CIAT

Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture
Consultative Group on International Agricultural Research

Annual Report 2009

SBA-4: RICE

For Internal Circulation and Discussion Only

May 2010

Pic by Neil Palmer (CIAT). A rice farmer in Kantuta, near Caranavi, Bolivia.

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CIAT's CORPORATE BUSINESS PLAN
JULY 2009 – DECEMBER 2010
RICE PROGRAM
RESEARCH ACTIVITIES

General description of the Rice Program:

- Rice is the leading food staple in South America and the Caribbean (LAC). While most of the world's rice is produced in Asia, which is home to IRRI, in LAC there are unique pests and diseases, as well as distinct grain types and cropping systems that require regional development of germplasm. The demand for rice in LAC is growing, and the region's abundant land and water resources give it the potential to be a growing supplier of rice to the world. During 1990-2004 rice production in LAC expanded annually at 3%, much higher than in any other region. Rice is grown under diverse agro climatic/soil conditions and production systems and the crop is subjected to diverse biotic and abiotic stresses, different from Asia and Africa. Mainly medium to small resource-poor farmers predominate in Central America and the Caribbean, and in the northern part of South America; some medium to large mechanized farms are found, especially in the Southern Cone. Therefore, there is a need to develop germplasm and technology appropriate for diverse type of rice production systems and different breeding strategies and rice materials are needed. Eco-efficient rice production systems, with high productivity and low impact on the environment are critical for the future. There are opportunities for growth in the rice sector in LAC. To meet challenges related to increasing yield potential, tolerance to biotic/ abiotic stresses, adapting to and mitigating the effects of climate change , improving the competitiveness of rice sector, reaching end-users, technology transfer and training of new rice scientists the Rice Program will focus on strengthening the rice sector of LAC by promoting a strong research and technology platform driven by CIAT-FLAR-EMBRAPA consortium including CIRAD-IRD partners in close collaboration with interested NARs, by building on IRRI-CIAT-WARDA strategic alliance, by promoting a win-win partnerships with private sector and advanced institutions/lab, and by combining breeding with biotechnology tools.

Goals: To improve rice production sustainability in LAC, generating food security, increasing income and improving health associated with Eco-Efficient production in the tropics with emphasis on increasing the options for the medium-small farmers. To develop stronger partnerships in the region, increasing the capacity to deliver technological products to targeted end users.

- *Objectives: Development of improved rice lines with higher yield and nutritional value*

- *Utilization of wild rice species and exotic germplasm for the development of resilient rice germplasm to respond to climate change with high yield potential, tolerance to main insect pest and diseases, and good grain quality*

- *Development of rice lines with improved water and nitrogen-use efficiency by complementing conventional approaches with advanced biotechnology tools that include molecular markers and transgenics*

- *Development of a regional hybrid rice project supported by a wide public-private partnership*

- *Promoting best management practices and technology transfer to close the yield gap by working with NARs, farmer's associations and private sector*

- *For whom? Rice consumers and farmers in Latin America, rice scientists, NARs breeding programs, FLAR, AgroSalud, INGER, and Grumega*

- *With whom? NARS and advanced institutions through the Generation Challenge Program , FLAR, CIRAD-IRD, and Embrapa-CNPAF*

- *Where? Latin America and the Caribbean*

- The Rice Program conducts research that is complementary to research carried out by both IRRI and WARDA especially in the development of breeding populations via the utilization of wild rice species through the Generation Challenge Program. CIAT is generating both segregating populations, parental, and advanced lines. These are transferred to partners through CIAT's nurseries, FLAR, GRUMEGA, AgroSalud, and INGER. In collaboration with JIRCAS (Japan), gene technology is being explored as an alternative to incorporate increased efficiency in water use for the irrigated rice ecosystems targeting reduction of water consumption. Close collaboration is formed with ARIs, NARS, and advanced laboratories to carry out strategic research to: generate, select , evaluate and release rice varieties ; enhance adaptation of rice lines to biotic and abiotic stresses; develop methods and tools for targeting, processing and evaluation of rice germplasm ; employ operational research principles to develop rice germplasm for specific production and market niches; explore the potential of wild rice species and exotic germplasm for adaptation to and mitigation of climate change and reduction of the negative impacts of agriculture on the environment. Capacity building including group/individual training and knowledge

management remains integral to our agenda to: a) strengthen/benefit from the research capacity of partners, and b) enhance our capacity to deliver research products in different environments.

In view of the dwindling number of alternative providers and the Center's expertise across different production systems, CIAT's Rice research has an explicit regional *focus*, with strategic research capacity in South America, Central America and the Caribbean. However, through our strategic alliance with IRRI-WARDA and INGER our germplasm is also available and evaluated in some regions in Africa and Asia. We will work together with IRRI and WARDA across themes and regions to address the major challenges and to deliver specific outputs. We recognize that the strongest interaction between research disciplines, partners and clients happens in the actual context of agricultural systems in the countries. Capacity building, strong partnerships and knowledge sharing are therefore integral parts of all three rice strategic research outputs.

- *Research Products and International Public Goods*. The research products of CIAT's Rice Program are in line with CIAT`s approach to eco-efficient agriculture for the poor and the mandate of the CGIAR to produce international public goods (IPGs). They can be grouped into the following categories: Development of Breeding (rice lines, parental sources, and populations), Genomic (introgression lines, CSSLs, maps, software, databases, protocols and markers), and Training Tools (reports, books, papers, conferences, and workshops).

- What we want to accomplish: In line with CIAT`s focus on eco-efficient agriculture for the poor (i.e., balancing between economic, ecological and social impacts), outputs have been further sharpened. The research agenda builds on our achievements in increasing productivity and lowering production costs of rice in LAC to make it more competitive, while enhancing efforts to realize the environmental benefits of a more eco-efficient rice production. People living in areas where rice consumption is high (Haiti, Nicaragua, Bolivia, Panama, Dominican Republic, Colombia, and rural Brazil) are suffering from a number of major nutritional problems. Women and children are especially susceptible to deficiencies in micronutrients, particularly vitamin A, iron and zinc. As a result, they are at risk of disease, premature death, lower cognitive capacity, and poor quality of life. The costs of these deficiencies are high and economic and health indicators in LAC are deteriorating. **Biofortified rice lines will be Rice Program's contribution to combat malnutrition and food security in LAC** through the development of and deployment of high iron and zinc rice lines having high yield potential and desirable agronomic traits (Output 1). The genetic base of both irrigated and upland rice in LAC is very narrow. To increase the genetic diversity of rice, we use different breeding strategies, including

interspecific crosses, composite populations, introgression and recombinant inbred lines, and we are developing biotechnology tools that allow the incorporation of traits more efficiently. Wild species are valued as a unique source of genetic variation. Since 1994 the CIAT Rice Program has been characterizing and utilizing wild rice species. The strategy in place makes use of molecular maps in combination with backcrossing to elite breeding lines or commercial varieties to develop populations that are used to identify and transfer quantitative trait loci (QTLs) associated with traits of agronomic importance to cultivated rice (Output 2). Rice production faces the difficult challenge of obtaining reliable yields under variable conditions, notably due to the prevalence of biotic and abiotic stresses exacerbated by climate stresses. Numerous genes of economic importance are transferred from one varietal background to another through conventional breeding approaches, a time –consuming effort. Sometimes, screening procedures are cumbersome and expensive, and require large experimental area. A lot of molecular data have been generated at CIAT in rice on different agronomic traits, including an anchor marker map, several mapping populations, a field phenotyping platform and transformed rice lines carrying DREB-gene associated with drought tolerance. CIAT has permission from the Colombian National Biosafety Committee and excellent biosafety and field facilities for the generation and testing of transformed rice. **Lines with better water and nitrogen-use efficiency will allow savings in water and fertilizer costs (Output3).** *To accomplish the goal and objective of the Rice Program, our research is being organized around three outputs:* Output 1 Rice germplasm for improving human health and nutrition in Latin America and the Caribbean; Output 2. Enhanced Gene Pools for Irrigated and Upland Rice in Latin America and the Caribbean, and Output3.Genotyping and Phenotyping Platforms for Rice Enhancement

Output 1 Rice germplasm for improving human health and nutrition in Latin America and the Caribbean:

Women and children are especially susceptible to iron and zinc deficiencies and vitamin A. The costs of these deficiencies are high and economic and health indicators in LAC are deteriorating. Biofortified rice lines will be Rice Program's contribution to combat malnutrition in LAC. Three Millenium Goals and food security are deal with this output. The main objective is the development of rice lines with 6-8ppm of iron and 22-25ppm of zinc in milled rice to combat malnutrition.

Activity 1. *Screening and field evaluation of local varieties and advanced breeding lines for mineral content to identify products that have immediate utility*

Activity 2. *Development of populations and lines having a high level of iron and zinc combined with good yield potential, tolerance to main diseases and insect pests, and good grain quality*

Activity 3. *Evaluation of promising lines and distribution to partners*

Activity4. *Evaluation of the nutritional status and acceptance of promising lines*

Target per output (Description of target). At least two lines with increased iron and zinc content in milled rice identified and ready for release by Agrosalud partners in 2009 and 2010 in at least two countries.

Target region	Production Constraints	Social constraints	Target traits	Research Priorities	Challenges	Technical Solutions	Social - Economical Impact
Colombia, Cuba, Bolivia , Brazil, Nicaragua, Dominican Republic, Panama	Tolerance to rice blast, rice hoja blanca virus, Tagosodes, grain quality	Malnutrition, anemia, poverty	High iron and zinc, good yield potential and improved agronomic characteristics	Combine good agronomic traits with enhanced nutritional value	Combining high yield potential and desirable agronomic traits with improved nutrition	Delivery and adoption of rice lines with improved nutritional and agronomic value	Reduced micronutrient deficiency and increased food and nutrition security among vulnerable populations in target countries

Output 2 Enhanced Gene Pools for Irrigated and Upland Rice in Latin America and the Caribbean:

Different breeding strategies and biotechnology tools are used to broaden the genetic base. Several traits of agronomic importance, and tolerance to biotic and abiotic stresses, have been transferred from wild rice species and exotic germplasm into improved rice cultivars following our participation in the Generation Challenge Program. Recombination of favorable alleles associated with desirable agronomic traits will allow the development of germplasm with specific adaptation for diverse types of production systems in line with an eco-efficient rice production.

Activity1. *Development of introgression and recombinant inbred lines using exotic germplasm and wild rice species*

Activity2. *Development of synthetic and composite populations using recurrent selection methods*

Activity3. *Development of a regional hybrid rice project supported by a wide public-private partnership*

Activity4. *Identification of promising breeding lines for a CIAT-ION nursery for distribution to partners*

Target per output (Description of target): A CIAT-ION nursery distributed to 10 partners made up of about 100-150 lines carrying genes for durable resistance to blast and other desirable agronomic traits derived from wild rice species or composite populations.

Target region	Production Constraints	Social constraints	Target traits	Research Priorities	Challenges	Technical Solutions	Social - Economical Impact
Latin America and the Caribbean	Rice blast, Rhyzoctonia, Hoja blanca virus(RHBV), Tagosodes, B.glumae, cold and heat tolerance , drought stress , and poor grain quality	Poverty, rice deficit, high food prices, and lack of sufficient trained rice scientists	Yield potential, grain quality, and tolerance to main biotic and abiotic constraints indicated in production constraints	1. Tolerance to rice blast, Rhyzoctonia , B.glumae, and RHBV. 2. Yield potential 3. Tolerance to heat and drought stress. 4. Good grain quality	1. Obtaining reliable yields under variable conditions 2. Combine phenotypic and genotypic data. 3.Increasing grain yield	1. Multidisciplinary team approach 2. Combining conventional and biotechnology tools. 3. Development of hybrid rice	Increased and eco-efficient rice production. Improved rice competitiveness via lower production costs and higher yields. More friendly rice production and trained personnel

Output 3 Genotyping and Phenotyping Platforms for Rice Enhancement:

A lot of molecular and phenotypic data have been generated in rice at CIAT on different agronomic traits, a field phenotyping platform is in place, and transformed rice lines with drought tolerance are available. The main objective is to integrate biotechnology tools available at CIAT and advanced institutions collaborating with us into the rice breeding program, especially via marker assisted selection and genetic engineering to improve eco-efficiency of rice production.

Activity1. *Devolopment of phenotyping platform for water and nitrogen-use efficiency*

Activity2. *Development of BC lines resistant to RHBV and Tagosodes via SRR markers*

Activity3. *Development of rice lines with better water and nitrogen –use efficiency*

Activity4. *Integration of conventional breeding methods with biotechnology tools for a marker assisted selection program*

Activity5. *Development of management practices and germplasm tolerant to B. glumae and potential new Pyricularia populations*

Target per Output (Description of target): 50 introgressions lines with chromosome segments substitutions from wild species and 20 BC lines resistant to RHBV and Tagosodes

Target region	Production Constraints	Social constraints	Target traits	Research Priorities	Challenges	Technical Solutions	Social - Economical Impact
Latin America and the Caribbean	Same as in Output 2.	**Poverty, rice deficit, high food prices, and lack of sufficient trained rice scientists**	Tolerance to rice blast, rice hoja blanca virus, Tagosodes, Rhyzoctonia, and B.glumae. Water and nitrogen use-efficiency.	Development of a marker assisted selection program, study the genetic and pathogenic diversity of *B. glumae* population, and the virulence spectra of new *Pyricularia* collection on rice breeding materials Evaluation of rice lines for water and nitrogen use-efficiency	**1. Obtaining reliable yields under a variable climate** 2. Adverse effects in rice production due to climate change 3.Increasing rice yield 4. Training of new rice scientists. 5. A marker assisted selection scheme in place	**1. Multidisciplinary team approach 2. Combining conventional and biotechnology tools.** 3. Capacity building	**Increased and eco-efficient rice production. Improved rice competitiveness via lower production costs and higher yields. More friendly rice production and trained personnel.**

General description of new initiatives:

1. A Unique LATIN AMERICAN RICE CONSORTIUM: All the elements are in place to propel LAC rice production- not only to become self-sufficient, but also to help solve future global rice food challenges. This CIAT-FLAR-EMBRAPA consortium will lead a research and extension efforts for a new Agronomic-Genetic revolution. This Consortium funded by the Latin American Development Bank (BID) will have four Outputs, each with different Activities and Outcomes.

Output 1: Increased yields and reduced costs

Activity1. Technology adaptation and transfer to implement proven agronomic practices to reduce production costs, increase yield, and improve net incomes of upland and irrigated farms

Activity2. Enhance technologies for water use efficiency, direct seeding and other validated crop management practices.

***Expected outcome*:** Yield increases (1 -3 t/ha), cost reductions (10 - 30%), less use of pesticides and fertilizers.

Output 2: Targeted delivery and refinement of new high-yield cultivars

Activity1. Increase efficiency of breeding programs to speed delivery new improved cultivars

Activity2. Integrate plant breeding with new genomic tools to develop varieties with superior nutritional content, resilience to climatic change, and more efficient water and nutrient use.

Activity3. Develop nutritious rice varieties with high iron and zinc

Activity4. Strengthen and facilitate a regional germplasm exchange network to increase breeding efficiency

Activity5. Develop public-private seed system partnerships to ensure delivery and adoption of improved varieties

Expected outcome: Higher yielding (10%), high milling and cooking quality more nutritious and resilient varieties with specific adaptation to the different LAC agro-ecosystems

Output 3: A new generation of LAC rice scientists

Activity1. In-service training of young scientists to obtain practical rice research experience.

Activity2. Exchange programs for NARS's scientists to advanced laboratories and universities (sabbaticals), for updating on new advances.

Activity3. Exchange programs and specific training for extension agronomists and farmer-leaders to speed co-development and transference of new crop management techniques.

Activity4. MSc. and PhD's theses in under-staffed scientific areas

Expected outcome: 200 persons /year including all areas

Output 4: Market efficiency and competitiveness

Activity1. Update the strategic vision for rice in LAC, taking into account future challenges, market trends and potential impacts of climate change.

Activity2. Analyze government and private sector policies on credit, tariffs, and commodity exchanges to improve regional performance and competitivity.

Activity3. Conduct ex-ante and ex-post impact studies to guide research and to justify investments.

Expected outcome: An active forum of rice scientists, marketing specialists, the private sector and governments that helps LAC improve competitiveness in global rice markets

	2009				2010			
	FTE	**Core $**	**Restricted $**	**Total $**	**FTE**	**Core $**	**Restricted $**	**Total $**
Research Execution								
Estimated research execution								
Research Headcount								
Personnel		**624**	**1,441**	**2,065**		**535**	**1,502**	**2,037**
IRS		288	448	**736**		268	461	**730**
NRS		336	993	**1,329**		267	1,041	**1,308**
Research Expenditures								
Operations		**143**	**1,582**	**1,725**		**102**	**1,620**	**1,721**
Supplies & Services		124	957	**1,080**		102	995	**1,097**
Parthership / Collaborators			318	**318**			308	**308**
Travel		20	266	**286**			276	**276**
Depreciation / Capital 6			41	**41**			41	**41**
Research Institutional Charges								
Direct and Indirect Charges		**479**	**547**	**1,026**		**444**	**312**	**756**
Facilities & Public Area (direct)		258	12	**271**		103	105	**207**
Depreciation Charges (direct)		48		**48**		48		**48**
IT Charges (direct)		168	5	**172**		45	59	**105**
Research & Technical Support (direct)		3	142	**145**		69	95	**164**
Admin/Overhead (indirect)		2	388	**390**		179	53	**232**
Total Expenses		**1,246**	**3,570**	**4,816**		**1,081**	**3,434**	**4,514**

Rice Program Senior Staff:

Cesar P. Martinez (100%)	Program Leader, Breeder
Marc Chatel (100%)	Plant Breeder CIRAD-France
Cécile Grenier (100%)	Associate of Staff CIRAD-France
Gloria Mosquera (70%)	Plant Pathologist
Edgar Torres (75%)	Plant Breeder CIAT-FLAR
Jagadish Rane (100%)	Plant Physiologist
Manabu Ishitani (25%)	Molecular Biologist
Carlos Bruzzone (50%)	Rice breeder Consultant INIA Perú
Mathias Lorieux (100%)	Molecular Geneticist –IRD France
Beata Dedicova (20%)	Transgenic Specialist and Tissue Culture

Description of staff needed to complete team (one paragraph description per staff needed)

1. **Agronomist.** Recent data collected by FLAR in several countries indicate that by using better agronomic practices and improved varieties farmers are getting 1-2 ton/ha more of rice lowering production cost/ton of rice. This experience needs to be transferred and adapted to other rice growing areas in LAC. This work has been done using soft money but in order to achieve a new Agronomic-Genetic revolution a more long-term commitment is needed.

2. **Training/ Technologist transfer specialist.** To contribute to the formation of a new cadre of rice scientists and to assist the agronomist in technology transfer.

3. **Full time economist.** To carry out impact assessment studies related to adoption of germplasm and improved agronomic practices.

4. **Half-time entomologist.** To assess changes in insect pests incidence and behavior due to climate change

5. **Half-time virologist.** To assess changes in rice hoja blanca and Tagosodes incidence due to climate change.

6. The Rice Program will also need input from **GIS Specialist** for quantifying the current land use of improved rice varieties and their risks for negative effects due to climate change and opportunities for increasing rice production in LAC.

7. **Transformation specialist**: To continue and supervise transformation activities carried out before by Zaida Lentini.

Rice Achievements in 2009

- Top 3 products-

- Three varieties (Azucena, Saavedra 26 and IACuba 30) with 30-50%more iron and 17-30% more zinc than commercial milled rice were released in Bolivia and Cuba. Eight varieties were released by FLAR's members in five countries.

- Five varieties from recurrent selection: Zafiro (Chile), Para and Yara (Bolivia-small holders), and Lines 1-INIA-FCI and 3-INIA-FCI (Venezuela-water saving)

- Improved crop management expanded and impacting yields and costs in Argentina, Venezuela, and Guyana. Direct seeding technology expanding in several tropical countries.

- Top 2 research – (tools/ methodologies)

- Establishment of drought screening methodology using rainout shelter for improved water use efficiency in rice; promising candidates using a transgenic approach were selected for further confirmation.

- Phenotyping using Infra-red thermography screening at field condition (Off-season. Santa Rosa Station). Alain Audebert *et al*

- Advances on MAS for RHBV and cold tolerance.

Proposed Solutions to the top 3 major bottlenecks in 2010-2011

- Insufficient budget. LAC s not a priority for donors.

  Proposed Solution: a) HP funding for rice biofortification; b) emphasis on a Rice Megaprogram; c) A new strategy to fund raising specially in LAC (private sector)

- Succession of senior scientists: Training opportunities for our Junior Staff.

  Proposed Solution: Careful selection of candidates to Monsanto-Beachell and IRRI's Scholarships (Two candidates identified)

- Introducing MAS into our conventional breeding program.

  Proposed Solution: Accelerate the use of MAS especially for RHBV and durable resistance to rice blast as an initial step towards gene pyramiding.

Product in pipeline for release in 2011

- Top 3 products

  Four lines with increased iron and zinc are in the pipeline for release in Brazil, Nicaragua, Dominican Republic, and Colombia in 2010 – 2011.One elite aromatic line from the temperate japonica site-specific aromatic synthetic population PCAQ-1. France & Chile. Temperate climate ecosystem. FLAR's nurseries with lines pyramiding valuable traits.

  First breeding product with resistance to RHBV developed by MAS and MAS for cold tolerance running at FLAR and partner's institutions.

  Heterotic hybrids identified and lines in process to be converted in male sterile

  Water harvesting pilot project demonstrates benefits of irrigated agriculture (FLAR). New resistance sources for blast races.

Product pipelines in context of climate changes and Eco-Efficient Agriculture for the poor

- Top 3 products for 2020

  Delivery of breeding lines with specific traits adapted to targeted areas in LAC to be used as parents in hybrid and/or conventional breeding by our partners. Synthetic population(s) enhanced through cycles of recurrent selection for drought tolerance.

  High yielding varieties from gene stacking of key traits such as RHBV, blast resistance genes, nutrition and milling quality, water and nitrogen use efficiency, heat tolerance, and yield components by the use of MAS in our breeding program.

  Improved crop management practices are combined with improved varieties, resulting in more competitive rice production in LAC (FLAR)

  Small resource poor farmers have access to irrigation, resulting in high and more stable income with reduced risks to climate change.

Partnerships to pursue

- For discovery: IRRI-Africa Rice, Universities, French ARIS(CIRAD, IRD), and Private Companies

  New valuable genes such as herbicide resistance, increased nutritional value, tolerance to biotic and abiotic stresses

  New male sterile systems, and heterotic groups

  New technologies for genotyping, and phenotyping

- For development: IRRI-Africa Rice-CIAT- FLAR-EMBRAPA, Private Companies

  IRRI or a Chinese Institute with CIAT, FLAR and some strong local institutions in the region to construct a competitive hybrid breeding program.

- For delivery: AgroSalud, FLAR and Grumega-NARS networks

  Public-private Institutional platform for improving seed systems in several countries where they are a bottle-neck for improved varieties impact

- FLAR in cooperation with CIAT establishes alliance with major development agencies to support transformation to irrigated agriculture using water harvesting.

New BOLD flagship projects

- Two Products

  Hybrids adapted to tropical LAC conditions

  Competitive Healthy Rice based on gene stacking of key traits such as RHBV, Blast, Grain Quality (Head recovery, tolerance to delayed harvest), lodging tolerance and water/nitrogen use efficiency and heat tolerance by 2020.

- One research product

  A POC-product carrying DREB gene associated with water use efficiency ready by 2011.

- One development product

  Transformation to irrigated agriculture in at least one country by 2015.

RICE PUBLICATIONS 2009

Articles in Refereed Journals

Liu, G.; Jia, Y.; Correa V., F.J.; Prado P., G.A.; Yeater, K.M.; McClung, A.M.; Correll, J.C. 2009. Mapping quantitative trait loci responsible for resistance to sheath blight in rice. Phytopathology 99(9):1078-1084.

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Zorrilla de San Martín, G. 2009. **FLAR synergy**. Rice Today 8(3):40-41.

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List of Partners

Institution	Country of project
ADECOAGRO	Argentina
Africa Rice Center (WARDA)	Benin
Agrocom (a seed producer)	Colombia
Asociación Cultivadores de Arroz, ACA	Uruguay
Asociación Guatemalteca del Arroz (ARROZGUA)	Guatemala
Asociación Hondureña de Productores de Arroz, AHPRA	Honduras
Asociación Nacional de Molineros de Honduras, ANAMH	Honduras
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CONAGRO S.A.	Panama
Consejo Mexicano del Arroz	Mexico
Consejo Nacional Arrocero, CONARROZ. Esta institución reúne y representa en el FLAR a las siguientes organizaciones: - Centro de Investigación Agrícola Tropical, CIAT - Asociación de Productores de Arroz, ASPAR - Cooperativa Agropecuaria Integral San Juan de Yapacaní, CAISY Ltda.	
- Federación Nacional de Cooperativas Arroceras de Bolivia, FENCA	Bolivia
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Institution	Country of project
Fundación para la Investigación Agrícola en Venezuela (DANAC)	Venezuela
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Génoplante	France
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Grupo de Mejoramiento Genético Avanzado en Arroz-Organización de las Naciones Unidas para la Agricultura y la Alimentación (GRUMEGA-FAO)	FAO
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Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH)	Colombia
Instituto de Investigaciones Agropecuarias (INIA)	Chile
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Instituto Venezolano de Investigaciones Científicas (IVIC)	Venezuela
International Atomic Energy Agency (IAEA)	Austria
International Network for Genetic Evaluation of Rice-International Rice Research Institute (INGER-IRRI)	Philippines
International Rice Research Institute (IRRI)	Philippines
Japan International Research Center for Agricultural Sciences (JIRCAS)	Japan
Kansas State University	USA
Louisiana State University	USA
Ministerio de Agricultura y Ganadería (MAG)	Panama
Ministry of Agriculture	Belize
Misión Alianza de Noruega en Bolivia (MAN-B)	Bolivia
Philippine Rice Research Institute (PhilRice)	Philippines
Plant Research International (PRI)	Netherlands
RiceTec	USA
Rutgers University	USA
Semillano (a seed producer)	Colombia
Semillas del Nuevo Milenio S.A. (SENUMISA)	Costa Rica
Texas A&M University	USA
United States Department of Agriculture (USDA)	USA

Institution	Country of project
Universidad Central de Venezuela (UCV)	Venezuela
Universidad de Antioquia	Colombia
Universidad de Córdoba	Colombia
Universidad de Costa Rica (UCR)	Costa Rica
Universidad de Panamá	Panama
Universidad del Tolima	Colombia
Universidad Nacional Autónoma de México (UNAM)	Mexico
Universidad Nacional de Colombia	Colombia
University of Arkansas	USA
University of Braunschweig	Germany
University of Hannover	Germany
University of Wageningen	Netherlands
University of Yale	USA

Output 1: Enhanced gene pools

1A IRRIGATED RICE

1A.1 Activities in Hybrid Breeding

Torres E.A.; Berrio J.E.; Carabali S.J.; Agrono T. and Martinez C.P.

Abstract

Hybrid rice, an emerging technology in rice, could be the key in the near future to face food security and to use modern technologies in commercial production. It consists in the exploitation of heterosis by crossing two inbreeds and using F1 seed for commercial planting. CIAT and FLAR program began this year some activities in hybrid breeding with two objectives: 1. To explore tropical japonica germplasm searching for maintainers using test crosses and 2. To produce pure seeds of five AB systems with IRRI origin.

Introduction

Hybrid rice cultivars have several advantages over inbred cultivars. They have a higher yielding ability and are more tolerant to stresses. Additionally, they are the ideal vehicle for innovations such as herbicide tolerance in rice (Byron D., 2009). For these reasons, hybrid rice utilization is growing very fast in some countries like US and Brazil.

To have a successful hybrid breeding program in Latin America new sources of germplasm must be developed. According with Virmani (2005) indica elite lines or indica/japonica lines have a 30-40% of restorers frequency. In consequence, these materials could be used to develop heterotic hybrids for tropical conditions. At the same time, Virmani (2003) indicate that maintainers frequency is high (70%) among japonica elite lines and very low (5%) in indica lines. Additionally, Long Pin (2003) mentions that indica/japonica hybrids have a better sink and source

than indica hybrids, and in consequence, better yielding ability. Irrigated CIAT-FLAR germplasm is very interesting because it is not pure indica or pure tropical japonica due to the use of tropical japonica as VHB, drought and Blast resistance donors. Several varieties coming from this germplasm like Fedearroz 50 that combines indica/japonica traits have showed excellent yielding ability and adaptation to LA tropical conditions. Additionally, because conventional breeding was focused in accumulation of traits, in the beginning it will be difficult to find heterotic hybrids. For these reasons the way to develop heterotic hybrids for LA conditions using CIAT-FLAR germplasm could be:

A. To develop maintainers using tropical japonica related germplasm which includes: FLAR lines related with Fedearroz 50, FLAR and CIAT materials that have African blood (TOX or IRAT) and CIAT acid soils germplasm.
B. To develop restores among indica related germplasm which includes: CIAT lines related with Cica 8 and Epagri 108, IRRI lines adapted to LA conditions, and FLAR and CIAT lines with few tropical japonica blood
C. These groups could be considerer as heterotic groups for LA

The objectives of this work were: To develop testcrosses using five IRRI lines as testers and to produce pure seeds of these testers.

Materials and Methods

Five male sterile lines (table 1) coming from IRRI were used as testers and nineteen CIAT lines (table 2) as pollen donors.

Table 1. Male sterile lines used as testers

No.	Pedigree	Cross	GID	CYTOPLASM	SOURCE OF CYTOPLASM
1	IR58025A	IR48483A/8*PUSA167-120-3-2//PUSA167-120-3-2	70962	WA	SHEN SHAN 97A
2	IR70369A	IR62829A/7*IR62849-110-13-8-7-4-5-6	90015	WA	SHEN SHAN 97A
3	IR79128A	IR68897A/7*IR71567-69-2-1	98683 6	WA	SHEN SHAN 97A
4	IR79156A	IR68897A/7*72798-42-1-2	10381 31	WA	SHEN SHAN 97A
5	IR80156A	IR75608A/4*IR71591-9-3-2	11377 44	??	KALINGA

Table 2. Pollen donors

Code	Origin	PEDIGREE
BCF 1106	WC5231	CT9998-41-12-M-4
BCF 1115	WC5140	CT6241-2-2-1-3
BCF 1175	WC5203	CT7244-9-2-1-52-1
BCF 1202	WC5232	CT10006-7-2-M-2
BCF 1203	WC5233	CT10035-9-3-M-6
BCF 1211	WC5242	CT10035-26-4-2-M
BCF 1217	WC5248	CT10006-26-3-M-3
BCF 1219	WC5250	CT10045-5-5-M-1
BCF 1224	WC5256	CT10006-1-1-M-8
BCF 1225	WC5257	CT10037-32-1-M-2
BCF 1528	WC332	CT10175-5-1-3P-1-3-2P
BCF 1542	WC346	CT9155-2-3-1-2M-4-1P
BCF 1543	WC347	CT9737-8-9-1-1-1P
BCF 1547	WC351	CT10865-CA-12-M
BCF 1549	WC353	CT11685-7-F4-6-2P-1
BCF 1550	WC354	CT11691-17-F4-1-1P-2
BCF 1551	WC355	CT11696-9-F4-10-2P-3
BCF 1565	WC369	CT9682-2-M-14-1-M-1-3P-M-1
BCF 1609	WC413	CT9748-13-2-1-M-M-1-1

To produce F1 seeds manual crosses were used. In order to synchronize flowering, pollen donors and testers were planted fives and two times respectively. In each plant date a single transplanted row with 17 plants was planted per each genotype. By the flowering time, pollen sterility in each male sterile plant was confirmed using IKI solution and observations under microscope; additionally, one panicle per each male sterile plant was bagged to observe sterility at maturity. After that, the glumes were cut and the flower manually pollinated in the greenhouse.
Pure seeds of these five AB systems were produced using single crosses between individual plants. Each AB system was transplanted (30x30 one plant per hill) in crossing blocks with two external B rows and three internal A rows. At flowering ten single crosses were made between individual A B plants. The pollen sterility was verified by microscope observations and in each pollen donors a panicle was bagged to produce pure seeds. Also, each crossing blocks was protected with a plastic barrier and natural cross pollinated seeds were collected.

Results and Discussion

Seeds from manual crosses and natural pollination were obtained. A total of 76 testcrosses with an average of 250 seeds and 42 AB crosses with 116 seeds in average, were obtained. Two parents in testcrosses do not germinate. This seeds will be used in the next season to identify maintainers, restorers and heterotic hybrids; and in the AB crosse to produce pure seeds.

The preliminary results obtained in the characterization indicated that Lines 4 and 5 have better characteristic to be adapted to LA conditions. They have the highest seed production under natural pollination, high amylose content, long slender grains, average milling quality and translucent grains under Palmira conditions (Table 3).

Table 3. Seed production and grain quality of five AB systems.

		Linea A		Línea B				
Number	**Pedigree**	**Grams/plant**	**Amy (%)**	**BT (%)**	**GE (%)**	**CB**	**Amy (%)**	**LG**
1	IR58025A	0.96		63.3	49.21	1.0	18.00	L
2	IR70369A	0.49		61.8	48.46	1.8	26.09	L
3	IR79128A	0.58		58.6	44.08	0.6	17.80	L
4	IR79156A	3.20	28.81	63.1	51.28	0.8	29.43	EL
5	IR80156A	2.35	28.14	64.3	51.23	0.8	27.69	L

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1A.1.1 Phenotypic evaluation of F2:3 families to VHB.

Torres E.A.; Carabali, S.J.; Morán R.; Cordoba, E.; Cuasquer J. and Martinez C.P.

Abstract

The rice Hoja Blanca virus in one of the most important threats to rice production in tropical Latin America and many efforts have been done at CIAT in breeding for resistance and develop of screening methodologies; however, a more efficient selection tool is needed. To validate molecular markers associated with the resistance, 900 F2:3 families coming from contrasting crosses, their parents and resistant and susceptible checks were evaluated under greenhouse conditions for hoja blanca virus in six experiments. Each experiment had three cages with nine trays (38 rows each) and experimental units were single ten plants rows. There was different rep number for genotypes: controls were repeated 27 times, susceptible parents were repeated 9 times and F3 families were repeated once per experiment. Parents were also evaluated under field conditions in the masal screening and the extremes susceptible or resistant per each population and the parents were evaluated in tubes. The results showed significant differences for genotypes, experiments and genotypes per experiment. The trend of segregation was almost continuous suggesting a quantitative trait. There was transgresive segregation for susceptible and resistant reaction in each population and a gradation from very susceptible to intermediate between the susceptible parents. Fedearroz 2000 the common resistant parent and resistant check was the most resistant genotype between parents. The reaction of parents in the field and the tubes was similar with Fedearroz 2000 as the resistant genotype. However, some resistant progenies were susceptible under tubes conditions.

Introduction.

Hoja Blanca virus, a disease which more important effect is the complete sterility of affected tillers, is caused by Rice Hoja Blanca Virus. This virus belongs to *Tenuivirus* genus (Morales & Neissen, 1983) and is transmitted by a planthopper from Delphacidae family, *Tagosodes orizicolus*. RHBV is an insect disease and the interaction between virus-insect-plant is very important in the genotypic reaction.

Because of the sterility, RHBV causes very important losses when farmers use susceptible varieties. For example in Colombia the outbreaks of RHVB have been documented since 1935, it appears in a cyclical way and the maximum virulence coincides with losses between 25 to 50% in fields. Additionally, farmers normally apply insecticides in order to control insects causing the opposite effect in planthopper population, resurgence because of killing the natural enemies of Sogata and environmental contamination.
Resistance to the virus and the insect has been the method to face RHBV; however, the massive screening method used now has many difficulties. To identify resistant strains a field infestation methodology has been developed (Zeigler et al, 1988). This method is effective to eliminate susceptible genotypes but it does not identify highly resistant genotypes. Additionally, there are factors related to the insect behavior that make difficult to have an uniform infection in the field and unknown micro environmental factors that cause genotype by environment interaction. For this reasons, a more effective method for selection is needed.

The genetic architecture of RHBV resistance has been studied recently. In 2006, Calvert *et at* reported in the population WC366xFedearroz 2000 four QTL's related with RHBV resistance. A QTL located in the short arm of chromosome 4 was highly significant. Additionally, a QTL in the chromosome five related with resistance to sogata was found. The chromosome 4 region associated with RHBV resistance was also found in the cross WC366xFedearroz 50.
To take advantage of this QTL's a practical method for marker assisted selection need to be developed. According with Bernado (2008) the vast majority of reported QTL's reside in journals rather in cultivars and it is necessary to understand how identified QTL's can be exploited in a breeding program. For do that, an important issue is to validate those markers in different populations.
The objective of this work was to evaluate the reaction of 900 F2:3 coming from contrasting crosses and their parents to RHBV under green house conditions.

Materials and Methods

Genetic Material
Six contrasting F2 populations were developed by crossing Fedearroz 2000 (male parent), a very resistant Colombian cultivar, with six elite susceptible materials (Table 1). In the F2 population 150 single plants were random chosen for advance to F3. To be sure about seed purity by the flowering time one panicle was bagged.

Table 1. Genetic material used in this study.

Code	Cross	Know Reaction (field)
CT21478	Fedearroz 174/Fedearroz 2000	****
CT21480	Fedearroz 60-132/Fedearroz 2000	****
CT21483	Federarroz 369-23/Fedearroz 2000	****
CT21484	CT 18685-10-3-1-2-2-M (12)/Fedearroz 2000	****
CT21485	CT18244-7-5-2-3-1-5-M (20)/Fedearroz 2000	****
CT21486	CT 18245-11-6-2-2-2-2-M(21)/Fedearroz 2000	****
Female 1	Fedearroz 174	Susceptible
Female 2	Fedearroz 60-132	Susceptible
Female 3	Fedearroz 369-23	Susceptible
Female 4	CT 18685-10-3-1-2-2-M (12)	Susceptible
Female 5	CT18244-7-5-2-3-1-5-M (20)	Susceptible
Female 6	CT 18245-11-6-2-2-2-2-M(21	Susceptible
Resistan Parent	Fedearroz 2000	Resistant
Susceptible Check	Bluebonnet 50	Highly Susceptible

Experimental Design

The evaluation of the materials was done using a multi evaluation with six experiments. In each experiment there were three cages (reps) and into each cage 9 trays (Blocks/Rep). In a tray were planted the resistant parent (also used as control) Fedearroz 2000, the susceptible control Bluebonnet 50, three susceptible parents and six F2:3 families from each population. According with this design, controls were repeated 27 times, susceptible parents and populations 9 times and individual families once per experiment. Reps for the individual families were the experiment over time.
The experimental unit was a single ten plants row. The infestation was done when the plants were 15 days old with an average of three second instar nymphs coming from a virulent (70%) colony. The nymphs feeding was permit by three days, after that they were eliminated using an insecticide. Thirty days after infestation the number of plants with symptoms was recorded to calculate the percentage of infection.

The substrate was a water vapor disinfected 1:1 Santander: CIAT soil mixture.
In the field evaluation the parents were planted in seed beds with six reps, each consisting of one single 50 cm row. The infestation was done with approximately 1.5 insects per plant at 18 days after seeding. Thirty days after infestation the symptoms were evaluated according with the scale from IRRI (Standard System for Evaluation).
To confirm the reaction in tubes, the parents, the five most resistant and the five most susceptible according with the reaction in cages were

chosen for evaluation. Ten plants were planted per each material. Each plant was put in an acetate tube and infested at 18 days with four insects. Symptoms were recorded thirty days after infestation.

Results and discussion

There were four important sources of variability in the phenotypic reaction to RHBV under greenhouse conditions. According with the combined analysis of variance, which is presented in table 2, the genotype effect is the most important source of variation. Also, the experiment, the interaction genotype by experiment and the cage effects were highly significant. These results mean that in the assessing of RHBV, the reaction of genotype is very important; however, to have a good estimate of the mean, it is necessary to repeat the experiments over time and to have several reps in each experiment.

Table 2. Analysis of variance for RHBV incidence (arsin (percentage)).

Source	DF	MS
Experiment	5	1.5758**
Cage (Exp)	12	0.2634**
Trail*Cage(Exp)	144	0.1023ns
Genotype	13	21.9623**
Experiment*Genotype	65	0.1742**
Cage*Genotype(Exp)	156	0.0591ns
Cage*Trial*Gen(Exp)	1332	0.0598ns
Error	4417	0.0649
Total	6144	
CV (%)		45.07
Mean of Incidence (%)		31.98

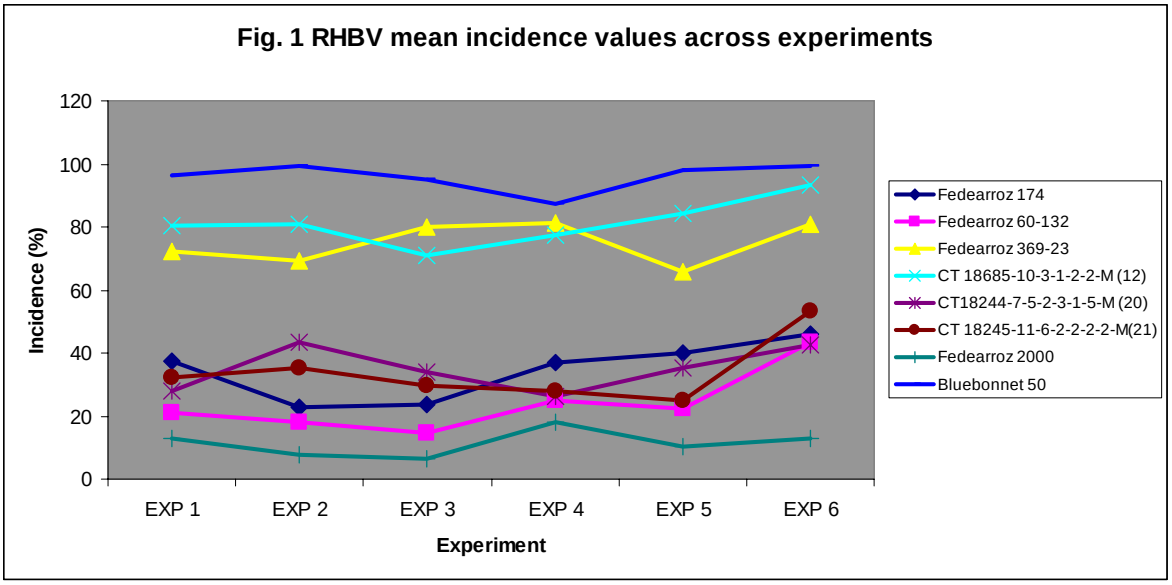
The genotypes under testing have wide range in RHBV reactions. Checks were the most extreme; Fedearroz 2000 with and average of 11.39% of incidence was the most resistant genotype confirming previous reports and Bluebonnet 50 with 95.90% of incidence was the most susceptible. Female parents were not uniformly susceptible in their reaction; for example females 4 and 3 were highly susceptible with 81.32% and 74.32% of incidence; females 5, 1 and 6 had 35.04, 34.68 and 34.53% incidence respectively and female 2 had 24.10% which is an intermediate reaction. The average incidence among F2 populations was almost intermediate between the reactions of parents involve in each cross.

Table 3. Least Square Means for percentage of RHBV incidence.

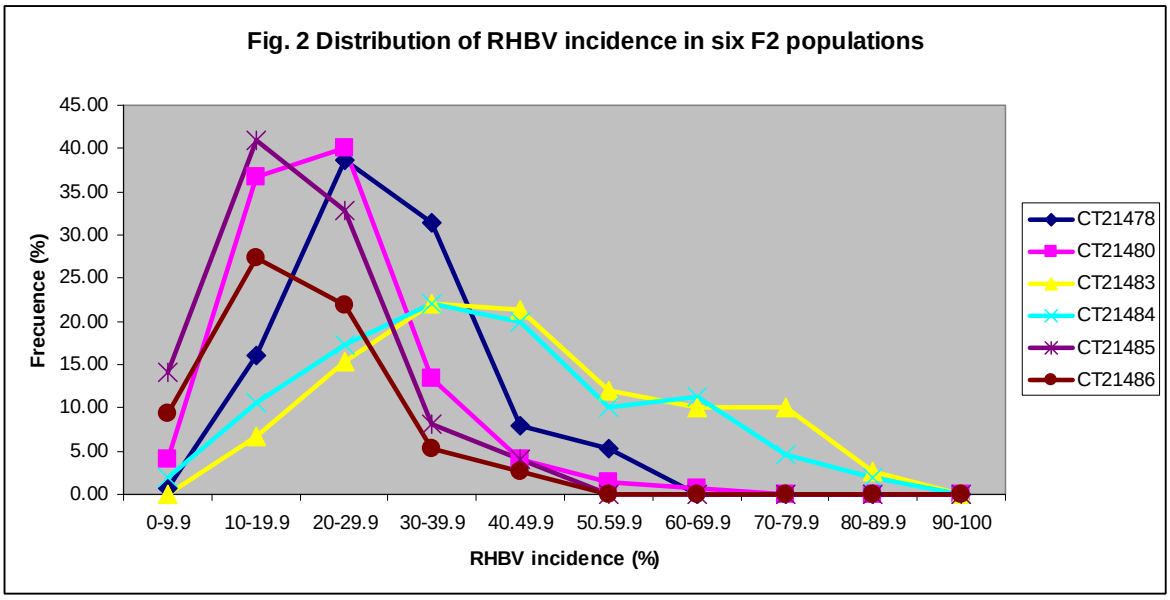
Code	Name	Incidence (%)	Scale‡
Susceptible Check	BLUEBONNET 50	95.90	9
Female 4	CT 18685-10-3-1-2-2-M (12)	81.32	9
Female 3	Fedearroz 369-23	74.92	9
CT21483	Fedearroz 369-23/F2000	44.63	7
CT21484	CT 18685-10-3-1-2-2-M (12)/F2000	40.37	7
Female 5	CT18244-7-5-2-3-1-5-M (20)	35.04	7
Female 1	Fedearroz 174	34.68	7
Female 6	CT 18245-11-6-2-2-2-2-M(21)	34.53	7
CT21478	Fedearroz 174/F2000	29.03	5
Female 2	Fedearroz 60-132	24.10	5
CT21480	Fedearroz 60-132/F2000	22.69	5
CT21486	CT 18245-11-6-2-2-2-2-M(21)/F2000	20.59	5
CT21485	CT18244-7-5-2-3-1-5-M (20)/F2000	19.31	5
Resistant Check	FEDEARROZ 2000	11.39	5

‡ Standard Evaluation System for Rice IRRI

The importance of genotype by experiment interaction is illustrated in fig 1. The reaction of each parent was variable depending on the experiment; however, there was a clear tendency Fedearroz 2000 always was the most resistant genotype and Bluebbonet 50 female 3 and female 4 the most susceptible. Other females always had less than 50% of RHBV incidence.



The reactions of individuals F2:3 families were distributed across the parent's reactions with not clear classes and transgressive reactions were observed in each population. The distribution of average RHBV incidence is showed in fig 2. Two populations CT21483 (female 3) and CT21484 (female 4) had the most wide distribution and the populations coming from less susceptible parents had a narrow distribution. There was considerable transgressive segregation and it was more common in narrow distribution populations and towards susceptibility. For example, in population CT21480 there was 18.3% of families more susceptible than susceptible parents and 4% more resistant than resistant parent.



† To see each cross parents refers to table 1
‡ F2:3 family reactions averaged over six experiments

In the field the performance of the genotypes was similar to the cages; however, the susceptible parents were more susceptible in the field than in the cages (table 4). For example female 2 was characterized as intermediate in cages and under field conditions was highly susceptible. This situation indicates that in the cages the reaction to the insect affects the phenotypic reaction to the virus and that probably the female 2 has non-preference resistance to the insect.

Table 4. Parents reaction to RHBV in field screening (IRRI scale).

Code	Genotype	I	II	III	IV	V	VI	VII	Mode	Mean
Female 1	Fedearroz 174	9	9	7	7	7	7	5	7	7.29
Female 2	Fedearroz 60-132	9	9	7	9	7	7	9	9	8.14
Female 3	Fedearroz 369-23	7	7	7	5	7	7	5	7	6.43
Female 4	CT 18685-10-3-1-2-2-M (12)	9	9	9	7	7	7	7	7	7.86
Female 5	CT18244-7-5-2-3-1-5-M (20)	9	9	9	7	9	7	7	9	8.14
Femlale 6	CT 18245-11-6-2-2-2-2-M(21)	7	7	7	7	7	7	7	7	7.00
Resistant Parent	Fedearroz 2000	1	1	3	3	3	1	1	1	1.86

When the most extreme genotypes and the parents were evaluated under tubes conditions a similar trend was observed. The susceptible parents and the susceptible families were susceptible in conditions, cages and tubes. The resistant parent Fedearroz 2000 had 30% of infection and the 37% (11 of 30) of F3 families that were highly resistant in cages were susceptible in tubes, with more than 60% of infection. Progenies with less infection than Fedearroz 2000 were also observed. Theses observations indicate that the phenotypic response to RHBV in cages is affected by the resistance to the insect.

In general these results indicate that RHBV resistant is a very complex trait, which is under the control of many genes affected by micro environmental conditions and that even thought Fedearroz 2000 is the most resistant genotype there are other genes dispersed among susceptible parents. Also, because of the differences in the reaction necessary to confirm the reaction of a resistant genotype.

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1A.1.2 Development and characterization of Ril´s for different traits.

Torres, E.A.; Carabali, S.J.; Amézquita N. and Martinez, C.P.

Abstract

With the objective of produce genetic material for further studies, near recombinant inbred lines from five populations were produced and initially characterized. Single crosses between contrasting parents for tillering ability, panicle length, leaf color, milling quality, grain shape, white belly, amylose content and tolerance to delayed harvest were made. These populations were advanced from F2 to F4 using single seed descendent and transplant. In F6 two rows per each line were transplanted and initially characterized. There was a reduction in the number of RIL´s from F2 to F6 because of sterility. Normal distribution was observed for almost all characters.

Introduction

Yield and grain quality are the most important traits for adoption of a rice variety by farmers. However, the study of these traits is very complex because the final phenotype is the results of other characters. For example; the sink, yield capacity or yield potential is the result of the number of grains per square meter; which in turn depend on the number of panicles, the number of grains per panicle and the percent of fertility; and the grain weight or full ripened grain mass. In the other hand, yield is also controlled by the source for grain filling that consists of non-structural carbohydrates stored before flowering and dry matter production during grain filling (Lubis *et al*, 2003). Additionally we must to consider the effect of genotype by environment interaction. In consequence to fully understand the phenotype it is necessary to have well designed experimental materials.

Recombinant inbred lines refers to a genotype created by crossing two inbred strains followed by repeated selfing which result is a new inbred line whose genome is a mosaic of parental genomes (Broman, 2005). This kind of populations has advantages over other mapping populations. A particular individual can be multiplied in the time without genetic changes, it is possible to have reps and it is essentially and eternal F2 population with unlimited mapping possibilities. According with

Reynolds *et al* (2001) RIL´s developed using SSD are one important tool to demonstrate genetic linkage between traits in homozygous lines. For these reasons, RIL´s are a useful tool to determine the genetic basis of a complex trait.
The objectives of this work were to develop several RIL´s populations for different important traits and to do an initial characterization.

Materials and Methods

The characteristics and the genotypes used in the crosses are showed in table 1.

Table 1. Pedigree and parents characteristics of RIL´s populations

Code	Cross	Female characteristics	Male characteristics	RIL´s Number
CT21374	BCF2390/FL01028	Low plant height High tillering ability Short panicles Normal leaf color	Intermediate plant height Intermediate tillering ability Long Panicles Dark green leaves	178
CT21375	FL04577-3P-11-4P-1P-M/Centauro	Normal panicles Dark green leaves RHBV resistant	Long panicles Normal leaf color RHBV susceptible	72
CT21376	CT11275-4-M-1-M/FL01028	Long panicles Normal leaf color	Normal panicles Dark green leaves	71
CT21378	Fedearroz 60/Fedearroz 473	Long grains High amilose Low white belly Tolerance to delayed harvest	Medium grains Intermediate amilose High white belly Susceptibility to delayed harvest	160
CT21379	Fundarroz PN1/FL02066-4P-1-1-1-2-4-4-2	Susceptibility to delayed harvest	Resistance to delayed harvest	124

Initially, single crosses in one direction were made between contrasting but adapted parents. In F2 individual plants were randomly chosen and advanced to F4 using single seed descend. In F5 one plant per line was transplanted and one panicle was bagged in order to avoid contamination. In F6, two rows, one plant per hill were transplanted in Palmira to increase seeds and initial characterization. Several traits such as flowering, yield, milling quality, tolerance to delayed harvest depending on the population were measured. To evaluate delayed

harvest, the methodology developed by FLAR was used. Its basically consist in: Harvest the seed at time, approximately when it achieve 24% humidity, reduce humidity until 12% using dry air, measure milling yield on time, submerge dry seeds on water for two hours, dry the seeds again and after 8 days measure milling yield again. To mill rice grains a McGill # 3 Miller was used.

Results and discussion

During the process of RIl's development several plants were lost because of sterility in the cross. It indicates that is necessary to begin the process with 400 to 500 plants to have an adequate population size by the end of the process.

Distribution for yield in the population CT21374 is showed in fig 1. There was a normal distribution indicating the quantitative nature of the trait as expected for yield and suggesting that the parents used in the cross are very different in terms of the genes carried out. This results show that this population will be adequate to study what is the contribution of different components on yield, the importance of gxe interaction and eventually to map QTl's. The best yield was obtained by the check Fedearroz 60 with 8080 kg ha^{-1} and 6 lines outyield this check in more than 10%. Additionally, 43 lines from this population were also evaluated in Santa Rosa to rice blast and in Palmira to RHBV. Nine lines were resistant to blast and 15 to RHBV. These results show that SSD method will be very interesting to develop elite lines in rice.

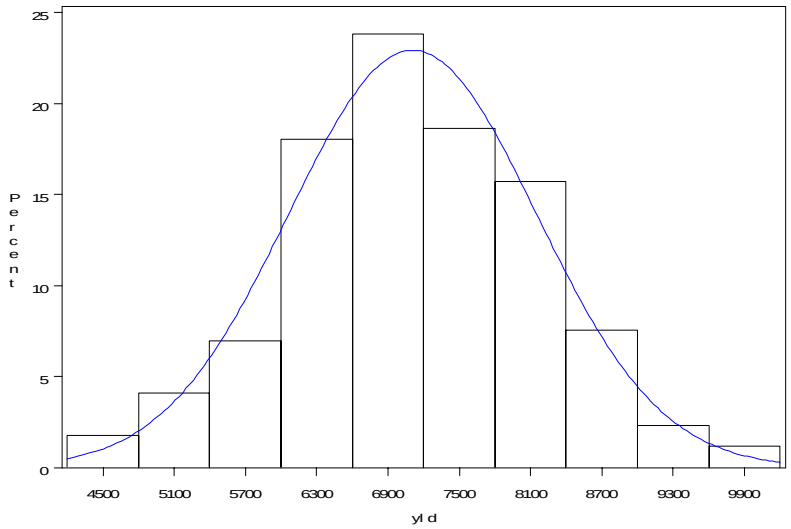

Figure 1 Grain yield distribution for RIL's from population CT21374 (Mutante FM/FL01028)

The distributions for yield in CT21375 and CT21376 populations were also normal; however, there was less variation than in CT21374 population. The CT21376 population was also evaluated to RHBV in the field screening using two reps, transgresive segregation with more resistant or susceptible material than parents and a continuous variation were observed suggesting dispersion of alleles in the parents and quantitative resistance in this cross. From CT21375 population, 23 lines were evaluated to rice blast and 13 were resistant. In the other hand, 7 of 20 lines were resistant to rice blast in CT21376 population.

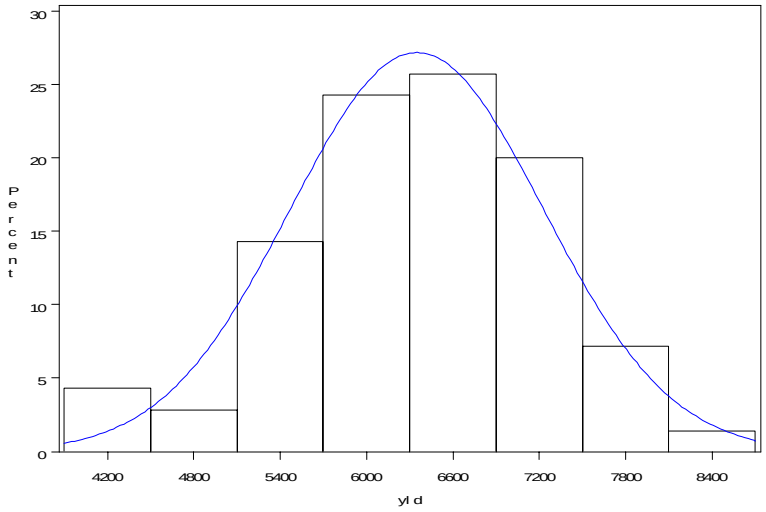

Figure 2. Grain yield distribution for RIL's from population CT21375 (FL04577/Centauro)

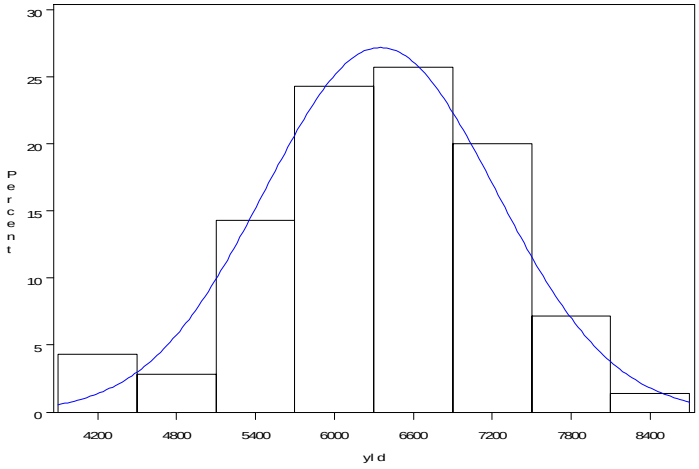

Figure 3. Grain yield distribution for RIL's from CT21376 (CT11275/FL01028 population)

The behavior of percentage of whole grain after milling in on time harvest of CT21378 population showed also a continuous distribution (fig. 4) suggesting a polygenic control of these trait. There was a difference of 21.82% between the maximum (63.08%) and the minimum (41.26%) values. Transgresive segregation for high and low values of whole grain percentage was also observed. This results indicate that this population could be adequate for further analysis.

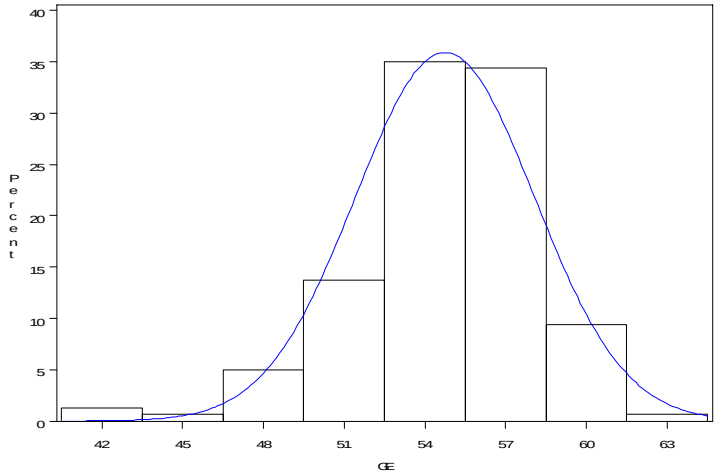

Figure 4. Whole grain (%) distribution for RIL's from CT21378 (FL03188-7P-5-3P-1P-M/ FEDEARROZ 473) population, normal harvest.

The percentage of whole grain after the delayed harvest treatment also showed a continuous distribution and a big difference between the maximum (56.64%) and the minimum (9.52%) whole grain yield. The resistant parent had a percentage of whole grain of 46.56% and the susceptible of 20% after the treatment indicating the presence of transgresive segregation.

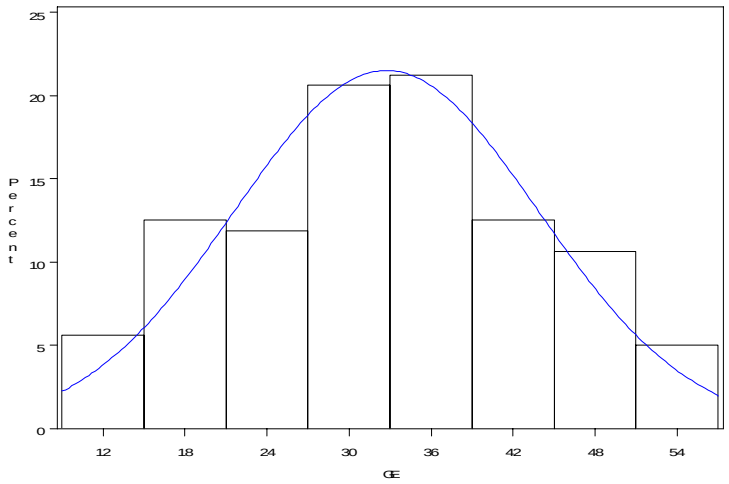

Figure 4. Whole grain (%) distribution for RIL's from CT21378 (FL03188-7P-5-3P-1P-M/ FEDEARROZ 473) population, after delayed harvest treatment.

In the case of CT21379 population a two peak distribution was observed suggesting the effect of major genes in the percentage of whole grains after delayed harvest treatment. The maximum observed value was 56.04% and the minimum was 8.56%. The resistant parent had 55.04% on time and 46.08% after treatment and the susceptible had 56.32% on time and 5.60% after treatment.

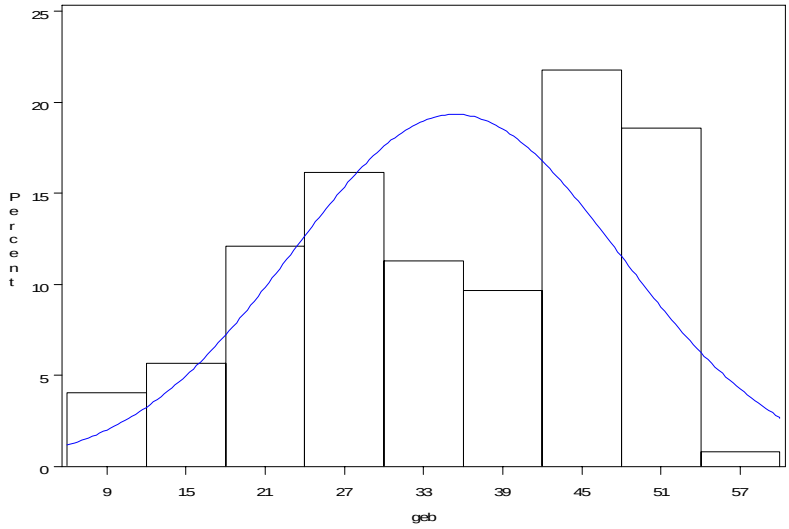

Figure 6. Whole grain (%) distribution for RIL's from CT21378 population after delayed harvest treatment.

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1A.2 Breeding Strategies to Increase the Content of Iron and Zinc in the Rice Grain

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Source of funding: CIDA-Canada and CIAT_Core

A fast-track approach was used to screen breeding lines and traditional/ improved rice varieties to find rice germplasm with higher iron and zinc content in milled rice. We planned to increase iron and zinc content in the rice grain using a breeding strategy in two phases. On a fast track, landraces and breeding lines conserved in the germplasm banks were screened for mineral content to identify products that could have immediate utility, as potential varieties or donors. For a second phase, a crossing program was started to combine high-iron and zinc with high yield potential, tolerance to main biotic and abiotic stresses, and good grain quality. This project was carried out in close partnership with research institutions in Colombia, Bolivia, Cuba, Brazil, Dominican Republic, Nicaragua and more recently Panamá. This is a summary report of main achievements realized by CIAT in close collaboration with AgroSalud's partners from 2005 to 2009. AgroSalud seeks to contribute to solving the problems of malnutrition in Latin America and the Caribbean through staple cultivars with improved nutritional value. Rice, beans and maize are primary staples of the diet in the greater part of the region.

Objective: Development of rice lines with 8 mg/kg of iron and 25 mg/kg of zinc to combat malnutrition in Latin America and the Caribbean. It was funded by CIDA-Canada. There were five main activities, including:

1. **Fast Track Mode**: Screening and field evaluation of local varieties and advanced breeding lines for mineral content to identify products that have immediate utility.
2. **Establishment of efficient screening protocols and methodologies** to identify rice lines having higher iron and zinc.
3. **Development of populations and lines having a high level of iron and zinc** combined with good yield potential, tolerance to

main diseases and insect pests, and good grain quality. This was done in conjunction with local breeders.
4. **Evaluation of promising lines** and distribution to partners for further evaluation under local conditions, including GxE studies.
5. Evaluation of the nutritional status and acceptance of promising lines. **Inform and educate the health sector** concerning the biofortification strategy, including seminars, workshops, conferences, field days, TV and press releases. Collaborate on the development of food products using rice flour in combination with other biofortified crops.

A summary of main accomplishments is presented:

1. **Fast Track Mode**: Screening and field evaluation of local varieties and advanced breeding lines for mineral content to identify products that have immediate utility.

a) Base line for iron and zinc: Brown and milled rice samples were collected in local stores and supermarkets in Colombia, Bolivia, Nicaragua, and Dominican Republic. Samples were analyzed by the CIAT Analytical Service Lab. using the atomic absorption method, and expressed as microgram/gram or ppm. Values found were around 2-3 ppm for iron and 16-17 ppm of zinc for milled rice, whilst values for brown rice were 10-11 for iron and 20-25 for zinc. Based on this data and consumers preference, base line for milled rice was established as 2 ppm for iron and 16 for zinc, and breeding targets were set up 8 ppm of iron and 25 ppm for zinc.

b) Nearly 11,400 rice samples from CIAT rice breeding program and NAR partners(CIAT-Bolivia/Aspar in Bolivia, Embrapa /CNPAF in Brazil, Fedearroz in Colombia, IIA in Cuba, IDIAF in Dominican Republic, INTA in Nicaragua, and IDAP in Panama)were evaluated for iron and zinc content in milled rice during the period 2006-2009. Average mean value for iron was 3.9(1.4 standard dev) with a high value of 8; 34% and 25 % of the samples were better than controls Fedearroz 50 and IR64, respectively.(Figure **1).** Average mean value for zinc during the same period was 14.3 ppm (3.6 std) and a high value of 29; 40% and 88 % of the samples were better than Fedearroz 50 and IR64, respectively **(**Figure **2).**

Figure 1. Distribution of iron content in milled rice grain in 11337 samples analyzed in the period 2007-2009 at CIAT.

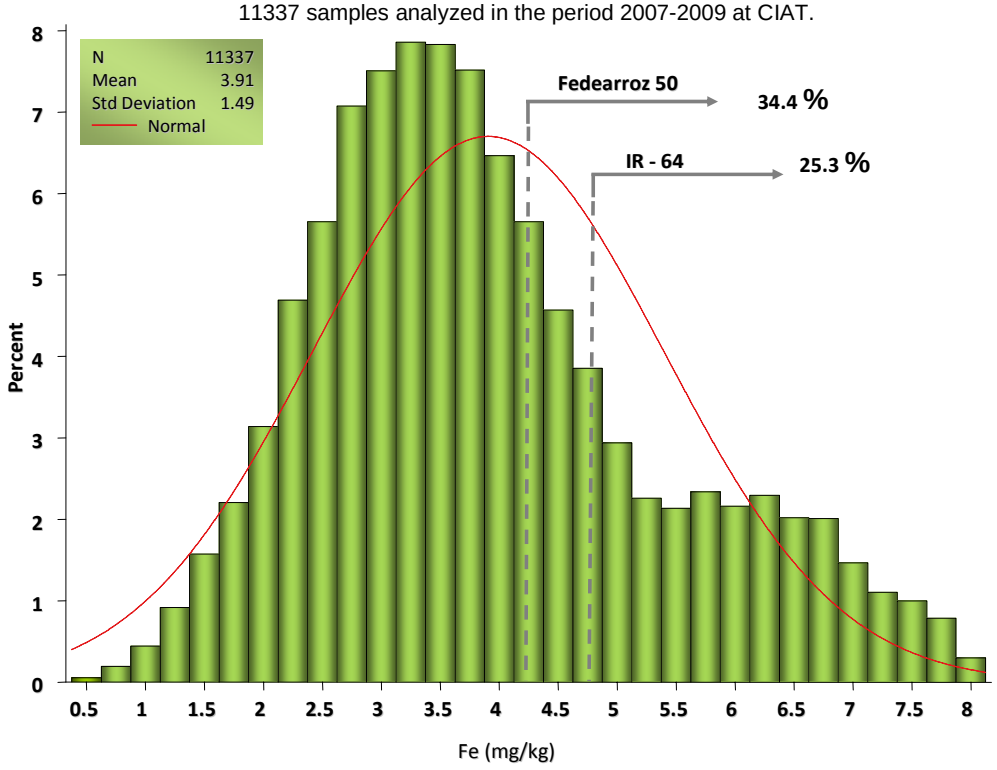
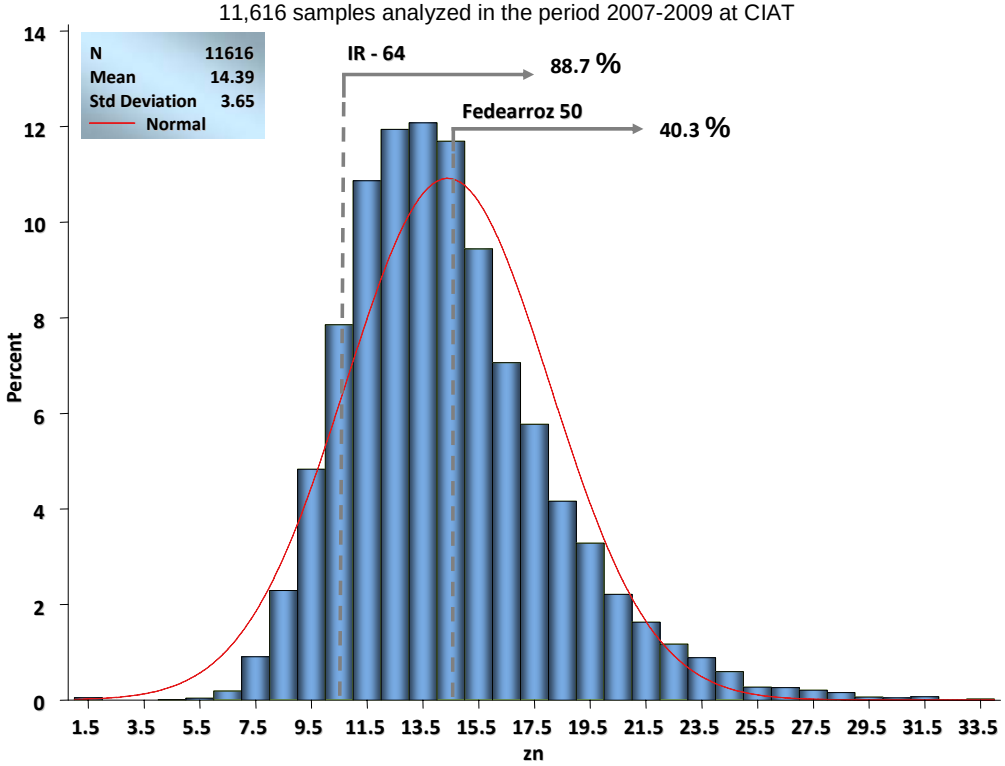


Figure 2. Distribution of zinc content in milled rice grain in 11,616 samples analyzed in the period 2007-2009 at CIAT



c) Figures 3 and 4 suggest that selection for iron and zinc over time has been effective, especially in the case of iron; the frequency of lines with higher iron content was increased, and the average iron content increased. In 2007 it went from 3.2 (1.26 std) to 3.92(1.2std) in 2008 and to 5.69(1.21 std) in 2009. In other words, in 2007 only 16 and 10% of samples did better than checks Fedearroz and IR64, whilst in 2008, 34 and 22 % of samples were better than the controls but in 2009, 84 and 22%, respectively did better than checks varieties (Figure 3). Progress has been slower in the case of zinc (Figure 4). In 2007, average zinc content was 14.2 (3.83 std), whilst in 2008 it moved to 15.2 (3.56 std), and went down to 13 (2.85 std) in 2009. Since the beginning, more attention was given to the identification of lines with higher iron content which could have affected progress in this trait. Another factor to keep in mind is that soils in our CIAT experimental area are high in pH, zinc content is very low and soil variability is also high. However, there are few lines combining good yield potential, and agronomic traits with 23 and 24 ppm of zinc, very close to the target of 25.

Figure 3.Progess observed over time in distribution of iron content in milled rice in samples analyzed in 2007,2008 ,and 2009 .

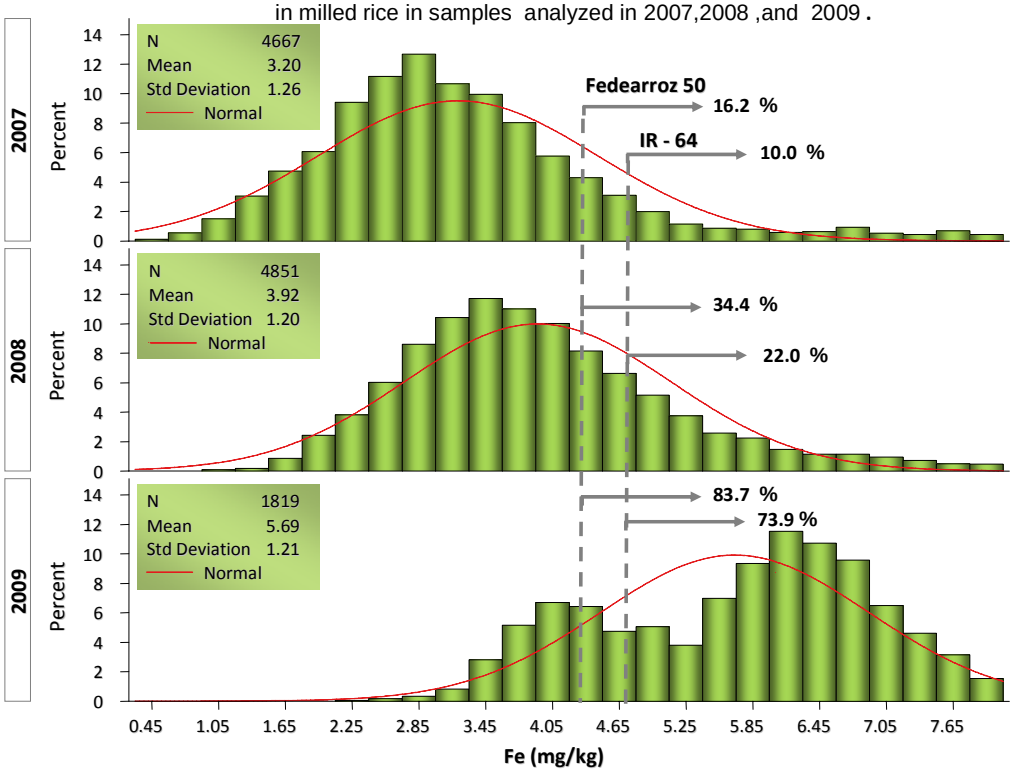
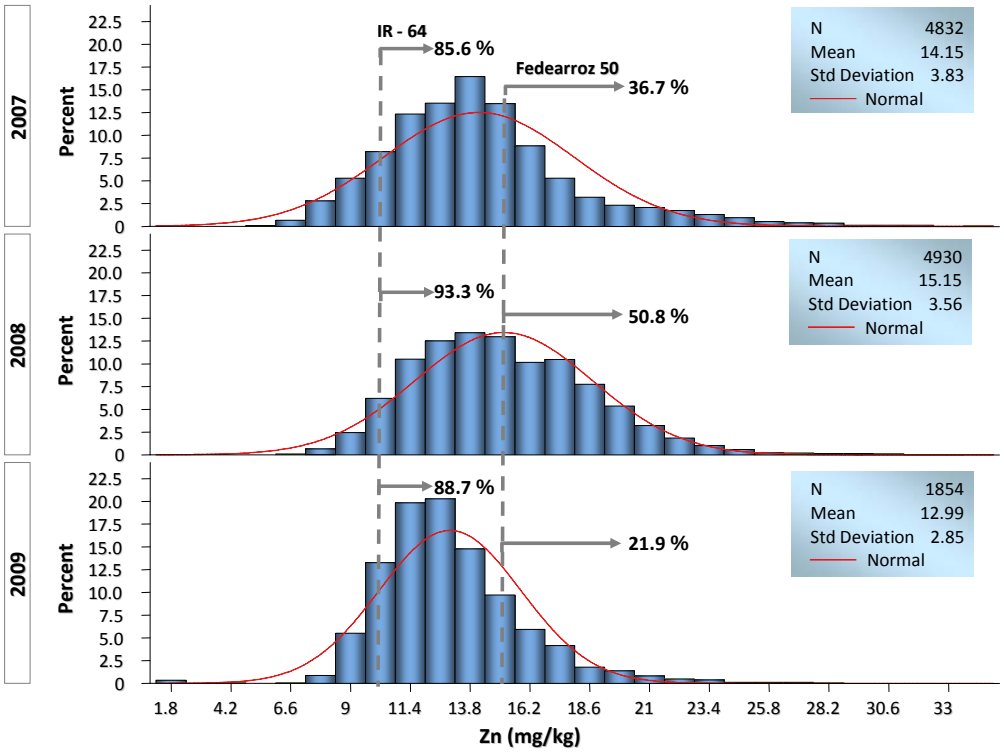


Figure 4.Progess observed over time in distribution of zinc content in milled rice in samples analyzed in 2007,2008 ,and 2009



d) Products delivered: After three years of evaluation, selection and field demonstrations in farmer's fields under local conditions two varieties (Azucena and Saavedra 27) were released in Bolivia and one in Cuba (IACuba 30) in 2009. Seed packages were delivered to 200 farmers in six rice growing areas in Bolivia and Cuba. Azucena with an average yield of 3 ton/ha (20% more than traditional varieties) is recommended for small-subsistence farmers. Saavedra 27, with an average yield of 7.3 ton/ha and good grain quality and tolerance to main diseases is recommended for irrigated and lowland conditions. Saavedra 27, with modest increases in iron and zinc, could contribute 197 µg of iron per day and 627 µg of zinc per day to the Bolivian diet. This represents 18% and 28% more iron than Paititi and Tari, respectively and 17% and 5% more zinc compared to Paititi and Tari, respectively. IACuba 30, was released in Cuba in 2003 but adoption by farmers was slow, representing a good case for the fast track method. Field evaluation carried out by the AgroSalud rice team following a participatory breeding approach showed its potential for low input small-rice farmers in five regions in Cuba because of its high yield potential (5-7 ton/ha), tolerance to salinity, and good grain quality. IACuba30 (5.3mg/kg of iron and 17.4mg/kg of zinc), and Cubana23(a bean variety), with modest increases in iron and zinc

could contribute 4139 µg of iron per day and 4888 µg of zinc per day to the Cuban diet. This represents 23% and 33% more iron and zinc.

e) New releases in the pipeline: One variety will be released by Embrapa in 2010; 2000 kg of seed of the variety Zebu Ligeiro, and 2000kg of variety Chatao Branco were distributed to small farmers in Maranhão State to test its acceptability, and questionnaires distributed in 15 communities to get feedback on its performance**.** Cuba will release Perla in July 2010**.** Line CT15679-1-1-1-4 was the preferred genotype by farmers in participatory breeding activities carried out in 10 locations in Nicaragua, and is ready for release as a commercial variety in 2010 by INTA, for upland and rainfed conditions due to its good performance in regional trails both in 2007 and 2008, more iron (4.1-5.54 ppm) and zinc (21 ppm) compared to the base line (2-3 ppm iron) and 17-18 ppm zinc. Panama joined Agrosalud in 2007 with its own funding, and just released four varieties in March 2010. Colombia and Dominican Republic will release one variety each in 2011.

2. **Establishment of efficient screening protocols and methodologies** to identify rice lines having higher Fe and Zinc.

a) **A clean room and milling /polishing facilities** were built at CIATin 2006 to assure good quality data and a fast turn over for our breeding program; several improvements were implemented in 2007 to reduce contamination at milling and grinding facilities, including setting up a double entry door at the lab. In addition, distilled and dionizided water is used for washing glassware, Teflon chambers, zirconium balls, and vials used to prepare rice flour. A lab manual with instructions for lab users was written, and a protocol (based on HP Manual 7) was developed for harvesting, drying, packing, and preparing rice samples for analysis.

b) **An experiment was set up to compare iron and zinc between labs**. Milled and brown rice samples of 10 genotypes, four replications each, were prepared and sent for iron/zinc analysis to both WAS(Waite Analytical Service, Adelaide University) and CIAT (Analytical Service Lab). In order to minimize contamination, two known rice lines (Icta Motagua and P5746-18-11-1-2-2A-1) having around 1 mg/kg of iron and 9 mg/kg of zinc were run as "blank controls" between samples of selected genotypes during the milling process; 12 grams of paddy rice were used per sample. A Suzuki rice mil was used to de-husk and milled the rice samples whilst a locally made mill shaker, already tested and validated by the bean AgroSalud project, was used to produce rice flour for iron and zinc

analysis. Data indicated that iron and zinc values obtained at both WAS and CIAT were very similar, and no indication of contamination was found. There was high and positive correlations between WAS and CIAT values. Data showed that Fedeaaroz 50, a commercially grown variety in several countries, had the highest iron content in milled rice followed by TOX 1859 and FL04052 (advanced breeding lines). These findings have the following implications: a) No contamination was found by WAS indicating that we already have in place at CIAT a protocol to handle, prepare and analyze rice samples for iron and zinc; b) Correlation between iron and zinc values obtained by WAS and CIAT were positive and very high; c) Fedearroz 50, a successful rice variety grown commercially in several countries in Latin America, had about 2-3 times the amount of iron found in rice bought by consumers(2-3 ppm).

c) **Establishing a base line for iron and zinc**. A total of 57 rice samples (brown, parboiled, and milled rice) were collected in supermarkets and stores in several places in Colombia(19), Bolivia(16), Nicaragua(5), and Dominican Republic(9). These samples represented the kind of rice bought by rice consumers for consumption and came directly from rice mills that use commercial rice mills in rice processing. It is expected that these samples have some kind of contamination. Samples were milled and sent for iron and zinc analysis by atomic absorption to the CIAT Analytical Service Lab; three reps per sample were used. There were statistical differences in the level of iron and zinc found in milled and brown rice samples collected in different locations. Brown rice had 11-13 mg/kg of iron and 20-25 mg/kg of zinc, compared to 2-3 mg/kg of iron and 17-19 mg/kg of zinc in milled rice. No significant differences were found in iron and zinc content in both brown and milled rice across countries, suggesting that these values can be taken as base line for iron and zinc. Some parboiled rice samples that were analyzed showed iron levels similar to milled rice, contrasting with what is reported in the literature. Similarly, some samples of rice sold as fortified rice had less than 2 mg/kg. Results suggest that the level of iron and zinc in milled rice used by consumers is low and similar to values reported by HP+ in Asia.

d) **Development of a iron and zinc database.** A database was developed to facilitate access of iron and zinc data as well as to main agronomic data of rice materials in a fast and structured form. This database is in Excel and constitutes a very easy, simple and friendly tool that can be handled by users; it is a dynamic

database, where the stored information can be modified and updated as new data becomes available. Main characteristics are:
-Fast and agile consultations by a series of agronomics characteristics including values of the content of micronutrients iron and zinc.
-It allows operations like updating, addition of data and consultations.
-It provides minimum and maximum values, averages values for iron and zinc of evaluated materials.
-It allows sorting the materials by anyone of the registered characteristics.
-It displays in graphic form the values of iron and zinc of the evaluated materials.
At present, the database has a record of 11,600 data points, and is being fine tuned with new applications aimed at offering more information to the users. The purpose is to upload it to the webpage of the project available to all cooperators.

e) **Assessment of NIR technology to speed up analysis of iron and zinc in the rice grain.**
A total of 400 rice samples (200 brown rice and 200 milled rice samples) representing 200 genotypes were sent to CIP (W.J. Gruneberg) for establishing a NIRS evaluation method for iron and zinc in rice in collaboration with Harvest Plus. These samples were scanned twice by NIRS, the spectra stored and samples sent to Adelaide to get reference values for iron and zinc. Based on this information and furher work done by Thomas Zum Felde at CIP it was possible to determine that NIRS can be used to estimate iron and zinc in rice. Two programs (rice meal and riceseed) were established and validated. Some work was done in our laboratory at CIAT to determine factors influencing the inconsistent results we are getting in iron and zinc values via the atomic adsorption method and to get familiar with NIRS before it is used as a high throughput technique. Results suggest that the size of the particles found in the rice flour is a key factor determining the mineral content in a given sample. Rice flour is not homogenous and different varieties produce rice flour having particles of different sizes. These results suggest that some adjustment in milling time, and selection of a representative size of the rice flour particles could produce more consistent data. In the case of NIRS, 1450 samples were run in an attempt to learn how to use this technique and to establish a calibration curve building on the preliminary work done by Thomas Zum Felde at CIP. Unfortunately, attempts to establish the calibration curve gave poor results. Additional work needs to be done with NIRS before using it as a high-throughput technique in rice.

3. **Development of populations and lines having a high level of iron and zinc** combined with good yield potential, tolerance to main diseases and insect pests, and good grain quality. This was done in conjunction with local breeders.

More than 500 crosses were made, and 1945 advanced breeding lines were sent to NARS in the period 2005-2009. Nearly 20.000 segregating breeding lines were evaluated in CIAT-Palmira and Santa Rosa. Based on HP data Azucena, Madhukar, Ketan Lumbu, Gundil Kuning, Perurutong, IR68552-100-1-2-2, and IR71703-657-3-1-2 were used as donor parents in crosses with improved lines and commercial varieties from the CIAT-germplasm bank. Out of 112 single crosses, a total of 147 backcrosses (BC1) were made and planted in CIAT-Palmira in May, 2005. High sterility and poor combining ability was observed in most crosses, especially in crosses with Azucena and Perurutong. A total of 366 F2 families were planted and evalauted in 2006 in CIAT-Palmira; based on agronomic traits and yield potential 1693 single plant selections were made for further evaluation in 2007.

Out of the 30 F2-F3 populations introduced from IRRI in 2004, a total of 2672 plant selections were further evaluated as F3-4 progenies in CIAT-Palmira in 2006. A total of 238 top crosses were made for evaluation in 2009. This crossing program was started to recombine desirable traits found in elite lines derived from diverse interspecific crosses. A total of 2738 segregating breeding lines were sown and evaluated at Ciat-Palmira and Santa Rosa for agronomic traits, tolerance to main insect pests and diseases, grain quality, and yield potential; 972 promising lines were identified. These are lines derived from crosses between *O.sativa* and other wild rice species. Two yield trials with selected lines were planted and harvested in April/2008 at CIAT-Palmira. Based on agronomic traits, yield potential and iron/zinc data best promising lines were selected for distribution to AgroSalud partners (Table 1).

Table 1. Evaluation of promising lines and distribution to partners

Promising lines evaluated in two contrasting sites

Pedigree	*Santa Rosa 2008*								*CIAT 2008*					
	VG	BL1	BL2	FL	LSC	BS	NBL	GD	VG	FL	HB	kg/ha	Fe mg/kg	Zn mg/kg
CT 15696-3-4-1-1-3SR-1-2	3	2	3	91	1	1	5	3	3	113	1	7296.6	6.45	9.28
CT 15717-7-1-1-1-2SR-M-2	3	5	6	87	3	5	5	3	3	113	3	9153.0	4.66	9.51
CT 14544-1-M-2-3-3-M-M	3	3	4	85	1	1	3	1	3	116	3	5518.7	5.99	19.42
CT 14544-1-M-2-4-1-M-M	3	3	3	97	1	1	3	3	3	109	3	4754.5	5.41	20.94
CT 18148-6-9-5-1-2-M	1	3	3	93	1	1	1	1	5	109	5	9149.4	7.12	9.78
CT 18148-6-9-5-1-3-4-M	1	2	3	93	1	1	3	3	5	109	5	7752.2	6.38	10.19
CT 18148-10-3-6-1-6-M	1	3	3	91	1	1	3	3	3	109	3	8441.3	4.24	10.18
FEDEARROZ 50 (check)	3	5	5	87	1	1	5	3	5	105	3	3159.6	5.29	17.29

Population breeding through recurrent selection methods.

Recurrent selection is being used by Embrapa, Fedearroz and CIAT to increase iron and zinc in the rice grain; preliminary data are encouraging. Four populations (PCT-8CG/1/CG/1, PCT-19, PCT-21, and PCT-22) developed by the CIAT Rice project and carrying cytoplasmic male-sterility gene were selected to start a population improvement program through recurrent selection for high iron and zinc. This is based on the successful use of recurrent selection by Dudley et al, 1974 to increase protein content in maize from 10.0% to 26,6%. Male -sterile plants were selected in each population and crossed to seven high iron/zinc cultivars (Azucena, Madhukar, Ketan Lumbu, Gundil Kuning, Perurutong, IR68552-100-1-2-2, IR71703-657-3-1-2). This activity was carried out in collaboration with Fedearroz, our partner in Colombia. F1 seed from each cross was planted and evaluated in 2006 and F2 seed was mixed in equal proportion to form new populations named as PCTBF1, PCTBF2, PCTBF3, and PCTBF4; after the first recombination cycle, the first two populations were mixed and named PCTBF1, whilst the other two populations were mixed and maned PCTBF**3;** 182 S_0fertile plants were harvested in PCTBF1 and after iron/zinc analysis 81single plants selections were selected whilst in PCTBF3 144 S_0 fertile plants were harvested and after iron /zinc analysis 93 were selected. Seed of the S_0 plants was mixed and planted for a second recombination cycle. Data are presented in Table 2.

Four new populations were assembled in 2007 to introgress some lines from IRRI (IR68144-2B-2-2-3-1-120, IR68144-2B-2-2-3-1-166, IR69428-1-1-3-3; IR75862-206-2-8-3-B-B-B); new populations are PCTBF-**5**, PCTBF-**6**, PCTBF-**7**, and PCTBF-**8**. Data is presented in Table 2.

Table 2. Number of S_0 plants evaluated and S_1 seed selected for recombination, and some statistical parameters measured in some populations developed by recurrent selection.

Populations	S_0 plants evaluated	S_1 seed used for recombination	Mean	Range (mg/kg)	*std*	Mean	Range (mg/kg)	*std*
			Fe			*Zn*		
PCTBF1	182	81	7.0	5.5 - 20.8	1.64	13.8	8.8 - 24.3	2.8
PCTBF3	144	93	6.9	6.1 - 8.8	0.44	12.7	8.3 - 19.7	2.1
PCTBF5	78	72	7.4	6.4 - 8.9	0.63	14.5	10.1 - 20.7	2.7
PCTBF6	162	57	6.8	5.9 - 8.0	0.40	12.9	8.7 - 21.5	2.3
PCTBF7	87	64	6.8	5.7 - 7.9	0.45	14.6	9.5 - 22.4	2.8
PCTBF8	176	78	6.9	6.7 - 8.5	0.53	13.6	10.1 - 23.9	2.3

Evaluation of S_1 lines: These lines were evaluated in CIAT-Palmira and 185 plants were selected for evaluation as S_2 lines in 2010.

Evaluation of S_3, S_4, and S_7 lines: 115 lines representing S_3, S_4, and S_7 generations were evaluated under field conditions in CIAT-Palmira y Santa Rosa-Villavicencio; a total of 149 lines were selected to be sent to our AgroSalud partners after evaluation for iron and zinc, and agronomic traits.

Mutation breeding: Five elite lines were selected and seed was sent to the International Institute of Atomic Energy for mutagenic treatment to start a mutation breeding program aimed at the identification of rice mutants with high levels of iron and zinc; two radiation treatments (150 and 250 GY) using Cobalt 60 as the mutagenic source were used. Aprox 27,000 seeds were planted in CIAT as M1 populations. A total of 8000 plants were selected at random and harvested individually; since iron and zinc analysis failed to find increases in iron and zinc, this activity was dropped.

4. **Evaluation of promising lines and distribution to partners**.

A total of 1945 advanced breeding lines and varieties were sent to NARS in the period 2005-2009 and about 540 traditional varieties and breeding lines were sent to CIAT for quality evaluation. The rice coordinator visited several AgroSalud partners at least four times a year to assess breeding progress, participate in local workshops or meetings, and field days.

GxE studies to understand climatic and soil factors affecting the expression of high iron and zinc in the rice grain. It has been shown by HP+ that there is a significant GxE interaction in the expression of iron and zinc in the grain; in wheat the expression of these minerals in the grain depends on soil conditions and fertilization practices. Similar findings have been reported in rice in Asia. Climatic conditions and crop management practices also affect the yield potential of rice. Therefore, there was a need to determine main factors affecting the expression of iron /zinc in rice to be able to better define best agronomic practices for rice in our regions of interest to assure not only high yield potential but also good expression of iron/zinc in the rice grain.

Field experiments were established by Fedearroz and at CIAT to estimate GxE interaction in the expression of iron and zinc in the rice grain due to soil, fertilization and climatic conditions. Different soil treatments (basal applications) and foliar applications of zinc were tested. Field experiments were harvested and data are being analyzed. **Table 3** shows data for iron and zinc of 12 rice lines and two controls in two locations in Nicaragua. Data suggest significant differences in iron and zinc in milled rice due to locations. More zinc was found under irrigated conditions whilst more iron under upland conditions. However, stable genotypes could be identified. Gx E studies are being conducted in Brazil by Embrapa-CNPAF in nine locations. As an MS thesis a GxE study was conducted in Dominican Republic using 10 genotypes and three locations differing in climatic and soil conditions to study the effect of rationing (a popular rice production system in this country) on iron and zinc in milled rice. Samples were analyzed at CIAT and data sent back to them.

Table 3. Genotype x Location Trials done by INTA. Nicaragua (2008)

Genotypes	Irrigated Sebaco				Upland Posoltega			
	Fe (mg/kg)	sdt	Zn (mg/kg)	sdt	Fe (mg/kg)	sdt	Zn (mg/kg)	sdt
FLO 3001-MP2-1P-3P-M	5.47	0.48	14.70	0.56	5.97	0.26	10.78	1.03
FLO 3724-3P-5-1P-M	6.00	0.20	17.68	1.63	6.43	1.73	14.60	2.95
FLO 3779-4P-9-3P-1P-M	6.63	1.42	16.29	1.22	5.61	0.28	14.65	3.86
FLO 3801-1P-1-1P-2P-M	5.23	0.69	17.17	0.94	5.29	0.11	15.07	0.98
FLO 4052-2P-3-2P-2P-M	5.47	0.32	16.01	0.33	7.25	0.91	14.62	0.92
CMVINI	4.74	0.74	13.81	0.63	5.28	0.45	11.67	0.80
CT15679-17-2-7-5-5-M	5.38	0.08	22.53	0.94	5.41	0.68	13.44	1.20
CT15679-17-1-1-1-4-M	4.09	0.41	20.99	0.57	5.54	0.77	12.27	1.08
CT15679-3-4-2-3-3-M	4.69	0.19	20.82	0.41	5.57	1.06	15.09	0.76
CT15691-4-5-2-2-1-M	5.78	1.36	16.24	0.74	7.53	0.67	13.04	1.41
ISA-40	5.30	0.41	15.44	0.23	7.51	2.81	12.24	0.34
CT15679-17-1-4-5-2-M	4.47	0.53	20.67	1.17	5.81	1.87	14.21	1.03
INTA DORADO	6.45	0.88	12.37	0.52	7.63	1.18	11.40	6.08
FEDEARROZ-50	5.36	0.62	20.20	0.25	6.92	1.06	12.76	4.48
Mean	4.98		16.05		5.77		12.36	

5. **Evaluation of the nutritional status and acceptance of promising lines. Inform and educate the health sector concerning the biofortification strategy**. Biofortified crops can improve food and nutrition security through two main mechanisms. First, biofortified crops are bred to be agronomically superior to their non-biofortified counterparts. This means crops with greater yields, resistance to pests, and tolerance to stresses which translates into more food available in farmer homes for consumption, sale, trade, etc. This, in turn, can diminish household food insecurity. Second, biofortified crops are bred to have greater nutritional content than non-biofortified crops.

Salomon Perez is conducting follow up studies in Bolivia and Cuba to assess farmer's adoption of nutritionally enhanced rice varieties released in these two countries, as well as estimating potential impact of these varieties in human nutrition in coordination with our nutritionist Helena Pachon. Studies were conducted in Bolivia, Nicaragua, Cuba, and Panama to evaluate acceptability of nutrient enhanced rice lines by consumers; except in Nicaragua, consumers did not find differences with non-biofortified controls and acceptability was high. These activities will be reported in more detail by our colleagues.

Lack of data has hindered carrying out *ex-ante* health and economic impact analyses, using the Disability-adjusted Life Years (DALYs) methodology, on more than a handful of LAC countries and for rice, beans and maize only (Salomón Pérez, CIAT, personal communication). The impact of biofortified rice and beans (singly and together), and

biofortified maize (singly) for northeast Brazil, Colombia, Honduras, Mexico and Nicaragua was calculated.
Compared with industrial fortification or medicinal supplementation (Baltussen et al., 2004), iron-biofortified crops that are more cost-effective (cost per DALY saved <US$20) in addressing iron deficiency are as follows: rice in Honduras and Nicaragua and beans in Honduras, Nicaragua and northeast Brazil (Salomón Pérez, CIAT, personal communication). The same is true for zinc-biofortified rice, beans and maize in northeast Brazil and provitamin A-biofortified maize in Mexico (Jacobsen, 2008a); these crops are more cost-effective (cost per DALY saved <US$20) in reducing zinc and vitamin A deficiency, respectively, than fortification or supplementation (WHO, 2006; Baltussen et al., 2004).
Multiple crops biofortified with the same nutrient increase the DALYs saved, a health proxy, as compared to single crops biofortified with the nutrient (Salomón Pérez, personal communication). However, the indicator of cost-effectiveness, cost per DALY saved, does not decrease substantially when multiple crops are biofortified with the same nutrient. This analysis suggests that these crop-nutrient combinations will have the most impact for the least cost (cost per DALY saved <US$50) in these countries:

• Iron biofortification of rice and beans together: northeast Brazil, Honduras, and Nicaragua.

Zinc biofortification of rice, beans and maize together: northeast Brazil and Nicaragua.

Information sharing was carried out by participating in meetings, workshops and conferences especially in Cuba, Dominican Republic, Bolivia, Nicaragua, Panama, and Brazil.

a) **Attendance to workshops, and conferences**: A workshop was conducted in La Habana, Cuba June 9, 2005 under the auspices of the III International Rice Conference organized by the Instituto de Investigaciones del Arroz. Two representatives each from the six target countries (Colombia, Brazil, Bolivia, Nicaragua, Dominican Republic, and Cuba) participated. Main objectives were to present and discuss national work plans, to establish priorities, and provide a forum for interactions among people involved in the project. Total attendance was around 40 people including colleagues from other institutions and the private sector in Panama, Colombia, Costa Rica, Cuba, Nicaragua, Mexico and the FAO Regional Representative based in La Habana. Main recommendations are presented: 1) It was agreed to use several breeding strategies such as pedigree, recurrent selection and mutation breeding methods, as well as anther culture techniques, including interspecific crosses in an attempt to maximize possibilities to obtain superior rice lines with high iron and zinc;2)

CIAT-Palmira should be the centre for seed multiplication and distribution; 3) Standardization of protocols for field evaluation of promising lines and for evaluation of iron and zinc; 4) Plant quarantine regulations established by national authorities must be followed in order to ensure seed distribution/exchange of rice germplasm; 5) Intellectual property rights need to be addressed properly; 6) It was suggested to consider level of iron and zinc not only in milled rice but also in brown rice. There are areas in LAC , specially in the rural areas where people eat brown rice; 7) Main emphasis should be on irrigated and favored-upland rice; 8) GxE studies should be carried out in every country including diverse growing conditions; 9) A multi-disciplinary approach was recommended including people with expertise in soil science, agronomy, plant physiology , pathology, etc.;10) Annual meetings should be conducted to review progress made, facilitate information exchange, review and update priorities, and adjust work-plans. Finally, a CD and a booklet with the presentations made by the participants were prepared and distributed.

A regional workshop was carried out in October 2005 in Posoltega, Nicaragua with the objective of re-inforcing the breeding capacity of NARs in the región (Nicaragua, El Salvador, Costa Rica) and to promote AgroSalud activities; 22 people from different organizations attended and selected breeding material for testing under local conditions.

b) Attendance to AgroSalud and HP Annual Program Reviews: We participated in all AgroSalud Review meetings and in three Annual Reviews organized by HP in IRRI (2005), Thailand (2007) and Singapore (2009). We also presented papers related to AgroSalud in workshops organized by IDIAF in Dominican Republic (2), CONARROZ in Costa Rica(1), IIA in Cuba(2), CIAT-Bolivia in Bolivia(2), and by Embrapa in Brazil (2). Papers were also presented in Rice Technical Working Group Meeting in USA (2), as well as in Training courses organized by IAEA in Chile, Cuba, and Nicaragua.

c) Our partners made efforts in promoting AgroSalud via conferences, workshops, publications and interviews in newsmedia. Brazil presented at least 20 papers in at least five international conferences, two TV shows, and articles in newsmedia; Cuba prepared five articles for local newspapers, two TV shows, technical bulletins for seed production and brochures of varieties grown in Cuba; Bolivia followed same trend. Dominican Republic prepared a brochure and two TV shows. All partners

prepared field days to present promising materials to farmers in their regions.

d) Product development. This activity is carried out in collaboration with Clayuca/Embrapa. Violeta Puldon in Cuba and Roger Taboada in Bolivia have led efforts to promote the development and use of a diverse portfolio of products based on rice; some of these products such as rice cheese, cookies, cakes, and milk are already being tried out at schools, nurseries and hospitals specially for treatments of elderly people and children.

Five main activities are proposed for the 2010-2012 period:

1. Coordinate rice activities by CIAT, taking into account the interface among breeding, genotype by environment (GxE), grain quality, seed multiplication, support to NARS, and economic and nutrition impact of nutrient-enhanced varieties.
2. Continue rice germplasm evaluation and selection according to desirable characteristics, as well as dissemination of the fast-track varieties selected in AgroSalud Phase 1.
3. Evaluate feedback provided by small farmers on cultivar acceptance.
4. Assure efficient functioning of an interdisciplinary, inter-institutional program with activities in six target countries (Brazil, Colombia, Bolivia, Cuba, Dominican Republic, Panama and Nicaragua).
5. Establishment of trials to study the influence of GxE fertilization on the grain.

1B UPLAND RICE

1B.1 Collaborative Project between CIAT and Cirad Rice improvement through the use of synthetic populations

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LAC Cooperators

Funding: Cirad, CIAT and LAC Cooperators

Abstract

The Cirad/CIAT rice collaborative project focuses on the development and enhancement of rice synthetic populations through recurrent selection (RS) with the goal to develop and diffuse improved material, populations or lines, for various rainfed ecosystems in Latin America and the Caribbean (LAC). Our breeding strategy is based on the development of broad-base populations, their improvement thought recurrent cycles of selection and recombination, and the exploitation of their genetic wealth for line development through conventional breeding.

Within our network of rice breeders from LAC, we released improved material as part of the breeding activities for upland rice in Colombia and shared germplasm of interest to LAC collaborators as part of pre-breeding activities.
We are developing large scale phenotyping methods adapted to field conditions to evaluate response to drought among synthetic populations. The thermographic infra-red technology was assessed for screening large number of families, as well as for single-plant evaluation within large populations. This evaluation method allowed us to identify interesting progenitors to develop new population with increased resilience to drought. Allelic variability measured in several synthetic populations revealed high level of neutral diversity.
These activities are part of a much broader mission on the management of synthetic populations for more efficient use of RS breeding and for the development of a marker assisted recurrent selection program.

Background

Recurrent selection (RS) method of breeding was designed to gradually increase the frequency of desirable alleles and allele combinations, while maintaining genetic variability for future selections in a genetically broad-based population. RS is a cyclical method of population improvement that consists in successive cycles of evaluation, selection and intermating. RS method is complementary to conventional breeding by which the variability of the population is exploited for variety development. RS has been used in plant species to enhance yield, alter seed or plant quality, increase pest resistance, and improve tolerance to the environment (Hallauer, 1985).

RS breeding was developed and has been used by Cirad rice breeders for almost two decades. Since 1996, the Cirad/CIAT rice project has worked on developing and enhancing rice germplasm, through RS and conventional breeding, with the aim to deliver genetic resources, training and networking with partners of the public and private research sectors in Latin America and Caribbean (LAC) (Châtel and Guimarães, 1997).

Among the panel of agronomic and adaptive traits considered by rice breeders, we chose to concentrate on resilience to water deficit. Drought stress is the most severe abiotic constraint in upland rice (Bernier et al., 2009) and a serious limit to crop production in LAC (AFP, 2009; FAO, 2005). This important trait is of concern to various rice growing areas reported with actual or future severe drought events. Furthermore, in the context of eco-efficient agriculture, water use efficiency is a critical characteristic to look for among breeding material. However, drought phenotyping can be a limiting factor when sizeable experiments are conducted. Phenotyping methods must follow the same trends as the advances in biotechnology in order to reduce the gap between genotyping and phenotyping capacity and to increase the discovery of true marker-trait associations.

Molecular makers are important tools for population improvement (Courtois et al., 2005; Ramis et al., 2005). They contribute to assist selection by using markers tightly linked to the gene of interest as screening tool. Furthermore, molecular markers are means to monitor diversity within populations. Synthetic populations being considered as a source of allelic richness, it is essential to insure high level of genetic diversity and avoid genetic erosion. Moreover, with targeted markers, populations can be evaluated for their functional diversity, to assess their content in particular allelic variants, and to monitor maintenance of various genes of interest within the populations.

Through the development and improvement of rice synthetic populations our goal is to respond to the need of broadening the genetic base of the material used in breeding programs, and to develop diverse and enhanced germplasm with given traits of interest. Our specific objective was to evaluate synthetic populations under drought conditions to, eventually, develop new population targeted for resilience to drought. Molecular markers tools were used to quantify and to characterize the genetic diversity encompassed in synthetic populations and to assess the breadth of their allelic richness.

Material and Methods

One population (PCT-4\0\0\1) was created in 1995 with a large set of progenitors (35 inbred lines). From this original population, four others were generated, one which was advanced for three cycles without selection (PCT-4\0\0\3), two with specific selection pressure and various recombination cycles (PCT-4\SA\8\1; PCT-4\SA\2\1-Bo\4\1), and one with a slightly different initial genetic constitution (PCT-11\0\0\2-Bo\4\1) (Table 1).

Table 1. Rice synthetic populations considered in our study

	Selection cycles	Recombination cycles	Generation
PTC-4\0\0\3	0	3	S0
PTC-4\0\0\3	0	3	S1
PTC-4\SA\8\1	SA (Acid Soils)	8	S1
PTC-4\SA\2\1,Bo\4\1	SA, Bolivia	7	S1
PTC-11\0\0\2,Bo\3\1	Bolivia	5	S1

Eco-physiology and breeding

Large scale phenotyping was set-up during the dry off-season 2008-2009 in the Experimental Station of Santa Rosa (EESR). Evaluation concerned 1000 plants coming from the PCT-4\0\0\3, and 400 S_1 lines coming from all four synthetic populations. Field screening methods consisted in canopy temperature detection by infra-red thermographic camera (NEC IR Camera). Water stress was applied after panicle initiation stage for 15 days. Readings were performed by Alain Audebert (Cirad) under stress condition and under no water limitation. Control of environmental

variation was achieved through the use of soil moisture monitor (Diviner 2000) and proper experimental design as suggested by Myriam Cristina Duque (CIAT).
In the Experimental Station of La Libertad (EELL) the same set of 400 lines was sown during the cropping season for phenotypic evaluation under the natural conditions of the experimental station (savanna type soils; pH 4.8, aluminum toxicity; high disease pressure).

Genetic diversity

Genetic analyses were performed on a random sample of 207 single S_0 plants for population PCT-4\0\0\3. For all four populations, hundred S_1 families per population were selected and analyzed as 10-S_1 plants bulk per family. Allelic diversity was estimated at 18 SSR loci.

Results and Discussion

A. Rice breeding through RS

As member of a rice breeders' network we develop and improve populations in centralized pre-breeding activities. Upland synthetic populations are observed, characterized and improved through RS in our program. Improved lines extracted from this diverse and enriched pool of germplasm are distributed to national programs in the region for further testing.

Germplasm enhancement

A set of 1664 lines at different stage of advancement (S_1 to S_8) were characterized in EELL-2009 and selected to follow the line development activity. Among these, 600 were advanced lines from S_4 to S_8 generation of which 480 were selected for further improvement through conventional breeding.

Population improvement

The four populations were advanced with one cycle of selection during the cropping season at EELL, and one cycle of recombination during the off-season in Palmira (EEP). No specific selection was performed on these populations, only selection for general plant adaptation under natural field conditions and for preserving a wide range of plant types to meet the diverse demand from our partners.

Germplasm release

Yara and Paya, two varieties extracted from the synthetic population PCT-11 were released by our partner from Bolivia - La Paz. These two varieties are specifically adapted to upland hillside small holders in Bolivia.

B. Breeding, eco-physiology and molecular markers

The phenotyping experiment in Santa Rosa (EESR-2008-09) and the results obtained will be described by Alain Audebert in another chapter of this CIAT-Rice project annual report. The outcomes were also reported in the InterDrought III meeting in Shanghai, China (Audebert et al., 2009).

The 400 S_1 families from the four populations were evaluated under drought conditions (EESR-2008-09) and the resulting 400 S_2 families were characterized in La Libertad (EELL-2009) under the natural soil conditions and heavy blast infestation. Seventy five S_1 families were found with low temperature differential between the stress and the control condition, indicating good transpiration potential under water deficit and a certain degree of resilience to drought. Nineteen of these families also showed good agronomic potential in the EELL09 evaluation. This 19 materials present great value traits and could be a group on which to concentrate efforts for line advancement.

Within the synthetic population that was evaluated under water limited conditions, 16 S_0 plants were found with great potential for response to drought. From these selected plants, eight fertile and with good seed set were selected to constitute the pool of progenitors to go through recombination for developing a new population. The recombination cycle occurred in Palmira (EEP) in absence of stress and 240 male sterile plants were harvested in EEP-2009 and pooled to constitute the new population with improved resilience to drought PCT-4\EF\1\3. This population will be further improved through RS to preserve and enhance its genetic variability and its adaptive characteristic.

Molecular markers were used to evaluate the genetic diversity encompassed in a subset of each of the four populations. The results from this study were presented at the symposium Recursos Genéticos para América Latina y el Caribe in Pucón, Chile (Grenier et al., 2009). The source of diversity used to develop the population resulted in high allelic richness. Averaged over the 16 loci, the

genetic diversity, expressed in terms of number of observed alleles per locus (*Na*) and Shannon diversity index (*I*), was high within the populations (Table 2). Across populations, *Na* was greater than 3.133 alleles per locus and the diversity index varied from *I*=0.693 to *I*=0.800.

Table 2. Genetic diversity assessed with the number of observed alleles (Na) and the Shannon diversity index (I)*. Mean and standard error (*SE)* per locus

Population	Na	I
PCT-4\0\0\3	3.133	0.695
	(0.401)	*(0.112)*
PCT-4\SA\8\1	3.200	0.704
	(0.527)	*(0.107)*
PCT-4\SA\2\1-Bo\4\1	3.667	0.800
	(0.607)	*(0.123)*
PCT-11\0\0\2-Bo\4\1	3.400	0.693
	(0.559)	(*0.118*)

* $I = -\sum p_i \ln p_i$

Across loci, *Na* was highly variable from 2 to 10 alleles per locus (Table 3). The three populations trace to specific evolution history, with different selection pressures and different events of recombination. Consequently they showed high level of diversity and significant (p≤0.05) allelic and genotypic differentiations were found between them (Table 3).

Table 3. Number of alleles observed (Na) and test for allelic and genotypic differentiation

		differentiation	
Locus	Na	allelic	genotypic
RM8068	6	***	***
RM6840	3	ns	ns
RM7382	2	***	***
RM5807	3	ns	ns
RM85	3	***	**
RM5608	3	***	***
RM507	2	***	**
RM5907	10	***	***
RM6775	5	***	**
RM5463	10	***	***
RM3394	9	***	***
RM420	2	***	***
RM408	3	***	***
RM477	2	**	**
RM23654	3	*	*
RM7492	4	***	***

ns: no significant; *, **, *** significant at the probability values p≤ 0.05, 0.01 and 0.001 respectively

Rare alleles (≤1%) were present in each population and taking the four populations all together, they represented 34% of the total alleles recoded (Table 4). Furthermore, 11 alleles were unique to any given population. These private alleles were found at various frequencies, and within the PCT-4\SA\2\1,Bo\4\1 four private alleles which were also frequent (>1%) were found.

Table 4. Allelic richness per population and locus. Frequency of observed alelles (Na) / frequency of rare (≤1%) alleles / frequency of "private" alleles

Locus	PCT-4 0\0\3	PCT-4 SA\8\1	PCT-4 SA\2\1-Bo\4\1	PCT-11 0\0\2-Bo\4\1
RM8068	4/0/0	4/1/0	4/0/1*	5/1/0
RM6840	3/0/0	3/0/0	3/0/0	3/0/0
RM7382	2/0/0	2/0/0	2/0/0	2/0/0
RM5807	3/1/1	2/0/0	2/0/0	2/0/0
RM85	2/0/0	2/0/0	3/1/0	3/1/0
RM5608	3/1/1	2/0/0	2/0/0	2/0/0
RM507	2/0/0	2/0/0	2/0/0	2/0/0
RM5907	6/2/0	7/2/0	8/2/1*	6/2/1
RM6775	2/0/0	3/1/0	4/1/1	3/0/0
RM5463	6/0/0	8/0/0	9/1/1*	7/1/1
RM3394	6/1/0	6/1/0	7/0/0	9/2/1*
RM420	2/0/0	2/0/0	2/0/0	2/0/0
RM408	3/1/0	2/0/0	3/0/0	2/0/0
RM477	2/0/0	2/0/0	2/0/0	2/0/0
RM23654	3/0/0	3/1/0	3/0/0	3/0/0
RM7492	2/0/0	2/0/0	3/0/1*	3/0/1*

* Private and frequent (>1%) alleles

Although there was selection pressure (approximately 20%) during the phases of population enhancement for three of the four populations, all four populations maintained high level of genetic diversity, including a significant portion of rare alleles. Because of the different way they were handled, these populations also appear to have fixed some specific and unique allelic variants, probably due to the selective pressure and hitchhiking effect. Functional diversity among these populations will soon be assessed.

Conclusion

RS in rice has proven to be a method with great benefits for rice breeding. Primarily, because the populations initiated more than 14 years ago still maintain high level of genetic diversity, as we could see looking at neutral genetic markers. Furthermore, the populations hold source of favorable alleles for resilience to dry conditions. Frequency of these favorable variants can be increased by population enhancement

through RS, or the favorable allele can be fixed in improved lines through conventional breeding. Besides showing the genetic wealth retained in these synthetic populations, our work presents an advance towards the integration of high-throughput phenotyping and use of molecular markers for improving RS breeding strategy.
Additionally, improved material with specific adaptation to particular ecologies was extracted from the synthetic populations and shared with partners; such as the two most recently released lines targeted for upland hillside small holders in Bolivia.

Future activities

The way forward in rice synthetic population improvement
The next step in our program is to further combine the use of eco-physiology and molecular markers to increase the efficiency of RS breeding. High quality phenotyping and molecular data will be used to study genome wide associations between allelic variants and phenotypes. Breeding scheme will be developed for marker assisted recurrent selection to facilitate selection in a contest of synthetic population improvement.

The monitoring of synthetic populations will be pursed, studying both neutral and functional diversity. Among many other purposes, this will provide an important assessment of the need to introgress other germplasm --exotic lines, improved genetic material, introgression lines, etc…--, in the population to broaden its genetic base; by adding source of allelic variability or new characteristics of interest.

Results and outcomes of these presented research activities conducted by the Cirad/CIAT project will be shared with LAC and others potential partners through the GRUMEGA network.

Additionally, we are fully engaged in a Cirad project “Genetic basis of adaptive diversity in rice; toward tools to pilot selection” for which the diversity in adaptive traits for drought and heat stress will be evaluated among a diverse set of 181 japonica and indica rice. Phenotypic and molecular data will be put together for genome wide association study and to provide rice breeders with parents and molecular markers to conduct marker assisted selection program for these traits. The collection was set-up in a phenotypic trial in Santa Rosa to evaluate response to a 15 days drought period applied at vegetative stage.

Networking

We are hoping to revive the rice breeders' network from LAC (www.grumega.org) through an activity we are launching to characterize the rice growing systems in LAC. This work should lead to a review of the current situation in the region, as well as to set-up observatories to obtain dynamic data base to ultimately monitor the future of rice growing in the region.

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1B.2 Physiological field evaluation for drought tolerance.Field phenotyping methods for the development of the new synthetic population

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Funding: Cirad, CIAT Cooperators

Abstract

The Cirad/CIAT collaborative project has developed a breeding program based on the recurrent selection (RS) for an eco-efficiency use of water. Selection was primarily based on yield and, in the course of the selection process, screening methods were refined with additional secondary relevant traits (morpho-pheno-physiological traits). The infrared (IR) thermography measures the plant canopy temperature, which gives an indication of the plant water status through leaf surface cooling capacity by transpiration along environmental conditions. We developed large scale phenotyping methods, adapting the IR technology to field conditions, to evaluate response to drought among synthetic populations.
An experiment was conducted during the 2008/2009 dry season in the Santa Rosa CIAT research station in Villavicencio (Colombia) to adjust the phenotyping method based on IR thermography and to evaluate 400 S_1 lines and 1000 S_0 plants derived from synthetic populations. This evaluation method allowed us to identify interesting progenitors to be integrated in the population improvement scheme with the goal to develop and diffuse improved material, populations or lines, with increased resilience to drought for various rainfed ecosystems in Latin America and the Caribbean (LAC).

Background

Enhanced crop production under limited water supply depends on a subtle dosage of various physiological mechanisms and plant traits according to timing, intensity and duration of the water deficit period. Unfortunately, knowledge on plant response to water deficit has only poorly impacted genetic improvement for crop productivity in drought-prone area. Indeed, the method based on direct selection for grain yield still produces the best-performing genotypes, particularly for rice. Facing

this situation, the Cirad/CIAT rice collaborative project intends to develop populations, lines and methodological tools to assist in the selection process. Integration of expertise from Cirad and CIAT on rice RS and plant physiology under water stress is expected to provide significant headway for selecting germplasm with better adaptation or resilience to drought conditions.

The project aims at creating new rice synthetic population, using available information on phenotypic traits related to drought tolerance in rice. With further knowledge on loci and alleles related to drought response, this population will also be used to develop breeding methods integrating molecular markers and ecophysiological criteria to enhance RS for drought tolerance and water use efficiency. Crop development models will also be used to analyze and predict the behavior of advanced breeding lines in the targeted environments. The improved genetic resources (population and lines) and methods developed in our project will be shared within the LAC rice breeders' network and with other CGIAR and national breeding programs. This new population will remain open to be enriched with new alleles at other target loci as and when available. The expected outputs are (i) new genetic resources (populations with a broad genetic base and advanced lines) with improved drought tolerance or improved water use efficiency, (ii) validated methods of molecular marker and crop model based selection for drought tolerance and (iii) better understanding of the physiological and genetic bases of drought tolerance mechanisms.

The experiment was conducted during the dry off-season 2008-2009 and its specifics objectives were:

-The physiological screening in field conditions of genetic material extracted from four synthetic populations
-Development of phenotyping methods based on IR thermography

Material and Methods

From an original synthetic population created in 1995 with a large set of progenitors (PCT-4) four populations were generated differing for the specific selective pressure and number of recombination cycles they were subjected to (cf. Grenier et al.'s chapter in this report).
A large scale phenotype screening was set-up during the dry off-season 2008-2009 at the Experimental Station of Santa Rosa (EESR) in two experiments. For both experiments, a 15-days water stress was applied after panicle initiation stage, and field screening methods consisted in evaluating canopy temperature with IR thermographic camera (NEC IR Camera) at the end of the water stress period (78-80 DAS).

-Experiment 1: Evaluation of 400 S_1 lines coming from the four synthetic populations previously presented. Two treatments were applied; one with the stress and one, used as a control, where irrigation was maintained during the entire experiment. Evaluations were conducted for both treatments. The experimental set-up followed an augmented split block design. The complete experiment consisted in a total of 32 blocs (16 blocs per treatment) with 25 S_1 lines and six controls varieties within each bloc. The control of environmental variation was achieved through the use of soil moisture monitor with Diviner 2000 equipment (Sentek). The soil moisture was monitored for two of the control varieties; Curinga and Moroberekan.

-Experiment 2: Screening for drought response among a population composed of 1000 individual S_0 plants originating from the population PCT-4\0\0\3. The same six control varieties as in experiment 1 were distributed regularly within the population. As in experiment 1 soil moisture was monitored for Curinga and Moroberekan.

The two experiments received nutrient application with SPT (basal application at 70 kg/ha), KCL (70 kg/ha in 2 application) and N (80 kg/ha of urea in 3 applications). Micronutrients were applied as 20 kg/ha Fertimex.

Results and Discussion

A. Experiment 1
   Leaf temperature at/around flowering stage exhibited strong and significant varietal differences that were negatively correlated with soil moisture content and yield. Figure 1 shows the effect of water stress on canopy temperature. In the Santa Rosa 2008-2009 dry season, the canopy temperature of rice populations under stress condition was in average 3.2°C greater than the in the control treatment.

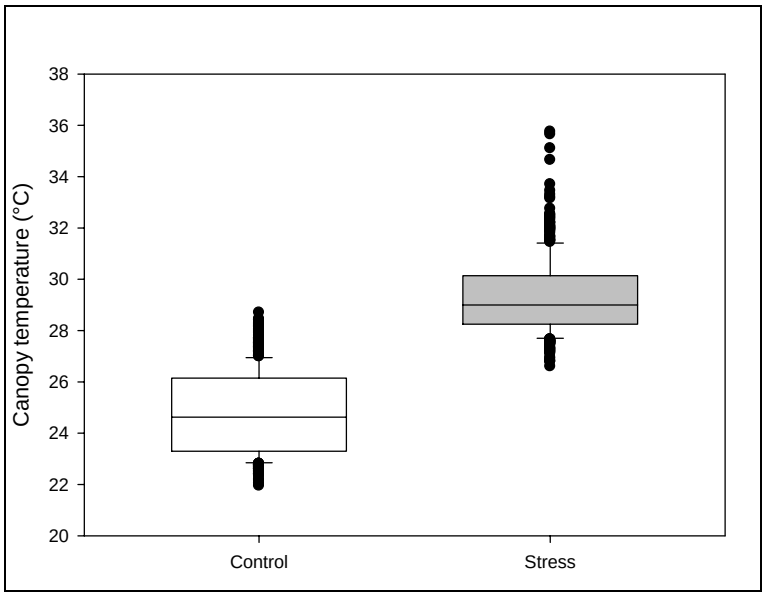

Figure 1: Effect of water deficit on the rice canopy temperature. n= 496 S_1 lines

The frequency histogram obtained for the canopy temperature also shows the existence of genetic variability for this trait (Figure 2). This diversity existed for the control as well as for the stress condition. The canopy temperature among lines did not rank similarly in both treatments. However, particular lines with low canopy temperature in the control treatment also showed a low canopy temperature in the stress treatment. Thus these materials seem to display better transpiration ability in all water availability conditions.

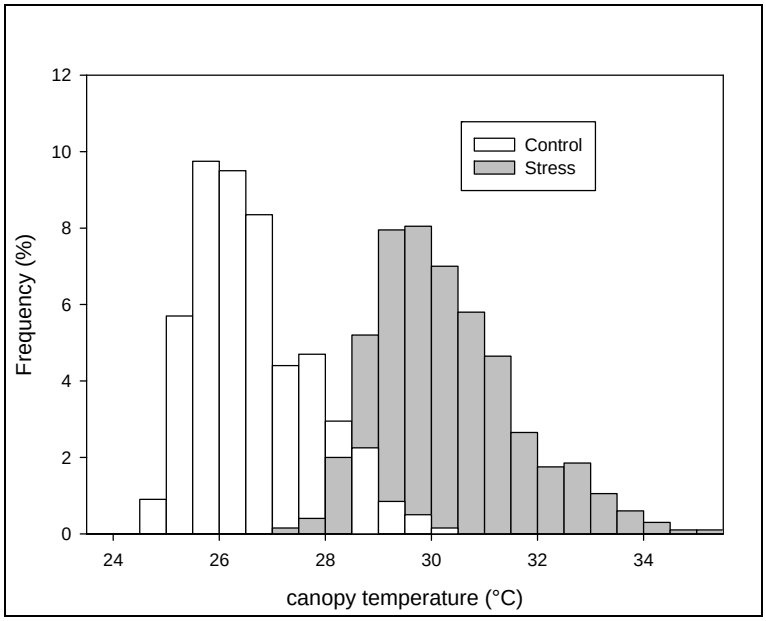

Figure 2: Frequency distribution of canopy temperature for all lines tested and for the two treatments. n = 496 S_1 lines.

This phenotyping approach permitted identifying genotypes with good maintenance of transpiration, and thus sustained growth, under drought stress. Preservation of this capacity to transpire is mainly related to limited water extraction and/or greater root depth. The IR phenotyping also enabled detecting genotypes that have high transpiration rate, and consequently higher potential growth rate, under irrigated control conditions. Selecting suitable combinations of both characteristics is expected to help identifying drought tolerant material with high yield potential.

Seventy five S_1 families were found with low temperature differential between the stress and the control condition, indicating good transpiration potential under water deficit and a certain degree of resilience to drought. Nineteen of these families also showed good agronomic values in a subsequent evaluation of the same 400 lines during the 2009 growing season under non-stress condition in the Experimental Station of La Libertad (Colombia). These 19 lines present valuable traits and could constitute a group of materials on which to concentrate efforts for line advancement and population improvement.

The outcomes of the phenotyping experiment EESR (2008-2009) and the results obtained were reported in the Inter Drought III meeting in Shanghai, China (Audebert et al., 2009).

B. Experiment 2

The use of the IR thermography under water limited conditions also permitted to detect individual plants with lower canopy temperature among a synthetic population. With this method, 16 S_0 plants were found with great potential for response to drought. From these selected plants, eight fertile ones with good seed set were selected to constitute the pool of progenitors to go through recombination for developing a new population. The recombination cycle occurred in Palmira (CIAT-HQ) in 2009 and a bulk of male sterile plants was collected to constitute a new population with improved resilience to drought (PCT-4\EF\1\3). This population will follow the recurrent selection to preserve and enhance its genetic variability and its adaptive characteristic for drought prone environment.

Conclusion

These experiments confirmed the effect of water deficit on canopy temperature. Canopy temperature could thus be used as a criterion to screen for drought tolerance and to serve as a selection tool for breeding programs. Furthermore, the frequency histogram proved the existence of genetic diversity for this trait. Despite a non severe drought and some methodological difficulties, lines presenting a good transpiration level under stress were selected to develop new populations with increased resilience to drought.
However, this phenotyping methodology based on IR imaging showed some drawbacks. One is that the method is time consuming. Without possibility to use a wide angle picture, the time required to photograph all the material becomes important for large experiment, thus affecting the comparison between images taken at different moments during the day. Another limit of the methodology is that the micro climatic conditions around the tested material are often not stable. Radiation and wind are fairly variable and have a direct effect on transpiration and canopy temperature. To resolve these limitations it is imperative to correct or standardize the canopy temperature measurements. The better way to do so is to use the Crop Water Stress (CWS) index. Unfortunately in 2009 no adapted equipment was available for us to assess the CWS for standardization of our IR readings.

Future activities

We are already making a new significant improvement in our high throughput phenotyping method for assessing response to water stress conditions. We recently acquired an equipment to assess the CWS that will thus allow standardizing IR readings. The next step in our program is to further combine the use of eco-physiology and molecular markers to increase the efficiency of RS breeding. High quality and high throughput phenotyping, and molecular data will be used for genetic association studies for a better knowledge of the drought adaptation mechanisms.

In addition to our Cirad/CIAT phenotyping project for breeding rice with improved efficiency of water use through RS, we have started a Cirad project entitled “Genetic basis of adaptive diversity in rice; toward tools to pilot selection”. For this project, a set of 181 highly diverse japonica and indica rice will be evaluated for their response to drought and heat stress. We have set-up an experiment in Santa Rosa for high throughput phenotyping of response to a 15-day water stress applied at vegetative stage, using the improved technology we suggested after our previous experiment. Genotyping with SNP markers will be performed on this

collection of germplasm and phenotypic and molecular data will then be put together for genome wide association study.

Publications

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 Inter Drought III Meeting: Shanghai-China 11 – 16 October 2009

1C BIOTECHNOLOGY ACTIVITIES RELATED TO ENHANTED GENE POOLS

1C.1 Exploring Natural Genetic Variation: Developing Genomic Resources and Introgression Lines for Four AA Genome Rice Relatives

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**These authors contributed equally to the work*
Partners: IRD-UMR5096, Cornell University, Embrapa-CNPAF, Fedearroz, WARDA Project funded by: The Generation Challenge Program, IRD, CIAT

Background

The future of crop improvement depends on the availability of genetic variation. Most modern crop varieties have undergone a genetic bottleneck associated with the process of domestication resulting in a restriction of the genetic options that are available to plant breeders. There is a larger pool of genetic variation available in landraces and wild relatives of crops. These resources are known to contain many interesting traits for breeding, including good to strong tolerance to abiotic and biotic stresses and various nutritional traits of interest (Sun et al., 2001). However, it is often difficult to utilize these natural sources of genetic diversity because of fertility barriers, linkage drag, the time and resources required to recover useful recombinants.

To take advantage of the unexploited reservoir that exists in the wild relatives of cultivated rice (*Oryza sativa* L.), we started to develop interspecific introgression lines that will be of immediate use to breeders and will simultaneously serve to enhance our understanding of the "wild alleles" that contribute favorably to plant performance under drought stress. These lines are called Chromosome Segment Substitution Lines (CSSLs).

CSSLs are particularly valuable when complex, quantitatively inherited phenotypes are the breeding target. Because they represent permanent (inbred) genetic resources that can be easily replicated by seed and

distributed to collaborators working in different environments. Each set of CSSLs consists of a relatively small number of lines that can be evaluated in replicated trials. They are constructed to provide maximum power of statistical analysis because each line can be compared to all others or may simply be compared to the recurrent parent, making it possible to extract a great deal of valuable information from a relatively small number of lines crops. For phenotypes that are difficult to measure, or require repeated evaluation over years and environments, the ability to focus quickly on a small number of lines is a critical component of success (Ghesquière et al., 1997).

In addition to the targeted introgression of traits that can be identified phenotypically in the wild material, such as biotic or abiotic stress tolerance, it has been demonstrated that alleles hidden in low yielding, agronomically undesirable ancestors can enhance the productivity of many of the world's most important crop varieties. These yield-enhancing alleles are the basis of 'transgressive variation' and may confer an advantage in both favorable (irrigated) and unfavorable conditions (drought and weed competition) (Moncada et al., 2000; Gur and Zamir, 2004). Thus, the use of wild and exotic germplasm for CSSLs construction carries with it the possibility that favorable transgressive segregants will be identified, providing the basis for studies aimed at understanding the genetic basis of transgressive variation associated with the trait of interest.

Wide spread utilization of *O. sativa* relatives remains limited due to the fact that: (1) no extensive study has been carried out to explore the range of allelic diversity in any of the *Oryza* AA genome relatives, (2) the genetic basis of heterosis or transgressive variation in interspecific crosses remains largely unknown, (3) interspecific crossing barriers have hampered full utilization of rice relatives for breeding and genetic studies, (4) very few genomic resources have yet been developed to facilitate breeding efforts using *O. sativa* relatives. In particular, the lack of a cost effective, high throughput marker system that targets gene-based polymorphisms impedes efforts to efficiently and systematically select the best introgression lines and to evaluate the gene content of those lines in the context of comparative cereal genomics.
The aim of the present work is to develop a genetic resource for four AA genome rice relatives. Here, we shown the developing of two cultivated x wild crosses, where the wild species are *O. meridionalis and O. rufipogon.* Two other populations with *O. barthii* and *O. glumaepatula* have been developed at WARDA, Benin and Embrapa-CNPAF, Brazil, respectively.

Results and Discussion

1C.1.1 *O. sativa ssp. tropical japonica* (cv. BRSMG Curinga) x *O. meridionalis* (acc. OR44)

Laura Moreno- CIAT

The development of a Chromosome Segment Substitution Line (CSSL) library for the interspecific cross between the cultivated rice *O. sativa* BRSMG Curinga and the wild species *O. meridionalis* OR44 is now at its final stage.

On 2008 a DH population of 848 plants was developed at CIAT through Anther Culture which represented 130 BC3DH families according to selected target fragment. Forward selection of 366 lines was done previously from double haploid lines development, in order to reduce the amount of plant material to be processed. Graphical genotypes of 130 lines with introgression target fragment obtained using CSSL finder software, are shown in Figure 1.

One fragment was lost at upper end of chromosome 2 and could not be recovered after forward selection of several additional lines.

One to three plants according to number of DH plants recovered from each family (130 BC3DH families) were selected from seed stock. A population of 376 BC3DH lines was used for DNA extraction and forward and background selection at Cornell University.

Forward selection of 376 BC3DH lines was done using the set of polymorphic SSRs and MITEs previously optimized. Three to four SSRs and MITEs markers were used to genotype each set of lines according to target fragment identified during forward selection at CIAT. Molecular techniques include 4% PAGE and 4% agarose gels. Preliminary results, showed a significant loss of target fragments in chromosome 1, 2, 3, 4,5,6, 7, 10 and 12. Only target fragments from chromosomes 7 and 9 were recovered completely. Introgression fragments from chromosomes 6 and 10 could not be recovered, during this forward selection.

Background selection of 62 lines with chromosome fragment introgressions and additional 32 lines selected to complete missing fragments were genotyped at Cornell University and CIAT, using 132 SSRs and MITEs markers. The selection criteria for the additional 32 lines was based on the BC2F1 data previously obtained. Thirty two lines which represent the best overlapping chromosome introgression fragments with the maximum background recovery, were selected using the CSSL Finder software (Figure 2).

Figure 1. Graphical genotypes from forward selection of 130 BC3F1 lines showing introgression target fragment (brown); fragment reduction or

background recovery (light pink); missing data (gray). Genotyping was done with 130 polymorphic markers (120 SSRs and 10 MITEs)

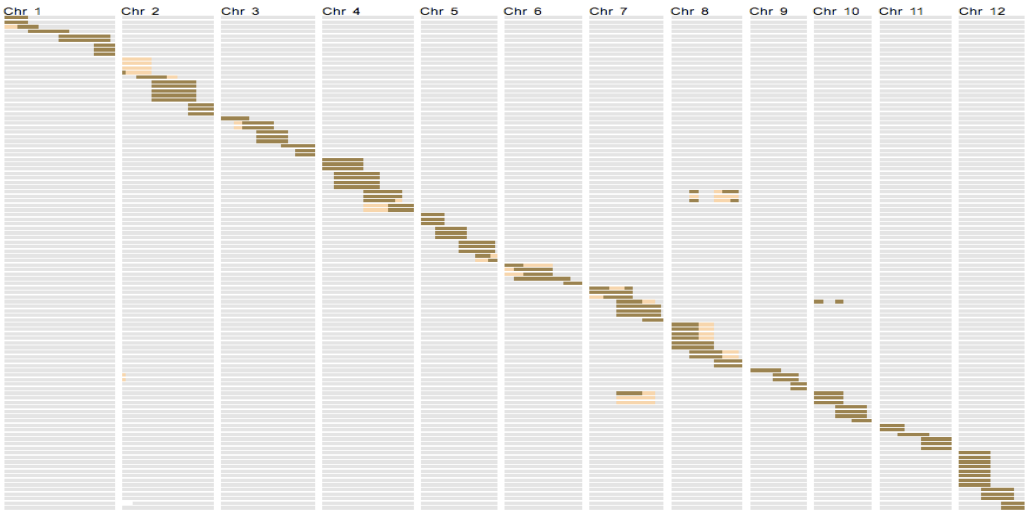
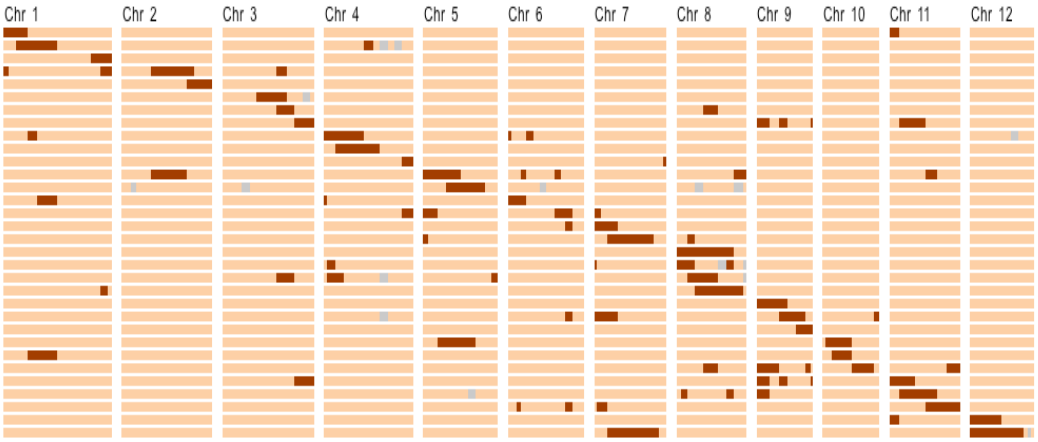


Figure 2. Graphical genotypes of 32 BC3DH lines Chosen from forward and background selection of 94 BC3DH lines. Lines were selected using the CSSL Finder software based on desire introgression fragment length and maximum background recovery: introgression target fragment (red); background recovery (light pink); missing data (gray)



Forward selection of additional 67 BC3DH lines is currently being done at CIAT. Lines were chosen to fill missing chromosome fragments in a last effort to complete the Curinga x OR44 CSSL library. Additionally, selected BC3F2 lines will be selfed for at least 4 more rounds for a second chance to recover fixed alleles in some missing target introgression fragments.

The desire income is to select around 64 CSSL lines with an average introgressed fragment size of ~50 cM or ~10Mb and an intermarker distance average of ~15cM or ~3Mb.

1C.1.2 *O. sativa ssp. tropical japonica* (cv. BRSMG Curinga) x *O. rufipogon* (acc. IRGC-105491)

Juan David Arbelaez – Fedearroz/ CIAT

Pursuing the development of this valuable germplasm, optimal for QTL analysis and plant breeding programs, is based on the construction of Advanced Backcross populations, Anther Culture technique, and the use of Molecular Marker Assisted Selection.

To accomplish this, during 2009 three main activities were carried out. The first was the *positive* checked of a BC_3F_1 population between Curinga (*Oryza sativa japonica*) and *O. rufipogon* (acc. IRGC-105491), those selected plants were process to generated *Double Haploid* plants, and third the BC_3F_1 DH population were *positive* and *negative* selected to developed the final CSSL library of 84 plants.

In the first semester of 2009, 360 BC_3F_1 plants (6 plants per family, 60 families) were chosen to be *positive* selected according to their target fragment. These plants contain a total of 50 desirable introgressed fragments from the wild parental *O. rufipogon* that represents its whole genome. Using a set of 130 SSRs markers, 180 BC_3F_1 plants (3 per family) were selected and then were processed through anther culture to generate *Double Haploid* plants.

Anther culture is an in-vitro procedure to fix segregants lines in one cycle. From the 180 BC_3F_1 plants selected, 468 BC_3F_1 DH were generated (~6 per family). DNA from these plants were extracted, dried, and shipped to Cornell University to be genotyped.

In the second semester of 2009, this BC_3F_1 DH population was genotyped for a *positive* selection implementing 130 SSR markers. This type of selection allowed to check those plants that keep the desirable wild introgressed fragment. From each family, around three plants (total of 184 plants) were chosen to be *negativ*e selected, selecting those with the less amount of donor genome in their background. These 184 plants were genotyped with an illumina 384 SNP platform, designed at Cornell. 248 SNP were polymorphic for the parents, with an average of 5 cM between them. Using the CSSL finder 84 plants were chosen according to their fragment of interest and background recovery to be the CSSL library between Curinga and IRGC-105491.

These lines have an average recover of 92% of the recurrent parent with an average desire introgressed fragment of 9 Mbp. This CSSL library is now being multiplied to be distributed to the partners and be evaluated for different traits of interest.

Conclusions

CSSLs were proven as very a powerful tool for gene discovery in different crops. They are of particular value for studies involving wild progenitors as they 1) often permit to overcome interspecific sterility barriers as a large part of the cultivated species is recovered in advanced generations, 2) allow a direct comparison of the introgressed lines to the cultivated parent, permitting to display the effect of the wild progenitor on the phenotype.

We hope that the development of full-genome coverage CSSL populations will contribute significantly to the set of genetic and genomic tools available for breeding and gene discovery in rice. This is the first time at CIAT that an interspecific population with a wild donor parent is used to produce Double Haploids.

To date, the project allowed us to obtain many important results. Among them, we may want to mention in particular the following:

- Four interspecific genetic maps were developed
- Four cultivated x wild CSSLs populations already finished
- A software (CSSL Finder) was designed for the specific purpose of helping at developing CSSL lines
- Several students and research assistants were trained
- Four students do shuttle research between their respective centers and Cornell University
- The international collaboration between several ARIs, CG centers and NARS was strengthened
- Several publications are in preparation

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1C.2 A Nested Association Mapping (NAM) population of rice

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Partners: WARDA, FLAR
Project funded by: The Generation Challenge Program, CIAT, IRD

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 J. *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.

Materials and Methods

We will produce twenty populations of Recombinant Inbred Lines (RILs), derived by Single Seed Descent (SSD), from F_1 hybrids between twenty carefully chosen rice accessions. Each population will contain 200 lines. The resulting meta-population, called a Nested Association Mapping (NAM) population of 4,000 recombinant lines, will be seed-increased and checked for homozygosity and allelic purity

Selection of parental lines

A diversity survey of 48 candidate lines as parents for the meta-population was done using a set of 24 SSR markers. 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 tropical *japonica* lines as parents that maximize the genetic diversity (Table 1).

Table 1: List of 15 selected varieties as parents.

Accession	***Pedigree***
IRAT 122	MAKALIOKA / CHIANAN 8
IRAT 146	IRAT 13 / DOURADO PRECOCE
ITA 164	LAC 23 (RED)/MULTIPLE PARENT 25
TOX 1011-4-1	IRAT 13/DP689//TOX490-1
CT6241-2-2-1-3	NGOVIE/TAIPEI 309//COL 1 X M312A-74-2-8-8
CT6241-19-2-1-3-1P	NGOVIE/TAIPEI 309//COL 1 X M312A-74-2-8-8
CT10011-5-4-M-M	CT6424-12-1-4-1-2//CT6515-18-1-3-1-6/CT8088-14-16
CT10006-7-2-M-2	CT6241-2-2-1-3//CT6516-23-10-1-2-2/CT8071-13-1
CT10048-6-3-M-2	CT6240-12-3-3-5//CT6516-23-10-1-2-2/CT8076-15-2
CT8556-37-1-3-1-M	TOX 1859-102-5M-7/COL 1 X M312A-74-2-8-8//TOX 1837-103-1-4
CT10035-26-4-2-M	CT6258-5-2-6-5//CT6129-17-2-1P-2/CT6196-33-10-2-1
CT10037-56-6-M-M	CT6424-12-1-4-1-2//CT6129-17-2-1P-2/CT6196-33-10-2-1
CT10045-5-5-M-1	CT6258-5-2-5-3-3P//CT6516-23-10-1-2-2/CT8060-2-1
Oryzica Turipana	P 4971/P 5004
Liderança	Not available

Production of F_1 hybrids

F_1 hybrids have been produced by crossing the *indica* IR64 accession as female with all 15 candidate lines. Ten F_1 seeds were sown per retained combination, and were checked for heterozygosity using 3 SSR markers. They were then brought to the field in order to be selfed and to produce the F_2 populations that will represent the starting point of the Single Seed Descent (SSD) process (Figure 1).

Figure 1. F_1 Plants selected for build the Nested Association Mapping (NAM) population.

Implications

We think that the NAM population will provide the rice research community with a highly efficient and powerful genetic resource that would allow to fully taking 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.

Next steps and/or challenges

From each F_2 population (Figure 2), 400 seeds will be sowed in order to make sure we obtain final F_7 population sizes of 200 individuals through the SSD process.

Figure 2. 4000 F_2 Plants representing 10 different populations.

Sequencing of IR64 will certainly increase very much the potential of this NAM meta-population, in allowing us to infer the ancient recombination events based on complete genomic information instead of partial (SNP) coverage.

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1C.3 Development of two Chromosome Segment Substitution Lines (CSSLs) populations derived from interspecific cross *Oryza sativa* L. x *Oryza glaberrima* Steud.

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Partners: IRD-UMR5096
Project funded by: The Generation Challenge Program, IRD, CIAT, The French Ministry of Foreign Affairs

Background

Developing new population types based on interspecific introgressions has been suggested by several authors to facilitate the discovery of novel allelic sources for traits of agronomic importance and to explore primitive and broad genetic resources in rice breeding (Zhang *et al.,* 2006). These populations have been named Introgression Lines (ILs) or Chromosome Segment Substitution Lines (CSSLs) (Eshed and Zamir 1995).

Basically, CSSLs populations contain one or few contiguous chromosomal segments of the donor genotype in the background of a recurrent genotype, limiting the interactions between donor alleles to those homozygous substituted tracts (Howell et *al.*, 1996). Also, these materials allow to make detailed analyses, as far as, marker assisted selection, map-based cloning, there represent a small number of lines that can be evaluated in replicated trials and provide a very good alternative to understand the genetic bases of reproductive barriers between species (Li *et al.*, 2005; Ebitani *et al.*, 2005).

We built two populations of Chromosome Segment Substitution Lines (CSSL) between the two cultivated species of rice; in which chromosomal segments of the African rice *Oryza glaberrima* replace the corresponding segments in the genome of the *Oryza sativa ssp. indica and japonica* and are available for distribution to partners. Furthermore, we present a QTLs detection analysis for yield, yield components and resistance to RSNV (Rice Stripe Necrosis Virus) in order to illustrate the advantages of using this kind of materials in genetic analysis and breeding of rice.

Materials and Methods

Population 1. Interspecific cross: *O. sativa* ssp. tropical *japonica* cv. Caiapo x *O. glaberrima* acc. IRGC103544 (MG12)

The recurrent parent Caiapo (*O. sativa ssp. tropical japonica*) is a commercial rice variety developed by EMBRAPA-CNPAF (Goiania, Brazil) and has been cultivated since 1992 in Brazil and other places in Latin America and the Caribbean. The donor parent MG12 (acc. IRGC103544) is an accession of the African cultivated rice species, *O. glaberrima*. This species is grown in West Africa and shows several negative characteristics with respect to the Asian *O. sativa.*

A BC_3F_1 population was obtained at CIAT HQs from the cross between Caiapo (an elite tropical *japonica* from Brazil) and *O. glaberrima* acc. IRGC103544 (MG12) (César P. Martinez). From these lines, anthers were collected and a population of 695 lines BC_3F_1 Doubled-Haploid (DH) was obtained through *in vitro* culture (Zaida Lentini). Subsequently, a subset of 312 BC_3F_1DH lines offering a good representation of the observed phenotypic variability was selected as mapping population for agronomic evaluation and molecular characterization.

The mapping population was evaluated in replicated field plots in Colombia in 2001. Five plants for each one of 312 BC_3F_1DH lines were randomized selected and then evaluated for six agronomic traits: plant height (PTHT), tiller number (TINB), panicle length (PNLG), percentage of sterility (ST), 1000-grain weight (TGRWT) and grain yield (YLD).

Total DNA was extracted from frozen leaf tissue based on a slightly modified version of the Dellaporta protocol. Subsequently, quality and quantity of DNA was evaluated on 0.8% agarose gel stained with ethidium bromide. A total of 200 polymorphic simple sequence repeats (SSR) loci distributed across the twelve rice chromosomes with an average spacing of 8.0 cM was used. Most of these SSR markers were selected from the Universal Core Genetic Map (UCGM) of rice developed at CIAT Rice Genetics and Genomics group. Polymerase chain reactions (PCR) were performed in a total volume of 15 μL. PCR products were separated on 4% agarose gels for markers that showed a polymorphism size higher than 10 bp, and stained with ethidium bromide. For polymorphism lower than 10 bp, PCR products were separated using 6% denaturing polyacrylamide gels followed by silver staining.

Selection of a subset of introgression lines that cover the entire donor genome was carried out with the help of the CSSL Finder v. 0.84

program. CSSL Finder was designed to search for a subset of CSSL. Subsequently, graphical genotypes of the candidate lines can be displayed. CSSL Finder is available at http://mapdisto.free.fr.
We used a genetic linkage map obtained from a BC_1F_1 population derived from the cross IR64 (*O. sativa ssp. indica*) x TOG5681 (*O. glaberrima*) (our unpublished data). The map was constructed using the computer program MapDisto v. 1.7 (http://mapdisto.free.fr). For each marker, a chi-squared test ($P < 0.01$) was performed to identify markers with segregation distortion. A QTL analysis for the evaluated traits was done using both the CSSL Finder v. 0.84 and the MapDisto v. 1.7 programs. Interval mapping (IM) and composite interval mapping (CIM) analyses using WinQTLCart v. 2.5 were also performed. Significant QTLs found using *F*-test, IM and CIM methods were compared with previous studies.

Population 2. Interspecific cross: *O. sativa* ssp. *indica* cv. IR64 x *O. glaberrima* acc. TOG5681

The recurrent parent IR64 (*O. sativa ssp. indica*) is a commercial rice variety developed by IRRI (Philippines). IR64 carries many valuable agronomic traits related to yield, plant architecture, grain quality, and tolerance to biotic and abiotic stresses. The donor parent TOG5681 is an accession of the African cultivated rice species, *O. glaberrima*.

A population made of BC_2F_4 and BC_3F_3 lines (Pre-CSSLs) was developed at IRD, Montpellier, France through marker-assisted backcrossing from the cross IR64 (*O. sativa* ssp. *indica*) x TOG5681 (*O. glaberrima*). These sub-populations were analyzed with 153 SSRs marker for its genomic content at CIAT. The microsatellite markers cover whole genome and were selected from Rice Universal Core Map data base (developed at CIAT).

For this population the above methodology for DNA marker and statistical analysis was used.

Results and Discussion

Popupation 1. Interspecific cross: *O. sativa* ssp. tropical *japonica* cv. Caiapo x *O. glaberrima* acc. IRGC103544 (MG12)

A subset of 312 BC_3DH lines were genotyped using 200 SSRs. Sixty-four lines that cover the *O. glaberrima* whole genome were selected as candidate for CSSL lines using CSSL Finder v. 0.84 computer program (Lorieux 2005) (Figure 1). The overlapping targeted chromosomal segment size was 10 cM on average.

A QTL analysis allowed to detect fourteen QTLs for plant height, tiller number per plant, panicle length, sterility percentage, 1000-grain weight and grain yield were located on chromosomes 1, 3, 4, 6, and 9. Furthermore, a highly significant QTL controlling resistance to the Rice Stripe Necrosis Virus (RSNV) was closely located on chromosome 11. Fine mapping of this major QTL can be envisaged using BC_4F_2/F_3 lines.

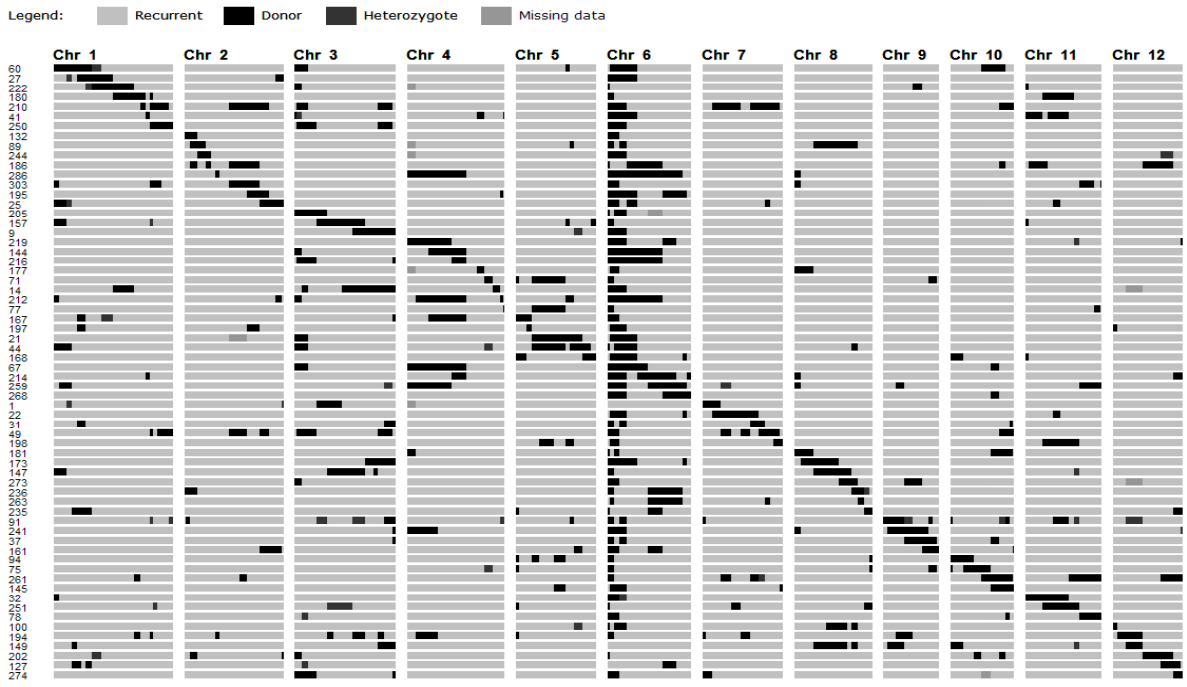

Figure 1. Graphical representation of the genotypes of 64 BC_3DH lines selected from a library of 312 lines. The 12 rice chromosomes are displayed vertically. There are covered by 125 evenly dispersed SSR markers. The genotypes are displayed horizontally. Colour legend indicates the allelic status of chromosomes, where "Recurrent" means homozygous for the Caiapo allele and "Donor" means homozygous for the MG12 allele.

On the other hand for to optimize and to purify the 64 CSSLs selected and to keep only one fragment introgressed from *Oryza glaberrima*, 59 of these lines were chosen and backcrossed to Caiapo and selfed to obtain BC_4F_2 lines. For each one of these 59 BC_4F_2 as minimum 60 individuals were planted in the field (in total 4200 BC_4F_2 individuals). With the aim to identify plants for only one target fragment, these 4200 materials were grouped in bulks and later they were evaluated with 2-3 microsatellite markers that flank the target segments of *Oryza glaberrima.* Forty-eight bulks were opened and selected the 48 single plants that containing the target fragment (Figure 2). These BC_4F_2 plants were planted and going to be selfed two or three times.

New bulks BC_4F_3 are being evaluated to fill small gaps on chromosomes 2, 4, 8, 9 and 12. Caiapo x MG12 CSSLs population is finished and available for distribution to partners.

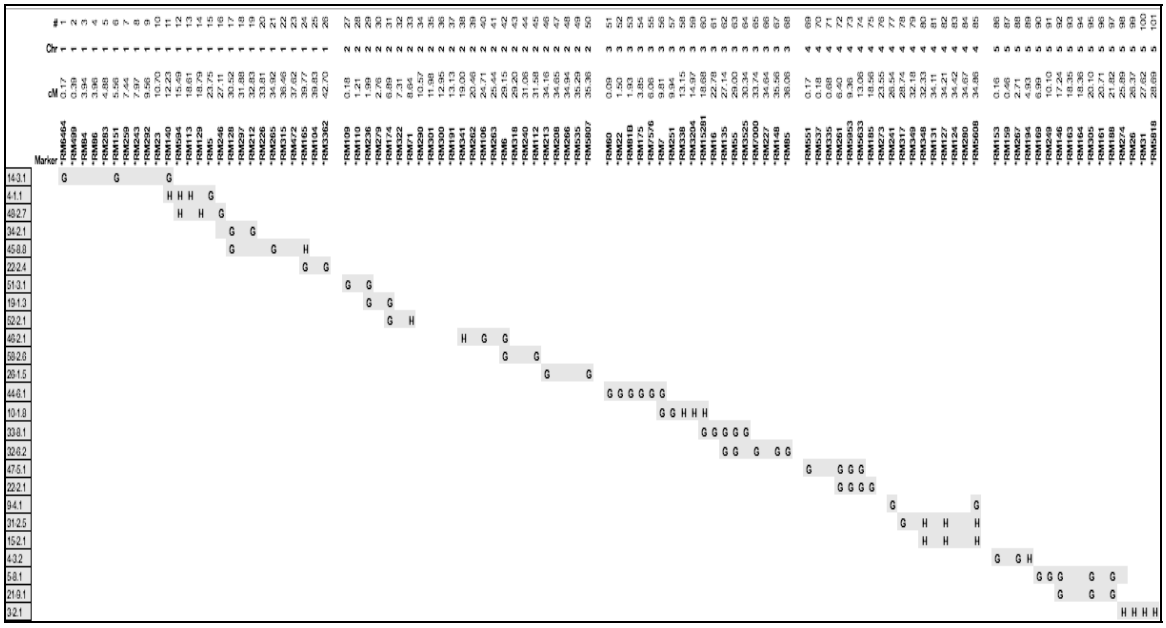

Figure 2. Single plants BC_4F_2 (CSSLs) with target fragment. On the left, CSSLs each one with a target fragment (gray rectangles) in homo or heterozygous status flanked by 2-3 SSRs marker (above). Chromosomes 1 to 5 are showing.

Population 2. Interspecific cross: *O. sativa* ssp. *indica* cv. IR64 x *O. glaberrima* acc. TOG5681

This work started at the IRD in Montpellier, France. From the cross IR64 (*O. sativa* ssp. *indica*) x TOG5681 (*O. glaberrima*) BC_2F_4 and BC_3F_3 populations were developed through marker-assisted backcrossing. These populations were analyzed for there genomic content at CIAT.

A set of 153 SSRs markers was use for the evaluation of the 237 BC_2F_4 and 117 BC_3F_3 sub-populations. With this evaluation the CSSL Finder program searched for candidate lines (Figure 3). As a result, 58 lines were selected using 123 of the 153 SSR markers showing an even distribution across the twelve rice chromosomes. These lines covered the whole *O. glaberrima* genome, except for two small regions of chromosomes 4 and 10.

A preliminary QTL analysis was carried out for various traits scored in the field at CIAT. We could identify several QTLs for tillering (Chr. 3, 4 and 5), panicle size (Chr. 3, 4 and 5) and plant height (Chr. 4 and 9). Each one of these QTLs are being compared to those obtained in the Caiapo x MG12 population and with QTLs for yield and yield components found in the literature and in the databases like Gramene (www.gramene.org).

We used the same methodology based on DNA bulks — the one used for the Caiapo x MG12 population — to choose single plants carrying the target fragment. The 58 lines were collected in bulks of 10 plants. In order to have a single plant per line, 10 plants of each bulk were planted and collected. Each group of plants (equivalent to a line) is being analyzed with the SSR markers used to trace the fragment of interest. Almost 580 plants are being analyzed with 123 SSR markers. This selection is done to obtain a CSSL library with single plants. With the single plant CSSL library a new backcross should be done with the corresponding screening for desired fragments.

When the 58 families of lines were planted in order to open de bulks, some germination problems came and just 259 plants germinated for 50 lines from the 58 selected. The plants without representation were planted in the BC3F2 and crossed again. For the 259 plants 143 SSR markers were evaluated. As a result 26 plants each one corresponding to a line (from the original 58) were selected because each plant has the original fragment. For the other 32, new crosses should be done with the BC3F2 seed.

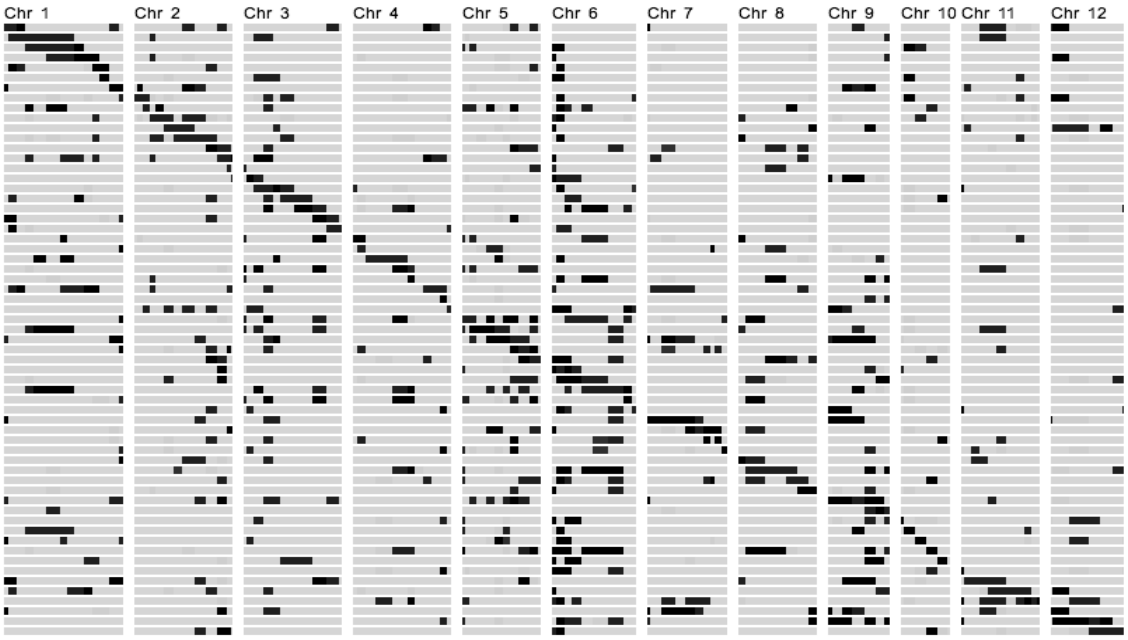

Figure 3. Graphical genotypes of 61 CSSLs lines selected by CSSL Finder. The 12 chromosomes of rice are displayed vertically. They are covered by 115 evenly dispersed SSRs marker. CSSLs lines are displayed horizontally. The black rectangles indicate homozygous introgressions from *O. glaberrima*, the light gray rectangles indicate homozygous fragment of the recurrent genotype IR64.

Conclusions

This work allowed us to advance significantly in the construction and evaluation of CSSLs libraries between the two cultivated species of rice, in both *indica* and *japonica* genetic backgrounds.

Caiapo x MG12 population is finished. IR64 x TOG5681 population is almost finished. Both populations are ready for distribution to partners.

CSSL Finder program, will allow us to easily search the best lines and to compare the gene or QTL locations discovered with those two populations.

Development and phenotyping of CSSL libraries with entire genome coverage represents a useful strategy for QTL discovery and a powerful breeding tool. It also helps in overcoming hybrid sterility barriers between species of rice.

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1C.4 A novel strategy to enhance nitrogen use efficiency in rice by exploiting the genetic diversity for biological nitrification inhibition.

Contributors: D. E. Moreta, X. Scheldeman and M. Ishitani.
Source of Funding: Bioversity International (Vavilov-Frankel Fellowship Program 2009)

Abstract

Human activities have profoundly influenced the global nitrogen (N) cycle and current global nitrogen fertilizer use has reached approximately 100 million ton N/yr in order to maintain agricultural production (IFA, 2005). Nearly 70% of the applied N fertilizer from managed ecosystems is lost through nitrification and associated processes (Raun and Johnson, 1999; Glass, 2003).

Nitrification, a key process in the global nitrogen cycle that generates nitrate through microbial activity, can lead to losses of fertilizer nitrogen by leaching and denitrification. In addition, nitrous oxide, a powerful greenhouse gas that contributes to global warming, is a by-product of denitrification. Certain plants can suppress soil-nitrification by releasing inhibitors in root exudates, a phenomenon termed as biological nitrification inhibition (BNI). Occurrence of this phenomenon has been reported in the tropical forage grass *Brachiaria humidicola* (Rendle) Schweick (Subbarao *et al.,* 2009). It is essential to extend this BNI research to other crops, mainly cereals, to develop cultivars with increased nitrogen use efficiency. This would reduce environmental pollution and can promote eco-resilient agriculture.

In preliminary experiments, we screened CIAT's rice germplasm that represents the rice diversity worldwide and observed genetic variability for BNI activity. The main objective of this BNI research is to identify contrasting rice genotypes and then to dissect genetic components (genes) associated with BNI. Standardized methodologies were used to screen more than 100 rice genotypes for BNI activity. We are also elucidating influence of environmental factors in the plant-soil system on BNI activity.

Materials and Methods

Under hydroponics conditions, we are studying the effect of two sources of nitrogen; i.e., NH_4^+ and NO_3^- as the main factors that control the

synthesis and release of BNI-compounds from rice roots. Contrasting rice genotypes with high (Line 32) and low (Line 19) BNI activity were identified and characterized along with *B. humidicola* 16888 as a positive control. Bioluminescence technique (Subbarao *et al.,* 2009) for detecting BNI activity in the root exudates of rice is being standardized.

In order to determine whether the release of BNI compounds is restricted to those roots that are directly exposed to NH_4^+, a split-root system was set up by dividing the root system equally in two separate containers with nutrient solutions containing either NH_4Cl or KNO_3. BNI activity in the root exudates will be then determined by bioluminescence (Subbarao *et al.,* 2009).
The BNI activity in response to rhizospheric NH_4^+ is being characterized in the soil by both soil incubation and molecular analyses. We are measuring NO_2^- and NO_3^- levels and quantifying ammonia-oxidizing (*amoA*) genes in bacteria and archaea populations. In these experiments we are testing if the release of BNI-compounds is exclusively induced by ammonium-based fertilizers applied to the soil and/or if it is affected by the type of soil (Santa Rosa farm and La Libertad farm from rice fields in "Los Llanos", Colombia).

Results

Exudates were collected from rice roots to determine the main environmental factors (N-form) that regulate synthesis and release of BNI-compounds (Figure 1). These exudates are currently being purified to determine BNI activity through bioluminescence (Subbarao *et al.,* 2009).

Figure 1. Split-root experiment with one half of the root in NH_4Cl and other in KNO_3 to find out whether the release of BNI compounds is restricted to those roots that are directly exposed to NH_4^+.

Molecular analysis was carried out to validate the primers to be used to quantify the target genes of bacteria and archaea and to characterize the BNI phenomenon in the plant soil-system. Figure 2 illustrates the PCR products profile of the genes that will be quantified through Real-Time PCR using soil DNA samples. No PCR products (bands) could be detected by using the primers for amplification of the ammonia-oxidizing bacteria (AOB) *amoA* gene. However, we are currently optimizing the technique to solve this problem. We were able to detect the expected PCR products (bands) for the other genes and primer sets are now ready for use in the subsequent experiments.

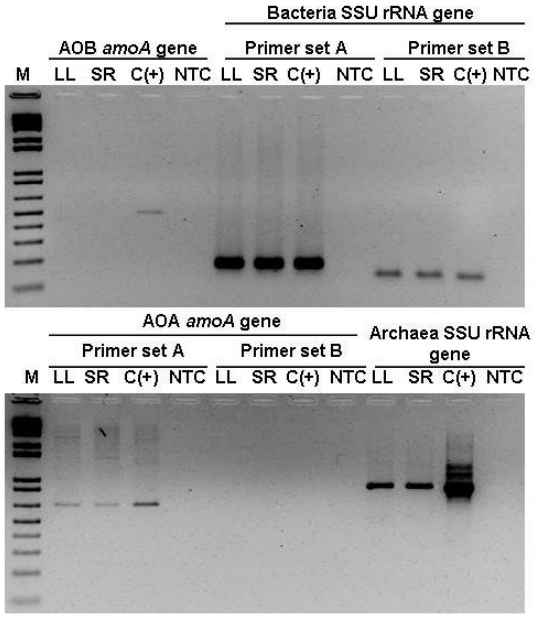

Figure 2. Primers validation through conventional PCR to amplify the *amoA* and SSU rRNA genes in bacteria and archaea populations of soil.

M: 1 Kb plus DNA size marker (Invitrogen, Cat. # 10787-018), LL: soil DNA from "La Libertad" location, SR: soil DNA from "Santa Rosa" location, C(+): soil DNA positive control, NTC: No Template Control. AOB: ammonia-oxidizing bacteria, AOA: ammonia-oxidizing archaea, *amoA*: ammonia-oxidizing gene, SSU rRNA: Small-Subunit ribosomal RNA.

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1C.4.1 Field Phenotyping Platform for evaluation of NUE in Rice Genotypes

Contributors: Rane, J., Manrique, C. E., Martinez, C.P. and Ishitani, M.

Abstract:

The use of nitrogen fertilizer will continue to increase substantially as global population and food requirements grow while global climate change is likely to add new dimensions to the issue. Only 30-50% of the applied nitrogen is used by crop plants and rest can substantially contribute to pollution of lakes, rivers and oceans. Unutilized N fertilizer can significantly contribute to accumulation of atmospheric N_2O, a potential green house gas. Improvement in nitrogen use efficiency (NUE) is one of the options to reduce these effects and to improve eco-efficiency of cultivation of crops such as rice. Hence, CIAT has initiated activities to establish a NUE evaluation field to facilitate genetic improvement for this trait in rice genotypes. After establishing the evaluation platform, we are planning to phenotype chromosome segment substitution lines (CSSL) carrying chromosomal segment of *O. glaberima,* African rice in the background of Caipo, a *O. Sativa* rice cultivar of Latin American. Identification of segments of *O. glaberima* in *O. Sativa* contributing to NUE may subsequently lead to identification of molecular markers and/or genes for marker aided breeding. In addition, the NUE evaluation fields will also be used to phenotype promising rice genotypes with efficient in biological nitrification inhibition and also the transgenic events carrying genes contributing to NUE.

Material and methods

With its focus on eco-efficient agriculture, CIAT has initiated activities to establish field experimental sites for NUE evaluation. To create N omission plots, maize was grown repeatedly and then preliminary experiments were conducted with a set of rice genotypes collected from low input environments of LAC. Our immediate objective is to scale up our capacity to evaluate NUE in rice genotypes under field environments then the evaluation of existing material in general and CSSL lines in particular.

CIAT has a unique set of 64 CSSL lines carrying contiguous chromosomal segments of African rice *Oryza glaberrima* MG12 (acc. IRGC103544) in the genetic background of *Oryza sativa ssp.* tropical japonica (cv. Caiapó). The introgression events in these lines have been genotyped with SSR markers and average size of the substituted

chromosomal segments in the substitution lines is about 10 cM and covers the whole donor genome, except for small regions on chromosome 2 and 4. Proportions of recurrent and donor genome in the substitution lines are 87.59% and 7.64%, respectively. Transgressive segregation has been observed for several traits measured in the population (Gutierrez et al. 2010). These CSSL lines will provide us an opportunity to decipher the role of chromosome segments of *O. glaberima* in regulation of NUE in cultivated rice.
Recent invention by CIAT scientists in collaboration with JIRCAS, Japan has revealed possibilities of Biological Nitrification Inhibition (BNI) as a novel approach to improve NUE in plants (Subbarao *et al.,* 2009et al, 2009). Promising lines of rice identified for high BNI activities in the preliminary experiments at CIAT will be evaluated for NUE in the field. We are also planning to evaluate transgene technology for NUE in rice as CIAT has an access to transgenic events generated in University of Alberta, Canada.

At least 3 overlapping experiments per year with split plot design will be followed. The initial screening will be carried out on the basis of grain yield and then promising lines will be precisely characterized for NUE components and their possible association with chromosomal segments. Performance of rice genotypes in N omission plots will be compared with the same under optimal and suboptimal dozes of N fertilizer. Promising lines with specific segments *O. glaberima* will be further used in backcross to decipher genes/QTLs contributing to NUE.
Once the efficiency of experimental site for evaluation of NUE is enhanced and demonstrated, this will set an example for a network of NUE field sites across LAC to broaden the Field Phenotyping Platform, which is an important component of long-term strategy of CIAT for eco-efficient agriculture. These activities will also enable us to identify promising lines as parental material that can serve as genetic resource for NUE genes. A student will be trained in phenotyping for NUE in rice in this process.

Results

Literature survey reveals that Colombia consumes about 5% of total fertilizers utilized in Latin America (Maene, 2000) while total N apparently consumed by Latin America in 2007 is about 6.3 million tons (Heffer, 2009). An optimistic research target of about 30% reductions in applied nitrogen fertilizer for rice in tropics, without compromising the crop productivity, can substantially reduce cost of rice production, which is one of the main concerns of rice farmers in Colombia.
Our efforts to reduce native N in the soil and to create N omission plots for NUE evaluation by repeatedly growing maize led to substantial

reduction in soil N and visible symptoms of N deficiency in some of the genotypes. Total soil N in the selected field site before growing maize ranged from 9 to 17 g/kg of soil which was reduced to about 1.5 g after two cycles of maize without N. Preliminary investigations with a set of 32 genotypes, which included collections from low input environment, revealed genetic variability in leaf chlorosis, plant biomass and grain yield under no N environment. At present the facility for evaluation is being created in the confined field and the similar procedure will be followed to establish a NUE evaluation field for non transgenic lines.

Figure 1. Preliminary investigation to assess genetic variability in low N adapted rice genotypes in N omission plots at CIAT, Palmira.

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1C.4.2 Identification and expression analysis of citrate transporter genes related to aluminum resistance in common bean

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Source of Funding: BMZ

Abstract

Aluminium (Al) resistance in common bean is known to be due to exudation of citrate from the root after a lag phase. The aims of this study were to identify the genes, which have the important role for exudation of citrate and to compare its functions between an Al-resistant (G35346-3Q (*Phaseolus coccineus)* and an Al-sensitive (VAX-1, *Phaseolus vulgaris*) genotype.
The citrate transporter gene *MATE* is a member of a large gene family that is known to regulate exudation of citrate in other plants. In this experiment, we identified putative MATE genes related to the citrate transporter in common bean through BLAST search against EST sequences for *Phaseolus vulgaris*. Using quantitative real-time PCR (qRT-PCR) the expression patterns of selected genes were assessed in the seedlings of an Al-resistant (G35346-3Q) and an Al-sensitive genotype (VAX 1) treated with Al for 4 hours. Four MATE homologous genes were isolated and the gene expression of three of them was induced by Al-treatment for 4 hours. However, there was no significant difference in the level of relative? expression of these genesbetween an Al-resistant (G35346-3Q) and an Al-sensitive genotype (VAX 1).

Materials and methods

Sequence homology search and phylogenetic analysis

EST sequences of *P.vulgaris* were collected from GeneBank (www.ncbi.nlm.nih.gov). Homologous sequences for citrate transporter gene were searched by tBLASTn against the EST sequences, using with the following gene as query, HvAACT1 gene (Accession number; BAF75822). Amino acid sequences of hit genes in BLAST search were aligned by PRANK program (www.ebi.ac.uk/goldman-srv/prank/prank/) and the phylogenic tree was constructed using the NJ method to find candidate sequences in common bean.

Plant materials, growth condition, harvest of root tips and RNA isolation

Common bean (*Phaseolus vulgaris*) genotypes proved to be Al-sensitive (genotype VAX-1) and Runner bean (*Phaseolus coccineus*) genotypes proved to be Al-tolerant (genotype G35346-3Q) were chosen according to the published results by Rangel *et al.,* 2005. They were also described as P-efficient and P-inefficient cultivars respectively under low P condition (Yan *et al.,* 2004). The seeds were germinated on Canadian peat in pH 5.5 and incubated for 3 days.
Subsequently, they were transferred to the nutrient solution containing 5mM CaCl, 0.5mM KCl, and 8 µM H_3BO_3 (Rangel *et al.,* 2005). During 3-days culture, the pH in the solution was gradually adjusted from pH 5.5 to pH 4.5 with HCl. Afterwards, Al was added into the nutrient solution to a final concentration of 20µM at pH 4.5. It was ensured that the pH (4.5) in the solution after Al addition was kept constant during the culture.
Root tips of 10 mm length and about 300 mg in each Eppendorf tube were collected in liquid nitrogen at 0h (non-Al treated) and 4 hours after the Al treatment. They were stored at -80°C and then RNA isolations were performed with a Trizol-based protocol following the manufacturer's guidelines. Four biological replications were used for each of the treatment and genotype combinations.

Quantitative real-time PCR

Total RNA was isolated from the root tips as described above. The isolated RNA was treated with DNase I and then first strand cDNA was synthesised by using the SuperScript III Reverse Transcriptase (Invitrogen, www.invitrogen.com). Random hexamer primers were used for this purpose. The reaction was stopped by heating at 70°C for 15 min. Quantitative real-time PCR (qRT-PCR) was undertaken using the Stratagene MX-3000p (www.stratagene.com). The SYBR Green detection system was used with Brilliant® II SYBR® Green QPCR Master Mix (Stratagene, www.stratagene.com). The constituents of the qRT-PCR reaction mix were 1x Master Mix, 150 nM each forward and reverse primers, 1 ul of synthesized cDNA template and Ultra pure DNAse/RNAse-free distilled water in a final volume of 20 µl. The qRT-PCR cycling stages had initial denaturation at 95°C (10 min), followed by 40 cycles of 95°C (30 sec), 57-62°C (60 sec) depend on the target genes, 72°C (60 sec), and a final melting curve stage of 82°C (1 sec), 55°C (30 sec) and 95°C (30 sec). The fluorescence signal was recorded during the strand elongation step at 72°C and the melting curve stag. Samples for qRT-PCR were run in three biological replicates and two technical replicates. Relative gene expression was calculated using the comparative $\Delta\Delta C_T$ method according to Livak and Schmittgen (2001). Control plants of the Al-sensitive genotype VAX-1 were used as calibrator and the actin

gene was used as internal standard. The PCR efficiencies of the actin and the target genes were comparable and thus relative gene expression was calculated without efficiency correction.

Results

Several ESTs of *P. vulgaris* which have similarity with known *MATE* genes were gathered and aligned to assess their homology. Based on the alignment result four homologous sequences were identified (MATE-a, MATE-b, MATE-c and MATE-d) and appropriate primers were designed by vectorNTI software (invitrogen). MATE-a, MATE-b, MATE-c and MATE-d have the predicted amino acid sequence similarities of 55%, 61%, 68% and 64%, respectively, with the HvAACT1 gene. Furthermore, MATE-a and MATE-b had the predicted amino acid sequence similarities of 66% and 73%, respectively, with the Arabidopsis *FRD3* (ferric reductase defective 3) gene (Locus: AT3G08040). Likewise, MATE-c and MATE-d has 77% and 65% similarity with the Arabidopsis *MATE* gene (Locus: AT1G51340).

The expression of three MATE genes was investigated using qRT-PCR. The expression levels of MATE-a, MATE-b and MATE-d were greatly enhanced by Al treatment for 4 hours in both bean genotypes (Figure 1). However, there was no significant difference of the expression level between an Al-resistant (G35346-3Q) and an Al-sensitive genotype (VAX 1).

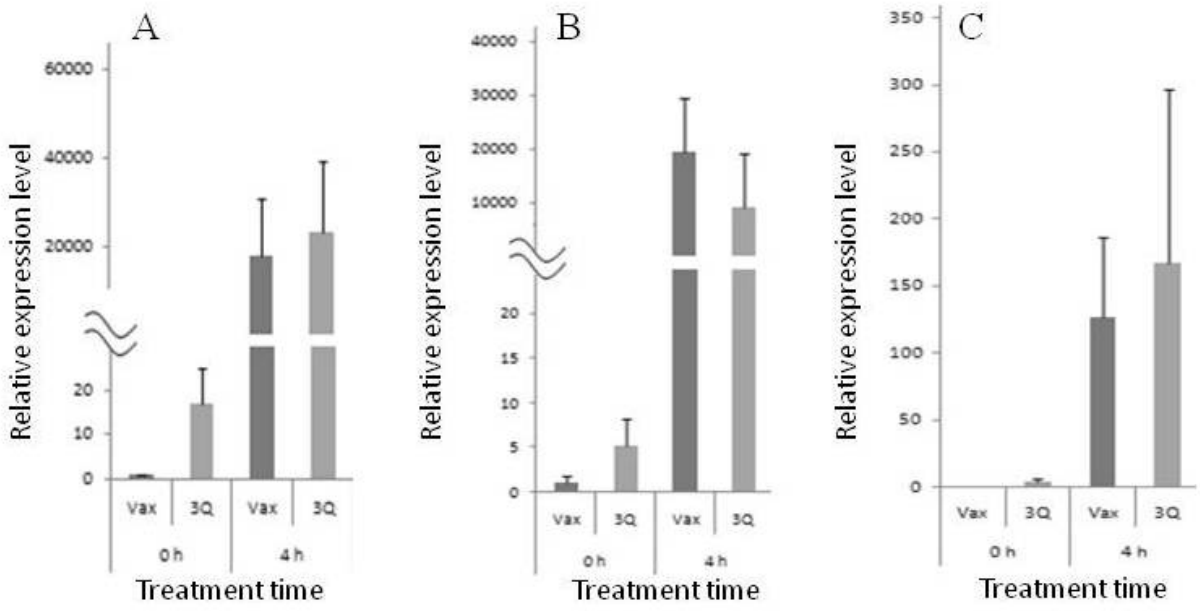

Figure 1. Expression of three *MATE* genes (A; MATE-a, B; MATE-b, C; MATE-d) under extended duration of Al treatment in the bean genotypes G35346-3Q (*Phaseolus coccineus*, Al resistance) and VAX-1 (*Phaseolus vulgaris*, Al-sensitive)

grown in nutrient solution treated without or with 20 µM Al for 4h. Total RNA was extracted from root tips. Quantitative RT-PCR was performed using the actin gene as internal standard and untreated plants of VAX-1 as calibrator. Relative gene expression was calculated from three biological and two technical replicates.

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Output 2: Integrated crop, pest and disease management

2A RICE PATHOLOGY

2A.1 Patotipic characterization of 32 Isolates of *pyricularia grisea* collected in diferent locations in Colombia

G. Prado, G. Aricapa, M.C. Duque, J. Cuasquer, and G. Mosquera

Introduction

After years of hard and continuous research rice blast, caused by *Pyricularia oryzae*, remains as the major biotic constraint affecting rice production worldwide. In the specific case of Colombia, it has been seen that fungal variability is very high and that major resistance genes effect is broken after few years of deployment. As a potential tool to achieve durable resistance, breeders have adopted the gene pyramiding as an effective strategy to delay the overcoming of major resistance genes effect by the fungus. As part of this strategy Oryzica llanos 5, with multiple major R genes; and Fedearroz 50, hosting similar R genes repertoire as O. Llanos 5, have been stayed as highly resistant after 20 and 11 years respectively of their released. On the other hand, Correa et al in 2002 reported the effectiveness of CT 13432-107 line harboring 3 major R genes. Even though some grade of infection has been seen in these materials in Santa Rosa experimental station, it is necessary to keep on track the evolution of pathogen virulence in the field to be able to anticipate the appearance of hyper-virulent strains.

For this reason a systematic evaluation of fungal population is mandatory. Monitoring system of Colombian blast population was interrupted in 2004 and our interest is to upgrade the collection of isolates and evaluate the potential changes in avirulence in the new isolated strains. In these report we are presenting preliminary results obtained with the first group of isolates collected in 2008. As shown in Table 1 these isolates were used along with isolates collected in previous years, for comparison effect. All described isolates were evaluated for

their ability to cause disease in 120 rice materials including differential varieties, isogenic lines, and commercial varieties.

Results and discussion

After processing all samples collected in 2008, we had 14 isolates from Meta and 10 from Tolima (Table 1). This group will be part of a more extensive collection planned to gather isolates until 2010 and assayed for phenotypic characterization. We are trying to have isolates representing not only the diversity inside a disease hot spot like Santa Rosa, but also include sampling other fields under different disease pressure and also with less variable germplasm.

Partial data analysis performed on 5 resent isolates of Tolima and 4 old isolates coming from Meta, shows that Pi-1, Pi-9, and Pi-kh are very effective as single genes (Table 2). Similarly, Pi1-Pi2-Pi33 pyramid is still effective against the tested isolates. On the other hand, analyzed new isolates were able to defeat 8 genes-pyramid present in O. Llanos5 and Fedearroz 50. Though this represents just partial information, is very intriguing to see how this group of isolates can overcome the resistant of O. Llanos5 and Fedearroz 50. However, we need to analyze the complete data to calculate the frequency of this type of isolates to evaluate if they could really jeopardize the effectiveness of multiple R genes. Another missing information is the genetic background of the cultivars from where these isolates were recovered, is possible that they could share the same arrange of R genes as O. Llanos 5 and Fedearroz 50. Other remarkable finding is the fact that not always more stacked genes provide more resistance. As shown in Table 2, three individual genes were able to resist the infection of these 5 isolates, meanwhile multiple genes combinations resulted ineffective. These isolates were not able to overcome the resistance imposed by Pi-kh and Pi-1 genes, which suggest that they have not lost the gene of virulence corresponding to these resistance genes yet, and that broken resistance on Fedearroz 50 and O. Llanos 5, could be due to the lack of Pi-1 R gene. We could also think that these isolates have not lost the virulence gene corresponding to Pi-1 gene because it is probably absent in the majority of materials that are being commercially cultivated. We can also notice that most of deployed resistance genes continue being defeated by the fungus, independently of the year of isolation (complete results in progress).

Conclusions

This preliminary study confirms that the population *P. grisea* can vary from one place to another as it occurred with the isolations obtained from Tolima, which seem more virulent than those obtained from Meta. However this theory needs to be proven by analyzing more isolates from this location. The resistance genes Pi-kh, Pi-1 y Pi-9 appear as good candidates for breeding programs, but we need to challenge more materials hosting these genes using more diverse isolates.

Future activities

We are expanding the pathogen collection, and will continue the phenotypic characterization of representative isolates. About 97 isolates collected in 2009 are ready to start the inoculation assays. Besides Meta and Tolima, this set of isolates includes multiple varieties and also geographical locations like Cesar, Cordoba, and Sucre. For this characterization it is proposed to use new differential varieties developed by IRRI and that are being used to characterize blast population in Asia.

Table 1. *P. grisea* isolates collected in different rice growing areas and used in this study.

Cons.	ISOALTION	ORIGIN VARIETY	COLLECTION SITE	DATE OF COLECTION
1	Improarroz 15-50 (1-1)	Improarroz 15-50	Meta;Puente de Oro	14-Julio-08
2	Fedearroz 369 (1-1)	Fedearroz 3699	Meta;Puente de Oro	14-Julio-08
3	Fedearroz 2000 (1-1)	Fedearroz2000	Meta;Puente de Oro	14-Julio-08
4	Fedearroz 369 (1-1)	Fedearroz 369	Meta,Caños Negros	16-Julio-08
5	Fedearroz 473 (1-1)	Fedearroz 473	Meta,Pompeya	14-Julio-08
6	Fedearroz 275 (1-1)	Fedearroz 275	Meta,Pompeya	15-Julio-08
7	Fedearroz 2000 (1-1)	Fedearroz2000	Meta,Pompeya	15-Julio-08
8	*CT13432-107-(1-1)	CT13432-107	Meta,Pompeya	15-Julio-08
9	Fedearroz 369 (1-1)	Fedearroz 369	Meta,Pompeya	15-Julio-08
10	Orquidea (1-1)	Orquidea	Meta, Caños Negros	16-Julio-08
11	Orquidea 1 (1-1)	Orquidea 1	Meta, Caños Negros	16-Julio-08
12	Orquidea 2 (1-1)	Orquidea 2	Meta, Caños Negros	16-Julio-08
13	Mutante 66 de col XXI (1-1)	Mutante 66 de col XXI	Meta, Puente de Oro	14-Julio-08
14	Fortaleza (4-1)	Fortaleza	Meta, Caños Negros	16-Julio-08
15	I-17 (1-1)	I-17	Tolima, Ibagué	8-Agosto-08
16	III-7 (1-1)	III-7	Tolima, bagué	8-Agosto-08
17	II-23 (1-1)	II-23	Tolima, Ibagué	8-Agosto-08
18	IV-22 (2-1)	IV-22	Tolima, Ibagué	8-Agosto-08
19	40130 (1-1)	40130	Tolima, Ibagué	8-August-08
20	40220 (1-1)	40220	Tolima, Ibagué	8-August-08
21	40389 (1-1)	40389	Tolima, Ibagué	8-August-08
22	40513 (2-1)	40513	Tolima, Ibagué	8-August-08
23	Fedearroz 50 (180-1)	Fedearroz 50	Tolima, Ibagué	8-August-08
24	Improarroz 15-50 (17-1)	Improarroz 15-50	Tolima, Ibagué	8-August-08
25	Fanny 54	Fanny 54	Palmira, Valle	13-Sep-93
26	Selecta 3-20 (1)	Selecta 3-20	Meta, Pompeya	9-Sep-94
27	Oryzica Yacu 9 (19-1)	Oryzica Yacu 9	Meta, Altillanura	June-96
28	Isolinea 6-7-1	Isolinea 6-7-1	Palmira, Valle	6-March-95
29	Isolinea 22-3-1	Isolinea 22-3-1	Meta, Santa Rosa	21-Dic-89
30	Oryzica Caribe 8 (17)	Oryzica Caribe 8	Meta, ICA	4-July-95
31	Metica 1 (33-18)	Metica 1	Meta, Santa Rosa	17-May-93
32	Oryzica Llanos 5 (237-2)	Oryzica Llanos 5	Meta, Granada	27-June-96
33	Cica 9 (151-1)	Cica 9	Meta, Santa Rosa	16-June-94
34	Cica 9 (52-1)	Cica 9	Meta, Santa Rosa	20-July-89
35	Cica 9 (15)	Cica 9	Meta, Santa Rosa	9-June-89
36	CT13432-107-(25-1)	CT13432-107	Meta, Santa Rosa	16-Nov-03
37	75-1-127 (7)	75-1-127	Meta, Santa Rosa	4-August-05
38	Fedearroz 50 (176-1)	Fedearroz 50	Meta, Santa Rosa	4-August-05
39	Fedearroz 50 (175-1)	Fedearroz 50	Meta, Santa Rosa	10-August-05
40	Fedearroz 2000 (18-1)	Fedearroz 2000	Cordoba, La doctrina	16-jan-06
41	Fedearroz 2000 (20-1)	Fedearroz 2000	Meta, Castilla la Nueva	24-Jan-06
42	Fedearroz 809 (4-1)	Fedearroz 809	Tolima, Ibagué	24-May-06
43	Fedearroz 60 (1-1)	Fedearroz 60	Meta, Puente de Oro	14-July-08
44	Fedearroz 369 (181)	Fedearroz 369	Meta, Puente de Oro	14-Julio-08
45	Orquidea (1-2)	Orquidea	Meta, Pompeya	15-Julio-08

Table 2. Compatibility /incompatibility reactions observed in a group of cultivars infected with different isolates

Compatibility between some isolation and the genes known to resistance to *Pyricularia grisea*										
Cultivate	Gen of Resistance	Isolations								
		Tolima					EESR – previous collection			
		19	17	15	16	18	36	37	38	39
Aichi Asahi	Pi-a	+	+	+	+	+	+	+	+	+
BL-1	Pi-b	+	+	+	+	+	+	+	+	+
Toride 1	Pi-zt	+	+	+	+	+	+	-	+	+
K 59	Pi-t	+	+	+	+	-	-	-	-	-
F 129-1	Pi-kp	+	+	+	+	+	+	-	+	+
F 80-1	Pi-k	+	+	+	+	+	+	+	+	+
F 98-7	Pi-km	+	+	+	+	+	+	+	+	+
F 124-1	Pi-ta	+	+	+	+	+	+	+	+	+
F 128-1	Pi-ta2	+	+	+	+	+	+	-	+	+
Rico 1	Pi-ks	+	+	+	+	+	+	+	+	+
Norin 22	Pi-sh	+	+	+	+	+	+	+	+	+
Nato	Pi-i	+	+	+	+	+	+	+	+	+
Ou 244	Pi-z	+	+	+	+	+	+	+	+	+
Tetep	Pi-kh	-	-	-	-	-	+	-		-
CT 13432-68	Pi-1	-	-	-	-	-	+	+	-	-
CT 13432-267	Pi-2	+	+	+	+	+	+	-	+	+
CT 13432-33	Pi-33	+	+	+	+	+	+	+	+	+
CT 13432-107	Pi-1, Pi-2, Pi-33	-	-	-	-	-	+	-	-	-
75-1-127	Pi-9	-	-	-	-	-	-	+	-	-
Fedearroz 50	Pi-2, Pi-33, Pi-z, Pi-z^t , Pi-ta^2, Pi-sh, Pi-k y Pi-b	+	+	+	+	+	-	-	+	+
Oryzica Llanos 5	Pi-2, Pi-33, Pi-z, Pi-z^t , Pi-ta^2, Pi-sh, Pi-k y Pi-b	+	+	+	+	+	-	-	+	+

(+) = Reaction susceptible; (-) = resistant Reaction

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2A.2 Establishment of the first Colombian collection of *Burkholderia glumae*, causal agent of panicle blight of rice and studies of disease distribution

D. Bravo, P. Fory, G. Prado and G. Mosquera

Introduction

The presence of *Burkholderia glumae* has been reported in Colombia since 1989 (Zeigler and Alvarez) as causal agent of grain discoloration of rice. Unfortunately, there is no detailed information about geographical location from where this bacterium was isolated at that time. As part of our activities, we are still giving support to national institutions for the diagnostic of this pathogen in areas where its presence has not been confirmed yet.

Due to the increased disease pressure in the last two years, a serious analysis of germplasm for tolerance identification needs to be conducted. Responses of the host against this pathogen under greenhouse conditions will give light on potential rice cultivars hosting tolerance genes that should be included on breeding programs as a disease control measure. As first step, we established a representative bacterial collection taking in account geographical distribution and host diversity. As a result, we have selected 36 strains isolated from affected rice fields, from 20 locations at 8 states. These isolates are being used on artificial inoculation assays aimed to identify pathogenic and genetic variability of this representative collection. This research will provide information about isolates that need to be included in germplasm evaluation for disease tolerance sources identification.

Objectives

- **A.** To confirm *B. glumae* as causal agent of grain discoloration affecting rice fields and study its geographical distribution
- **B.** To establish a pathogen collection for phenotypic and genetic characterization
- **C.** To implement a reliable screening methodology for the identification of tolerance sources for this disease in a germplasm collection

Results

Field samples processing and bacterial isolation

Rice panicles showing suggestive symptoms and collected nationwide were processed for *B. glumae* molecular detection and colony isolation. As is shown in Table 1 we have processed 157 samples coming from different national locations. Each sample was tested for *B. glumae* and *B. gladioli* using PCR molecular technique.

Origin	**# Sites**	**# Samples**	**Positive for *B . Glumae***	**Positive for *B. galdioli***
	1	2	1	0
Atlántico	1	1	0	0
Bolivar	2	4	3	0
Casanare	1	2	0	0
Cesar	2	3	2	0
CIAT	1	90	33	3
Cordoba	4	12	7	0
Huila	1	1	0	0
Meta	2	14	10	3
Santander	1	2	2	0
Sucre	1	1	1	0
Tolima	12	24	15	0
Valle del Cauca	1	1	1	1
Total	**30**	**157**	**75**	**7**

From all tested samples only those collected at Atlantico and Casanare were not reported as positive for any Burkholderia. Interestingly, two locations, Santa Rosa and CIAT were positive for other Burkholderia specie associated with grain discoloration, *B. gladioli*. This is the first report of the presence of this bacterium in Colombia. Our prediction is that a bacterium has been introduced by contaminated seeds coming from Asia where this bacterium has been widely reported. Another interesting finding was that about 48% of the samples were negative for both tested bacterial species, what means that there are still other causal agents involved in grain discoloration what remain to be identified.

Disease distribution in Colombia

Since 2007, when panicle Blight, caused by *B. glumae*, was reported in Monteria and la Doctrina (Correa et al 2007), our studies showed that the disease is spreading along the main rice growing areas in Colombia. So far, the disease has been confirmed in 8 states what means that pathogen is affecting rice in almost every rice cultivated location.

Bacterial colonies obtained from positive samples were stored at -80 C to establish a bacterial collection. For a first approach, isolates coming from different locations and isolated from different rice varieties were chosen as group of strains representing the Colombian geographical and host diversity (Table 2). These strains are being used in a phenotypic and genetic diversity assay.

Table 1. *B. glumae* isolates chosen for characterization

#	Strain	Origin		Variety
1	3193-No 4	Cordoba	Monteria	Fedearroz 2000
2	3193-No 5	Cordoba	Monteria	Fedearroz 2000
3	3200-12	Cordoba	Monteria	Fedearroz 2000
4	3252-8	Antioquia	Nechi	Unknown
5	3455-1	Bolivar	San Jacinto	Fedearroz733
6	3459-4	Bolivar	Maria la baja	Fedearroz737
7	3460-1	Bolivar	Maria la baja	Fedearro473
8	3880-7	Cesar	Tamalameque	Fedearroz2000
9	3643-2	Cordoba	Lorica	arroz rojo
10	3646-1	Cordoba	Lorica	Fedearroz 2000
11	3650-3	Cordoba	Lorica	Fedearroz 733
12	3656-1	Cordoba	Montería	MixtureFedear
13	2965--2-2	Meta	Sta rosa	Unknown
14	3196-10	Meta	Sta rosa	Oryzica llanos6
15	3847B10(S)	Meta	Sta rosa	Fedearroz 174
16	3848-5 (S)	Meta	Sta rosa	Fedearroz 134
17	3861-2	Meta	Sta rosa	RD- FLAR 18
18	3862-3	Meta	Sta rosa	RD- FLAR 28
19	3864-4	Meta	Sta rosa	RD- FLAR 68
20	3883-1	Meta	Villavicencio	Improarroz1551
21	3866B-10	Nte Santander	Cúcuta	Fedearroz 733
22	3161-2	Sucre	San Marcos	Fedearroz2000
23	3718-2	Tolima	Ambalema	OR 370
24	3722-1	Tolima	Guamo	cimarron barinas
25	3807-2	Tolima	Espinal	Orizya 1
26	3809-2	Tolima	Guamo	Fedearroz60
27	3818-8	Tolima	Venadillo	clearfield 370
28	3825-14	Tolima	Venadillo	Fedearroz60
29	3826-2	Tolima	Venadillo	CF-370
30	3846-10	Tolima	Purificacion	cimarron barinas
31	3853-4	Tolima	Saldaña	cimarron barinas
32	3854-2	Tolima	Prado	cimarron barinas
33	3855-4	Tolima	Saldaña	Coprosem304
34	3856-5	Tolima	Purificacion	cimarron barinas
35	3858-2	Tolima	Purificacion	Coprosem304
36	3859-5	Tolima	Saldaña	cimarron barinas

Inoculation assays and pathogenicity spectrum evaluation

We established a new panicle inoculation method to improve data analysis. Briefly, one fully emerged panicle per plant (Colombia XXI susceptible cultivar) was spray inoculated using 5ml of bacterial suspension (OD_{600}=0,2). Four independent panicles were evaluated in each experiment. Sterile water was used as negative control. Strain aggressiveness was evaluated according to the percentage of affected grains out of total grains in each panicle, after 14 days after inoculation. According to the number of affected grains, differences in aggressiveness over Colombia XXI were observed among tested strains, so far (Figure 1). Control strain, 3200-12, showed higher aggressiveness than other tested strains like 3807-2 and 3718-2. Under experimental conditions used here, strains 3193-8, 3859-5 and 3196-10 were weakly aggressive on Colombia XXI cultivar. These results correlates with pigment production of bacterial strains on artificial culture media, the most pathogenic were pigment positive meanwhile less aggressive ones produce no pigmentation. Same results have been previously reported by Yuan, 2004.

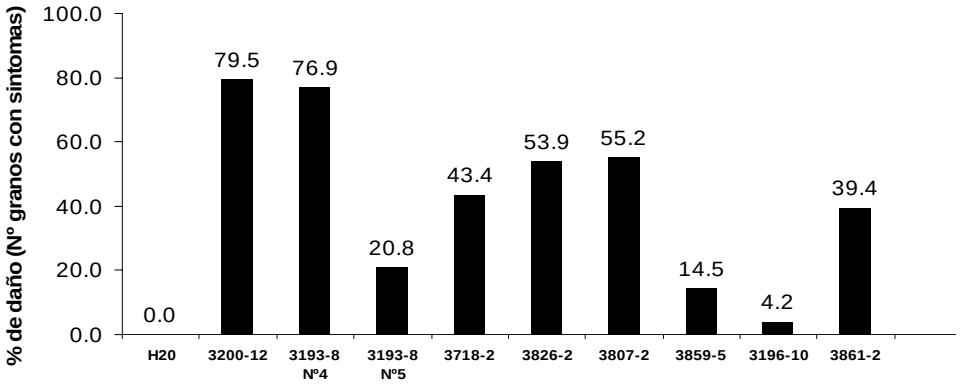
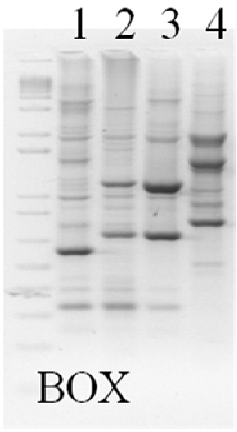


Figure 1. Differences in aggressiveness found in different *B. glumae* strains.

Preliminary results on genetic diversity assays

To assess the genetic variability of *B. glumae* isolates we are in process of standardizing three different P-CR-based techniques, REP, ERIC, and BOX. So far we have identified BOX as a potential tool for discriminating among different isolates using 4 different strains. As is shown in Figure 2, this technique displays well-defined band pattern that can be used to study genetic relationships among different isolates of *B. glumae* found in Colombia.



Bacterial strains

1. 3200-12
2. 3193-8 No 4
3. 3193-8 No 5
4. 3407p8 (22)

Figure 2. Band pattern obtained from different isolates of *B. glumae* using BOX PCR.

Future Activities

- Collection of germplasm will be evaluated using isolates described in Table 2. The purpose of these assays would be the identification of potential sources of disease tolerance.
- Genetic variability will be assessed using REP, ERIC, and BOX. So far only one of these tools has been standardized, once this step is completed for all them the analysis will be run with all isolates describe in Table 2.
- A journal paper is writing process.

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2B RICE VIROLOGY

2B.1 Introgression of QTLs for resistance to Rice Hoja Blanca Virus (RHBV) in elite germplasm through Marker-Assisted Backcrossing

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Introduction

Rice Hoja Blanca virus (RHBV) is a disease that considerably had affected the yield of rice with losses until 80% in several regions of Latin America (Calvert et al. 1996). The evaluation of lines with resistance to RHBV and obtain varieties resistant implies greenhouse and field assays, as well as availability of insect vector colonies (*Tagosodes orizicolus)* with a high transmission percent.

Understanding the genetic model of resistance, we can to develop a strategy of selection more efficient and more effective in terms of save money and time-working. We propose to use Molecular-Assisted Backcrossing (MAB), in order to fasten facilitate the identification and selection of resistant candidates, and to reduce the costs of the breeding process. In a previous study carried out at CIAT in Dr. Calvert's team, three QTLs that contribute to RHBV resistance were identified, using two F2/F3 populations derived from the crosses between the highly susceptible line WC366 and the varieties Fedearroz 2000 (FD2000) and Fedearroz 50 (FD50) that show different levels of resistance to RHBV and *T. orizicolus* (Calvert et al. 2006).

On this sense, the aim of this work is (i) to better localize and estimate the QTLs resistance parameters in both populations, (ii) introgress the RHBV resistance QTLs into two susceptible elite materials through MAB and (iii) fine map the most important QTLs in order to optimize future marker-based selection strategies.

We hope to obtain an acceptable level of resistance/tolerance together with a good recovery of the recurrent genetic background in 3 generations of backcrossing, using the MAB strategy. This would represent a drastic reduction of the costs associated to the selection for this trait in rice breeding.

Materials and methods

Two elite lines: Fedearroz 174 and CT18685 were selected from 12 susceptible materials to RHBV but with good yield traits. All materials belong to FLAR and CIAT breeding programs. The selection was carried out using 428 SSRs molecular markers well distributed in the whole rice genome. After that, crosses of each one of this elite lines were done with Fedearroz 2000 and Fedearroz 50, which have the resistant alleles for the QTLs identified. The F1 seeds were sown at the greenhouse and fifteen days after, the leaf tissue was collected for DNA isolation and posterior molecular evaluation by PCR to identified the heterozygous plants that will be used in the first backcross toward the corresponding elite line and then obtaining four BC1F1 populations. Homozygous lines were discarded.

The molecular evaluation of 652 DNA samples from the BC1F1 populations was done of the following way: 366 samples from crosses CT22550: Fedearroz 174/Fd2000//Fedearroz 174 with 14 SSRs (7 located on chromosome 4, and 7 located on chromosome 5, see Table 1); and 286 samples from crosses CT22553: CT18685/Fedearroz 2000//CT18685, with 9 SSRs (4 SSRs located on chromosome 4, and 5 SSRs located on chromosome 5, see Table 1). The DNA bands were visualized in agarose (4%) and acrylamide (6%) gels. Once finished the genotyping, the individuals with genotype "H" (Heterozygous) with the resistant allele (Fedearroz 2000) were selected for a new backcross toward the corresponding elite line to obtain BC2F1 populations.

The same process above was carried out for the donor Fedearroz 50. After that, will be do the corresponding backcrosses toward each one of the elite lines until obtain BC4F1 of the four established crosses. Then, will be evaluated for search resistant alleles into the QTLs identified and after, the lines with the favorable alleles will be selfed. BC4F2 populations with homozygous genotype for the resistant allele to RHBV will be selected to obtain a BC4F3 populations, then will be screened in the field. By means of self crossing, the lines more resistant will be fixed and multiplied with the purpose to be incorporated into the breeding programs.

Results and discussion

Polymorphism survey

428 SSRs markers (108 additional markers to the 320 markers before evaluated) well distributed in the 12 chromosomes of rice genome were evaluated in 12 varieties: Fedearroz 2000, Fedearroz 50, Fedearroz 60 selection 132, Fedearroz 60 selection 154, Fedearroz 174, Fedearroz 369 selection 23, Fedearroz 369 selection 67, Centauro, CT18685-10-3-1-2-2-M, CT18244-7-5-2-3-1-5-M, CT18245-11-6-2-2-2-2-M and WC366. The percentages of polymorphism in the elite varieties selected for the backcrosses proposed were: CT18685-10-3-1-2-2-M (31,2%) and Fedearroz 174 (25,8 %). These molecular data also revealed that the selected progenitors are closely related, which is traduced by low levels of polymorphism compared to the reported data for *indica* x *japonica* crosses (45.82%) (Orjuela, 2006). This result is congruent with the pedigree analysis, which shows that these progenitors have many common ancestors.

Validation of F1 hybrids

Using two polymorphic markers (RM16353/RM3708 and RM6770) depending of the crosses, were evaluated 242 F1 plants obtained from the cross (Fedearroz 174 and CT18685, with the donor parent Fedearroz 2000) and 243 plants F1 obtained from the cross (Fedearroz 174 and CT18685, with the donor parent Fedearroz 50). As results, 100 F1 heterozygous plants from the cross with Fedearroz 2000 and 143 heterozygous plants from the cross with Fedearroz 50 were identified. These results shows that 50% of the selfing was successful compared with the percentage of individuals selfed.

In the BC1F1 population (CT22550 crosses) were selected 140 heterozygous individuals with allele form Fedearroz 2000, in the loci of interest. On the same way, 98 heterozygous individuals for Fedearroz 50 were identified. These results will allow us to do the backcrosses with these plants toward the corresponding elite line for to obtain BC2F1 populations in each cross.

Table 1. SSRs evaluated in BC1F1 crosses (CT22550 and CT22553)

Chromosome 4	SSR	Position (Mb)
	RM16335	1.69
	RM518	2.02
	RM16353	2.04
	RM16368	2.44
	RM6770	2.81
	RM16413	4.03
	RM16416	4.17
	RM627	4.43
	RM1305	5.63
Chromosome 5	**SSR**	**Position (pb)**
	RM5374	1.23
	RM413	2.19
	RM592	2.77
	RM13	2.89
	RM5874	3.51
	RM17959	3.82
	RM18054	5.85
	RM7118	6.06
	RM4691	7.00
	RM5140	13.46

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