivered within the IB–MYC, and necessary consultation and support services were provided via email and other remote means.

Genetic Resources Support Service: This service was put in abeyance, following severe challenges with the validation of the GCP reference sets, and the uncertainty engendered by the CGIAR reforms. The reference sets remain viable but access will be directly through the originating CGIAR Centres. To serve the needs of breeders in the interim, a webpage has been established on the IBP portal that provides a convenient link to GENESYS – the leading genetic resources service.

Trait and metabolite service: Development of this service was not pursued given that it is not a priority with partners. However, contact information for selected service providers is maintained on the IBP portal.

Phenotyping sites and screening protocols: Infrastructure improvements at selected field sites were largely completed in 2012 – targeting national institute field stations in nine African countries (Burkina Faso, Ethiopia, Ghana, Mali, Mozambique, Niger, Nigeria, Kenya and Tanzania) and two Asian countries (China and India).

Intellectual property and policy service: There was limited demand for this service; the few queries received were referred to relevant experts.

Challenges and lessons learnt

The implementation of a detailed project management system has helped in tracking activities and communicating expectations to the partners, a challenge that has dogged the project from the beginning. GCP also invested in the development of a single simple middleware layer to be used by all development teams to overcome the challenge of ensuring compatibility between applications and avoiding duplication of functions.

Collaborating partners, mainly CGIAR Centres, continued to suffer difficulties with recruitment and retention of appropriate staff. It became necessary to contract more of the development work to private providers to ensure adherence to timelines. However, IBP continued to work hard to keep the larger CGIAR Centres engaged, to safeguard future adoption, development and sustainability. The team of 'early adopters' involved in rigorous testing of IBP tools include many scientists at these Centres.

The need for training in molecular breeding, data management and statistical analysis remained high. IB–MYC was initiated to address this, and was well received.

Conclusions perspectives on 2013

Good overall progress was made in 2012. However, delays in the development of the IB FieldBook forced a postponement of the official launch of IBP. A generic version of the IB FieldBook will be ready in the first quarter of 2013, allowing the release of a pilot version of the consolidated IBWS to early adopters, providing more opportunities for improvements and customisation on a crop-by-crop basis. Existing crop databases will be converted to the new format, and the redesign of the IB phenotypic database is also expected to be completed by mid-2013.

The services and capacity building areas of the IBP have continued to perform well and meet the project objectives. The whole IBP web portal will be redesigned to better fit and represent the IBWS concept, with its focus on breeders.

The remaining year and a half of the GCP is expected to be characterised by a diligent adherence to milestones, the release of stable user-friendly informatics tools and provision of quality services that will together effectively underpin the adoption and sustainability of IBP into the future.

Integrated Breeding Platform portal and helpdesk

#	Projects: Axapta No	Projects: Project Title	Principal Investigator	Target Countries	Target Crops	Target Traits	Partners	Project Dates: Start	Project Dates: End
	Component 1: Integrated Breeding Platform portal and helpdesk								
	G8009.01 – Objective 1.1: Establish and manage the Integrated Breeding Platform (IBP)								
1	*G8009.01.01*	*Activity 1.1.1: Establish and manage the IBP*	Xavier Delannay, GCP	Global	Global	Global	AAFC, Agropolis–INRA, BI, CAAS, CIAT, CIMMYT, CSIRO, ICRISAT, IRRI, KUL, UQ, WUR	July 2009	July 2014
2	*G8009.01.02*	*Activity 1.1.2: Develop and deploy the IBP web portal*	Fred Okono, GCP	Global	Global	Global		July 2009	July 2014
3	*G8009.01.03*	*Activity 1.1.3: Establish IBP Helpdesk and coordinate training and communication activities*	Ndeye Ndack Diop, GCP	Global	Global	Global		July 2009	July 2014
4	*G8009.01.04*	*Activity 1.1.4: Establish and support crop molecular breeding communities of practice*	Ndeye Ndack Diop, GCP	Global	Global	Global		July 2009	July 2014

Integrated breeding information system

#	Projects: Axapta No	Projects: Project Title	Principal Investigator	Target Countries	Target Crops	Target Traits	Partners	Project Dates: Start	Project Dates: End
	Component 2: Integrated breeding information system								
	G8009.02 – Objective 2.1: Make existing tools for data management and breeding logistics available to molecular breeding projects through the IBP								
	G8009.02.01	*Activity 2.1.1: Identify, deploy and support tools to facilitate the management of germplasm lists, pedigrees, intellectual property and other passport data*	Shawn Yates and Fran Clarke, AAFC	Global	Global	Global	AAFC, Agropolis–INRA, BI, CAAS, CIAT, CIMMYT, CSIRO, ICRISAT, IRRI, KUL, UQ, WUR	July 2009	COMPLETED IN DECEMBER 2012
	G8009.02.02	*Activity 2.1.2: Identify, deploy and support tools to manage phenotypic characterisation and evaluation*	H Sánchez, CIMMYT	Global	Global	Global		July 2009	COMPLETED IN JULY 2012
	G8009.02.03	*Activity 2.1.3: Identify, deploy and support tools to manage genotypic characterisation*	Trushar Shah, ICRISAT	Global	Global	Global		July 2009	COLLAPSED INTO ACTIVITY 2.2.4 IN JUNE 2012
	G8009.03/G8009.04 – Objective 2.2: Develop and deploy an integrated breeding (IB) configurable workflow system								
5	*G8009.03.01*	*Activity 2.2.1: Develop and deploy an IB WorkBench Administration & Configuration Application*	Graham McLaren, GCP	Global	Global	Global	AAFC, Agropolis–INRA, Bioversity, CAAS, CCAFS, CIAT, CIMMYT, CSIRO, ICRISAT, IRRI, KU Leuven, UQ, WUR	July 2009	July 2014
6		*Subactivity 2.2.1.1: Develop an IB Workflow Administration Application*	Graham McLaren, GCP	Global	Global	Global		July 2009	July 2014
7		*Subactivity 2.2.1.2: Develop IB Project Configuration Application*	Graham McLaren, GCP	Global	Global	Global		July 2009	July 2014
8		*Subactivity 2.2.1.3: Develop Database back-end for the IB workflow*	Graham McLaren, GCP	Global	Global	Global		July 2009	July 2014
9	*G8009.03.02*	*Activity 2.2.2: Develop and deploy an IB Management System Application*	Graham McLaren, GCP	Global	Global	Global		July 2009	July 2014
10		*Subactivity 2.2.2.1: Develop Breeding Manager Application*	C Carreiro, CIMMYT	Global	Global	Global		July 2009	July 2014

#	Projects: Axapta No	Projects: Project Title	Principal Investigator	Target Countries	Target Crops	Target Traits	Partners	Project Dates: Start	Project Dates: End
11		*Subactivity 2.2.2.2: Develop Geneaology Manager Application*	William Eusebio, IRRI	Global	Global	Global		July 2009	July 2014
12		*Subactivity 2.2.2.3: Develop Query Manager Application*	Graham McLaren, GCP	Global	Global	Global		July 2009	July 2014
13	*G8009.03.03*	*Activity 2.2.3: Develop and deploy an IB Field Trial Management System Application*	Graham McLaren, GCP	Global	Global	Global		July 2009	July 2014
14		*Subactivity: 2.2.3.1 Develop a Trial FieldBook Application*	C Carreiro, CIMMYT	Global	Global	Global		July 2009	July 2014
15		*Subactivity: 2.2.3.2 Develop an Environment Characterisation System Application*	G McLaren, GCP	Global	Global	Global		July 2009	July 2014
16	*G8009.03.04*	*Activity 2.2.4: Develop and deploy an IB Genotypic Data Management System Application*	G McLaren, GCP	Global	Global	Global		July 2009	July 2014
17		*Subactivity 2.2.4.1: Develop a Genotypic Data Management Application*	Trushar Shah, ICRISAT	Global	Global	Global		July 2009	July 2014
18	*G8009.03.05*	*Activity 2.2.5: Develop and deploy an IB Analytical Pipeline Application*	Mark Sawkins, GCP	Global	Global	Global		July 2009	July 2014
19		*Subactivity 2.2.5.1: Develop a Data Management Application*	Mark Sawkins, GCP	Global	Global	Global		July 2009	July 2014
20		*Subactivity 2.2.5.2: Develop a Phenotypic data Analysis Application*	FA v Eeuwijk (WUR)	Global	Global	Global		July 2009	July 2014
21		*Subactivity 2.2.5.3: Develop a Molecular Genetic Analysis Application*	FA v Eeuwijk (WUR)	Global	Global	Global		July 2009	July 2014
22		*Subactivity 2.2.5.4: Develop a Selection Indices Application*	J Crossa (CIMMYT)/FA v Eeuwijk (WUR)	Global	Global	Global		July 2009	July 2014
23		*Subactivity 2.2.5.5: Develop a Selection Indices Application*	J Crossa (CIMMYT)/FA v Eeuwijk (WUR)	Global	Global	Global		July 2009	July 2014
24	*G8009.03.06*	*Activity 2.2.6 Genstat Breeding View*	Mark Sawkins (GCP)	Global	Global	Global		July 2009	July 2014
25		*Subactivity 2.2.6.1: Develop Breeding Decision Support Applications*	T Shah (ICRISAT)	Global	Global	Global		July 2009	July 2014
26		*Subactivity 2.2.6.2: Develop MARS Decision Support Application*	Alain Charcosset (CIRAD)	Global	Global	Global		July 2009	July 2014
27		*Subactivity 2.2.6.3: Develop Simulation Application*	M Dieters (UoQ)	Global	Global	Global		July 2009	TERMINATED JULY 2013
Component 3: Integrated breeding services									
	G8009.05 – Objective 3.1: Provide access to critical molecular breeding services								
28	*G8009.05.01*	*Activity 3.1.1: Genetic Resources Support Service*	Larry Butler, GCP	Global	Global	Global	AAFC, Agropolis–INRA, BI, CAAS, CCAFS, CIAT, CIMMYT, CSIRO, ICRISAT, IRRI, KU Leuven, UQ, WUR	July 2009	July 2014
29	*G8009.05.02*	*Activity 3.1.2: Marker Services*	Chunlin He, GCP	Global	Global	Global		July 2009	July 2014
30	*G8009.05.03*	*Activity 3.1.3: Trait and metabolite services*	Chunlin He, GCP	Global	Global	Global		July 2009	July 2014

#	Projects: Axapta No	Projects: Project Title	Principal Investigator	Target Countries	Target Crops	Target Traits	Partners	Project Dates: Start	Project Dates: End
	G8009.06 Objective 3.2: Provide assistance with a range of molecular breeding support services								
31	*G8009.06.01*	*Activity 3.2.1: Breeding Plan Development*	Mark Sawkins, GCP	Global	Global	Global	AAFC, Agropolis–INRA, BI, CAAS,	July 2009	July 2014
32		*Subactivity 3.2.1.2: PMB (Planning tool for molecular breeding projects – project appraisal and cost-benefit analysis to support decision-making)*	Mark Sawkins, GCP and Jiankang Wang, CIMMYT/CAAS	Global	Global	Global	CCAFS, CIAT, CIMMYT, CSIRO, ICRISAT, IRRI, KU Leuven, UQ, WUR	July 2009	July 2014
33	*G8009.06.02*	*Activity 3.2.2: Information Management*	Arllet Portugal, GCP	Global	Global	Global		July 2009	July 2014
34	G8009.06.03	*Activity 3.2.3: Data curation*	Arllet Portugal, GCP	Global	Global	Global		July 2009	July 2014
35	*G8009.06.04*	*Activity 3.2.4: Design & analysis*	Marcos Malosetti, WUR	Global	Global	Global		July 2009	July 2014
36	*G8009.06.05*	*Activity 3.2.5: Phenotyping sites & screening protocols)*	Xavier Delannay, GCP	Global	Global	Global		July 2009	July 2014
37	*G8009.06.06*	*Activity 3.2.6: Genotying Support Service*	Chunlin He, GCP	Global	Global	Global		July 2009	July 2014
38	*G8009.06.07*	*Activity 3.2.7: IP & Policy Helpdesk*	L Butler, GCP	Global				July 2009	July 2014
Projects reported under Theme 3 – Crop information systems									
39	*G4009.03/ G4010.06/ G4011.01/ G4011.10*	*Enhancement and implementation of the Crop Ontology for data integration and data interoperability, and expanding its use within communities of practice and to partners to integrate datasets for GCP priority crops through the IBP*	Elizabeth Arnaud, Bioversity	Global	Global	Global	CIMMYT, CIP, IRRI, CIAT, IATA, NCRI, Plant Ontology Consortium, NERC Environmental Bioinformatics Centre, University of Manchester	January 2010	December 2012; NCE: October 2013
40	*G4009.04/ G4010.05/ G4011.05*	*Devt of Integrated SNP Mining and Utilization (ISMU) pipeline based on next generation sequencing (NGS) and high-throughput (HTP) genotyping technologies for facilitating molecular breeding*	Rajeev Varshney and Trushar Shah, ICRISAT	Global	Global	Global	ICRISAT, NCGR, SCRI, UoQ	September 2010	October 2013
41	*G4011.09*	*Developent of a Genotyping Data Management System (GDMS)*	Trushar Shah, ICRISAT	Global	Global	Global	CIMMYT, GCP, Hutton, IRRI, NCGR, SCRI	December 2011	November 2013

Projects reported under Theme 3 – Crop information systems (not included in IBP report above)

43. G4009.03/G4010.06/G4011.01/ G4011.10: Enhancement and implementation of the Crop Ontology for data integration and data interoperability, and expanding its use within communities of practice and to partners to integrate datasets for GCP priority crops through the IBP

- *January 2009–December 2012; NCE: October 2013*

Principal Investigator and lead institute

Elizabeth Arnaud, Bioversity international; email: e.arnaud@cgiar.org

Collaborating scientists and institutions

- Bioversity International: Inge van den Bergh, Rhiannon Chrichton, Luca Matteis, Milko Skofic , Tom Hazekamp (consultant)
- CIAT: Alberto Fabio Guerrero
- CIMMYT: Rosemary Shrestha
- CIP: Simon Reinhard
- CIRAD: Jean Francois Rami
- IITA: Peter Kulakow, Moshood Bakare, Antonio Lopez-Montes , Sam Ofodile, Ousmane Boukare, Hesham Agraba
- ICARDA: Ramesh Verna, Shiv Kumar Agrawal, Fawzy Nawar
- ICRISAT: Praveen Reddy T. Ibrahima Sissoko, Prasad, Suyash Patil, Trushar Shah
- IRRI: Nikki Frances Borja, Mauleon Ramil

Collaborators from other GCP projects:

- Graham McLaren, Project Leader
- Delphine Fleury , Analytical Pipeline Coordinator
- Ndeye Ndack Diop , Communities of Practice
- Mark Sawkins , Configurable Workflow System Manager
- Glenn Hyman, Herlin Espinosa (CCAFS-CIAT)

External (self-funded) collaborators:

- Pankaj Jaiswal and Laurel Cooper (Plant and Trait Ontology, www.plantontology.org)
- Lukas Mueller, Naama Menda (Sol Genomic Network, Boyce Thompson Institute, USA)
- Pierre-Yves Chibon (University of Wageningen, NDL)
- Rex Nelson, Soybase, (USDA-ARS, USA)
- Cyril Pommier, Institut National de Recherche Agronomique (INRA, FRA),
- Ramona Walls, Damian Gassler (iPlant Collaborative, USA)
- Mark Wilkinson (Centro de Biotecnología y Genómica de Plantas UPM-INIA, SPA)

The GCP Crop Ontology (CO) is published online (www.cropontology.org) and currently includes: cassava, banana, chickpea, common bean, cowpea, groundnut, maize, pearl millet, pigeon pea, potato, rice, sorghum, wheat and yam.

The objective of CO is to provide the reference lists of traits along with their methods and scales which are necessary to harmonize the capture and the annotation of breeders' data. The project gives particular emphasis to breeders'trait dictionaries developed within the framework of the Integrated Breeding Platform (IBP), primarily for providing standard lists of the most frequently measured traits for the IB Fieldbook. The workflow is presented in figure 1. The Crop Ontology is curated by the managers of the central crop databases located in the crop lead centers. Aside the ongoing curation of existing CO concepts, breeders wish to add new crops like barley, lentil and also expand the current standard lists because priority traits vary by region following the predominant environmental pressure or breeding needs. The concepts of CO are cross referenced or submitted to Plant Ontology (PO) and Trait Ontology (TO). This year, an environmental and experimental design ontology working group will be formed with GIS specialists, teams from phenotyping platforms and crop modelling projects. Additionally, CO will integrate the IBP services through the iPlantCollaborative platform (http://www.iplantcollaborative.org/) and provide access to a production site publishing stabilized versions of the ontology.

A Programmatic Application Interface (API) enables the direct use of the CO concepts by 3rd party web sites like : the international cassava database (http://www.cassavabase.org/), the Global Agricultural Trial Repository (http://agtrials.org/), Eu-Solanaceae at University of Wageningen, NDL (https://www.eu-sol.wur.nl/), the Phenomics Ontology Driven Database (PODD: http://150.229.2.236/podd/about) of the Australian Plant Phenomics Facility (APPF) and the Australian Phenomics Network (APN). In collaboration with FAO, the CO concepts will be aligned with the thesaurus AGROVOC to integrate crop data into the OpenAgris web site (http://aims.fao.org/openagris). This site uses semantic web technologies like Linked Open Data (LOD).

CGIAR centers, European phenotyping platforms and US crop communities have developed the vision of a consortium contributing to an international semantic framework for the integration of phenotypic and genotypic data. CO will be a key element for providing crop traits, environmental variables and data annotations to this framework.

Bibliographic references

Arnaud E. et. al., (2012). *Towards a Reference Plant Trait Ontology for Modeling Knowledge of Plant Traits and Phenotypes*. In Proceedings of the International Conference on Knowledge Engineering and Ontology Development, pages220-225, SciTePress. DOI: 10.5220/0004138302200225

Shrestha R. et. al., (2012) *Bridging the phenotypic and genetic data useful for integrated breeding through a data annotation using the Crop Ontology developed by the crop communities of practice*. Frontiers in Plant Physiology v. 3 Article 326: doi: 10.3389/fphys.2012.00326 , ISSN: 1664-042X

Shrestha R., Arnaud E., Mauleon R., Senger M., Davenport G.F., 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*. AoB Plants (2010) Vol. 2010 first published online May 27, 2010 doi:10.1093/aobpla/plq008 (http://aobpla.oxfordjournals.org/content/2010/plq008.abstract)

Shrestha R., Davenport G. F, Bruskiewich R. and Arnaud E.*Development of crop ontology for sharing crop phenotypic information* In : Monneveux Philippe and Ribaut Jean-Marcel, eds (2011). *Drought phenotyping in crops: from theory to practice* CGIAR Generation Challenge Programme, Texcoco, Mexico. ISBN: 978-970-648-178-8. 475pp.

44. G4009.04/G4010.05/G4011.05: 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–October 2013*

Principal Investigators and lead institute

Rajeev Varshney & Abhishek Rathore, ICRISAT

Collaborating institutes and scientists

- ICRISAT: Trushar Shah, Sarwar Azam
- INRA, France: Alain Charcosset
- Cornell University: Jean-Luc Jannink, Ed Buckler, Mark Sorrells
- The James Hutton Institute, UK: David Marshall
- University of Queensland, Australia: Dave Edwards

Marker-Assisted Recurrent Selection (MARS) and Genomic Selection (GS) have gained much momentum in plant breeding application due to their potential to enhance genetic gain per cycle in comparison to

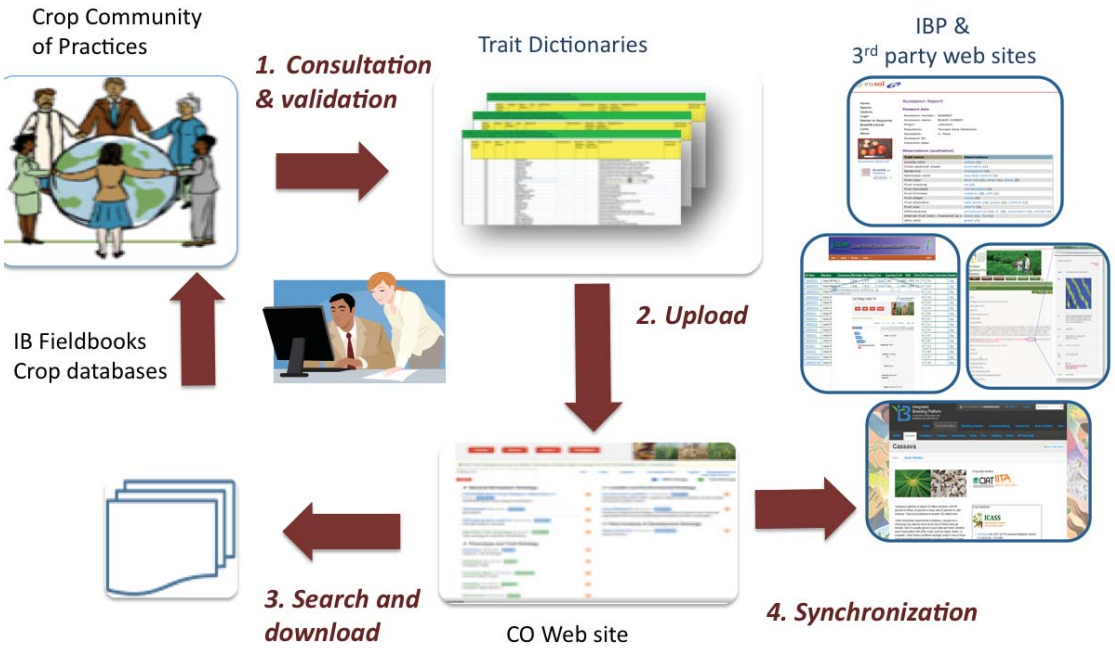


Figure 1: Workflow of the Crop Ontology project

conventional breeding. However many breeding programmes don't have access to specialized tools and algorithms to deploy MARS and GS in their breeding programmes. Therefore it was decided to identify and integrate best modules for MARS and GS in to the existing Integrated SNP Mining and Utilization (ISMU) pipeline developed earlier at ICRISAT. In this direction, 3rd International Workshop on Next Generation Sequencing Data Analysis and Modern Breeding workshop was organized at ICRISAT during Aug 29- 31, 2012 in which 16 scientists representing eight countries participated (http://www.icrisat.org/ceg/3-ngs.htm). As a result OptiMAS was identified as best tool to integrate in the ISMU pipeline for MARS. For GS, several algorithms/tools from Cornell University and The Edinburgh University, UK have been identified as most suitable candidates. Some of these scripts have been obtained from corresponding collaborators and at present are being implemented in ISMU. These scripts have been tested on existing datasets on chickpea and groundnut. In parallel, existing pipeline (ISMU) was further improved for parellel computing to deal with larger amount of data in reasonable time frame and GUI were more simplified by plug-in a Java based interface for Desktops. The Desktop version of the pipeline has been packaged in CD/DVD and tested on Fedora core 13, RHEL 5 and CentOS 6 on 64 bit architecture. Capabilities of ISMU were presented in three meetings: (i) XV Meeting of EUCARPIA Section "Biometrics in Plant Breeding", (ii) "XXI Plant Animal Genome Conference, and (iii) Genotyping-by-Sequencing (GBS) workshop organised by Cornell University at Hyderabad. In these meetings, >50 CDs of the Desktop edition were distributed to interested users. Efforts are underway to develop new interface that will be ideal tool for breeders to apply new molecular breeding approaches.

Objective 1. Identification of most appropriate modules for MARS, GWS and GBS

Activity 1.1. Organize a workshop of international experts for brainstorming on MARS and GWS

Activity 1.2. Expert review for MARS and GWS

3rd International Workshop on Next Generation Sequencing Data Analysis and Modern Breeding workshop was organized at ICRISAT during Aug 29- 31, 2012 with an objective of identifying best algorithm and programme for MARS and GWS. A total of 50 participants including 16 scientists from 8 countries participated in this workshop. The workshop had several presentations dealing MARS and GS. OptiMAS developed by INRA France (Alain Charcosset's group) was found the most suitable tool for MARS. For GS, several algorithms/tools developed by Cornell University and The University of Edinburgh, UK were found suitable to be added in pipeline. These tools include methodologies such as Ridge Regression Best Linear Unbiased Predictor (RR-BLUP), LASSO, Bayesian Ridge Regression, Bayesian LASSO, BayesA, BayesB and BayesCπ, Empirical Bayes and Weighted Bayesian Shrinkage Regression. Additional review of literature suggested use of machine learning algorithms including Random Forest Regression (RFR) (Breiman, 2001), and Support Vector Regression (SVR) (Drucker et al., 1997) specially for non-additive genetic effects.

Objective 2. Integration of MARS module in the ISMU pipeline

Activity 2.1. Identification of suitable MARS module

Activity 2.2. Integration of module in the existing ISMU pipeline

OptiMAS tool (http://moulon.inra.fr/optimas/) developed as a part of GCP projects and under the framework of Integrated Breeding Platform (IBP) was identified as suitable tool to be used as MARS module for pipeline. OptiMAS is an IDE based MARS software and provides several capabilities including scoring of crosses, generating different crossing schemes, ranking them based on genotypic scores, plotting and generating several crossing lists. In OptiMAS algorithms have been deployed to trace parental QTL alleles identified as favorable throughout selection generations, using information given by markers located in the vicinity of the estimated QTL positions. OptiMAS is programmed in C language and is available on Linux and Windows. ISMU will generate input file for OptiMAS through GDMS. Generation of input file for OptiMAS through GDMS is on final stage. In this direction, we have made a beta interface which generates input files for OptiMAS and also executes it.

Objective 3: Integration of GWS module in the ISMU pipeline

Activity 3.1. Identification of suitable GWS module

Activity 3.2. Integration of module in the existing ISMU pipeline

As a result of brainstorming in the workshop and literature survey, several algorithms used in GS have been identified suitable for integrating in ISMU pipeline. R and Fortran scripts has been customised to be implemented in ISMU. These scripts includes different methods of GS including Ridge Regression BLUP (RR-BLUP), Kinship GAUSS, BayesA, BayesB, Bayes Cπ, Bayes LASSO and Random Forest. In GS pre-processing of data is very important and hence code to have a control over allele frequency and polymorphic information content has

been implemented in pipeline. As enough resources are not available to move ahead in this direction, efforts are being made to achieve this milestone at large-scale through some other projects.

Objective 4. Linking of ISMU pipeline with Data Management System (DMS) of IBP

Activity 4.1. Develop an interface to upload HTP genotyping / GBS data in DMS of IBP

The Genotyping Data Management System (GDMS) which is an integral part of the IBP Workbench is being used to store the genotyping data matrix coming from the genotyping service providers. The GDMS currently supports upload of SNP data through templates that have been developed. During the upload a number of validations and quality control checks have been incorporated so that the standards of minimal information requirement are met. Once the data has been uploaded it can be queried and exported out into the Flapjack format which form the link as the input to OptiMAS and other analysis tools within the ISMU pipeline and the IBP workbench.

PROJECTS COMPLETED IN 2012

I. Research Initiatives

Cassava

1. 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 activities:
Community of practice project

2. G4012. 02.01: Participation to the Travel Grant Program of the GCP21-II conference

- *February–August 2012*

Principal Investigator and lead institute
Claude M Fauquet, Danforth Plant Science Center

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborators insitutes and scientists
NaCCRI: Yona Baguma

Location: Speke Resort, Kampala, Uganda, Africa

Summary
The second GCP21 triennial international scientific meeting was held 18th-22nd June 2012, at the *Speke Resort* & Conference Centre, Munyonyo, Kampala, Uganda and has focused on urgent issues related to cassava, most especially the impact and challenges of global climate change and state of the crop in sub-Saharan Africa. GCP21-II was organized by the local host institution, the National Crops Resources Research Institute (NaCRRI), Namulonge, Uganda. Dr. Yona Baguma, (Principal Research Officer at NACRRI) acted as lead organizer supported by Drs. Claude Fauquet and Joe Tohme with contributions from local and international organizing committees. The Local Organization Committee is composed of 12 members from NaCRRI and its parent organization, the National Agriculture Research Organization (NARO), and took care of all issues pertaining to the meeting within Uganda and particularly for the NaCRRI station visit on Wednesday June 20th. The International Organization Committee (IOC) consists of 15 highly experienced cassava researchers from the cassava growing regions of Africa, Asia, China and South America, in addition to scientists from Europe and North America. The IOC determined the overall structure of the meeting, its major sub-themes and chose the invited speakers. In addition to highlighting state of the art research on cassava, sessions will focus on the cassava marketing and value chain, processing and R&D prioritizations. The overall goal is to unify and strengthen the international community to improve cassava productivity and value.

The meeting took place at the Speke Resort & Conference Centre, Munyonyo, Kampala, Uganda (http://www.spekeresort.com/). And the third Conference of GCP21 will be in Nanning, China in October 2015 and it will be organized by CATAS.

Over the last decade international meetings organized by CBN and GCP21 have attracted 200-300 people. We anticipated similar attendance for the 2012 meeting due to major ongoing research projects associated with cassava genomics, molecular markers, double haploids, BioCassava Plus, Harvest Plus, Agra, Great Lakes Initiative, VIRCA, Bioinnovate, Cassava Processing, Cassava Regional Center of Excellence (CRCoE) in Uganda and increasing R&D programs in Japan, Thailand, Vietnam and China. By basing GCP21-II in Africa we expected significant attendance by African NARs, research organization, industry and NGOs. It was facilitated by a travel grant program supported by funds raised from a variety of public and private organizations. GCP21-II partially or completely supported more than 140 participants from developing nations.

GCP21-II was overall an overwhelmingly successful conference. First of all the attendance almost reached 500 people, which was much more than expected and secondly there was a lot of interest and enthusiasm for all events but most certainly for the evening workshops. The rooms were full and the workshops well lasted after 10pm. This demonstrated the profound interest and commitment to the improvement of the crop. It also made clear that GCP21 has a role to play, and actually does play a role already in the development of science and technology for the improvement of cassava. This conference will empower GCP21 way more and we will take action and be proactive to develop a number of small but very important workshops to come up with decisions and action.

Conference agenda

The conference consisted of an approximately 131 oral and 204 poster presentations, in the format of:

Day 1 - plenary presentations on i). major achievements in cassava research and development over the last 3 years, ii). immediate challenges facing cassava in Africa – for example, the impact of climate change and the cassava brown streak disease (CBSD) epidemic in East Africa. In the evening the second Golden Cassava Award was awarded to Dr Mike Thresh, a renown plant epidemiologist, specialized in cassava viral diseases.

Days 2 and 4 – twelve scientific and technical sessions including; Climate and social changes impacting cassava, Biodiversity and Genetic Resources, Cassava Field Production, Genomics and Gene Discovery Platforms, CBSD, CMD and CBB (biotic stress), Enabling Transgenic and In vitro Technologies (includes haploids), Metabolic Engineering (includes starch, biofuels, nutritional enhancement), Field Breeding, Modern Breeding (includes MAS, heterosis, transgenics), Physiology and Abiotic stress (includes PPD), Cassava Delivery Chain, Value Chain and Processing. Speakers were selected from registered attendees submitting abstracts under each session. Interactive poster sessions took place in the late afternoons and round tables discussions on "hot topics" after dinner. In this manner an estimated 465 attendees presented their work to the community.

In addition we had several pre-satellite meetings as well as post-satellite meetings and one training workshop on Integrated Breeding Platform. All these peripheral meetings were well attended and very successful. Furthermore during the week on Tuesday June 19th, Thursday June 21st and Friday June 22nd, a total of 9 workshops were organized at the proposal of registered scientists, predicted to focus on issues such as access to and use of cassava biodiversity, new technology platforms, biosafety, landrace identification etc. (see program attached).

Day 3 – The day started with a special session on Cassava priority settings, organized by CRP-RTB, the new organization of the CG system dealing with tuber crops and cassava in particular. Then we had a field trip to farmers' fields with special emphasis on the impact of cassava brown streak disease and afternoon visit to NaCRRI, Namulonge station, tour of its facilities and confined field trial site. In the evening the attendants were offered an African banquet with African dancers and it was a very relaxing evening!

Day 5 - dedicated to the future of cassava R&D, with distinguished speakers invited to make presentations on non-cassava topics that could be of importance if developed on cassava in the immediate to near future. There were several reports on cassava genomics which were quite impressive relative to the recent progress made on cassava.

Legumes

Beans

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

- *November 2008–October 2011; NCE: October 2012*

Principal Investigator and lead Institute

Jonathan Lynch, Penn State University, USA; JPL4@psu.edu. Dept Horticulture, Penn State University, University Park, PA, USA; 814-863-2256; fax 814 863-6139

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- Stephen Beebe, CIAT, Colombia
- Kathleen Brown, Penn State, USA
- Rowland Chirwa, SABRN, Malawi
- Celestina Jochua, IIAM, Mozambique
- Magalhaes Miguel, IIAM, Mozambique
- Idupulapati Rao, CIAT, Colombia

Summary

This project focuses on basal root traits of common bean that have promise for improving adaptation to drought and poor soils. Basal root whorl number (BRWN) has been phenotyped in several germplasm collections and mapping populations, and a QTL map has been generated. Identified QTLs have been confirmed in a further RIL set and markers for MAS are being evaluated in different genetic backgrounds. An associated trait, basal root number (BRN) has been phenotyped and mapped concurrently and may provide a more reliable estimate of the potential for soil exploration by roots in this class. The impact of BRN and BRWN on crop performance has been evaluated in multiple trials at multiple sites, and in general, increased BRN and BRWN was associated with improved tolerance to drought and low phosphorus. BRN, BRWN, and root hair length and density (RHLD) are being deployed in bean breeding programs in Mozambique. Future work will include additional assessment of the value of these traits and additional phenotyping of several populations to improve the quality and reliability of associated QTL. The second trait, basal root growth angle plasticity (BRGAP) has proven less reliable than anticipated. BRGA has proven to be a valuable trait, but the plasticity, or change in angle with phosphorus availability, has proven unreliable in multiple field trials. We will therefore focus on BRGA itself rather than plasticity, since the genetic variation in BRGA is significant and the trait is associated with performance under stress.

Conclusions

Basal root whorl number (BRWN) has proven to be a promising trait for adaptation to drought and nutrient deficiency. Basal root number and other measures of basal root growth and development may also hold promise for defining this trait genetically and determining its agronomic value. Basal root growth angle (BRGA) is important for soil exploration and interplant competition. BRGA plasticity has proven to be unreliable, therefore future mapping efforts and experiments on agronomic impact will focus on BRGA, BRWN, and basal root number. Several new traits and concepts have been developed during the course of this project.

Quantifiable Outputs

1. 165 accessions of reference collection evaluated for BRWN
2. 120 accessions of P. coccineus and P. dumosus evaluated for *BRWN*. Based on these results P, coccineus and 7 P. vulgaris were evaluated for root and shoot traits in solution culture.
3. 100 interspecific RILs and lines evaluated for BRWN
4. 100 RILS from cross of DOR364 x G19833 evaluated for BRWN and BRGA in greenhouse
5. Improved QTL analysis for *BRWN* performed for population of DOR 364 x G19833 and G2333 x G19839.
6. 150 RILs genotyped in key genomic regions for *BRWN* in crosses DOR364 x G19833 and AND696 x G19833
7. 6 segregating populations derived from 3 commercial varieties (Pinto Villa, PVA773, SUG47) used for marker assisted selection
8. 4 QTL for *BRWN* validated in backcross or recurrent selection populations through marker screening
9. 16 common bean genotypes evaluated in the rainout shelter over two seasons in CIAT for shoot and root traits under drought
10. 16 common bean genotypes evaluated in greenhouse at PSU for BRWN and BRGA traits under drought and/or P stress
11. 16 common bean genotypes evaluated in the field over two seasons at PSU for BRWN and BRGA traits under drought and/or P stress
12. 16 common bean genotypes evaluated in the field under rainfed conditions over two seasons in Mozambique and Malawi for shoot and root traits under drought
13. 16 common bean genotypes evaluated in the field under rainfed conditions over two seasons in CIAT-HQ (Palmira) for shoot and root traits under drought

14. 16 common bean genotypes evaluated in the field under rainfed conditions in CIAT-Darién over two seasons for shoot and root traits under drought with and without low P
15. Evaluation of 3 multiline combinations under nutrient and water stress at URBC

Chickpeas

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

- *January 2009–December 2011; NCE: June 2012*

Principal Investigator and lead institute

Pooran Gaur, ICRISAT, Patancheru, Hyderabad 502324, AP, India. E-mail: p.gaur@cgiar.org, Tel: +91-40-30713356/+91-9866080915, Fax: +91-40-30713074/30713075

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- ICRISAT, Patancheru, India: Rajeev Varshney; Mahendar Thudi; L Krishnamurthy; Vincent Vadez, S Srinivasan
- 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

Summary

This project builds on Tropical Legume I project, where efforts were made to map QTLs for drought tolerance related root traits, yield and yield component traits. Genetic linkage map comprising 240 marker loci was constructed for recombinant inbred line (RIL) population ICC 4958 × ICC 1882 and population was phenotyped for different agronomic, phenology, yield and yield component traits for two years under rainfed and irrigated environments at five locations (ICRISAT-Patancheru, RARS-Nandyal, ARS- Durgapura, UAS Bangalore, Hiriyur and RAKCA, Sehore). Based on extensive phenotyping data and the genotyping data generated a genomic region on LG04 harbouring QTLs for various drought tolerance related traits was identified. The major QTLs identified in ICC 4958 × ICC 1882 were validated in an intra-specific RIL mapping population ICC 283 × ICC 8261. The QTLs for drought tolerance related traits explained about 36% phenotypic variation. Further, to enhance drought tolerance among elite cultivars of chickpea by introgressing this genomic region, a set ten elite cultivars each from India, Kenya and Ethiopia were genotyped using markers in QTL region. Based on marker polymorphism the recipient genotype were identified for each region and efforts are underway to introgress the genomic region into Indian cultivars (ICCV 10, sponsored by Department of Biotechnology, Government of India), and into Kenyan and Ethiopian cultivars (ICCV 97105, Ejere respectively, as a part of TLI Phase II).

Conclusions

Genetic maps with 240 and 171 loci were constructed for two recombinant inbred line populations ICC 4958 × ICC 1882 and ICC 283 × ICC 8261. QTLs for various drought tolerance related root traits, morphological, phonological yield and yield component traits identified in ICC 4958 × ICC 1882 were also validated on ICC 283 X ICC 8261 mapping population. Farmer preferred verities identified under TL I and TL II related projects were tested for marker polymorphism and recipient parents were identified for marker-assisted introgression of the validated QTLs.

Key Products Developed by the Project

1. Linkage map of chickpea based on intra-specific mapping population
2. QTLs for drought tolerance traits in chickpea

Quantifiable Outputs

1. Phenotypic data available on yield, biomass and HI for ICC 4958 × ICC 1882 RILs from multiple locations (3-4) in India.
2. Phenotypic data available on δ13C for ICC 4958 × ICC 1882 RILs
3. Genotypic data on at least 100 SSR markers available for 250 RILs of ICC 4958 × ICC 1882 cross.
4. QTLs identified for root traits, δ13C and HI from ICC 4958 × ICC 1882 RILs
5. About 10 cultivars, 5 each from South Asia and Sub-Saharan Africa identified for introgression of drought tolerance traits through marker- assisted breeding (already identified in phase I of TL-I and TL-II projects).
6. Donor parents each for root traits, δ13C and HI identified (tentatively identified, but these will be finalized after results of year II).
7. QTLs identified for root traits, δ13C and HI from ICC 4958 × ICC 1882 RILs
8. QTLs identified for root traits, δ13C and HI from ICC 283 × ICC 8261RILs

9. A validation population consisting of minimum 100 germplasm/breeding lines with variable levels of drought tolerance established.
10. Genotypic data available on SSR markers linked to drought tolerance QTLs in the validation population
11. Phenotypic data available on root traits, δ13C and HI for the validation population
12. Information on association of markers with drought tolerance traits available from the validation population
13. About 10 cultivars, 5 each from South Asia and Sub-Saharan Africa identified for introgression of drought tolerance traits through marker- assisted breeding
14. Donor parents each for root traits, δ13C and HI identified
15. Minimum 100 polymorphic markers identified between the parents of each intended cross.
16. Minimum 10 crosses made and backcross generations developed for marker-assisted breeding of root traits.

Legumes: Capacity-building activities

5. G4012.02.02: Travel grant: VI ICLGG – Sponsorship

- *April–October 2012*

Principal Investigator and lead institute

- Rajeev K. Varshney, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru - 502 324, Greater Hyderabad, India Tel: 040-3071 3305, Fax: 040 3071 3074; Email: r.k.varshney@cgiar.org

EXTRACT FROM FINAL TECHNICAL REPORT

Summary

The VI International Congress on Legume Genetics and Genomics (*ICLGG*) was held at the Hyderabad Marriott Hotel and Convention Center, Hyderabad, India, from October 2 to 7, 2012. The VI ICLGG was the biggest ICLGG held so far as well as the largest event hosted by ICRISAT.

About 520 delegates from 44 countries participated in the conference. Drs Larry Butler and Ndeye Ndack have represented Generation Challenge Programme (GCP) at VI ICLGG.

The conference had 10 sessions, namely:

1) Next Generation Genomics;
2) Symbiosis and Development;
3) Evolution and Diversity;
4) Harnessing Germplasm Resources;
5) Abiotic Stress;
6) Pathogenesis and Disease Resistance;
7) Genomic Resources and Trait Mapping;
8) Genomics - Assisted Breeding;
9) Nutrition and Quality; and
10) Translational Genomics.

Of the 63 presentations more than half were selected from the abstracts that had been submitted earlier. In addition to these presentations, 293 posters were exhibited at the poster session as well as in the 1-minute window.

Additionally, there was one exclusive session entitled "Special Session: Young Scientists in Legume Genetics and Genomics" (co-sponsored by GCP, GRDC, NSF and SPG), which was devoted to talks from young scientists representing different countries, disciplines and crops. This session was co-chaired by the representatives from GRDC,NSF, GCP and SPG. Dr Ndeye Ndack from GCP was a co-chair at this session.

There was a balance of different topics at this conference ranging from very basic subjects, such as symbiosis and developmental biology, to very applied aspects such as genomics-assisted breeding and harnessing germplasm resources. In much the same manner talks and posters covered not only model legume species such as Medicago and Lotus, but also crop species such as soybean, cowpea, chickpea, lentil, common bean, pea, faba bean, mung bean, etc.

The VI ICLGG was financially supported by many organizations including Generation Challenge Program, GRDC, Illumina, Spinco Biotech, Xcleris, The Noble Foundation, Saskatchewan Pulse Crop Growers, Krishidhan Seeds, Macrogen, BGI, Premas Biotech, Life Technologies, The Peanut Foundation, MARS Inc., Monsanto, Pioneer Dupont, Sci Genome, and others. More details are available at http://www.icrisat.org/gt-bt/VI-ICLGG/Homepage.htm

Maize

6. 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
James Gethi, KARI; jgethi@wananchi.com

NO UPDATE SUBMITTED

Rice

7. 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: April 2012*

Principal Investigator and lead Institute
Arvind Kumar, International Rice Research Institute (IRRI); akumar@cgiar.org
DAPO BOX 7777, Metro Manila, Philippines; (63) 2-580-5600; Fax: (63) 2-580-5699

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- IRRI: R. Anitha
- Indira Gandhi Krishi Vishwavidyalaya, Raipur, India: S.B. Verulkar
- Central Rice Research Institute, Cuttack, India: O.N. Singh, P. Swain
- Central Rainfed Upland Rice Research Station, Hazaribag, India: N.P.
- Mandal
- Narendra Dev University of Agriculture and Technology, Faizabad, India: J.L. Dwivedi
- University of Agricultural Sciences, Bangalore, India: S. Hittalmani, Venkatesh Gandhi
- Tamil Nadu Agricultural University, Coimbatore, India: R. Chandrababu, A.
- Senthil, S. Robin
- Birsa Agricultural University, Ranchi, India: Krishna Prasad
- Jawahar Lal Nehru Krishi Vishwavidyalaya, Jabalpur, India: P. Perraju
- Barwale Foundation, Hyderabad, India: H.E. Shashidhar, Abhinav Jain
- Yunnan Academy of Agricultural Sciences, Kunming, China: D. Tao
- University of Alberta, Edmonton, Alberta, Canada: Dean Spaner

Summary
During the four years and eight months of the project period, a study was conducted on fine mapping of four large-effect QTLs for grain yield under drought, introgression of *qDTY12.1* in Vandana and the development of Vandana NILs with improved grain yield under drought, introgression and pyramiding of qDTY QTLs in IR64 to improve its yield under drought, interaction of major QTLs with other loci, demonstration of bulk segregant analysis (BSA) to be an effective approach to identify large-effect QTLs, testing of breeding lines at partners' sites as well as in national systems, release of drought-tolerant varieties for cultivation by farmers, simulation modeling to determine an efficient strategy to pyramid DTY QTLs, including standardization of population size and optimum number of crosses required to combine multiple QTLs in an efficient and economical way, and training of Ph.D. scholars and national scientists from participating partners' institutes in the project.

Conclusions
The present project helped the investigators, scholars as well as national partners not only to get a better understanding of drought tolerance but also helped breeders to refine conventional as well as molecular breeding approaches so as to be able to move ahead towards higher assured probability of improving yield under drought. The project not only established the existence of major QTLs for grain yield under drought in rice but through demonstration of development of product following marker assisted introgression of identified QTLs and the superior yield of developed QTLs introgressed lines under drought over the recipient variety has provided increased confidence to the associated researchers to implement marker assisted breeding for drought tolerance as a large scale strategy to improve rice yield under drought. The precise phenotyping and genotyping, number of plants/lines to be selected at each generation during QTLs pyramiding in order to get sufficient number of plants with desired combination of QTLs will be of immense help to implement similar breeding program to improve yield under drought of other popular varieties. One of the most important lessons learnt from this project is that it is very important for breeder during the course of MAB to precisely understand the combination of QTLs that shall provide the highest yield advantage under drought without any adverse effect on yield under irrigated situation and related traits.

Key Products Developed by the Project

1. IR64 QTL pyramided lines with yield advantage of 0.5 tha-1 to 1.5 tha-1 over IR64 under drought developed.
2. Vandna NILs with qDTY12.1 with yield advantage of 0.5tha-1 and 0.4tha-1 under drought and irrigated control situation respectively developed.
3. qDTY1.1, the first large effect QTL with stable effect against different high yielding genetic background identified.
4. Four breeding lines have been released as varieties for cultivation in different provinces of India: in Tamil

Nadu, PMK3; in Orissa and Jharkhand, Sahbhagi dhan; in Chhattisgarh, Indira Barani dhan 1; and in Uttar Pradesh, Shusk Samrat.
5. Two Ph.D. scholars and two national partners trained on marker assisted breeding for drought tolerance

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

- *November 2008–October 2011; NCE: October 2013*

Principal Investigator and lead institute
Eduardo Blumwald, Department of Plant Sciences, University of California, Davis,
eblumwald@ucdavis.edu
One Shields Ave, Davis, CA 95616, USA; (01)530-752-4640; Fax: (01)530-752-2278.

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists
- International Rice Research Institute (IRRI):
- Abdelbagi Ismail, abdelbagi.ismail@cgiar.org;
- Rachid Serraj, r.serraj@cgiar.org

Summary
Drought is the major constraint to rice production in the drought-prone rainfed environments, and enhanced drought tolerance and crop water productivity are major targets for improving and sustaining food security in these areas. We hypothesized that drought-induced plant senescence is due to a type of cell death program naturally activated during drought. Down- regulating such program could therefore enable plants to acquire vigorous acclimation responses to stress, resulting in enhanced drought tolerance with reduced yield losses. We generated plants overexpressing an *IPT* gene (mediating the synthesis of cytokinins) under the control of *SARK*, an inducible maturation- and stress-dependent promoter, and demonstrated that the suppression of drought-induced leaf senescence results in significantly enhanced drought-tolerance of the plants. These plants maintained relatively high relative water content, retained photosynthetic activity and survived longer periods without irrigation, Moreover, the plants overexpressing PSARK-IPT were able to grow under restrictive water supply with a lower yield penalty compared to controls and displayed minimal yield losses when watered with only 30% of the amount of water used under control conditions.

Based on all previous results, in this proposal we will test the efficacy of stress-induced cytokinin synthesis in conferring drought tolerance in upland and lowland rice varieties overexpressing IPT. The general objective is to identify genes with significant roles in conferring drought tolerance in rice, and the generation of drought-tolerant and water-use-efficient rice plants in different genetic backgrounds. We will use forward-, reverse-genetics and TILLING to assess and confirm the roles of the identified genes in drought tolerance. The development of drought-tolerant rice varieties able to grow and produce higher biomass and yield under restricted water regimes would considerably minimize drought-related losses and increase food production in water-limited rainfed rice lands.

Conclusions
We have demonstrated the feasibility of the project and the suitability of the promoter and transgene to generate drought tolerant rice plants. The development of drought-tolerant rice varieties able to grow and produce higher biomass and yield under restricted water regimes would considerably minimize drought-related losses and increase food production in water-limited rainfed rice lands.

Quantifiable outputs
1. Twenty-five independent lines of each rice variety expressing PSARK-IPT will be developed.
2. Copy number will be determined in each line.
3. Transformants with 1 to 2 insertion copies will be selected and grown to maturity.
4. Ten to 15 lines (of each variety) will be evaluated phenotypically.
5. RNA from 3 lines (from each variety) showing enhanced drought tolerance will be isolated for further microarray analysis.
6. Five lines (of each variety) showing a drought tolerant phenotype in the greenhouse will be evaluated at IRRI for drought tolerance under field conditions.
7. Identification of key genes playing significant role(s) during the adaptation to drought and reduced water treatments.
8. Ten key determinant genes (whose expression were activated or reduced during drought and/or reduced watering) are being characterized.
9. Lines expressing the individual selected genes are being generated in both backgrounds (upland and lowland rice).
10. In addition, knockout-mutants have been be identified from existing knockout collections and knockdown-mutants are being generated by RNAi expression.
11. Non-transgenic drought-tolerant rice mutants will be identified and tested (both in the greenhouse and in the field). Breeding of these lines into commercial varieties will accelerate the development of non-transgenic drought tolerant rice.

Sorghum

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

- *January 2008–December 2010; NCE: May 2012*

Principal Investigator and lead institute
HD Upadhyaya, Principal Scientist, ICRISAT Patancheru PO, 502324, AP, India
h.upadhyaya@cgiar.org; Telephone: +91 40 3071 3333, Fax: +91 40 3071 3174

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- ICRISAT, Patancheru, India: Shivali Sharma; V Vadez; CT Hash; SL Dwivedi, L Krishnamurthy
- ICRISAT, Bamako, Mali: E Weltzien-Rattunde
- 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

Summary
Sorghum reference set has been characterized completely for phenotypic diversity. Evaluation of this reference set under well-watered (WW) and post-flowering water-stressed (WS) conditions showed a large variation for various morpho-agronomic traits, and for SPAD chlorophyll meter reading (SCMR) across locations. Evaluation of this reference set for seed micronutrient contents, Fe and Zn at ICRISAT, Patancheru showed variability for Fe and Zn contents. Multi-location evaluation of promising accessions selected based upon stay-green character, SCMR, transpiration efficiency (TE) and rate of water loss per unit of leaf area under terminal drought conditions across locations in Asia and Africa revealed enormous variability for morpho-agronomic traits including grain and stover yield among these accessions. Promising high yielding drought tolerant accessions have been identified for different locations. At ICRISAT, Patancheru, 14 accessions had <10 % stover and grain yield reduction under WS compared to WW condition. When compared with the best control cultivars, 4 accessions (IS 14556, 62(73)509, IS 12531 and IS 9303) for stover yield, 5 accessions (IS 33173, SSM 215, IS 8882, IS 21124 and IS 24786) for grain yield, and one accession (IS 929) both for stover and grain yield has been identified as promising accessions at ICRISAT. Evaluation of the promising accessions for Fe and Zn content at ICRISAT, Patancheru showed seven accessions, IS #s 35, 5720, 10876, 15752, 29638, 30335 and 30409 having high Fe and Zn contents both under WW and WS conditions as well as when pooled. Similarly, at Dharwad, Karnataka, India, 2 accessions (IS 929 and IS 26833) for grain yield; in Senegal, one accession (IS 15443) for grain yield; in Mali, 5 drought tolerant accessions (452(411)510, 393(421)659, IS 3963, IS 22287 and IS 24009); and in Kenya, 2 accessions (IS 1284 and IS 28389) for dry ear weight and grain weight have been identified as promising accessions.

Conclusions
The sorghum reference set accessions showed large variations for various agronomic traits as well as for Fe and Zn contents. Multilocation evaluation of promising reference set accessions for stover/grain yield and component traits by Asia and Africa NARS has resulted in the identification of high yielding drought tolerant accessions for different locations. At ICRISAT, Patancheru, India, 4 accessions (IS 14556, 62(73)509, IS 12531 and IS 9303) for stover yield, 5 accessions (IS 33173, SSM 215, IS 8882, IS 21124 and IS 24786) for grain yield, and one accession (IS 929) both for stover and grain yield; at Dharwad, Karnataka, India, 2 accessions (IS 929 and IS 26833) for grain yield; in Senegal, one accession (IS 15443) for grain yield; in Mali, 5 drought tolerant accessions (452(411)510, 393(421)659, IS 3963, IS 22287 and IS 24009); and in Kenya, 2 accessions (IS 1284 and IS 28389) for dry ear weight and grain weight have been identified as promising drought tolerant accessions. Evaluation of the promising accessions for Fe and Zn content at ICRISAT, Patancheru showed seven accessions, IS #s 35, 5720, 10876, 15752, 29638, 30335 and 30409 having high Fe and Zn contents both under WW and WS conditions as well as when pooled.

Key Products Developed by the Project/Quantifiable Outputs

1. Germplasm accessions having high Fe and Zn contents both at flowering and 30 DAF, under WW and post-flowering WS conditions
2. Germplasm accessions having high SCMR, both at flowering and 30 DAF, under WW and post-flowering WS conditions
3. Germplasm accessions with higher transpiration efficiency than stay-green QTL recurrent parental lines, R16 and S35
4. Germplasm accessions with higher water extraction ability than stay-green QTL recurrent parent lines, R16 and S35
5. Post-flowering high yielding drought tolerant accessions identified for different locations for use in breeding programs

Comparative Genomics

10. 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; NCE: October 2012*

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

EXTRACT FROM FINAL TECHNICAL REPORT

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

Summary
A QTL map for sorghum drought tolerance was developed based on the RIL population developed from lines BR007 and SC283. The phenotypic data was grain yield and stay green, assessed under controlled conditions and under drought stress. A genetic map was constructed with 255 Diversity Arrays Technology (DArT) markers, 83 SSRs, 5 sequence-tagged site (STS) and one RFLP marker. QTL detection for stay green and grain yield was done using QTLs for flowering time and plant height as cofactors. All but one of the flowering time QTL were detected near yield and stay green QTLs. Similar co-localization was observed for two plant height QTLs. A QTL analysis for yield, using flowering time/plant height as cofactors, led to yield QTL on chromosomes 2, 3, 6, 8 and 10. For stay green, QTL on chromosomes 3, 4, 8 and 10 were not related to differences in flowering time/plant height. The physical positions for markers in QTL regions projected on the sorghum genome, suggest that the previously detected plant height QTL (*Sb-HT9-1, and Dw2*), in addition to the maturity gene, *Ma5*, had a major confounding impact on the expression of yield and stay green QTL. Co-localization between an apparently novel stay green QTL and a yield QTL on chromosome 3 suggests that there is potential for indirect selection based on stay green to improve drought tolerance in sorghum.

Conclusions
Therefore, we can conclude that Al tolerance *qALT6* significantly improved maize yield stability under acid soil conditions in lines *per se* (NILs) and in hybrid combinations (NIHs). Even considering that this experiment was conducted in only one year, most of the NIHs introgressed with *qALT6* showed grain yield levels similar to the commercial checks under 40% of Al saturation. Additionally, there was a clear tendency of a grain yield enhancement for both NILs and NIHs carrying *qALT6* cultivated in Cerrado soil without Al saturation in the surface. NILs showed 13 to 29% higher grain yield than L53 whereas NIHs exhibited up to a 17% yield improvement when compared to crosses with L53 under corrected soil. The benefits of *qALT6* on grain yield are likely to be due to the improved Al tolerance conferred by *ZmMATE1*, along with other possible smaller contributions that will require further investigations. Indeed, marker-assisted introgression of *qALT6* is expected to improve maize adaptation to acidic soils that can be expanded to other tropical regions.

11. G3008.04: Drought from a different perspective: Improved tolerance through Phosphorus acquisition

- *November 2008–October 2011; NCE: October 2012*

Principal Investigator and lead institute
Sigrid Heuer, International Rice Research Institute, replaced 01st April 2013 by
Tobias Kretzschmar, International Rice Research Institute, t.kretzschmar@irri.org DAPO Box 7777, 1301 Metro Manila, Philippines, Tel: +63 (2) 580 5600 ext 2761/2253

Collaborating Institutes and Scientists

- International Rice Research Institute: Abdelbagi Ismail
- International Rice Research Institute: Stephan Haefele
- Japan International Research Center for Agricultural Sciences: Matthias Wissuwa
- Institute for Agricultural Biotechnology & Genetic Resources Research and Development: Masdiar Bustamam
- Institute of Biochemistry and Biology, University of Potsdam and MPI of Molecular Plant Physiology: Bernd Mueller-Roeber

Almost 50% of rice soils are currently deficient in phosphorus (P), yet resource-poor farmers in drought-prone upland and rainfed lowland environments typically apply little fertilizer. 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. Such negative nutrient × drought interactions have not been examined sufficiently, yet they may in part explain the large G × E interactions typically encountered under drought and the difficulty in improving yields following approaches that focus entirely on drought tolerance in its narrowest definition. We have shown repeatedly that rice lines with the major P uptake QTL *Pup1* maintain higher root growth rates under P deficiency, whereas intolerant genotypes fail to reach their full root growth potential. This observation has prompted us to further investigate the potential benefits of *Pup1* under combined drought × P-deficiency stress. To confirm this hypothesis and to devise an efficient strategy for the use of *Pup1* in developing genotypes with dual tolerance, it is essential to proceed with the molecular and physiological characterization of *Pup1* and the functional assessment of *Pup1* genes.

Identification and functional assessment of the tolerance gene(s) at the Pup1 locus.

The major rice QTL *Phosphorus uptake 1* (*Pup1*) confers tolerance of phosphorus (P) deficiency under field conditions. The major gene in the *Pup1* locus has now been cloned and functionally characterized in overexpression (35S) and RNAi plants. The gene was named *Phosphorus starvation tolerance 1* (*OsPSTOL1*) and codes for a Ser/Thr protein kinase. Our current understanding of the gene function suggests that it acts as an enhancer of root growth and that the *OsPSTOL1*-mediated larger root system provides better access to P and other nutrients. The data were published in August 2012 in *Nature* (Ref 1) and received considerable international attention by the scientific community as well as public media.

Three other genes with putative implications in phosphorous starvation and/or drought tolerance were also cloned from the Pup1 region. One in particular, a dirigent-like (OsPupK20-2) gene is currently being studied in depth for biochemical and physiological implications in Pup-1 mediated multiple tolerance.

Collaborators in Germany are currently investigating metabolic profiles under phosphorous starvation and/or drought with the aim to identify metabolic markers and metabolic pathways of significance.

Pyramiding of additional QTLs that complement Pup1 and multiple location trials to assess dual tolerance of Pup1

Pup1 is currently being pyramided with two of the most promising yield-under drought QTLs, DTY2.2 and DTY4.1 (Ref 2) in the IR64 background.

A new allele of the *PSTOL1* gene has been identified in African germplasm and markers have been developed to facilitate allele differentiation and selection of recipient parents.

The IR64-Pup1 and IR74-Pup1 breeding lines have been further advanced and evaluated in two field experiments at IRRI. Likewise, the Indonesian Pup1 breeding lines have been further evaluated at ICABIOGRAD and at IRRI. The data are now being compiled for final analyses and selection of the best lines for seed dissemination.

In order to disseminate the *Pup1* molecular marker technology and initiate breeding of tolerant rice varieties across Asia, a training workshop with participants from seven Asian countries was held in August 2012. Dissemination of Pup1 seed material to participants of the Pup1 workshop is in its final stages. Once finalized, it will lead to relevant field trials being conducted in Vietnam, Thailand, India (multiple locations), Nepal and Tanzania as well as further locations within the Philippines.

Pup1 lines are currently tested in several countries in East and West Africa for performance under limited phosphorous availability in combination with drought.

References

Gamuyao R, Chin JH, Pariasca-Tanaka J, Pesaresi P, Dalid C, Slamet-Loedin I, Tecson-Mendoza EM, Wissuwa M, Heuer S. 2012. The protein kinase pstol1 from traditional rice confers tolerance of phosphorus deficiency. Nature 488:535-541.

Dixit S, Swamy BP, Vikram P, Ahmed HU, Sta Cruz MT, Amante M, Atri D, Leung H, Kumar A. 2012. Fine mapping of QTLs for rice grain yield under drought reveals sub-QTLs conferring a response to variable drought severities. Theor Appl Genet. 125(1):155-69

12. 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 2012*

Principal Investigator and Lead Institute
Robert Schaffert. EMBRAPA Maize and Sorghum; schaffer@cnpms.embrapa.br; and reschaffert@hotmail.com
Caixa Postal 151, 357001-970 Sete Lagoas, MG, Brazil; tel: +55-31-3027-1176, cel: +55-31-9986-1698, fax:+55-31-3027-1188)

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- Institut National de la Recherche Agronomique du Niger (INRAN BP 429 Niamey, Niger): Maman Nouri, Soumana Souley, Magagi Abdou, Adam Kiari, Fatouma Beidari, and Issoufou Kapran
- ICRISAT – Niamey, BP 12404, Niamey, Niger: Bettina Haussmann and Tom Hash
- ICRISAT – Bamako, BP 320, Bamako, Mali: Eva Weltzien Rattunde and Fred Rattunde
- Embrapa Maize and Sorghum: Jurandir Magalh**ães**, Alvaro Vilela Resende and, Jo**ão** Herbert M. Viana

Summary
This project (ALTFIELD) was designed to provide a link between the outputs of ALTSORGHUM (G3007.04) and sorghum breeding programs in Niger and Mali. The exchange of germplasm; INRAN and ICRISAT-Mali to Embrapa and Embrapa to INRAN/ICRISAT, was delayed during the first 24 months of the project delaying the initiation of some activities. The germplasm exchange was completed in 2009 and 2010. The germplasm from INRAN (164 accessions) and ICRISAT Mali (187) was phenotyped for tolerance to Al toxicity in nutrient solution at Embrapa Maize and Sorghum (Embrapa MS) and genotyped with molecular markers for the aluminium tolerant gene AltSB The frequency of landraces showing tolerance to Al toxicity from INRAN was low (approximately 1%) and Al tolerance levels in nutrient solution were intermediate and heterogeneous requiring an extra selection step in developing homogeneous Al tolerant landraces for repatriation for field validation, whereas the frequency of Al tolerant selections from Mali was greater (approximately 9%) and Al tolerance levels were more tolerant in nutrient solution and more homogeneous. Seed of selected Al tolerant plants of seven intermediate Al tolerant INRAN accessions were repatriated to Niger. However this process was not necessary for the ICRISAT Mali germplasm, allowing the highly tolerant selected accessions from Mali to be used directly for field validation and potential use by farmers. MABC was completed by Embrapa using two elite Al sensitive accessions from INRAN germplasm. About 400 BC1 (Back Cross 1) seedlings from two crosses (recurrent parents 90SN-7 and IRAT204) were genotyped and four or five plants were selected for *AltSB* and background markers to make the BC2. The BC2 generation was completed for these two entries. About 500-700 BC2F1 seedlings from these crosses were genotyped with both AltSB and background markers and five to ten plants were selected for self-pollinating to fix the Al tolerant gene with over 95 recovery of the recurrent parent. These isogenic lines were repatriated to Niger and Mali for field validation in late 2011 and 2012.

One of the bottlenecks in establishing uniform phenotyping sites is the low level of interaction and collaboration of plant breeders and soil scientists. Initially in this project, we encountered criticism in selecting Mali and Niger in West Africa to carry out the objectives of this project by scientists and research administrators claiming that aluminium toxicity would not occur in regions of semi-arid (SAT) and arid tropics. Our collaboration with the ICRISAT Centers in Mali and Niger, which have conducted detailed on station soil analysis, have shown serious constraints of soil acidity and Al toxicity at both locations. Unfortunately, soil maps of the region have not identified these problems. The sandy soils of Western Niger, which are acid with damaging levels of Al toxicity, have been recognized as constraints for peanut production but not for sorghum production (Karlheinz Michels *and* Charles I. Bielders, Pearl millet growth on an erosion-effected soil in the Sahel (Expl Agric. (2005), volume 42, pp. 1–17). In fact the level of toxic aluminium is quite low, but the aluminium saturation can be very high and toxic to plant growth because of the low levels of cation exchange capacity (CEC). As changing levels of organic matter (OM) in the soil strongly influence the CEC the levels of aluminium saturation can also change, contributing to the difficulty of developing and maintaining uniform aluminium toxicity phenotyping sites. Considering that the spacial variability of aluminium distribution in the soils of the SAT is very high and that OM content of the sandy soils can change rapidly depending on site management practices, we had difficulty in fully meeting the objective of establishing aluminium toxicity phenotyping sites in Mali and Niger.

The development of adequate phenotyping sites and phenotyping for field validation was and continues to be a significant bottleneck in finalizing the ALTFIELD project. We requested a no cost extension until December 31, 2012 to provide Embrapa consultants to continueevaluation and improvement of the phenotyping sites and finalize the field validation of the effect of AltSB with contrasting selected sorghum lines, landraces and isogenic hybrids (repatriation of improved and selected germplasm from Embrapa to Mali and Niger).

Field validation of isogenic Embrapa germplasm at Embrapa Al Phenotyping Sites has clearly validated the positive effect of *AltSB* on yield with Al saturation between 20 and 40% with no apparent yield drag when Al toxicity is not present. The gene *AltSB* is induced with the presence of Al in the soil solution, consequently there is no diversion of photosynthates to citrate production in the absence of Al. In general the tolerance gene can increase yield in the presence of Al toxicity by one ton of grain per hectare or more than 25%. Field evaluation of Embrapa germplasm and Niger germplasm at the ICRSAT Center at Sadoré, Niger in trials with and without lime (Al toxicity) was not conclusive, but there was a trend for Al tolerant germplasm to perform better than the Al susceptible material in 2010 and 2011. The two Al tolerant reference materials from Embrapa, SC566-14 and SC283, both converted lines (SC) from Africa landraces had top performance at the Sadoré trials. Some of the introduced Al tolerant germplasm was from temperate sources and probably not adapted to Niger, resulting in poor performance.

Conclusion

The development of adequate phenotyping sites and phenotyping for field validation was and continues to be a significant bottleneck in finalizing the ALTFIELD project. We requested a no cost extension until December 31, 2012 to provide Embrapa consultants to continue evaluation and improvement of the phenotyping sites and finalize the field validation of the effect of *AltSB* with contrasting selected sorghum lines, landraces and isogenic hybrids and repatriation of improved and selected germplasm from Embrapa to Mali and Niger. The development of the African phenotyping sites has not progressed satisfactorily, principally due to extreme spacial variability for Al toxicity in soils of the Sahel and lack of resident expertise in the area of soil fertility and establishment of uniform phenotyping sites at the Sahelian Institutions. Embrapa scientists with expertise in developing soil stress phenotyping sites worked closely with scientists at the African institutions to improve Al phenotyping sites. Improved management at the ICRISAT Center at Sadoré, Niger by maintaining adequate soil organic matter by alternating between two adjacent areas in alternate years was a solution in Niger. A cover crop is planted in the alternate year to maintain adequate organic matter for the following year. The organic matter maintains a higher and uniform CEC and lower level of Al saturation. The risk of very high CVs or even complete data loss in dry land sites due to drought is high in West Africa and we concluded that an irrigated site in necessary to produce adequate results in validating the superiority of Al tolerant cultivars.

Field validation of isogenic Embrapa germplasm at Embrapa Al tolerant Phenotyping sites has clearly validated the positive effect of *AltSB* on yield with Al saturation between 20 and 60% with no apparent yield drag when Al toxicity is not present. In general the tolerance gene can increase yield in the presence of Al toxicity by one ton or more of grain per hectare or more than 25%. Field evaluation of Embrapa germplasm and Niger germplasm at the ICRSAT Center at Sadoré, Niger in trials with and without lime (Al toxicity) was not conclusive, but there was a trend for Al tolerant germplasm to perform better than the Al susceptible material in 2010 and 2011. The two Al tolerant reference materials from Embrapa, SC566-14 and SC283, both converted lines (SC) from Africa landraces had top performance at the Sadoré trials. Some of the introduced Al tolerant germplasm was from temperate sources and probably not adapted to Niger, resulting in poor performance.

The process of germplasm selection for the objectives of this project has been finalized and the appropriate germplasm has been exchanged and is being increased at Embrapa and at ICRISAT. The INRAN germplasm set of 164 accessions has been both genotyped and phenotyped and approximately five percent have some level of tolerance to Al toxicity. MABC was conducted at Embrapa with the INRAN material (two elite breeding lines for West Africa selected (recurrent parents 90SN-7 and IRAT204)) from Niger to introduce the AltSB gene for tolerance to Al toxicity. The landraces from Niger identified as having tolerance to Al toxicity were heterogeneous and were purified with phenotyping and genotyping selection and repatriated to Niger.

Seven entries from the Mali panel; Sambalma (1), IS 23767, Sambalma (4), IS 7978na, Zabuwa (5), SSV MD Sorg 20006, and Yarfalgori (23) are recommended for use by farmers where Al toxicity may be a problem. An additional ten entries also had tolerance to Al toxicity and can be used by farmers in Mali.

II. Themes
Theme 1 – Comparative and applied genomics

13. G4008.07: Genomic resources and mapping populations developed and assembled for pearl millet to enable trait/gene identification

- *January 2008–December 2009 (final report submitted in 2011)*

Principal Investigator and lead institute
Tom Hash, ICRISAT; c.hash@icrisatne.ne

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- ICRISAT: T Nepolean, RK Varshney, S Senthilvel, FR Bidinger, V Vadez
- ILRI: M Blümmel
- AICPMIP: IS Khairwal
- CARZRI: OP Yadav
- Rajasthan Agril. Univ.: PC Gupta

Report

Pearl millet (*Pennisetum glaucum*) is a dual-purpose grain and fodder crop that is an essential component of dryland crop-livestock production systems of sub-Saharan Africa (*e.g.*, Nigeria, Niger, Burkina Faso, Mali, Senegal, Sudan, and Chad) and South Asia (*e.g.*, India) in areas that are too hot, too dry, and/or have soils that are too acid or too infertile for reliable production of maize, sorghum or any other cereal crop. The crop is also increasingly used as the mulch component of sustainable minimum tillage crop production systems in the humid tropics (*e.g.*, Brazil), where its acid soil tolerance, deep root system, and high vegetative growth rates under high temperature conditions often make it the best option for retrieving soil nutrients from depth, smothering weeds, and producing a mulch that protects the soil surface from erosion by rain drop impact or surface water movement. There are limited genomic tools available for this orphan crop despite pearl millet being the 6th most important cereal crop globally and being likely to be, along with sorghum, an important source of genes and alleles that will enable plant breeders to engineer other crops (*e.g.*, rice, wheat and maize) to better tolerate higher temperatures and increased frequencies of drought stress that are predicted to arise from on-going global warming.

This project proposed to strengthen genomic resources for pearl millet, developing EST libraries from the parents (841B-P3 and 863B-P2) of a well-characterized pearl millet drought tolerance mapping population, identifying EST sequence polymorphisms between the parents of this population, and mapping these polymorphisms using the 150 RIL progenies of this population. The augmented linkage map of this population, combined with information on the positions in the completed sorghum and rice genome sequences of homologues of the pearl millet ESTs from which these newly mapped markers are derived, was used to refine the rice- pearl millet comparative map and develop at sorghum-pearl millet comparative map.

In addition, we used STS and SSR markers to skeleton map two new conventional biparental pearl millet mapping populations of random inbred lines, and conduct initial testcross hybrid evaluations of these populations for terminal drought stress tolerance (measured in terms of grain and stover yield maintenance under stress conditions) and grain and stover nutritional value (measured in terms of digestibility and metabolizable energy content). Finally, we advanced eight additional bi-parental pearl millet mapping populations to random inbred line sets that are ready for map saturation with DArT and SSR markers, would permit development of a high density consensus linkage map for pearl millet.

Tangible outputs delivered

- A set of nearly 400,000 short-sequence pearl millet ESTs from drought-stressed root and shoot libraries of a pair of elite, genetically diverse pearl millet inbreds has been developed and mined for EST-SSRs and SNPs (annotation is still on-going).
- A set of 136 pearl millet EST-SSR primer pairs were developed capable of producing scorable amplification products using a touch-down PCR protocol. Of these, 119 detected polymorphism between parental inbred pairs of one or more of four newly completed pearl millet RIL populations, and 99 of these new pearl millet EST-SSRs were mapped on one or more of these four RIL populations. This increased the number of mapped pearl millet SSR markers by >50%, and greatly improved pearl millet genome coverage by SSR markers.

- Moderately-well saturated SSR-based genetic linkage maps of three pearl millet RIL populations, and a partially saturated SSR-based genetic linkage map for a fourth pearl millet RIL population, were produced and then merged to provide a pearl millet consensus linkage map comprised of 174 markers (172 SSR and 2 STS markers) and estimated to span 898 cM (Haldane).
- A total of 14 recombinant inbred line sets derived from pearl millet biparental crosses were advanced and are now available for use. Compared to the intended 12 populations advanced to F7 seed generation, we completed production of F7 seed for eight RIL populations, F6 seed for two RIL populations, F5 seed for three RIL populations and F4 seed for a single RIL population. DNA samples of five of the F7 populations are available, and SSR-anchored DArT-saturated linkage maps are available for four of these five. The fifth population for which DNA samples are available has an SSR-based skeleton linkage map, but this does not appear to provide full genome coverage.

Data Availability

The nearly 400,000 short sequence (average read length = 240 bp) ESTs from leaf and root tissue samples of stressed plants of the two mapping population parental lines, ICMB 841-P3 and 863B-P2 are being submitted to GenBank and GCP databases. Annotations for these will follow in due course.

Data on the EST-SSR markers developed for pearl millet, which includes primer sequence information, a functional PCR protocol, and information on PCR product amplification from template DNA of 8 mapping population parental lines, is being uploaded to the GCP central registry at http://gcpcr.grinfo.net/index.php?app=datasets&inc=files_list.

Marker data sets for four RIL populations, that were used to produce the pearl millet SSR consensus linkage map, is being made available through the GCP central registry at http://gcpcr.grinfo.net/index.php?app=datasets&inc=files_list.

Theme 2 – Integrated crop breeding

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

- *January 2008-December 2010, NCE: June 2012*

Principal Investigator and lead institute
Arvind Kumar, Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute (IRRI); a.kumar@cgiar.org
IRRI, DAPO Box 7777, Metro Manila, Philippines; Telephone: +63 (2) 580-5621; Fax: +63 (2)580-5699

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists
- International Rice Research Institute (IRRI), Philippines; KL McNally
- Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France; M Dingkuhn, D Luquet, B Courtois
- African Rice Center (AfricaRice), Nigeria; S Mande
- BIOTEC, Thailand; T Theerayut
- Central Rice Research Institute (CRRI), Cuttack, India; P Swain
- Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India; S Robin
- Birsa Agricultural University (BAU), Ranchi, India; BN Singh

Summary
During the report period (2009-11), germplasm accessions were screened at IRRI, Philippines; BIOTEC, Thailand; and CRRI, TNAU, and BAU, India, under drought stress and non-stress conditions. A majority of the yield trials showed moderate to high stress with a yield reduction in stress experiments ranging from 41% to 80% compared with non-stress experiments. The lines were divided into six maturity groups and planting was staggered in the field under reproductive- stage drought stress. These experiments have enabled the classification of a large set of germplasm accessions into different maturity groups. Apart from this, several landraces with high yield under reproductive-stage drought stress and/or non-stress conditions were identified. These lines will serve as valuable genetic material for developing high-yielding drought-tolerant rice varieties. At CIRAD, experiments were undertaken to assist in phenotyping the regulation of early morphogenesis and source-sink relationships of rice under vegetative-stage drought. Under non-water-limited conditions, plant development rate, DR (inverse of phyllochron), was shown to be the main or one of the main factors explaining shoot dry weight (SDW) and corresponding relative growth rate (RGR). This relationship between DR and RGR was confirmed under drought conditions on the same genotypes: DR both before and during stress was positively correlated to RGR estimated during the whole experiment (from transplanting to the end of stress), pointing out the key role of DR as a constitutive and adaptive trait in the maintenance of early vigor across a severe, short drought event. The positive relationship observed experimentally between DR and RGR was confirmed by optimized *plasto* (plastochron) 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. Fifty of the 203 genotypes were first analyzed for sugar content in source and sink leaves. Interestingly, carbohydrate patterns confirm the functional analysis of source-sink relationships pointed out through morphogenetic data on the 203 genotypes.

Conclusions
A total of 17 field experiments were conducted from 2009 to 2011 at IRRI, BIOTEC, CRRI, TNAU, and BAU. The number of entries in each experiment ranged from 402 (*indica* accessions only) at IRRI in 2011 to 688 (*indica* + *aus* accessions combined). In 2009 at IRRI, *indica* and *aus* accessions were screened in separate experiments, whereas the two sets were combined for all the other experiments at other sites. Moderate to severe stress was seen in a majority of the experiments, in which yield in the stress experiments declined from 41% (at IRRI 2009) to 80% (at IRRI 2010). The only exception to this was the experiment conducted at BIOTEC in 2011, where the stress and non-stress experiments had similar mean yield and no yield reduction was observed. Mean yield under stress conditions ranged between 634 kg/ha (at CRRI) and 2,185 kg/ha (at BIOTEC). A combined analysis was conducted with yield data sets from stress and non-stress experiments conducted at IRRI, BIOTEC, and CRRI for 588 common accessions. Twenty lines with high yield under both stress and non-stress conditions were identified through the combined analysis: Bathuri, Chmar Reth, Garia, Wanni Dahanala, Pratao, SR26B, B44141 F-MR-6-3, Tadukan, nan Te 113, IR75870-5-8-5-B-2, Kalia, Bang Pra, Bhasamanik, IR64-21, Masuran, Zalcha, Gorbai, CSR-89-IR-15, Co 25, and DA 5.

These germplasm accessions can serve as valuable sources of tolerance for a drought breeding program because of their performance across a wide range of environments in South and Southeast Asia.

At CIRAD, early vigor was understood as the accumulation of biomass during the exponential growth of rice seedlings. Under non-water-limited conditions, plant development rate, DR (inverse of phyllochron), was shown to be the main or one of the main factors explaining SDW and corresponding relative growth rate, RGR (g/g × °C/d), together with tillering and leaf size. Under both water-sufficient and stress situations, a sensitivity analysis confirmed the key role of DR in seedling early vigor.

Key Products Developed by the Project

1. Development of purified seed stocks in progress
2. List of accessions developed for association studies
3. Accessions characterized for phenology at IRRI, BIOTEC, TNAU, CRRI, AfricaRice, and CIRAD and under reproductive-stage drought stress at IRRI and BIOTEC, and is under characterization at TNAU, BAU, and CRRI
4. Phenology of around 1,000 accessions determined
5. Standardized managed field drought-stress phenotyping protocol for application in association studies targeting reproductive-stage drought tolerance in rice developed

Quantifiable Outputs

1. Phenology determined for around 1,000 accessions at IRRI
2. Accessions evaluated at BIOTEC and IRRI and evaluation in progress at TNAU, BAU, and CRRI
3. Detailed data on rainfall obtained from sites; soil characterization in progress based on phenology and site rainfall pattern; sowing plan for drought screening worked out
4. Physiological parameters recorded for japonica accessions under dry-down experiments at vegetative stage at CIRAD
5. Characterization of plant development, assimilate partitioning, and response to vegetative-stage drought under field conditions carried out
6. Data in EcoMeristem model confirmed

Theme 4 – Capacity building

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

- *September 2006–December 2011; NCE: September 2012*

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

NO UPDATE SUBMITTED

Theme 5: Product delivery

16. 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; NCE: June 2012*

Principal Investigator and lead institute
George W. Norton, Virginia Polytechnic Institute and State University, VT; gnorton@vt.edu
Department of Agricultural and Applied Economics 205B Hutchenson Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24060-0401; (540) 231-7731; Fax: (540) 231-7417

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- International Food Policy Research Institute, IFPRI: Stan Wood
- Virginia Polytechnic Institute and State University, VT: Elizabeth Phillips

Summary
This report provides an assessment of the potential net economic benefits for eight ongoing Challenge Initiative projects that utilize the tools, services and applications offered by the Integrated Breeding Platform (IBP). One project targets rice in Burkina Faso, Mali and Nigeria; two target sorghum in Mali; two target wheat in China and India; and three focus on cassava in Ghana, Nigeria, and Tanzania. The assessment predicts the cumulative net economic benefits for each project over 20 years, from the start of the project. The potential gains are projected based on what the economic benefits would be with and without the IBP. Economic surplus analysis is used to project the net economic benefits. That analysis uses published data on base production, prices, and markets for the crops in each country, and scientist opinions on projected yield and cost changes due to the new technologies. Other parameters used in the economic analysis include the estimated time for discovery, development, and deployment of improved technologies, probabilities of success or failure, and cost changes. These parameters were obtained via questionnaires sent to scientists involved with the projects, asking them how the probabilities of success, total costs and time frames were affected by the use of the IBP tools, services, and applications.

Gains in benefits due to the IBP are presented for the most likely, minimum projected, and maximum projected yield changes, for rice, sorghum, and wheat, and the most likely projected yield changes for the cassava projects. For the most likely scenario, wheat in China has the largest projected benefit gains ($1,188.4 billion) due to the high base value of production and the projected adoption rate as compared to the other countries. The large cassava production base in Nigeria results in projected benefit gains of $256.9 million to $749.0 million for the three cassava projects in that country. The most likely benefit gains for cassava range from $62 million to $129.9 million in Ghana and from $43.3 million to $67.5 million in Tanzania. The smaller cassava benefits in the latter two countries (compared to Nigeria) are due to the smaller production bases, despite improvements in probability of success, timing, and cost. Likewise rice has a projected large benefit gain in Nigeria of $419.8 million due to the large production base as the change in parameters are the same across the three target countries. The rice benefit gains are projected at $26.4 million in Mali and $5.8 million in Burkina Faso. The relatively smallproduction base and high input cost change result in estimated benefit gains of $17.9 million and $21.1 million for the two sorghum projects in Mali. The two sorghum projects are parallel activities with one projected to have higher benefits than the other.

Projected economic benefit gains for most crops are substantial, with differences (with and without IBP) in probabilities of research success (risks of failure), changes in time frame, release year, and total cost of each project considered. Scientists were asked how much those factors were affected by use of the IBP. For the rice project, when utilizing the tools, services and applications from the IBP, the probabilities of success in Mali, Burkina Faso, and Nigeria were projected to increase by 9 percent. In other words, if the rice project did not utilize the services offered by the IBP, the probabilities of success for Mali, Burkina Faso and Nigeria were projected to decrease by 9 percent. The changes in probabilities of success for Sorghum were projected to be 25 percent higher with the use of the services of the IBP. For wheat, the projected increases in probabilities of research success were 5.5 percent for China and 16 percent for India. The overall projected benefit gains are still high, despite small changes in probabilities of research success, because of the large

production bases. For cassava, the projected changes in the probabilities of success due to utilizing the services offered by the IBP were between 6 and 16 percent depending on the project and country. Cassava project 2 incurs the higher percent improvement while project 1 and 3 incur lower percent improvements. If one were to rank GCP research by commodity, based on the projected increases of probabilities of success due to the IBP, the highest would be sorghum, followed by wheat in India and cassava project 2, with cassava project 3 and rice in third, and wheat in China and cassava project 1 last. In other words, wheat in China and cassava project 1 have the lowest expected increase to the probability of success by using the IBP. However, ranking each commodity by a total of expected gains to the overall net economic benefits, for the most likely yield change scenario, wheat and cassava rank first followed by rice and sorghum.

Results

Projected gains in economic benefits for the various research projects due to the presence of the IBP are presented in Table 2. Cumulative net benefits were projected over 20 years from the start of each project, with and without the IBP. The estimated gains in benefits are presented for the most likely, minimum projected, and maximum projected yield changes for rice, sorghum and wheat, and for the most likely projected yield change for cassava. Focusing on the most likely benefits, wheat in China has the largest projected gain in benefits ($1,188.4 billion) for two reasons: (1) the base value of production is the highest for all commodities, causing a small percentage increase to be a sizable total increase and (2) the adoption rate projected is relatively large.

Second, the large production base in Nigeria also resulted in large projected gain in benefits of $749 million for cassava project 2 (Nigeria), and $419.8 million for rice.

Third, the relatively small production base and high input-cost change for the two sorghum projects in Mali resulted in most-likely estimated benefits from the IBP of $17.9 million and $21.1 million. The two sorghum projects are parallel activities.

Fourth, the three cassava projects appear to have large benefits from the IBP when considered as a whole. While these projects are not complete substitutes (parallel), they also are not complete complements. If they were complements, the gains could be added to together. In addition, there is undoubtedly conventional breeding research currently underway that is also at least partly parallel and might have had benefits without the GCP projects. The estimated benefits from the IBP for each cassava project (Nigeria) are: $256.9 for cassava project 1, $749 million for cassava project 2, and $348.7 for cassava project 3. The largest benefits of the IBP occur in Nigeria, the lowest in Tanzania.

For rice, the IBP GCP project is projected to have a wide range of benefits depending on the country. The primary reason for the large difference in benefits by country is the size of the production base, even with the changes to probability of success, timing and cost.

PROJECTS COMPLETED IN 2011

I. Research Initiatives

Cassava

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

- *September 2007–February 2010; NCE: February 2011*

Principal Investigator and lead institute

Pablo Rabinowicz, Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore; prabinowicz@som.umaryland.edu
801 West Baltimore Street, Baltimore, Maryland 21201, USA; Tel: 1- (410) 706-6714; Fax: 1 (410) 706-1482

Collaborating institutes and scientists

- African Centre for Gene Technologies (ACGT), University of Pretoria, Pretoria, South Africa: Jane Morris
- ACGT, Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa: Alexander Myburg
- ACGT, School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa: Chris Rey
- University of California, Davis, CA, USA: Mingcheng Luo

Summary

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, 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 the first SNP-based genetic map of cassava to our knowledge.

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.

Conclusion

Our project has substantially increased the amount of publicly available genomic resources for cassava, in the form of a physical map that will be anchored to the genetic map. This resources will not only accelerate the translation of genomic technologies to the development of molecular breeding tools, but will also contribute to substantial improvement of the cassava genome sequence generated during the course of our project by the project led by the University of Arizona.

We have delivered a substantial number of validated SNP markers that are being converted to a versatile platform and will be made available to the cassava breeding community. We have also provided training in cassava SNP genotyping and genomic technologies to National Programs through a capacity building workshop. The genetic and physical maps of cassava, as well as the genetic diversity study will result in at least two publications that are currently in preparation.

Legumes

Chickpeas

2. 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

L. Krishnamurthy, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT); L.Krishnamurthy@cgiar.org Patancheru 502 324, Andhra Pradesh, India. Tel: +914030713657 Fax: +914030713074

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

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

Summary

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 chickpea reference set of accessions were chosen to be phenotyped for $\Delta^{13}C$, the best opted estimate of TE, and SLA and SPAD chlorophyll meter readings two other proxies for TE. The field experiments were conducted at ICRISAT, Patancheru and University of Agricultural Sciences (UAS), Bangalore during 2008-09 for this purpose. The trial at UAS, Bangalore failed due to very late sowing. Therefore, the trial was repeated at both the locations on a no-cost extension of this project during 2009-10 but again the data collected at UAS, Bangalore was with large gaps and was less useful. This has lead to the availability of two season's data from ICRISAT. The trials were grown with 280 accessions of the reference collection under drought stressed and optimally irrigated conditions. The leaf samples collected at 40 DAS and 63 DAS were used for both SLA estimation and for carbon isotopes. SPAD chlorophyll meter readings were made on intact leaves. There was large range of variation for days to 50% flowering and maturity. The $\Delta^{13}C$ analysis displayed a good range. The yield, yield components, SLA, SPAD readings and $\Delta^{13}C$ of the accessions displayed a vast range and $\Delta^{13}C$ was significantly associated with seed yield or WUE in both years under drought stress. Also $\Delta^{13}C$ recorded under terminal drought as well as under optimal irrigation was closely associated indicating that this trait is a constitutive one. In 2009-10 $\Delta^{13}C$ also was found to be correlated with other phenological and yield components. However these relationships could not be seen under optimally irrigated conditions during 2009-10. There were two DArT markers (cpPb-680078, cpPb-490776) that were identified to be closely associated with the $\Delta^{13}C$ trait under terminal drought prone condition. The SLA and SPAD chlorophyll meter reading were found not to be associated with $\Delta^{13}C$ but these were significantly related to each other.

A training cum capacity building workshop was conducted at JIRCAS and Hokkaido university, Japan between 25-29 May 2010 and four NARS scientists involved in drought tolerance breeding in chickpea from Asia and Africa had participated besides 7 collaborators. This training workshop included visits to five related institutions/industry and presentation of six highly relevant lectures.

Conclusion

The analysis of data from field experiment conducted at ICRISAT, Patancheru during 2008-09 postrainy season sowed a good relationship of per day productivity under drought stress with $\Delta^{13}C$. Also $\Delta^{13}C$ recorded under terminal drought as well as under optimal irrigation was closely associated indicating that this trait is a constitutive one. Though not committed in the proposal, the data collected in 2009-10 has not only confirmed this relationship to be very close, but also was correlated with other phenological and yield components. However these relationships could not be seen under optimally irrigated conditions during 2009-10. There were two DArT markers that were identified to be associated with the $\Delta^{13}C$ trait under terminal drought prone condition.

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

- *November 2009–October 2010; NCE: October 2011*

Principal Investigator and lead institute

Doug Cook, UC–D; drcook@ucdavis.edu

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- ICRISAT: Rajeev Varshney

Summary
Recent efforts under Tropical Legume 1 (TL1) and allied projects have yielded a significant increase in molecular marker resources for chickpea. Nevertheless, there remains a pressing need to identify polymorphisms that discriminate cultivated accessions, especially the elite germplasm that will form the foundation of phase 2 of TL1 (e.g., MARS parents and the focus of current breeding and QTL analyses). The objective of this proposal was to use Next Generation sequencing for deep re-sequencing of cDNA libraries from a select set of elite accessions.

We describe the development of a genotyping by sequencing protocol that samples the chickpea genome at ~80K loci and returns an estimated >3,900 unique SNP between cultivated accessions and ~10,000 SNP in inter-specific comparisons. The method is considerably less expensive that current SNP genotyping platforms and has been optimized specifically for chickpea, both at the bench and for downstream bioinformatics analyses.

Conclusion
This protocol has the potential to substantially change the nature of molecular genetic analyses in chickpea, because it generates large quantities of genotype data, at densities that are ideal for mapping and breeding purposes, and at very low cost.

By way of example, we are currently constructing a detailed genetic map for the inter- specific mapping population of ICC4958 and PI489777. Within this pair of genotypes, we can use the existing RAD data to calculate that 87% of 83,044 RAD loci will be scorable and that polymorphism rates will be in the range of 1.6 SNP per kb. Thus, 8,088 SNP loci represent the lower boundary for the expected molecular marker data.

The cost of the analysis, across 96 individuals (8,088 X 96 = 764,448 SNP assays), is ~$2K for sequencing and $2.5K for library preparation and QC, bringing the cost per data point to $0.005, which is ~3% (a 33-fold savings) the cost of GoldenGate SNP assays performed at a similar scale. Moreover, because library preparation costs increase only slightly as additional quantities of 96 genotypes are added, the cost per data point will decline further as population sizes increase. But note that for low polymorphism populations, cost per data increases as the number of informative loci declines.

The reduced representation aspect of this protocol provides great cost savings in sequencing, due to the reduced effective genome size and the ability to multiplex many samples per lane. Data analysis is also greatly simplified, because the entire genome is "reduced" to the subset of 83K unique loci that are pre-determined to be polymorphic between parental genotypes. In the example of ICC4958 and PI489777, above, this equates to ~8K loci, which for assay purposes we convert to the corresponding 8K SNP. As a consequence, the bioinformatics task no longer involves invoking stringent or loose criteria, but rather simple presence/absence of validated, informative SNPs. We anticipate that with minimal effort this bioinformatic protocol could be converted to a simple, user-friendly software program.

Quantifiable Outputs:
1. 21 cDNA libraries
2. ~1 Gbp of cDNA sequence
3. ~10 thousand DNA alignments and 5-10 thousand SNPs for elite genotypes
4. SNP haplotypes for parents of RIL populations that are currently the focus of TL1 phase 2 and the chickpea challenge initiative.

Cowpeas

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

- *January 2008–December 2010, NCE: March 2011*

Principal Investigator and lead institute
Jeffrey Ehlers, University of California, Riverside; jeff.ehlers@ucr.edu; Dept. of Botany and Plant Sciences, 900 Univ. Ave., Riverside, CA 92521-0124 USA; TEL. (951) 323-5918; FAX (951) 827-4437

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists
- International Institute of Tropical Agriculture, Kano, Nigeria: Satoru
- Muranaka, Ousmane Boukar
- University of California, Riverside, Riverside, CA, USA: Timothy Close, Philip Roberts
- Institut Senegalais de la Recherches Agricole, Senegal: Ndiaga Cisse
- Institut de l'Environmentet des Recherches Agricole, Burkina Faso: Issa Drabo
- Texas A&M, College Station, TX, USA: William Payne and Bir B. Singh

Summary
This project was designed to: 1) provide baseline drought tolerance information for early and medium cycle cowpea varieties and assess the importance of genotype x environment (G x E) interactions for grain yield under drought across a range of environments; 2) determine the relationship between grain yield under drought and various traits, and select applicable methodologies, including thermal imaging analysis, for practical and efficient indirect measures of drought tolerance relevant to the major cowpea production zones in Africa; and 3) provide some preliminary information on relationships between drought tolerance and shoot and root traits, and identification of potential drought tolerant genotypes with beneficial root characteristics.

Activities conducted during the project were 1) completion of 34 field trials (Table 1) in Burkina Faso, Senegal, Nigeria and California with a common set of 30 early maturing or 30 medium maturing cowpea varieties (Appendix E Table 1), 2) evaluation of transpiration efficiency (TE) of medium maturing lines using the dry down method and 3) a preliminary evaluation of thermal imaging of field plots and seedlings of 40 selected drought tolerant and susceptible cowpeas. Based on the results collected that have been analysed so far, we could identify several breeding lines and varieties with lower drought-induced grain yield reductions. Major conclusions include that GxE effects are very strong across the West African environments investigated, that deeper rooting is one of the characteristics of drought-tolerant cowpea lines, that differences in drought induced reduction of stomatal conductance could be a key physiological symptom/criteria in detecting difference of drought tolerance in cowpea. Our results also indicate that the delta 13C value of leaf and grain are not reliable indicators of drought tolerance for selection.

Conclusions
Based on the results collected that have been analysed so far, we could identify several breeding lines and varieties with lower drought-induced grain yield reductions. Major conclusions include that GxE effects are very strong across the West African environments investigated, that deeper rooting is one of the characteristics of drought-tolerant cowpea lines, that differences in drought induced reduction of stomatal conductance could be a key physiological symptom/criteria in detecting difference of drought tolerance in cowpea. Our results also indicate that the delta 13C value of leaf and grain are not reliable indicators of drought tolerance for selection. The thermal ismaging work has begun to shed light on the relationship between canopy temperature and the possible mechanisms and levels of drought tolerance. Ongoing experiments will continue to elucidate this relationship. Limitations of using thermal imaging for drought phenotyping have been found, and a method of overcoming these limitations has proved successful. Further experimentation will serve to better understand how to better interpret canopy temperature with a view towards developing a reliable selection criteria for breeders.

Key Products
1. Drought tolerant check varieties identified
2. Efficient tools for drought tolerance screening

Groundnuts

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

- *January 2008 to December 2008; NCE: December 2011*

Principal Investigators and lead institute
Steven J. Knapp and Peggy Ozias-Akins, College of Agricultural and Environmental Sciences, The University of Georgia (UGA), pozias@uga.edu, 2360 Rainwater Rd, Tifton, GA 31793-5766, USA; (1) 229-386-3902; Fax: (1) 229-386-7274

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists
- ICRISAT: David Hoisington; Rupakula Aruna; Rajeev Varshney

Summary
DNA marker resources have been expanded to enable 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' (NCBI TSA project PRJNA49471), has provided a database for SNP discovery. These genotypes included *A. hypogaea* ssp. *hypogaea* (Florunner, Dixie Giant, Gregory, Georgia Green, C76-16, NC3033, SunOleic 97R, Florida-07, Bailey HO, Basse, Overo Chiquitano, SSD6), *A. hypogaea* ssp. *fastigiata* (Olin, New Mexico

Valencia A, Georgia Valencia), *A. monticola* (GKBSPSc 30062), and an interspecific hybrid-derived line (SPT06-06). Reduced representation sequencing targeted root, leaf, and developing pod transcriptomes of each genotype with Roche 454-Ti next generation technology. Although other technologies were debated, 454 could provide the best balance of read length and depth at the time in order to reduce the probability of assembling homoeologous sequences. Over 350 Mb of 454 sequence from the 17 genotypes has been assembled along with Sanger and 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. Since there is no peanut genome sequence available to use for predicting intron positions, intron sites were inferred from alignments with *Medicago truncatula*, *Glycine max*, *Vitis vinifera*, and *Arabidopsis thaliana* genome sequences.

Although the research described here generated high-quality SNPs to efficiently increase DNA marker density in groundnut, the levels of polymorphism between parents of tetraploid mapping populations is extremely low. The assay platform is, however, quite robust, providing the ability to genotype several hundred progeny in only a few days instead of the weeks or months required for producing an equivalent number of molecular data points (MDPs) by genotyping SSR markers. With GoldenGate arrays, SNPs are genotyped using genomic DNA samples, thereby greatly reducing labor costs and increasing genotyping throughput. The challenge in groundnut and other plants is the up-front expenditure and effort needed for SNP discovery. This problem is compounded in groundnut by low SNP frequencies (narrow genetic diversity) and limited DNA sequence resources. The narrow genetic diversity has been reaffirmed by the results of these experiments (see examples under IV. Scientific activities). The current SNP array has demonstrated the feasibility of SNP genotyping in tetraploid peanut but will have limited applicability for genetic mapping of crosses between elite genotypes. The strategy of SNP discovery by transcriptome sequencing in groundnut will likely need to be extended to genome sequencing in order to capture more variable regions of the genome. SNPs that have been validated by this study can be converted to a low throughput SNP assay platform and used immediately as markers for genetic mapping and possible association with traits of interest.

Conclusion

A set of single nucleotide polymorphisms has been identified for assay with the Illumina GoldenGate platform. The assay has served for genotyping of 80 tetraploid inbred lines, amphidiploids, and several diploid accessions. SNPs between tetraploid genotypes are rare unless the tetraploid is synthetic. Nevertheless, the validated SNPs can be 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.

Rice

6. G4010.04: Enhancing capacity for use of advance genotyping for fine-mapping and pyramiding of major salt tolerant QTLs through MABC for the development of durable saline tolerant rice varieties

- *July 2010–June 2011; NCE: November 2011*

Principal Investigator and lead institute
Zeba I. Seraj, University of Dhaka, zebai@univdhaka.edu

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- International Rice Research Institute: Abdelbagi M. Ismail, Michael J. Thomson
- Bangladesh Rice Research Institute: Md. Sazzadur Rahman

Summary
Out of 9 million ha of cultivatable land in Bangladesh, about 1 million ha in the coastal areas are affected by salinity. Due to the high sensitivity of modern rice varieties, resource-poor farmers living in these areas can grow only one crop of rice during the monsoon season when salinity is low. Therefore, the development of salt tolerant rice varieties with wider adaptability in these areas is the most sustainable and cost effective way to improve the livelihood of marginal farmers living off these lands and to sustain sufficient rice production for the country. Recent developments in molecular genetics and genomics made available modern breeding tools that can speed up the development of salt tolerant genotypes in the background of high yielding varieties. Through the previous GCP projects, substantial progress was made in the use of MABC to introgress *Saltol*, the major QTL associated with salt tolerance, into Bangladeshi mega rice varieties BR11 & BRRI dhan28, using FL378 as the donor, and also in mapping of salt tolerance QTLs from the Bangladeshi landrace Boilam.

From this one year capacity building grant, introgression of *Saltol* QTL into BRRI dhan28 was completed with clean and minimum background introgression. Here we tested 4-different sized donor introgression across the *Saltol* region to identify the optimum introgression for maximum tolerance. By comparing the phenotypic SES values at seedling stage it was clear that larger introgression could give better tolerance at seedling, suggesting that there are probably more than one gene involved at this locus. One major QTL (5S) for reproductive stage tolerance from Boilam background was mapped at the 33.1 cM region with linked marker RM516. Related traits are filled grain numbers, panicle exertion, spikelet fertility, number of degenerated spikelets, flag leaf Na^+ and flag leaf Na^+-K^+ ratio.

Conclusions
The project helped in the completion of introgression of *Saltol* QTL into BRRI dhan28 using FL378 as the donor. From this work 4-different sized introgressions at the *Saltol* region were isolated, with the larger introgressions seem to confer better tolerance at the seedling stage, suggesting multiple genes at this locus. All the four different-sized introgressions in of BRRI dhan28-*Saltol* are available for further characterization under controlled and field conditions. Moreover, Fine-mapping a reproductive stage tolerance QTL from Boilam on chromosome 5 was completed with the help of high-throughput facilities available at IRRI, confining it to comparatively narrower region, and linked markers were identified. This will facilitates the development of NILs for validating the actual effects of the QTL and the development of a MABC system for pyramiding this QTL with the *Saltol* locus to enhance tolerance at multiple stages. Furthermore, this grant helped in training of Mr. Sazzadur Rahman, from BRRI, in all aspects of QTL mapping, MABC and in the use of various software.

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

1. *Saltol* introgression lines (BRRI dhan28-*Saltol* lines) by using donor FL378.
2. One fine-mapped reproductive tolerance QTL from Boilam background.

Milestones/Quantifiable Outputs

1. Better understanding of the use of advance genotyping facility of IRRI for quick delivery of outputs: Completed
2. One BRRI dhan28-*Saltol* line with clean background: Completed
3. One fine mapped reproductive stage tolerance QTL with flanking markers

7. 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; NCE: June 2011*

Fellow and lead institute
Honoré Kam, Institut de l'Environnement et de Recherches Agricoles; kamhonore@yahoo.fr
Farako-Ba, Bobo-Dioulasso, 01 BP 910, Burkina Faso; (226) 20 98 23 29; Fax: (226) 20 97 01 59

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- CIRAD, France: Nourollah Ahmadi
- IRD, France: Alain Ghesquière
- Africa Rice Center, Benin: Marie-Noelle Ndjiondjop
- University of KwaZula Natal, South Africa: Mark Laing

Summary
In Burkina Faso, rice is the fourth most important cereal crop after sorghum, millet and maize. However, *Rice Yellow Mottle Virus* (RYMV) disease that emerged in Kenya in 1966 has become a serious threat for many rice growing countries in Africa. The main goals of this study were to investigate the genetic diversity of 332 rice landraces from Burkina Faso with 26 microsatellites, and to exploit this diversity in a search for varieties resistant and tolerant to RYMV, for their utilisation in rice breeding. The microsatellites confirmed the existence of two substructures, namely *O. glaberrima* and *O. sativa*, and the presence of hybrid accessions. A core collection of 52 accessions was established based on 22 microsatellites. This core collection includes 13 *O. glaberrima* and 39 *O. sativa* accessions and reflects the genetic diversity of the sub-clusters present in each species. This core collection contains 89% of the allelic richness of the entire collection. However, the screening of the entire collection with different RYMV isolates exposed the susceptibility of most of the accessions in the collection. Most of the *O. s. indica* accessions were highly susceptible. However, ten *O. glaberrima* accessions displayed a delay of symptom expression, and moderate resistance. Nonetheless, their resistance was overcome later by a particularly virulent RYMV isolate BF1. Remarkably, a single moderately resistant cultivar, BM24, showed that partial resistance and tolerance to RYMV can be found in an *O. sativa* variety. Serological evaluation of this local variety in comparison with the partially resistant variety, Azucena, showed that BM24 and Azucena expressed similar resistance patterns. A genetic profile of both varieties showed that both had an identical allele status at RM101, which is a marker bracketed in the same zone as QTL12.

Conclusion
This project "Study of Burkina Faso rice landraces diversity and breeding for resistance to *Rice Yellow Mottle Virus* (RYMV)" contributed to the molecular characterization of Burkina Faso rice landraces and to the molecular screening of the resistant and tolerant accessions with microsatellites at CIRAD and IRD Montpellier. The Burkina Faso rice core collection was established and resistant and tolerant accessions against RYMV were identified. The results of the activities achieved were used as part of our PhD thesis. The thesis was already sent to the higher degree of University of KwaZulu Natal for submission to external reviewers and two papers are in press for publication in international journals. The PhD is not completed yet. I am waiting for the outcomes of the external reviewers. Along with the project we gained experience in the use of molecular markers in plant breeding that is of a great interest for INERA rice breeding program.

Quantifiable Outputs

1. 26 polymorphic SSR identified
2. 332 rice accessions genotyped
3. Population structure of Burkina Faso rice determined
4. Genetic diversity of Burkina Faso rice germplasms established
5. Burkina Faso rice core collection established
6. Results on diversity study to be published in an international journal
7. Ten resistant and one tolerant varieties against RYMV identified
8. The 10 resistant accessions do not bear any of the known RYMV1 alleles
9. Results on RYMV resistance study to be published in an international journal

Sorghum

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

- *August 2008–July 2010; NCE: February 2011*

Principal Investigator and lead institute
Tom Hash, ICRISAT; c.hash@icrisatne.ne

This project was discontinued in early 2011 because the crossing of the original parental lines of the intended MAGIC population was unsuccessful. The chosen lines were too divergent in their maturing and flowering times. The PI was also transferred to a different position and was no longer working on sorghum.

Wheat

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

- *January 2006–December 2010; NCE: March 2011*

Principal Investigator and lead institute
Susanne Dreisigacker, Global Wheat Program, CIMMYT; sdreisigacker@cgiar.org
El Batan, km 45 Carretera México-Veracruz, CP 56130 Texcoco, México; (52)5558042004; Fax: (52)5558047558

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- CIMMYT: M. Reynolds, Y. Manes, T. Payne, H-J. Braun, J. Crossa, M. Zaharieva
- INRA Morocco: R. Dahan, N.Nasrolha, H. Ouabbou
- CIMMYT-IRAN, DARI: J. Kamali,
- SPII, Iran: AR. Nikzad, S. Tahmasbi, S. Sarikhani

Summary
Association mapping (AM) is increasingly being adopted as a method complementary to bi-parental linkage mapping to identify genotype-phenotype associations. Association mapping was used in this project to dissect the genetic basis of grain yield and related agronomic and physiological traits in a collection of 191 hexaploid spring wheat accessions included in the GCP spring wheat reference sample. The panel was genotyped with Diversity Array Technology (DArT) and gene specific markers for major genes responsive for vernalization requirement, photoperiod sensitivity and plant height. Phenotypic data on the panel were collected for 14 different traits under rainfed conditions in four environments during 2008 and 2009. Agronomic traits included ear emergence (EARE), days to anthesis (ANTH), days to maturity (MAT), plant height (PH), grain number (GN), percent grain filling (GF), thousand kernel weight (TGW), and grain yield (GY). Physiological traits included canopy temperature at the vegetative (CTv) and grain filling (CTg) stage, water soluble carbohydrates of the stem (WSC), chlorophyll content during anthesis (CHL), normalized difference vegetative index at the vegetative stage (NDVIv), normalized difference vegetative index at the grainfill stage (NDVIg).

The AM panel encompassed a broad range of genetic and functional diversity expressed by the large genotypic variation for the investigated traits, a large number of marker polymorphism and low linkage disequilibrium (LD). Significant variances for environment and genotypes were measured for most of the traits. A total of 2815 DArT markers were polymorphic across the AM panel and LD declined fast and within 10 cM below the significant threshold. On the basis of DArT markers and principal component analyses the AM panel was structured into three groups, the biggest group including CIMMYT derived lines. Using single-locus analyses with the method of efficient mixed-model association (EMMA) and the mixed linear model (MLM) in TASSEL, the number and consistency of marker trait associations (MTA) across the four rainfed environments was generally very low, with in total only 1.14% to 1.56% detectable associations with *P* values less than 0.01. Only 18 associations survived the Bonferroni correction for multiple testing. Thus, significant marker effects were consistently detected in only a rather limited number of cases with R^2 values below 10% suggesting that the power to detect the relevant loci for grain yield and its agronomic and physiological components via AM decreases, most likely because under such conditions similar yield values can be attained by different genotypes through different adaptive strategies corresponding of significant MTAs. None the less, AM revealed the influence of several chromosome regions on the variability of the targeted agronomic and physiological traits such as for EARE, PH, TKW, and CT. Some of the regions have already been described in bi-parental populations. The most robust MTA with the highest R^2 for PH was detected on chromosome 6A. MTAs robust across more than two environments for CT were identified in linkage groups 1B, 4A and 6A, chromosome regions that have been previously reported elsewhere. The project emphasised the relevance of these chromosome regions and highlighted their importance for setting up new mapping and breeding populations. The AM panel segregated for major flowering time genes, with a consequent varying phenology. MTA for phenolgy were therefore partly co-located with performance MTA although MAT was considered as a covariate in mixed model analyses when computing genotypic variation. Any AM panel with high genetic and functional should be screened for synchronized phenology cycles in advance to more precisely study the effect of minor genes underlying grain yield and related adaptive traits.

Conclusion

Our results indicate the suitability of using gene bank core collections to investigate the genetic control of agronomic and physiological traits under rainfed conditions. The AM panel based on the GCP reference sample did encompassed a broad range of genetic and functional diversity expressed by large genotypic variations for the investigated traits, a large number of marker polymorphism and low LD. The number and consistency of MTA across four rainfed environments was generally very low, with only 1.56% detectable associations. Significant marker effects were consistently detected in only a rather limited number of cases suggesting that the power to detect the relevant loci for grain yield and its agronomic and physiological components via AM decreases, most likely because under such conditions similar yield values can be attained by different genotypes through different adaptive strategies corresponding of significant MTAs. None the less, AM revealed the influence of several chromosome regions on the variability of the targeted agronomic and physiological traits such as EARE, PH, TKW, CT. Although some of the regions have already been described in bi-parental populations the project emphasised their relevance and highlighted QTL for setting up new mapping and breeding populations. The panel segregated for major flowering time genes, with a consequent varying phenology. MTA for phenolgy were therefore partly co-located with performance MTA although MAT was considered as a covariate in mixed model analyses when computing genotypic variation. Any AM panel with high genetic and functional should be screened for synchronized phenology cycles in advance to more precisely study the effect of minor genes underlying grain yield and related adaptive traits.

II. Themes
Theme 2: Integrated crop breeding

10. 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
WJ Grüneberg, CIP; w.gruneberg@cgiar.org

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- CIP: Ian Barker (left CIP in 2010), Kelvin Huamani
- NaCCRI: Robert Mwanga
- CSIRO: Andrzej Kilian

Summary
Sweetpotato is an important food crop and, owing to its extremely high pro-vitamin A content, orange-fleshed sweetpotato (OFSP) can alleviate vitamin A deficiency. The sweetpotato virus disease (SPVD) often causes serious yield losses, especially in high virus pressure zones within Sub-Saharan Africa, and introduced OFSP material has failed because of high SPVD susceptibility. The critical component within SPVD (which is usually a co-infection of sweetpotato clorotic stunt virus [SPCSV] with other sweetpotato viruses) is SPCSV, because this virus breaks down the resistance of sweetpotato against other sweetpotato viruses. Sweetpotato virus pressure varies extremely from year to year and from location to location, and NCM-ELISA tests to determine resistant clones are not very reliable. This should make marker-assisted selection a very useful strategy in applied breeding programs.

The project developed two populations that are useful for marker association studies. Two major problems occurred in this project. The first problem was that the "resistant" clone to SPCSV (DLP3163) did not have the high resistance as originally expected. The second problem was that extreme difficulties occurred in phenotyping SPCSV and sweet potato feathery mottle virus (SPFMV).

The major outputs of the project until end of June 2011 were:

- The finding of resistance to SPSCV, which is more pronounced then every found before,
- Clearly improving SPVD screening methods for SPVD phenotyping
- Clarifying the inheritance of SPCSV resistance on basis of a self-compatible clone and SPCSV resistant clone (VJ08.330)
- Confirmation that the resistance to SPVD - on basis of SPCSV resistance found - is pronounced in high virus pressure fields of East Africa
- Broadening the genetic basis of SPCSV resistance (at least 12 SPCSV resistant clones are available now and by a new cycle of recombination we perhaps increase this to 500 seed and genotypes, respectively
- A small reference population has been developed for marker association studies
- The finding of six potential AFLP markers, seven potential SSR markers, and one potential DArT marker associated with SPCSV resistance.

On the basis of these results we think that the project was successful. There is one important output missing before the project can be declared a success for marker assisted selection in sweetpotato or not and this is to validate the potential markers in applied breeding material. The development of marker assistant selection for SPVD will continue at CIP in the frame of a project funded by the Bill & Melinda Gates Foundation (at least until mid 2014) with the target to implement the technology developed in this project in applied breeding programs.

Conclusion
By the end June of 2011, the project had achieved five notable successes: (1) the finding of resistance to SPSCV, which is more pronounced than ever found before, (2) clearly improving SPVD screening methods for SPVD phenotyping, (3) the confirmation of a apparently recessive inheritance of SPCSV resistance, (4) developing a small reference population for marker association studies, and (5) the finding of six potential AFLP markers, seven potential SSR markers, and one potential DArT marker. We think most problematic in SPVD research is appropriate phenotyping, which need years. However, we believe this a good phenotyped reference population – extended from year to year with carefully phenotyped clones – molecular markers will come into application for SPVD screening, because it is the only option for rapid SPVD resistance determination. Fortunately the work of this project can be continued until June 2014 in the frame of a larger sweetpotato project for Africa funded by the Bill & Melinda Gates Foundation. We will keep GCP

informed about further achievements in the context of marker-assisted selection for sweetpotato virus disease (SPVD). The next two targets are:

- Validation of markers in the population VZ08.
- Evaluations of the auto-fertilized offspring of VJ08.330 (Table 5) under field conditions at NaCCRI Uganda.
- Application of the 14 molecular markers found so far for SPVD selection in a pre- breeding population derived from crossings described in Table 10.

The major lesson learned is that NCM-ELISA is not useful to develop good reference populations for marker association studies aiming at SPVD; without real-time PCR or DAS- ELISA and TAS-ELISA, such studies should not be undertaken.

Quantifiable Outputs

1. Four parents carrying the resistant allele with q = 0.5, elevated agronomic value (>15 t/ha storage root yield) and food quality (>100 ppm beta-carotene, >24ppm iron, > 13 ppm zinc) for back crossings
2. Frequencies of SPVD resistance phenotypes in a population of 12 genotypes derived from self-fertilizations and frequencies of SPVD resistance phenotypes in a population of 610 genotypes derived from crossings with the clone "Resistant"
3. At least 40 new SSR loci identified
4. Four backcross populations developed each with at least 250 seeds
5. Frequencies of SPVD resistance phenotypes in 4 backcross populations
6. Two backcross population selected for mapping studies
7. An average map resolution of 2 cM based on AFLP marker
8. At least one SSR or SNP marker for SPVD resistance
9. At least 300 potential parents with elevated agronomic value and food quality screened for the frequency of the resistant allele
10. At least 400 germplasm clones screened for the frequency of the resistant allele

Theme 4: Capacity building

11. 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

EXTRACT FROM FINAL TECHNICAL REPORT

Collaborating institutes and scientists

- CropGen International, Robert Koebner

Summary

A number of new training materials have recently been developed by the GCP, training courses held, and software and bioinformatics tools developed, all directed towards facilitating the use of molecular markers and genomic information by plant breeders. However, all these materials have been based on the assumption that a working knowledge of core plant breeding concepts is already firmly in place. Without this, any positive improvement in plant breeding practice, with or without molecular markers, is unlikely, and much of the effort in exposing trainees to "molecular breeding" will have been wasted. Furthermore, the growing cadre of scientists trained in molecular biology, genetic diversity and other related fields, all too often lack any appreciation or knowledge of basic plant breeding techniques, thereby limiting the potential for fruitful interaction and collaboration between disciplines.

This new learning module aims to fill this gap, via the development of a resource covering basic plant breeding concepts and techniques. It seeks, in much the same way as previous SP5 modules have done, to complement, rather than to replace more conventional learning materials; and to supply the content in a way which will be readily accessible for institutions which lack the resources to support comprehensive and up-to-date printed literature. The module will be useful either as a teaching tool or as a self-learning tutorial.

The draft module was reviewed by external reviewers (the Cornell Plant Breeding Department and Geneva Agricultural Station) and the slides sent to GCP in December 2010. It was formatted into the online module, reviewed and edited by Robert and Theresa, and the final product is now (as of September, 2011) available at http://www.generationcp.org/plantbreeding/ (Now at https://www.integratedbreeding.net/plant-breeding-concepts-methods)

Conclusion

The final product is now available http://www.generationcp.org/plantbreeding/ (Now at https://www.integratedbreeding.net/plant-breeding-concepts-methods). A limited number of CDs were made available at the GRM-India and they were quickly gone, so it seems interest is high.

Theme 5: Product delivery

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

- *January 2007–December 2008; NCE: Oct 2011*

Principal Investigator and lead institute
Jean-Francois Rami, CIRAD; rami@cirad.fr
UMR AGAP, TA 108/03, Av Agropolis 34398 Montpellier CEDEX 5, FRANCE, +33 4 67 61 44 65, +33 4 67 61 56 05

Collaborating institutes and scientists
- CIRAD: Claire Billot, Sarah Mc Grath
- ADNid: Fabienne Moreau
- ICRISAT: Hari D. Upadhyaya, Tom Hash
- IRRI: Ken McNally
- CIP: Marc Ghislain, Wolfgang Gruneberg
- CIMMYT: Jill Cairns, Susanne Dreisigacker
- CIAT: Martin Fregene, Steve Beebe
- ICARDA: Michael Baum
- Bioversity: Nicolas Roux
- CIRAD: Luc Baudouin
- IITA: Robert Asiedu, Sarah Hearne
- UCR: Jeff Ehlers

Summary
The scientific community involved in the SP1 sub-programme of the Phase 1 of the Generation Challenge Programme has contributed one of the biggest efforts of characterisation of genetic diversity on 21 crop species. This characterisa- tion was based on the utilisation of microsatellite markers, which constitute a powerful marker system for such a purpose. However, this work was by na- ture composite, involving different species and different partners using different technologies. For each crop, one of the main products of this exercise was a "ref- erence set" of representative germplasm to serve as a material for international coordination in the future.

The present project was designed to assess the different microsatellite datasets produced in SP1 by having the reference set re-genotyped by an external geno- typing facility (service provider) to validate and certify the genotypic informa- tion attached to the reference set.

Out of the 21 mandate crops of the GCP, re-genotyping has been performed for 17 species for which a reference set was available. The data produced as part of this project (validation data) was compared to the data produced initially (original data) following a standard protocol in order to decipher various source of difference between original and validation data. Major differences in terms of technical marker problems or accessions conformity issues have been reported for most species in a detailed analysis report. The validation and original datasets have been stored in a database and made available online.

Conclusion
The project provided a complete assesment of reference set genotyping infor- mation for most of the crops of the GCP, including the priority crops of second phase of GCP. Meanwhile, it included a comparison of currently available refer- ence set biological material with material previously genotyped when included in the global composite set. Difficulties in the analysis were mainly related to the necessity of partitioning genotyping errors, and consequently assessing genotyping quality, from biological differences. It can be generally reported from the exercise conducted as part of this project that SSR genotyping is very challenging in terms of protocols standardization across laboratories. Finger-printing toolkits, defined as a set of well characterized control varieties, must be developped as a prerequisite to any collaborative effort of SSR diversity. The validation conducted as part of the project allowed to propose such toolkits for the various crops that have been analysed. More generally, we may antic- ipate that considering the rapid evolution of genotyping technologies toward SNP markers this technology may facilitate the production of lab-independant reference genotyping datasets that can be used for further conformity checks.

The comparison conducted with original data initially produced as part of GCP SP1 pointed out potentially questionable accessions for which germplasm management issue may have occured during the reference set production process. These accessions should be further verified. From the validation dataset that as been generated as part of this project and from its comparison with original data, it is recommended that, for each species, a consolidated validated dataset be defined by the institute in charge of the reference set distribution and made accessible through the GCP central registry.

Key Products Developed by the Project
1. A stabilized genetic stock for each accession constituting the reference samples in each of the 21 crop species
2. A genotypic dataset for the reference samples of the 21 crop species

III. Services
G8009 Integrated Breeding Platform/Theme 3 – Crop information systems

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

- *July 2008–June 2009; NCE: July 2011*

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

EXTRACT FROM FINAL TECHNICAL REPORT

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

Summary
This report provides the background in presenting an open question to the plant breeding community and those that resource it, "would more formal quality management systems be valued in plant breeding and, if yes, how would we achieve a voluntary Community of Best Practice (CoBP) standard". Some key benefits are noted in terms of client needs, cost efficiency, staff succession and transfer of knowhow, winning business, data legacy and delivery of synergy across projects. These benefits sum to ensure delivery of elite varieties and in maintaining critical knowledge for the future. The report runs with the assumption that a quality system is required and proceeds to identify critical points of leverage in effecting behavioural change amongst stakeholders of a plant breeding pipeline.

Case examples are provided from different situation as may serve good example such as the accreditation of the CIP germplasm to named pests and the quality assurance that structure the development of pesticide development. Positive example from industry is also highlighted in contrasting working practise to institute research. A particular consideration is also given to some of the unique features about Research for Development in meeting needs of development that have to be accommodated within a quality system and require specific consideration.

A critical need is identified in placing quality assurance as a condition, or at least a review factor, for accessing funding. The Consultative Group for International Agricultural Research (CGIAR) is identified as the most likely champion to articulate this condition for funding 'downwards' to the research community and 'upwards' through their reporting responsibility to donors. The CGIAR entities most suitably placed to fulfil this role are seen as the Integrated Breeding Platform (IBP) of the Generation Challenger Programme (GCP) or alternatively, but less suited because of proximity to delivery and potential conflicts of interests, the emerging CGIAR Research Programmes.

In progressing a quality management framework the critical gap is identified in setting the expectation of the quality metrics as will form the CoBP. Discussion on the standard recognises the need for the standard to be pragmatic, flexible and not overly burdensome. Furthermore, consideration is given to how best to value existing breeding tools, designed for breeding or for other purpose but with value. It is recommended that the expectation should be set independently of what is existing so that any tool can qualify to be part of the scheme. Accordingly, some preliminary 'expectation' and 'measure' are suggested at different stages of a generic plant breeding pipeline that require further elaboration.

The report concludes that it is currently premature to think too deeply on the implementation of the standard until such time as the standard is clear. Accordingly, a brief guidance to implementation is provided only. It is recognised that implementation of the standard at the institute level will be on a case-by-case bases and that the quality systems for any two institutes, whilst meeting the same standard, are unlikely to be exactly the same.

By this project it has been possible to sow a seed for quality assurance amongst the breeding community for 'why' such measures can be beneficial to them in their professional careers, their institutes in their business growth and continuity and to the poor in delivering with greater certainty elite varieties that perform under evermore challenging growing conditions in meeting the needs of a growing and demanding population. The report identifies the below next steps for implementation as a mechanism to progress a stepwise development and acceptance of the CoBP.

Generation Challenge Programme (GCP)
Hosted by CIMMYT

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

Mailing address:
Apdo Postal 6–641
06600 México, DF, México

Physical address:
Km 45 Carretera México-Veracruz
El Batán, Texcoco, México, CP 56130

Tel: +52 55 5804 2004 • **Fax:** +52 55 5804 7558

Email: generationcp@cgiar.org or info@generationcp.org
www.generationcp.org