Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

Annual Report 2006

Project IP-4:
Improved Rice Germplasm for Latin America and the Caribbean

For Internal Circulation and Discussion Only

April 2007

OUTPUT 1. ENHANCING GENE POOLS (1208 kb)

OUTPUT 3. ENHANCING REGIONAL RICE RESEARCH CAPACITIES AND PRIORITIZING WITH EMPHASIS ON THE SMALL FARMERS (120 kb)

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EXECUTIVE SUMARY

PROJECT IP-4: IMPROVED RICE FOR LATIN AMERICA AND THE CARIBBEAN

PROJECT MANAGER: FERNANDO CORREA

Project Description

Goal

To generate food security and employment associated with rice production with emphasis on improving the options for the small farmers.

Objective

To produce robust high yielding rice varieties requiring lower inputs, we will provide well-characterized progenitors and advanced materials with an ample genetic base as well as information and training.

Target Ecoregion

Low and Mid Altitude Regions of Latin America and the Caribbean.

Principal Collaborators: France CIRAD, IRD & Genoplante, FLAR, IRRI, WARDA, Japan JIRCAS, Korea RDA, Brazil EMBRAPA, Colombia FEDEARROZ & CORPOICA, Peru INIA, Venezuela INIA & DANAC, Cuba IIA, Nicaragua INTA, Bolivia CIAT Santa Cruz, Chile INIA, Uruguay INIA, Argentina U. Corrientes & U. Tucumán, China, US Universities: KSU, Cornell, Purdue, LSU, U. Arkansas, Texas A&M and Yale.

CGIAR system linkages: Enhancement and Breeding (50%); Protecting the Environment (20%); Saving Biodiversity (15%); Transfer of Technologies (10%); Crop Systems (5%). Linked to IRRI and WARDA.

CIAT project linkages: Germplasm conservation SB-1, genomics SB-2, participatory research SW-3 for upland in hillsides PE-3 and cropping systems SW-2 for the savannahs.

1. IP-4 Project Log Frame (2006)

PROJECT: **IMPROVED RICE FOR LATIN AMERICA AND THE CARIBBEAN**
PROJECT MANAGER: **FERNANDO CORREA**

	Outputs	Intended users	Outcome	Impact
Output 1: Enhanced gene pools				
Output Targets 2006 .	Enhanced gene pools and advanced lines with disease resistance to rice blast and rice hoja blanca complex that are high yielding with good grain quality for both irrigated and upland rice	Rice researchers, FLAR, and breeding programs throughout the region	Rice breeding methods and strategies for development, evaluation and selection of promising rice lines that result in varieties released by the rice sectors resulting in higher rice yields.	A robust rice sector will generate employment and maintain low rice prices for the consumers.

	Outputs	Intended users	Outcome	Impact
Output 2: Integrated crop, pest and disease Management				
Output Targets 2006 .	Characterization of the diversity of rice pathogens, resistance genes, and transfer of technology to partners	Rice pathologists and breeders	Better practices in place to reduce losses caused by pathogens as well as decreased use of agrichemicals.	The ecosystem will be less contaminated and the workers will be healthier.
	Integrated Pest, Disease, and Crop Management strategies adapted for at least 5 countries .	Rice scientists, extension agents and farmers.	Prerequisite for developing information based system as it confirms efficacy in local production systems	A more competitive rice sector with lower negative impact on the environment.

	Outputs	Intended users	Outcome	Impact
Output 3: Intensification and diversification of rice systems for small farmers				
Output Targets 2006 .	Varieties including specialized high value rice (ethnic) and management practices (organic) developed for small rice farmers using participatory methods in two countries in Central America.	Small holders who produce rice and extension agents.	Better-organized small farmers. Increased yields and options that allow crop diversification including high value crops.	Improved livelihoods of small farmers. A dynamic and robust rice sector.

2. Achievements of 2006 Output Targets

Output 1

Output targets for the output on Enhanced Gene Pools were achieved on 100%.
The CIAT-ION nursery with 130 rice lines was distributed to different partners in Colombia, Nicaragua, Costa Rica, and Bolivia. Several sets of advanced rice lines were distributed to partners in the USA (Cornell University, Louisiana State University, U. of Arkansas, and RiceTec), WARDA, and University of South Africa. Rice lines from the biofortification project were sent to: Brazil (EMBRAPA-CNPAF, and University of Rio Grande Do Sul), Colombia, Cuba, Nicaragua, Republica Dominicana, Bolivia, Panamá, and WARDA.
In Bolivia, the first upland/aerobic commercial rice variety selected from the enhanced composite population PCT-4 (recurrent selection) was officially released in 2006 as the variety "ESPERANZA". The variety is adapted both to manual upland and mechanized aerobic rice ecosystems. In Chile, the first commercial variety, "RQuila 28", adapted to the temperate irrigated rice ecosystem, coming from the enhancement and selection of the population PQUI-1 was selected in 2006 and proposed for official release in early 2007. A total of 983 upland rice lines were multiplied and distributed to 25 Institutions from 12 countries.
Four additional commercial rice varieties were developed within the CIAT-FLAR consortium and released in different Latin American countries: ANAR 2006 (Nicaragua), IDIAP 54-05 and IDIAP 145-05 (Panamá) and CENTAURO (Venezuela).

Output 2

The Output Targets for the Output 2: Integrated crop, pest and disease management, were achieved on more than 90%.
This output considers the study of several rice pathogens including the rice blast pathogen (*Pyricularia grisea*), sheath blight (*Rhizoctonia solani*) and the new rice disease detected in Latin America, bacterial panicle blight (*Burkholderia glumae*). For each pathogen, significant advances were made in studying the pathogen diversity and in identifying resistance sources. For the rice blast pathogen, evolution studies allowed to identify potential mutants able to break

resistance gene combinations being used in the breeding program. The advanced detection of these mutants allows the identification of resistance genes that need to be incorporated in the new varieties to move ahead of future changes of the pathogen in natural populations (annual reports 2005-2006). Molecular markers associated to blast resistance genes were identified and implemented for marker assisted selection allowing the introgression of three blast genes into improved rice germplasm (see references). Lack of funding has not allowed the evaluation of large populations for the identification of rice lines carrying the three genes. Advanced lines with durable field blast resistance up to the eight generation were identified and are being multiplied to be given to our FLAR partner for their distribution to partners in the region. These lines can give origin to new varieties or be used as sources of stable resistance. Reliable screening techniques for the identification of resistance sources to the sheath blight pathogen under controlled greenhouse conditions were developed. Commercial rice varieties from Latina America were screened for resistance and resistance sources were identified. Rice populations (recombinant inbreed lines and double haploids) were screened and the information will be used to identify quantitative trait loci associated with resistance as part of a USA study funded by USDA. Characterization of the disease complex mite-fungus-bacteria affecting rice production in Central America and the Caribbean was initiated. A bacterial pathogen (*Burkholderia glumae*) was identified as the major cause of disease symptoms and responsible for economic losses (reference). The role of the mite seems to be more a way for spreading the bacterium. A reliable screening method to identify resistance sources to the bacterium is being developed under controlled conditions and preliminary results suggest that resistance genes to this pathogen are available. We initiated the screening of our germplasm bank as well as our elite and progenitors rice lines. All actual commercial cultivars planted were susceptible. Results will be confirmed under field conditions. Seed treatments using hot water and antibiotics suggest positive control of this pathogen in infected seeds, which is the main source for pathogen infection. Adoption of crop management practices by rice farmers in the region (lower seeding rates, hot water seed treatment, planting date, and adequate fertilization) to favor a healthier crop is also helping to reduce or minimize the presence of this disease complex. This research is being conducted as part of a FONTAGRO project and implementation and adoption practices are being conducted together with FLAR scientists working with different rice farmers in Central America. Activities within this output will continue during 2007.

Output 3

Selection of high performing well-praised rice varieties for different upland cropping systems and agro-ecological areas of Central America through participatory methods were achieved on more than 80%.
Two varieties in process of registration and three to be proposed for registration and release in 2007 in Nicaragua: two for low inputs upland cropping systems of the north-eastregion (Serviteca); one for the mechanized upland cropping systems of the north-pacific region (INTA); two very early upland lines for dry areas and/or new cropping systems (INTA). Farmers, NGO and extension technicians and NARS scientists trained on PCI approaches methods. Germplasm exchange and training course on PCI approaches with Guatemala, El Salvador and Costa Rica (germplasm in intermediate on-farm trials in Guatemala). No significant breeding work achieved on high value rice (just starting): 20%. Outputs on management practices: 20% (because of budget limitations, climatic and partnership constraints.

Another line derived from the cross Caiapo x *O. glaberrima* (African cultivated species) was identified trough participatory breeding by small farmers in Nicaragua as a variety and seed is being multiplied for commercial planting. Small farmers in Bolivia have identified a traditional variety called "azucena" as a potential variety for special markets.

3. Research Highlights 2006

- Understanding of meiotic process of F1 hybrids between *O. sativa* x *O. latifolia* including abnormalities in spindle formation, chromosome segregation and cytokinesis leading to polyads formation, which give rise to unviable pollen and sterility, and chromosome elimination. Evaluation of about 13,000 breeding lines and identification of promising lines for CIAT-ION nurseries. Promising interespecific breeding lines with high yield potential, tolerance to main diseases and good grain quality were identified and included in the CIAT-ION nursery made available to NARs in 2006. Out of the 194 lines, 65 were from interspecific crosses. Two varieties for low inputs upland cropping systems in process to be registered by a private partner of the project (launching at mid-2007). Following convincing validation trials carried out in 2005 and 2006, future launching by the Nicaraguan agriculture research institute (INTA) of a very early line for upland areas with drought constraints and a line for favorable upland mechanized cropping systems. Follow-up of the participatory plant breeding schemes with associated farmers and NGOs in two areas of Nicaragua. Creation of a national Participatory Crop Improvement (PCI) network in Nicaragua.
- The blast resistance in the cultivar Oryzica Llanos 5 (durable blast resistance for more than 15 years) was found to have very complex inheritance. The durable broad-spectrum resistance in the rice cultivar Oryzica Llanos 5 is associated with multiple genes of major and minor effects that induce resistance to different blast isolates. Twenty-one QTL present in nine chromosomes were detected and associated with resistant traits in Oryzica Llanos 5. Most but not all of the QTL occurred in the same genomic regions of other genes that had been reported in the literature. None of the QTLs was effective against all blast isolates and all were isolate specific. One QTL mapped to a region on chromosome 9 where no blast resistance genes had yet been mapped. Another QTL near the bottom of rice chromosome 11 was found to be significantly associated with partial resistance. Advanced breeding lines (generation F_7-F_{11}) with transgenic-resistance to RHBV combining high yield potential, good grain quality, tolerance to *Rhizoctonia* and characterized profile for strain resistance to pyricularia were developed. These plants are ready to be evaluated by peers and to decide potential process for deployment to farmers.
- Chloroplast and nuclear sequences selected and tested for genome and species characterization of *Oryza* allowing characterization of species composition and direction of gene flow in samples collected in farmers' fields in Colombia and Venezuela. High through-output methodology PCR-real time based for analysis of gene flow in rice at landscape level optimized. Set up international collaboration on experimental design and data collection for gene flow at landscape level that may allow adaptation of expert model systems for tropical conditions, applicable tool for biosafety decision process by competent authorities. Near-completion of a clean lab for handling and preparing rice samples for iron and zinc analysis, establishment of a methodology for running iron and zinc analysis in rice at CIAT, and establishment of base lines for iron and zinc.

Validation of SNP markers to be used for the screening of rice genotypes having contrasting levels of iron content in the polished grains. Identification of rice cultivars having 2-3 times more iron than commercial milled rice bought by consumers

4. Major outcome of the Rice Project in 2006: Commercial Rice Varieties Released

Nearly 13,000 breeding lines in different stages of development were evaluated in Santa Rosa and Palmira; percentage of selected material varied depending on the type of cross combinations. About 1224 advanced lines were selected by participants from diverse NARs in a Breeder's Workshop held in Santa Rosa. Wide segregation for desirable traits including grain quality and a good number of plant selections were made for further testing in crosses involving different wild species of rice. Elite lines derived from crosses with *O. latifolia, O. glaberrima*, *O. barthii*, and *O. rufipogon* showed good field performance and high yield potential in replicated trials run by several partners including Fedearroz, our main local partner. As a result and main outcome of the Rice Project activities developed in collaboration with our Latin American Partners, the following rice varieties were released in the region during 2006:

In Bolivia, the first upland/aerobic commercial variety selected from the enhanced composite population PCT-4 was officially released in 2006 as **ESPERANZA**. The variety is adapted both to manual upland and mechanized aerobic rice ecosystems. This variety has the Pedigree CT8240-1-5-2P-M-1P/CT8008-3-12-3P-1X//CT9509-17-3-1-1-M-1-3P-M

In Chile, the first commercial variety, **RQuila 28**, adapted to the temperate irrigated rice ecosystem, coming from the enhancement and selection of the population PQUI-1 was proposed for official release in early 2007

In Salvador, the commercial variety **CENTA A-8** was released by ANAR. This variety came from the cross CT 11519/CT 11492 and Pedigree CT 122249-3-4-3-3P-1P

In Nicaragua, the commercial variety **ANAR 2006** was released by ANAR. Pedigree CT8240-1-5-2P-M-1P/CT8008-3-12-3P-1X//CT9509-17-3-1-1-M-1-3P-M

In Panamá, the commercial variety **IDIAP 54-05** was released by IDIAP. This variety originated form the cross CT9682-2-M-14-1-M-1-3P-M-1/CT10825-1-2-1-3-M//CT8222-7-6-2P-1X. The variety **IDIAP 145-05** was also released in the country. This variety originated form the cross CT8008-16-31-3P-M//CT9682-2-M-14-1-M-1-3P-M-1/CT11008-12-3-1M-4P-4P

In Venezuela, the commercial variety **CENTAURO** was released by FUNDARROZ, INIA and FLAR. This variety originated from the cross ECIA38-2-4-2-5-6/CT822-7-6-2P-1X/FB0007-3-1-6-1-M and Pedigree FL00984-8P11-2P-2P-M-M

5. Publication List

Refereed Journal

1. Flórez-Ramos C.P., Z. Lentini*, M.E. Buitrago, and J. Cock. 2006. Somatic Embryogenesis and Plantlet Regeneration of Mango (*Mangifera indica* L.). *Acta Horticulturae* (In Press)
2. Ruiz J.J., Z. Lentini*, V. Segovia, M. Buitrago, C. Flórez, and J. Cock. 2006. *In vitro* Propagation and Regeneration of *Solanum quitoense* (Lulo) Plants and their Use as Elite Clones by Resource Farmers. Somatic Embryogenesis and Plantlet. *Acta Horticulturae* (In Press).
3. Ceballos*, H., M. Fregene, Z. Lentini, T. Sánchez, Y.I. Puentes, J.C. Pérez, A. Rosero and A.P. Tofiño. 2006. Development and Identification of High-Value Cassava Clones. Acta Horticulturae 703:63-70.
4. Fuentes, J.L., Correa-Victoria, F.J., Escobar, F., Prado, G., Aricapa, G., Duque, M.C., and Tohme, J. 2006. Microsatellite markers linked to the blast resistance gene *Pi-1* in rice for use in marker assisted selection. Euphytica (accepted)
5. Jia, Y., Correa-Victoria, F.J., McClung, A., Zhu, L., Wamishe, Y., Xie, J., Marchetti, M., Pinson, S., Rutger, N., and Correll. J. 2006. Rapid determination of rice cultivar responses to the sheath blight pathogen *Rhizoctonia solani* using a micro-chamber screening method. Plant Disease (accepted)
6. Lopez-Gerena, J., Correa-Victoria, F.J., Prado, G., Tohme, J., Zeigler, R., and Hulbert, S. 2006. Mapping QTL affecting partial resistance and identification of new blast resistance genes in rice (*Oryza sativa*). Theor. Appli. Genet. (submitted)
7. Trouche, G.; Narváez-Rojas, L.; Chow-Wong, Z.; Corrales-Blandón, J. 2006. Fitomejoramiento participativo del arroz de secano en Nicaragua: metodologías, resultados y lecciones aprendidas. Agronomía Mesoamericana (CR) 17(3): 307-322.
8. Trouche, G.; Hocdé, H.; Aguirre-Acuña, S.; Martínez-Sanchez, F.; Gutiérrez-Palacios, N. 2006. Dinámicas campesinas y fitomejoramiento participativo: el caso de los sorgos blancos (Sorghum bicolor, L. Moench) en la region Norte de Nicaragua. Agronomía Mesoamericana (CR) 17(3): 407-425.

Book Chapters

1. Calvert L.A. and Z. Lentini. 2007. Rice Hoja Blanca Virus. *In:* Characterization, Diagnosis and Management of Plant Viruses. Vol. 4: Grain Crops and Ornamentals. Govind P. Rao, Claude Bragard and Benedicte S.M. Lebas (Editors). Stadium Press ILLC, Texas, USA. ISBN 1-933699-34-5. p: 85-99.
2. Marc Châtel, Yolima Ospina and Gilles Trouche. 2006. Impact of the rice synthetic population breeding project for Latin America and the Caribbean. In: France and the CGIAR. Delivering Scientific Results for Agricultural Development. Chapter 1: Scientific Partnerships. Producing more and better food. Publication coordinated by Daniel Rocchi, Liaison Officer at the CGIAR Secretariat in Washington.Washington, U.S.A. CGIAR, p.44-47.

Other publications

1. Correa-Victoria, F.J. 2006. Improving Blast Resistance for Upland Rice in Colombia: a Challenging Task. 31st Rice Technical Working Group Meeting. The Woodlands, Texas, February 26-March 1, 2006.
2. Correa-Victoria, F.J. 2006. Identification of molecular markers for pyramiding rice blast resistance genes. Second Research Coordination Meeting. Nanjing, China, April 10-14, 2006.
3. Correa-Victoria, F.J. 2006. Avances en la investigación en enfermedades del arroz: *Pyricularia grisea.* II Congreso Brasilero de la Cadena Productiva del Arroz. VIII Reunión Nacional de Pesquisa de Arroz. EMBRAPA, Brasilia 26-28 de Abril, 2006. (Invited speaker).
4. Correa-Victoria, F.J. 2006. Situación del complejo acaro-hongo-bacteria en el arroz. Segundo Congreso Arrocero. San José, Costa Rica, Junio 29-30, 2006. (Invited speaker).
5. Correa-Victoria, F.J. 2006. Using rice differentials with known blast resistance genes for pathogen characterization and improving rice cultivars in Latin America. Rice Blast Workshop IRRI-JIRCAS. IRRI, Los Baños, Philippines, August 29-30, 2006.

Workshop and Conferences

- Lentini, Z*. 2006. Biotecnología y Riesgos Fitosanitarios *Invited Key-note lecture*. 2do Curso Internacional sobre Riesgos Fitosanitarios para la Agricultura Colombiana. Cali, Colombia December 2006. Funded by MADR Colombia.
- Coordination and Execution of Course: Capacitación para el Fortalecimiento de la capacidad institucional del Ministerio de Ambiente, Vivienda y Desarrollo Territorial y Autoridades Ambientales Regionales en materia de Biotecnología y Bioseguridad Ambiental de OGM con énfasis en Plantas Transgénicas. Abril 26, 27, y 28 de 2006. Funded by Colombia GEF/WB Biosafety Project.
- Trouche, G.; Hocdé, H.; Aguirre S. 2006. Sélection participative des sorghos au Nicaragua : approche et dispositifs. *In:* Lançon J., Weltzien E., Floquet A. Eds. Gestion du partenariat dans les projets de sélection participative. Actes de l'atelier Recherche 14-18 Mars 2005, Cotonou, Benin: 159-173.
- Lancon, J.; Bertrand, B.; Clément-Demange, A.; Hocdé, H.; Nouy, B.; Trouche, G. 2006. What determines the stakeholders'participation in plant breeding programs? Cases studies in the South. *In:* Lançon J., Weltzien E., Floquet A. Eds. Gestion du partenariat dans les projets de sélection participative. Actes de l'atelier Recherche 14-18 Mars 2005, Cotonou, Benin: 179-193.
- Taller de selección de material genético de arroz de secano y de riego. Villavicencio-Colombia. August 15-18, 2006. 61 participants from 12 countries (Bolivia; Colombia; Costa Rica; Cuba; Dominican Republic; France; Guatemala; Madagascar; Nicaragua; Panama; Peru and Venezuela)
- Correa-Victoria, F.J. 2006. Improving Blast Resistance for Upland Rice in Colombia: a Challenging Task. 31st Rice Technical Working Group Meeting. The Woodlands, Texas, February 26-March 1, 2006.

- Correa-Victoria, F.J. 2006. Identification of molecular markers for pyramiding rice blast resistance genes. Second Research Coordination Meeting. Nanjing, China, April 10-14, 2006.
- Correa-Victoria, F.J. 2006. Avances en la investigación en enfermedades del arroz: *Pyricularia grisea.* II Congreso Brasilero de la Cadena Productiva del Arroz. VIII Reunión Nacional de Pesquisa de Arroz. EMBRAPA, Brasilia 26-28 de Abril, 2006. (Invited speaker).
- Correa-Victoria, F.J. 2006. Situación del complejo acaro-hongo-bacteria en el arroz. Segundo Congreso Arrocero. San José, Costa Rica, Junio 29-30, 2006. (Invited speaker).
- Correa-Victoria, F.J. 2006. Using rice differentials with known blast resistance genes for pathogen characterization and improving rice cultivars in Latin America. Rice Blast Workshop IRRI-JIRCAS. IRRI, Los Baños, Philippines, August 29-30, 2006.

6. List of proposals funded in 2006, dollar value of contract and donor

- Gene Flow Analysis for Environmental safety in the Tropics. CIAT – University of Costa Rica – Hannover University and BBA, Germany. Donor: EURO 450,000 (2005-2007).
- Development and evaluation of drought-tolerant rice transgenic plants. GCP SB3 USD 70,000 (2005-2006)
- The Latin America: Multi-country capacity-building for compliance with the Cartagena Protocol on biosafety. PDF-B: Development of PAD (Project Appraisal Document). Donor: GEF-World Bank. USD 260,000 (Nov 2005-April 2007)
- Latin America: Multi-country capacity-building for compliance with the Cartagena Protocol on biosafety (Brazil, Colombia, Costa Rica, Peru). USD 5 million. Donor: GEF-World Bank
- Impacto ambiental de la adopción del arroz resistente a las imidazolinoas en sistemas productivos contrastantes de América Latina (AL). INIA-UCV-CIAT. USD 420,000. Donor: Fontagro.
- Capacitación en fitomejoramiento genético e intercambio de germoplasma para utilizar los recursos genéticos del arroz en América Latina y el Caribe TCP/RLA/3102 (A) USD 340,000.00. FAO
- High iron and zinc rice lines. AgroSalud. CIDA-Cananda US$230,000.
- Interspecific bridges to get full access to genetic diversity found in O. glaberrima: GCP , US 300,000 total; about US$ 80,000 for CIAT. To get started in 2007.
- Cenicafe. Technical assistance to the Coffee Genome funded by MADR: US30,000.
- Identification and expression analysis of genes important for iron translocation to the rice grain , hp+ us 15,000.
- Reducción del uso y desarrollo de resistencia a plaguicidas en el cultivo del arroz y fríjol en Colombia, Venezuela y Ecuador. FONTAGRO. US$ 224,000 (2006-2008)
- Manejo del complejo acaro-hongo-bacteria, nuevo reto para arroceros centroamericanos. FONTAGRO. US$ 360,000 (2006-2008)
- Identify and use candidate genes and other molecular markers linked to quantitative trait loci which control milling quality and resistance to sheath blight disease. USDA National Research Initiative Competitive Grants Program. CIAT US$ 47,000 (2005-2007).

- Phenotype evaluation of mutant collection for sheath blight resistance within the commissioned research project PI: Dr. Mathias Lorieux/Dr. I. Manabu (2006-2007). US$ 8,000
- Rice breeding for disease resistance and grain quality in Cuba. IAEA. US$ 30,000 (CIAT US$ 3,750). Within a Project on Pyramiding of mutated genes contributing to crop quality and resistance to stress affecting quality. Project for 15 countries and several crops (US$ 750,000 for five years).

7. Problems encountered and their solutions

- In July 2006, CIBIOGEM (Mexico National Biosafety Secretary) indicated the impossibility of Mexico to participate in implementation of the project entitled: The Latin America: Multi-country capacity-building for compliance with the Cartagena Protocol on biosafety. USD 5 million. Donor: GEF-World Bank. The decision was communicated to the World Bank, and modifications of activities were jointly adjusted without affecting the outcome of the multi-country project.
- The main problem encountered for 2006 was the elimination of funding from the Colombian Government to the Rice Project and the reduction of core budget assigned to the Project by CIAT. Special Projects were funded during 2006 that will help to cover part of the gap in funding but will not be enough for future budget reduction expected to be implemented for 2007-2008. Special projects do not generally fund costs of personnel, which do not solve all the problems of budget reductions at the Center
- Transaction costs continue to be too high. It is very hard to keep up with breeding, coordination and supervision of diverse activities, and field work due to too many meetings, travel and paper work. Even my support team feels overloaded. We have tried to more carefully divide and assign responsibilities among support staff and field workers to do our job without sacrificing efficiency, quality and quality of life.
- As last year this year we managed to keep going our core breeding activities by using other sources of funding, especially from AgroSalud, and Cenicafe.
- The stability and sustainability of the Rice Project continues to be a major concern. We have to be more creative and original in approaching the Latin American rice sectors to obtain additional funding for our core activities. There are sectors that actually are not contributing to funding research activities but that are willing to contribute if an adequate mechanism is proposed to them.

8. Staff List (2006)

Principal Staff	Allocation of time IP-4	Other	Affiliations	Location
Dr. Lee Calvert	70%		CIAT	CIAT HQ
Dr. Marc Chatel	100%		CIRAD/CIAT	CIAT HQ
Dr. Fernando Correa	100%		CIAT	CIAT HQ
Dr. Zaida Lentini	20%	80% SB-2	CIAT	CIAT HQ
Dr. Mathias Lorieux	50%	50% SB-2	IRD/CIAT	CIAT HQ
Dr. César Martínez	50%	50% SB-2	CIAT	CIAT HQ
Dr. Rafael Meneses	20%		IIA Cuba/CIAT	CIAT/Cuba
Dr. Gilles Trouche	100%		CIRAD/CIAT	Nicaragua/CIAT
Principal Staff positions in IP-4: 5.1 Associated projects 1.8				
Dr. Carlos Bruzzone Works as a consultant			(INIA)	INIA/CIAT Peru

9. Summary Budget

ACTUAL EXPENDITURES 2006

PROJECT IP4: Improved Rice for Latin America and the Caribbean

SOURCE	AMOUNT US$	PROPORTION (%)
Unrestricted Core	388,100	57%
Restricted Core		0%
		0%
Sub-total	**388,100**	**57%**
Special Projects	287,959	43%
		0%
Total Project	**676,059**	**100%**

ANNUAL REPORT 2006

OUTPUT 1. ENHANCING GENE POOLS

1A. Broadening the Genetic Base of Rice in Latin America and The Caribbean

- **Rice synthetic population breeding project for Latin America and the Caribbean**

Châtel Marc, Ospina Yolima, Rodríguez Francisco and Guzmán Daniel
Funding: CIRAD, CIAT and FAO

Abstract

The year 2006 was the 10th anniversary of the development of rice population breeding activities at regional level in Latin America and the Caribbean (LAC). Centralized (in Colombia) and decentralized (with LAC NARS) breeding activities, the adoption and inclusion of population breeding into LAC partners' breeding projects and the release of varieties are the main outputs of the sustained efforts made by the CIRAD-CIAT rice project to promote the use of a new breeding method in the region. Two commercial varieties were released in Bolivia and Chile for upland/ aerobic and temperate irrigated rice ecosystems respectively. Promising lines are in the pipeline for release in others countries. The collaborative project has established and nurtured a Working Group on Advanced Rice Breeding (GRUMEGA by its Spanish acronym) for collaborative research with national scientists based on capacity building, germplasm development and sharing, workshops for germplasm evaluation and selection, conferences, and joint publications.

Introduction

Since the 1960s, commercial rice cultivars have been developed by conventional crossbreeding, often from breeding populations derived by crossing two inbred lines. This approach encourages inbreeding and so narrows the genetic base of breeding materials. Narrow genetic diversity is of major concern to breeders, geneticists and the agricultural community in general. In LAC, the genetic diversity of rice cultivars depends on a small core of landraces (Cuevas-Pérez et al. 1992). This finding led the rice project at CIAT in Colombia to direct its efforts toward broadening the genetic base in rice.

In 1996, a collaborative project between the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), CIAT and LAC NARS was established to develop and enhance at regional level, synthetic rice populations for the different rice ecosystems (upland, aerobic and irrigated) . The objective was to broaden the genetic base of Latin American rice by assessing genotype × environment interactions to identify specific potential parents and pooling them to create site-specific synthetic rice populations with a broader genetic base (Guimarães and Châtel 2005).

The CIRAD-CIAT project set out to develop collaboration with rice breeders throughout LAC and took the lead in creating and sharing synthetic populations and providing training. In 1999, the Working Group on Advanced Rice Breeding (GRUMEGA by its Spanish acronym) was set up during a regional rice breeders' conference organized in Brazil by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), CIRAD and CIAT and sponsored by the Food and Agriculture Organization of the United Nations (FAO). The leadership in networking activities of the group is assumed by the rice projects of CIRAD-CIAT and EMBRAPA's Arroz e Feijão Center (**http://www.grumega.org**).

In 2002, CIRAD and CIAT established a new collaborative project in Nicaragua on participatory breeding of upland rice and sorghum for poor farmers in Central America. This project is developing and testing breeding schemes, including population improvement methods, in which farmers are fully involved, to develop varieties that are better adapted to the farmers' specific cropping conditions and needs (Trouche 2005). It is expected that participatory breeding methods and the genetic materials developed with this approach in Nicaragua will be applicable to other Central American countries.

Upland and aerobic rice

Population breeding by recurrent selection is efficient for traits that show low heritability. Through short cycles of selection and recombination, linkage barriers are broken down and favorable genes are accumulated. This is a smooth process of continuous improvement. Basic populations were improved using recurrent selection in centralized pre-breeding activities (Châtel et al. 2005; Ospina et al. 2005). Upland composite populations are observed, characterized and improved by recurrent selection in Colombia, and improved lines are distributed to national programs in the region for further testing.

Lowland irrigated rice

Lowland irrigated rice improvement is carried out in close collaboration with partners in the LAC region. In Colombia, the CIRAD-CIAT rice project started developing basic populations targeting the various lowland rice ecosystems present in LAC, in partnership with scientists in Colombia, Venezuela and Cuba for the tropical ecosystem; Argentina for the subtropics; and Chile and France for the temperate zone. The basic populations were shipped to regional partners and evaluated locally. Most of the cooperators used this material to develop site-specific populations by introgressing additional variability to meet their specific breeding objectives. They then used these populations in their rice-improvement programs by recurrent selection.

Integrating population breeding with applied breeding

The main purpose of a breeding project is to create variability and develop breeding materials that may lead to identifying promising lines and new cultivars for release. Recurrent selection methods can contribute to meeting the goals of continuous genetic improvement but should be integrated with other breeding methods to deliver superior breeding materials and improved varieties. Recurrent selection breeding should not be considered a separate phase of an applied breeding program (Hallauer and Miranda 1982).

Selfed progenies extracted from recurrent populations are evaluated and then recombined to obtain improved populations. Superior progenies also have to be including in the applied breeding program, passing through cycles of selection and agronomic evaluation. Advanced lines are the starting point for developing commercial varieties and are donors in crossbreeding programs.

Commercial line release in 2006

In **Bolivia**, the CIRAD-CIAT project has been collaborating with both the public sector (CIAT Santa Cruz) and the private rice sector (Consejo Nacional del Arroz – CONARROZ). The first upland/aerobic commercial variety selected from the enhanced composite population PCT-4 was officially released in 2006 as **ESPERANZA**. The variety is adapted both to manual upland and mechanized aerobic rice ecosystems. For the smallholders, earliness and drought tolerance associated with good yield potential are important traits the variety has. The new variety allows rotation with other food or cash crops. Earliness also allows farmers to place rice to the market early in the season, when prices are high. The variety produces long, slender grain praised by the rice industry (Taboada et al. 2005.)

In **Chile**, the population-breeding project uses site-specific populations developed from the introgression of locally adapted material into the population GPIRAT-10 (Hernaiz et al. 2005). The Chilean populations were being improved for cold tolerance at the vegetative stage and for other agronomic traits. Segregating lines were developed during the improvement process, and passed through regional yield trials. The first commercial variety, **RQuila 28**, adapted to the temperate irrigated rice ecosystem, coming from the enhancement and selection of the population PQUI-1 was proposed for official released in early 2007.

Promising line for release

In **Cuba**, with the Instituto de Investigaciones en Arroz (IIA), one advanced line from the population PCT-4 has completed the process of evaluation and selection and is now in regional yield trials (Pérez Polanco et al. 2005). It shows good yield potential, good resistance to rice blast and lodging, and broad adaptation to the various cropping situations of the so called "popular rice" ecosystem that ranges from upland and aerobic conditions to irrigated.

In **Nicaragua**, with the Instituto Nicaraguense de Tecnología Agropecuaria (INTA) and farmers groups, the project has tested upland composite populations and advanced and segregating lines shipped from Colombia. Promising lines selected from the population PCT-4 are being evaluated in regional participatory varietal selection yield trials, and one (PCT-4\SA\1\1>1479-M-1-M-1) was identified as very promising. Introduced composite populations were used to start participatory plant breeding as well as the source of male-sterile gene and good genetic background. New site-specific populations for Nicaragua and Central America are being developed (Trouche 2005).

In **Venezuela**, 43% of the advanced lines from the Fundación para la Investigación Agricola DANAC rice breeding project come from selections made in various introduced and site-specific

composite populations. A line from the population PCT-16 was identified as a candidate cultivar for release as a commercial variety in 2007 (Jayaro et al. 2005).

Networking with LAC breeders

GRUMEGA

This is a framework for collaborative research built on five pillars: (i) capacity building, (ii) germplasm development and sharing, (iii) workshops for germplasm evaluation and selection, (iv) conferences to present results and advances, and (v) publications with and by collaborators. The IV GRUMEGA Conference was held in January 2006 in Chile and organized by INIA-Chile, CIRAD-CIAT and FAO. (**http://www.grumega.org**)

Regional Technical Cooperation Project (TCP)

The TCP *"Capacitación en fitomejoramiento genético e intercambio de germoplasma para utilizar los recursos genéticos del arroz en América Latina y el Caribe"* involving 7 countries (Argentina, Bolivia, Chile, Cuba, Guatemala, Nicaragua and Dominican Republic) and CIRAD and CIAT was accepted and funded by FAO for 2 years, starting January 2006. In this framework a Breeders' Workshop and a breeding training course were organized in 2006 in Colombia and Cuba respectivelly.

The wokshop 2006 was organized by CIRAD-CIAT and held in Villavicencio Colombia in August 2006. Sixty one rice breeders from 14 countries attended the workshop and selected upland and irrigated segregating and fixed lines. The participants selected a total of 983 and 1310 upland and irrigated lines respectively. These numbers show the importance for the region in having access to public genetic resources developed by international centers.

The Training course 2006 was organized in Cuba to attend Central American and Caribbean rice breeders. CIAT and CIRAD staff were present as instructors.

The way forward in rice population improvement

Since 1996, improving rice biodiversity has become an important breeding alternative in LAC because of investment in capacity building, breeders' confidence in the outcomes, and regional networking between international and national programs. The next step is to take advantage of new molecular tools to increase the efficiency of recurrent selection breeding. Molecular tools are now used, for example, to better assess the level of genetic diversity in a population (Ramis et al. 2005).

Conclusion

Improving rice synthetic populations by recurrent selection is not intended to replace conventional breeding but to supplement other techniques in the breeders' arsenal for developing improved varieties.

Starting a rice recurrent selection breeding project was a challenge for the collaborative rice project between CIRAD and CIAT; a greater challenge was to make it available and useful for the region. Thanks to the enthusiasm and constant dedication of LAC rice breeders, the regional

rice recurrent selection breeding project has been adopted, developed and implemented in several countries.

References

Châtel M, Guimarães EP. 1996. Selección recurrente con androesterilidad en arroz (Motta F, ed.). CIAT publication No. 246. CIAT, Cali, Colombia. 70 p.

Châtel M, Ospina Y, Rodríguez F, Lozano VH. 2005. CIRAD/CIAT Rice Project: Population improvement and obtaining rice lines for the savannah ecosystem. In: Population improvement: A way of exploiting the rice genetic resources of Latin America (Guimarães EP, ed.). FAO, Rome, Italy. pp 237-253.

Cuevas-Pérez FE, Guimarães EP, Berrio LE, González DI. 1992. Genetic base of irrigated rice in Latin America and the Caribbean, 1971 to 1989. Crop Sci. 32:1054-1059.

Guimarães EP, Châtel M. 2005. Exploiting rice genetic resources through population improvement. In: Population improvement: A way of exploiting the rice genetic resources of Latin America (Guimarães EP, ed.). FAO, Rome, Italy. pp 3-17.

Hallauer AR, Miranda JB. 1982. Quantitative genetics in maize breeding (2nd edition). Iowa State University Press, Ames, Iowa, USA. pp. 468.

Hernaiz S, Alvarado JR, Châtel M, Castillo D, Ospina Y. 2005. Improving irrigated rice populations of the temperate climate in Chile. In: Population improvement: A way of exploiting the rice genetic resources of Latin America (Guimarães EP, ed.). FAO, Rome, Italy. pp 129-143.

Jayaro Y, Gamboa C, Graterol E. 2005. Avances del programa de mejoramiento poblacional de arroz mediamte selección recurrente en la Fundación DANAC, Venezuela. Proceedings of the III Encuentro Internacional del Arroz, Havana, Cuba, 6-10 June 2005. p 2.

Ospina Y, Guimarães EP, Châtel M, Duque MC. 2005. Effects of selection and of recombinations on an upland-rice population. In: Population improvement: A way of exploiting the rice genetic resources of Latin America (Guimarães EP, ed.). FAO, Rome, Italy. pp 331-350.

Pérez Polanco R, Châtel M, Guimarães EP. 2005. Acquiring a basic understanding of rice population improvement for use in Cuba. In: Population improvement: A way of exploiting the rice genetic resources of Latin America (Guimarães EP, ed.). FAO, Rome, Italy. pp 205-220.

Ramis C, Badan AC de C, Díaz A, Gamboa CE. 2005. Molecular markers as tools for rice population improvement. In: Population improvement: A way of exploiting the rice genetic resources of Latin America (Guimarães EP, ed.). FAO, Rome, Italy. pp 75-93.

Singh RJ, Ikehashi HI. 1981. Monogenic male-sterility in rice: Introduction, identification and inheritance. Crop Sci. 21:286-289.

Taboada R, Guzman R, Viruez J, Callaú VH, Châtel M, Ospina Y, Rodriguez F, Lozano VH. 2005. Bolivia lanza la primera variedad de arroz de secano originada del mejoramiento poblacional. Proceedings of the III Encuentro Internacional del Arroz, Havana, Cuba, 6-10 June 2005. p 1.

Trouche G. 2005. Participatory rice breeding, using population improvement: A new methodology adapted to the needs of small farmers in Central America and the Caribbean. In: Population improvement: A way of exploiting the rice genetic resources of Latin America (Guimarães EP, ed.). FAO, Rome, Italy. pp 99-109.

- **Rice breeding for drought tolerance. Molecular marker and ecophysiological model based recurrent selection for population improvement.**

Collaborators
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Source of funding
CIRAD, CIAT

Abstract

Cirad and CIAT develop a new breeding project based on the recurrent selection for drought. Selection is primarily based on yield and, in the curse of the selection process, refines the screening methods by additional secondary relevant traits (morpho-pheno-physiological traits). The 2006 preliminary experiment conducted during the growing season, in La Libertad research station in Villavicencio, is used for characterized a group of potential recurrent population genitors. The thirteen cultivars used showed the morpho-pheno-physiological diversity for rice crop behaviour in rainfed conditions. Three groups with homogeneous morphology traits were define according the roots-shoot allometric rate. The experiment was also used for obtained specific cultivar parameter for SaraH rice crop growth model and for ecophysiological methodology training course for CIAT field observers.

Key words: Drought, Recurrent selection, Oryza sativa, Upland rice, Latin America

Background

Enhanced crop production under limited water supply depends upon a subtle dosage of various physiological mechanisms and plant traits according to timing, intensity and duration of the water shortage period. Regrettably, the real impact of the knowledge on plant response to water deficit for genetic improvement of crop productivity in drought-prone area is not substantiated with data. On the contrary, the direct selection for grain yield method continues to produce best-performing genotypes in particular for rice. Facing this situation, the CIRAD-CIAT project intends to develop populations, lines and, secondarily, methodological tools to assist the selection process. Integration of CIRAD and CIAT's expertise in rice recurrent selection and drought physiology will be expected to provide significant headway for drought adapted rice selection. Expected progress in plant modelling should help to achieve this dosage on request.

The project aim is to create new rice breeding population (BP), using already available information on loci, alleles, phenotypic traits of interest regarding drought tolerance in rice, in order to develop molecular marker and ecophysiological knowledge based breeding methods for enhancing recurrent selection (RS) for drought tolerance and water use efficiency. Crop developments models will also be used to analyzed and predict the behaviour of advanced breeding lines over the targeted environments. The improved genetic resources (population and advanced lines) and methods developed by this project will be shared with the Latin America's recurrent breeding network and with other CGIAR and national breeding programs. The new BP will remain open to be enriched with new alleles at new target loci as and when available. The expected output will be (i) new genetic resources (population with broad genetic base and advanced lines) with improved tolerance to drought or improved water use efficiency, (ii) validated methods of molecular marker and crop model based selection for drought tolerance and (iii) a better understanding of the physiological and genetic bases of mechanisms of drought tolerance.

Cirad and CIAT decided to start the project in 2006 with preliminaries studies. The objectives were to

- Physiological field characterisation of references cultivars
- Selection of potential parents (wide spectrum of genitors) for the development of the new synthetic population based on their agronomic and physiological characters of interest and also based on molecular evaluation of their genetic diversity.
- Trained field observers to ecophysiological measurement

Materials and Methods

The water deficit constraint must be reasoned in a wide range of situation. The use of crop growth model simulating the grain yield is an essential tool for this objective. The crop growth model SarraH developed by Ecotrop cirad Team seems to be well adapted well to develop screening methodologies integrating a wide range of agro-environmental conditions.

To confirm the generic ability of this model for different environments, we realized field experiments in "La libertad" research station at Villavicencio, Colombia. The objectives were to obtain data to adjust crop model parameter for Latin America conditions.

A phenological monitoring was done to describe development of cultivar used. The growth was monitored with key dates destructive samplings (45 day after sowing, 70 DAS and 90 DAS). Two experiments were managed, caused by the importation delay of some cultivars seeds coming from Europ.

In the Experiment 1, 10 varieties were tested in a complete randomized bloc design with 4 replications. Elementary plot had a 15 meter square dimension. Seed were sowing in lines distant from 0.25 meter. Sowing date was april the 21st.

Three cultivars were sowing with same conditions in the experiment 2. For space condition, 3 replications could be realized. Sowing date was may the 15th.

The both 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 application). Micronutrients were applied with 20 kg/ha of Fertimex.

5 plants were used for the destructive sampling. Different plants organs (leaves, stem, roots, and panicle) were separate dry and weight. Total leaves surface was measure with the Licor 3000

area meter. At harvest, a sub plot (6 m²) was used for grain yield calculation. The yield components were measured on a 20 panicles sample.
Statistical analyses were performed individually for each trial with Statistix software using the General Anova model. Treatment means were separated with Fisher's protected least significance difference (LSD) test at $P<0.05$.

Results and Discussion

Table 1 shows some cultivars characteristics measured in the experiment. The crop duration of the thirteen cultivars used was short or medium (from 105 to 144 days). The morphological characteristics diversity differs according the trait. Diversity was low for plant height with CV around 11. Tiller number and panicle number were more heterogeneous with CV around 30.
Cultivars grain yield (Figure 1) varied from 1200 to 3800 kg per ha. Cica 8 and Irat 216 cultivars showed the best yield production. The Irat 170 and Bouaké 189 cultivars did not present a well adaptation the Villavicencio rainfed rice conditions. Esperanza cultivar (a new improved variety selected in Villavicencio during the last 5 years did not show a good grain production. This result could be explained by a very high sterility rate (Figure 2).
The Figure 3 presents the roots and shoots dry matter evolution along the time (days). For Root and shoot at flowering stage (95 days) cultivars could be separate in three groups. The 3 lines from PCT4 population had a low root dry matter. The high root production group is represented by IRAt 170, Saquarema and Fedearroz 50 (three typical japonica type). The other cultivars were in the medium root production group. PCT4 lines presented the higher shoot production. Irat 170, WAB 775977, Cirad 409 Sabana 8 and Esperanza were in the medium shoot production group and Cica 8, Bouaké 189, Fedearroz 50, Saquarema and Irat 216 were in the low production group.
The allometric study (Figure 4) confirmed the general decreasing of the roots proportion along the growth period. For some cultivars the allometric dynamic showed a roots proportion increasing at the 95 days after sowing. This result could be explained by the proportion of leaves senescence. However, the figure presented also 3 groups of morphology. For Saquarema and Irat 170 roots represent around 50 % of the shoot dry matter. In the medium group with Fedearroz50, Irat 216, Cica 8, Sabana 6 Wab 77597 and Esperanza, the roots/shoots allometry is around 30%. And the PCT4 lines and Cirad 409 roots represent 10% of shoots dry matter**.**

Conclusions

The 2006 Villavicencio experiment, showed cultivar diversity for all plant growth and development variable. This diversity also exists for morphological aspect. Saquarema and Irat170 cultivars witch had high roots system did not produce good grain production in the experiment conducted without water deficit period. Their morphology with high roots system seems to be very well adapted for drought constraint in rainfed condition.
The experiment gave us the opportunity to adapt the SarraH rice growth model to Latin America condition and to have specific parameters for the cultivars tested.

Future Activities

The collaborative Cirad-Ciat project will continue in 2007. The planed activities were :

- Build-up of the breeding populations by Allofecundation enablement of each CGs accession through crossing with source of genic male sterility.
- Melting and first recombination of allofecundation-enabled segregating material of adequate set of SGs to establish the 2 new breeding populations.
- Characterization of genitors

Table 1 : Cultivar list and characteristics, Villavicencio, 2006

Cultivars	Duration (days)	Linear meter tillering	Height (cm)
SABANA 6	**122**	**78**	**99**
CIRAD 409	**106**	**102**	**94**
WAB77597-2-2-HB-2	**122**	**77**	**103**
(127)	**144**	**91**	**83**
FEDEARROZ 50	**132**	**61**	**88**
SAQUAREMA	**144**	**137**	**71**
CICA 8	**122**	**73**	**101**
ESPERANZA	**109**	**106**	**102**
PCT-4\SA\1\1>975-M-2-	**113**	**87**	**93**
M-3	**105**	**111**	**93**
PCT-4\SA\1\1>1479-M-1-	**126**	**108**	**90**
M-1			**95**
PCT-4\SA\1\1>540-M-3-	**126**	**56**	**81**
M-3			
IRAT 216			
BOUAKE 189			
IRAT 170			

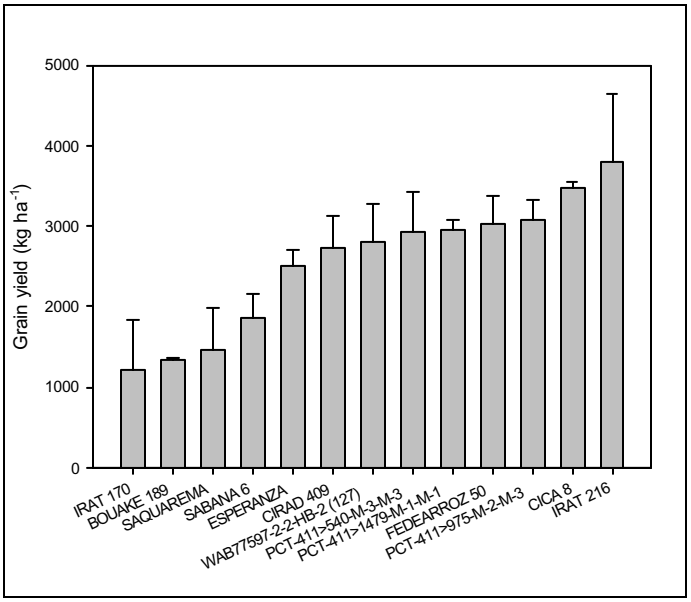

Figure 1 : Grain yield, Villavicencio, 2006

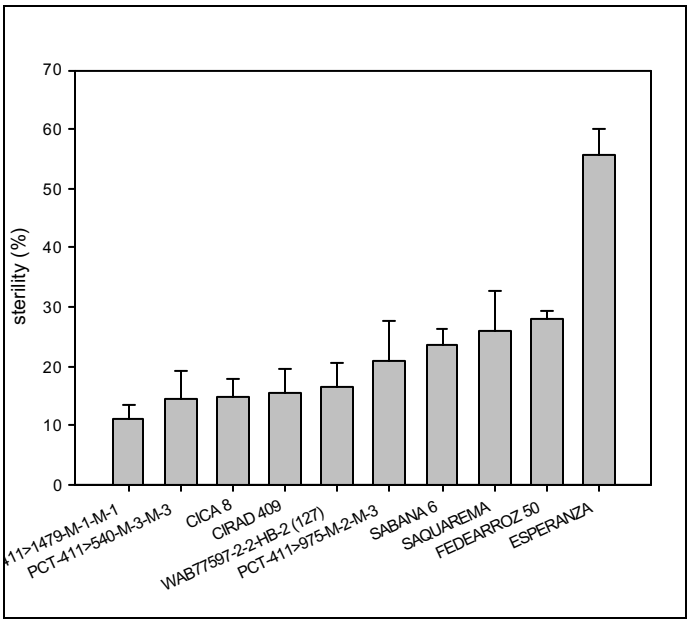

Figure 2 : Sterility diversity for the experiment 1 cultivars. Villavicencio, 2006

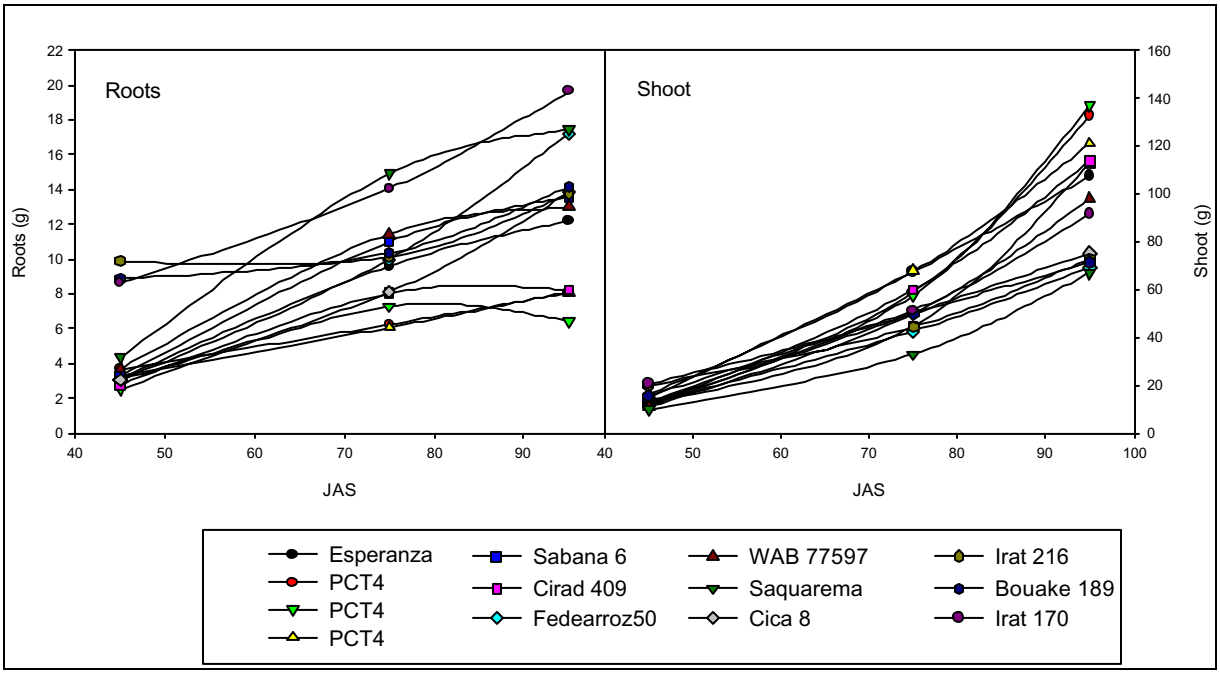

Figure 3 : Shoot and roots evolution, Villavicencio, 2006

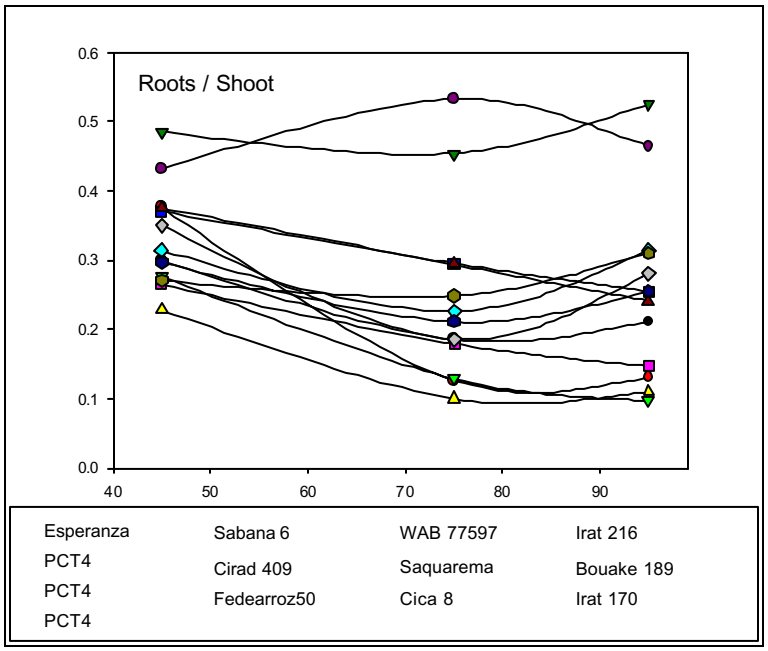

Figure 4 : Allometries between roots and shoot. Villavicencio, 2006

References

Guimarães E.P. 2005 population improvement : Away of exploiting the rice genetic resources of Latin America, FAO, Rome, Italia. pp349

1B. Broadening the Genetic Base of Irrigated Rice in Latin America

- **Chromosome elimination in the diploid *Oryza sativa* L. x tetraploid *O. latifolia* Desv.**

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Abstract

Meiotic behavior of *O. sativa* L. (2n=2x=24), *O. latifolia* Desv. (2n=4x=48) and their F_1 *O. sativa* x *O. latifolia*, BC_2 and BC_3 was evaluated. All meiotic phases were normal and pollen viability was high in both parents. Whole meiotic process of F_1 hybrids presented abnormalities in spindle formation, chromosome segregation and cytokinesis leading to polyads formation, which give rise to unviable pollen. In addition, chromosome elimination mediated by microcytes was observed. Some BC_2 and BC_3 plants were completely diploid (2n=24) and fertile, while others showed tendency to diploidy with one or two additional chromosomes, which were eliminated throughout segregation mechanism in the next generation. We discuss here meiotic behavior that take place for fertility restoration and the importance of chromosome elimination in this type of interspecific cross.

Key words: chromosome elimination, interspecific cross, meiotic behavior, *Oryza sativa*, *Oryza latifolia.*

Introduction

According to Vaughan *et al*. (2003), *Oryza* genus counts with 23 species formed by 10 genomes, six diploids (AA, BB, CC, EE, FF and GG) and four tetraploids (BBCC, CCDD, HHJJ, and one unknown genome). The interspecific cross between individuals with different genomes generates abnormal embryos and therefore abortion. The F_1 individuals are totally sterile, being necessary several backcrosses for fertility restoration (Brar and Khush, 1997). To avoid this, hybrids embryos are recovered in culture medium. Many works have been achieved in order to obtain alien introgression from wild species with different genomes to the cultivar *O. sativa* L. (AA). For instance, *O. officinalis* (CC) (Jena and Khush, 1989), *O. australiensis* (EE) (Multani *et al*., 1994), *O. minuta* (BBCC) (Mariam *et al*., 1996), *O. malapuzhaensis* and *O. punctata* (BBCC) (Kaushal and Ravi, 1998), *O. latifolia* (CCDD) (Multani *et al*. 2003), and *O. grandiglumis* (CCDD) (Kim *et al*., 2003) were used, but the sterility problem remained in all cases, which is surpassed by backcrossing. Despite it, efforts for fertility restoration is necessary since the pairing and recombination among distant genomes, although limited, are the mean for interest wild traits transfer (Voss *et al*., 2000; Chen *et al*., 2001; Multani *et al*., 2003).

Meiotic behavior defines the possibility of producing viable pollen. Therefore, its evaluation is important for understand the process that give rise to fertile and sterile individuals. It could be more crucial in the case of intergenomic crosses, where cell cycle is under different genomes control, in special in those between diploid and allotetraploid species. The extra chromosome

load may be generate genetic unbalance and therefore prevents embryo development. In this view, these additional chromosomes are generally eliminated. This strategy has been reported in intergeneric hybrids of *Brassica napus* and *Orychophragmus violaceus* (Cheng *et al*., 2002), interspecific hybrids of *Hordeum* (Linde-Laursen and Von Bothmer, 1993), somatic hybrids of *Oryza sativa* and *O. punctata* (Shishido *et al*., 1998), and in polyploids as *Paspalum subciliatum* (Adamowski *et al*., 1998) and *Avena sativa* (Baptista-Giacomelli *et al*., 2000).

Meiosis evaluation in rice cultivars was widely described by Chen *et al.* (2005), while chromosome pairing in prophase and metaphase I in individuals arising from interspecific crosses of *Oryza* has been focused by Gopalakrishnan and Shastry (1966) and Katayama (1995). On the other hand, meiotic behavior of progenies resulting from crosses of the wild tetraploid *O. latifolia* and the diploid *O. sativa* has been poorly studied (Li *et al*., 1962; Multani *et al*., 2003). We discuss here the meiotic behavior of *O. sativa*, *O. latifolia* and the progeny arising of crosses among them, and the implications of chromosome aberrations observed in these materials.

Materials and Methods

Seeds of wild tetraploid accession of *O. latifolia* were collected in the Colombian Pacific Coast South. The first cross was achieved using the Fedearroz 50 variety of *O. sativa* as pollen receptor and the wild tetraploid as pollen donator. BC_1 was obtained from the one F_1 individual named CT18228-8 as pollen receptor and two CIAT improved lines of *O. sativa* and the Fedearroz 50 variety as pollen donors. BC_2 and BC_3 were produced from only one BC_1 individual named CT18487, with BCF1720, Pi9, Fanny and Fedearroz varieties. All F_1 and BCs were obtained by embryo rescue in MS medium (1/4), about 12 days after pollination to avoid abortion.

Chromosome number was counted in mitotic cells from roots tips obtained from parents and F_1, pretreated with 8-hidroxiquinoline 2mM during six hours and fixed in Farmer's solution (ethanol–acetic acid 3:1) for 24 hours. Cell wall was digested in HCl 1M to 60ºC for 10 minutes. The cells were stained with modified fuchsine.

Meiotic behavior was evaluated in panicles of parents, F_1, BC_2 and BC_3, in the ideal stage for meiosis studies, which were fixed in Farmer's solution for 24 hours at room temperature and then transferred to a new fixer solution and stored under freezing. Anthers were removed. Meiocytes were released and stained with 1% acetic-carmine for evaluation under light microscope. A minimum of 100 meiocytes per phase was considered. Pollen viability was determined using the same 1% acetic carmine. Black and white photography was taking with a Wild MF 45 system in Leica microscope on Kodak T-Max 100 films, scanned in a Nikon Coolscan V ED system and edited with the Software Adobe Photoshop CS2.

Results

O. sativa and *O. latifolia* genotypes showed a normal behavior in all meiosis phases (Table 1, Figures 1a and b). Some cells of *O. latifolia* presented two nucleoli in both prophases, which did not affect fertility. Because seed development was impossible, F_1 plants obtained by embryo rescue showed intermediate morphology between their parents. Some traits such as purple stigma of *O. latifolia* were dominants, and the heterosis effect was seen in the awns size. In general

plants structure didn't show important irregularities. However, all individuals were sterile, although a few mature seeds were obtained in F_1 plants. Chromosome number in mitotic metaphase was 36 in 20 cells of three individuals, which may indicate the presence of 12 chromosomes from *O. sativa* (A genome), and 24 chromosomes from *O. latifolia* (C and D genomes). The same 36 chromosomes were counted in all diacinesis, mainly as univalent. In this case, bivalents ranged from 1 to 8 per cell (Figure 3a). In prophase I and II a high number of cells had two nucleoli, but one of them disappeared before the other. Due chromosome accumulation in some regions, cytoplasm extensions involving cytoskeleton were observed in prophase I of some PMCs (Figure 3b).

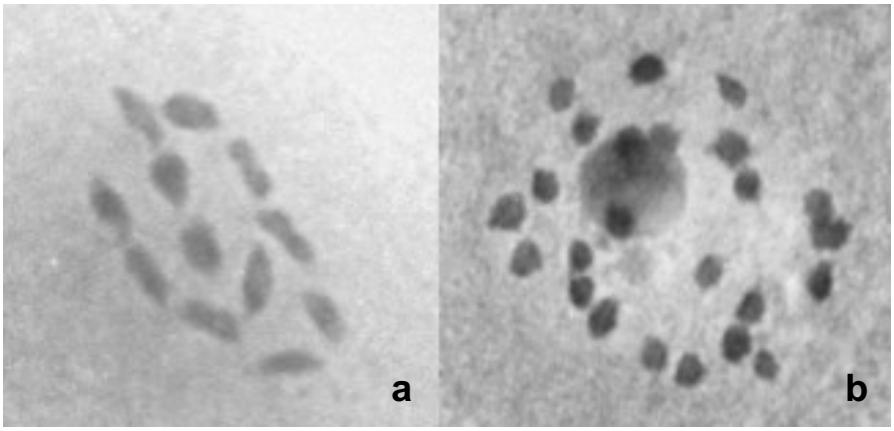

Figure 1. Chromosome number in *O. sativa* and *O. latifolia*. **a**. Prometafase in *O. sativa* exhibiting 2n=24. **b.** Diacinesis in *O. latifolia*, with 2n=48.

An abnormal chromosome arrangement throughout cytoplasm was observed in both metaphases of the F_1 PMCs. Although chromosomes may congregate in the mid cell plate, spindle formation was irregular and therefore segregation in both anaphases was altered. Due abnormal disposition of chromosomes, it can give rise to multiple spindles in metaphase I (Figure 3c). Abnormalities in the spindle no only was seen in the number but also in the orientation pattern during metaphase II, contrarily to the parallel disposition which is the common pattern in all metaphases of a normal meiosis rice. In these hybrids cells, convergent, perpendicular and lineal patterns were found (Figure 2a-c). These patterns were also observed, although in a low percentage, in aneuploids BC_2 and BC_3. In some F_1 meiocytes there is no spindle formation. Micronuclei resulting by abnormal chromosome segregation and microcytes originated by extra cytokinesis were observed from telophase I to metaphase II (Figure 3d). Microsporocytes and microcytes could present several micronuclei (Figure 3e). In addition, asynchronous cell behavior during meiosis II occurred at regular frequency. While some cells were in metaphase or anaphase II, others were in 'tetrad of microspores' stage, accompanied by microcytes rising from abnormal cytokinesis (Figure 3e). As a consequence of this type of cytokinesis, polyads presenting a varied number of microspores and microcytes were observed (Figure 3f). The number of microcytes ranged from one to four. Finally, pollen viability was very low (0.01%). Pollen grains were highly polymorphic.

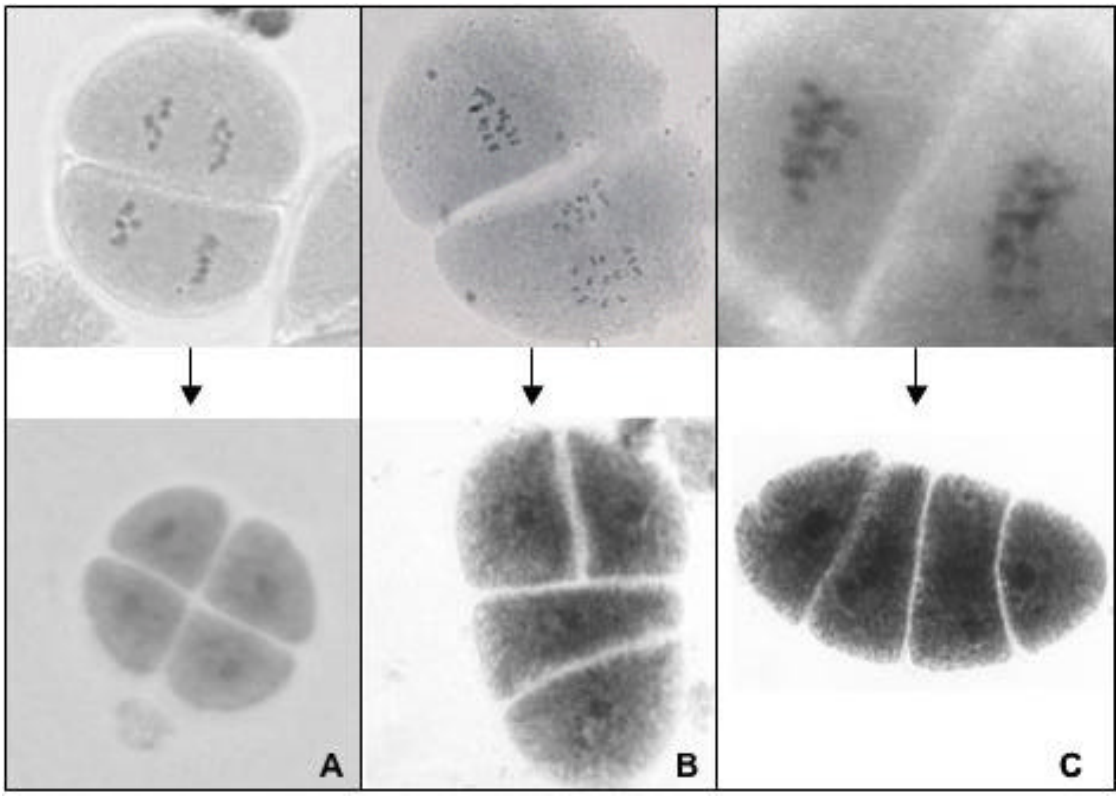

Figure 2. Polyads resulting by irregular spindle formation and cytokinesis. Upper meiocytes in metaphase II and anaphase II, down tetrads produced by the each type of segregation. **A.** Spindles in parallel disposition produce normal tetrad. **B.** Perpendicular disposition produce abnormal tetrad. **C.** Lineal disposition.

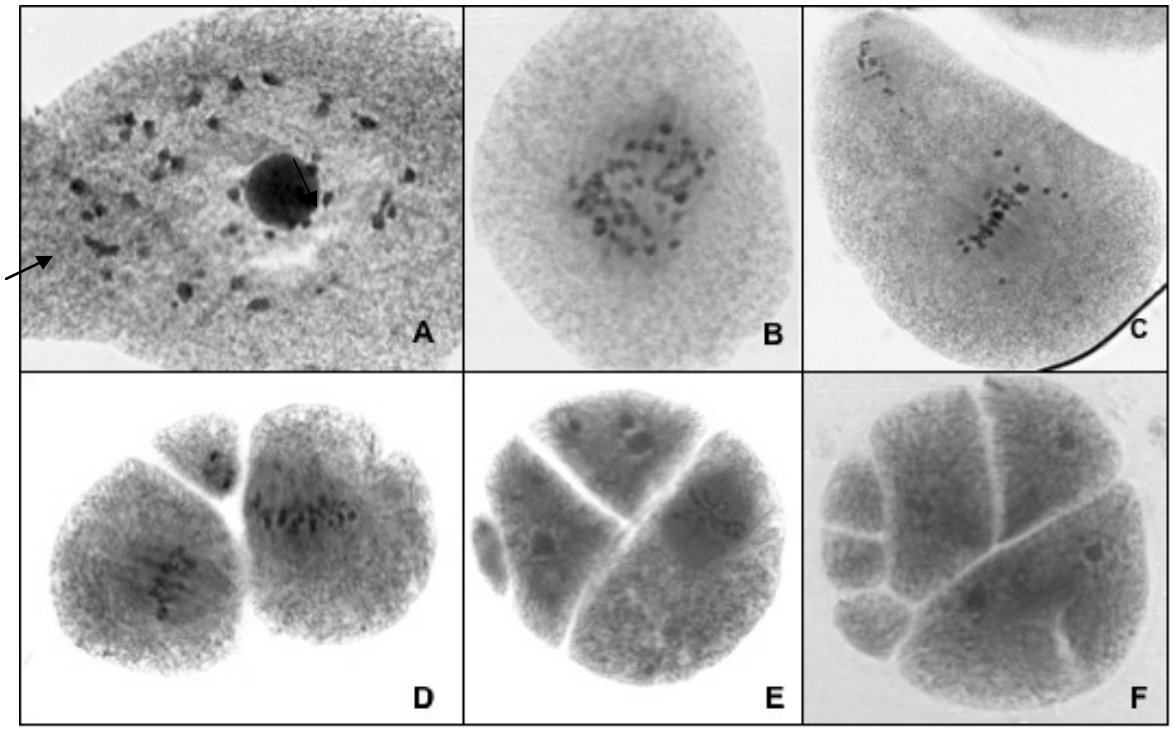

Figure 3. Meiotic abnormalities in F_1 *O. sativa* x *O. latifolia*. **a.** Diacinesis showing bivalents (arrows), while others chromosomes are distributed as univalent. **b. C**ytoplasmatic extensions in prophase I induced by random chromosome presence **c.** Asynchronous chromosome segregation during anaphase I. **d.** Multiple spindles and irregular chromosome segregation in the same metaphase II cell. **e.** Microcyte formed during metaphase II by additional cytokinesis induced by multiple spindles and irregular chromosome position. **f.** Abnormal cytokinesis leading to microcyte formation; micronuclei were observed in one microspore (arrow head). One of de cells exhibits phase asynchrony, as anaphase initial (arrow). Polyad resulting by irregular spindle formation and cytokinesis.

Twenty eight BC_2 and BC_3 individuals were very similar in its architecture to the varieties of *O. sativa*, although presented traits of *O. latifolia*, such as stigma purple, presence of awn, color of grain and height. Eleven plants of both BC_2 and BC_3 were 12 II+ 1I = 25, 10 were 12 II+ 2 I = 26 and seven were 12 II = 24 (Table 1). Meiotic behavior in these plants was normal except by segregation problems of additional chromosomes (Figures 3a-c). These showed precocious chromosome migration (Figures 3d-f). Individuals with two additional chromosomes showed pollen viability average of 37.85% (ranged from 9 to 65%), while individuals with one additional chromosome 33.22% (ranged from 12 to 72%). Diploid showed an average of 78.86% (ranged from 59 to 94%). Size polymorphism of pollen grain in BC_2 and BC_3 individuals with additional chromosomes was also observed.

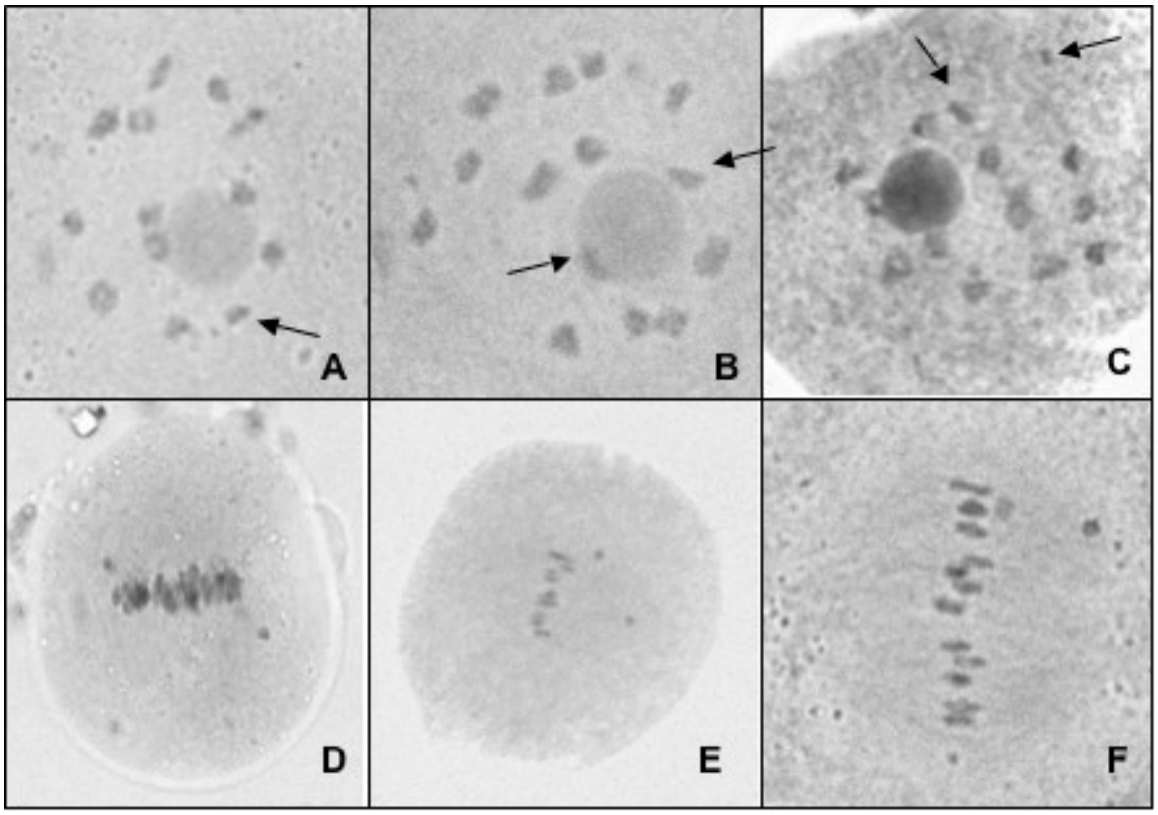

Figure 4. Additional chromosomes in BC_2 and BC_3 plants. Diacinesis with 12 II + 1 I (arrow, **a**), 12 II + 2 I (**b**), 12 II + 1I (arrow) + chromosome fragment (arrow head; **c**). Irregular segregation of additional chromosomes during metaphase I (**d-f**).

Discussion

O. sativa presented a higher meiotic stability, as such as described by Chen *et al*., 2005. Meiotic behavior in *O. latifolia* was normal, despite of its tetraploid condition, composed by two different genomes. Although homeologous pairing may reduce the fertility (Ma and Gutafton, 2005), this pairing in the first step of the development of allotetraploid species is quickly suppressed by changes in the genomes next to the early generations, after duplication (Kashkush *et al.*, 2002; Blanc and Wolfe, 2004).

The way to obtain F_1 individuals in the present case was embryo rescue in culture medium, by providing to embryo the nutrients that not were received by the fail in the endosperm development. According to Vinkenoog *et al*. (2003), difficulty to obtain embryos and impossibility to develop seeds in the first cross are caused by gene dosage in tetraploid - diploid hybrids, which affects the ratio of parental genomes, and therefore producing genomic imprinting. These could explain the reduced number of embryos obtained from the tetraploid *O. latifolia* and the diploid *O. sativa*.

Behavior of F_1 individuals was completely aberrant in all meiosis phases. A higher number of univalents and a variable frequency of bivalents were observed in diacinesis, which was hoped, because the presence of three different genomes. Several genes and proteins that act in the homologous pairing in rice are known, as PAIR 1 (Nonomura *et al*., 2004), PAIR 2 (Nonomura *et al.*, 2006), and cohesin protein OsRad21-4 (Zhang *et al*., 2006). However, the process of the homeologous pairing is no understood very well. In grasses, chromosome regions conserved among species maintain colinearity (Bowers *et al*., 2005), but chromosome arrangements and changes in the chromosome size generally prevent homeologous recognition and therefore recombination process. The comparative mapping of *O. sativa* and *O. latifolia* has shown that exists a high conservation between both species (Huang and Kochert, 1994). This idea is reinforced by pairing and introgressions works from *O. latifolia* to *O. sativa* (Multani *et al*., 2003) and by the high detection of introgressions of species nearly to *O. latifolia*, as *O. grandiglumis* to *O. sativa* cv. (Kim *et al*., 2003). Moreover, preliminary results using molecular markers in the lines originated of our cross, confirm the introgression of *O. latifolia* in nine of the 12 chromosomes of *O. sativa* (unpublished data). It suggests that recombination could be affected by fails in the synaptonemal complex (SC) between homeologous chromosomes. Expression of two different genomes from the formation of SC could inhibit correct formation of this structure. In the case of co-expression, the structural protein subunits could fail in the recognition of subunits generated by the other genome, and in the recognition protein-DNA. Thus, recognition of homologous regions of both genomes would be given by others different mechanisms to SC or randomly encounters among chromosomes with homologous sequences. The random encounters could explain the variable number of bivalents by cell. This is possible since SC cannot be strictly required for meiotic exchange (Dernburg *et al*. 1998). May exist proteins involved in the homology searching such as Rec102 and mei4 in yeast, but in absence of these there exist alternative mechanisms that allow the pairing (Nag *et al*. 1995). Comparison among the protein involved in the SC formation in several species of *Oryza* genus with different genomes could contribute to understand homeologous pairing and genome evolution. In addition, comparison of physical maps of the *Oryza* genus, achieved in the Oryza Map Alignament Project (OMAP) (Wing *et al*., 2005) also would help.

The protein OsRad21-4 orthologue of yeast Rec8 protein is required for homologous pairing and for the joint of sister chromatids in rice, but contrarily to the mutants from this protein evaluated by Zhang *et al*. (2006) and in maize meiotic mutants by Chan and Cande (1998), which showed chromosomes of the interspecific hybrids between *O. sativa* and *O. latifolia* in metaphase I, does not fail in the cohesion of sister chromatid, and the segregation is give in a ramdom distribution of the univalents towards the poles according to the orientation of kinetochore (figure 2B), taking place at separation of sister chromatids in anaphase II, such it occur in haploids of durum wheat (Jauhar *et al*., 2000).

In many cases, meiotic division take place on acentriolar spindles. After to the nuclear envelope breakdown, microtubules are established as an array of microtubules around condensed chromatin in absence of centrosomes or discrete microtubule-organizing centers (McKim and Hawley, 1995). From here the microtubules are self-organized and is defined the polarity. In this way, the spindle formation independent of centrosomes could explain the appearance of multiples congregations of chromosomes in metaphase I, multiple spindles and later several micronuclei. The segregation in anaphase I depend of the interaction between the kinetochore

and the microtubules. The normal segregation in meiosis I involved the kinetochores of both homologous chromosomes of a bivalent, each kinetochore is oriented towards one of the poles give rise to a bivalent bi-oriented, and therefore, equal distribution of chromosomes in the daughter cells. However, in the F_1 hybrids from *O. sativa* and *O. latifolia*, each univalent is oriented toward anyone side, only depending of the kinetochore positioning, this make that the distribution of homeologuos chromosomes be asymmetric in the daughter cells, except for the chromosomes that formed bivalents, while that in meiosis II the distribution among the daughter cells may be equal, since the distribution depend of the segregation of the sister chromatids. Is possible that these cytologic phenomen also are present in the first steps of natural polyploidization and would be surpassed by the duplication and later stabilization of the genomes.

Chromosome congression in the metaphase plate in mitosis is give when chromosomes begin to be bi-oriented, when the congression is finalized, begins the anaphase of a synchronous way towards each pole (Kapoor *et al*., 2006), is possible that in meiosis the transition among metaphase I and anaphase I work in the same way, but in meiocytes with a high number of univalents the bi-orientation is no possible. Therefore, metaphase and anaphase I transition does not exist, and the segregation is completely asynchronous, as well as the later phases (Figure 2E). This is also true in the individuals BC_2 and BC_3 with one or two additional chromosomes, when the univalents have irregular segregation (figure 3), although in some cases its segregation agree with the other chromosomes.

Incorrect orientation and the fail in the congression make that the chromosomes be distributed in a disorder way in the cytoplasm during metaphase I, the later accumulation of microtubules around of separate chromosomes groups can to form meiotic spindles for each group, generating multiple spindles for cell (figure 2C). This behavior is similar to the described one by Caetano-Pereira and Pagliarini (2001) in maize, however in this case the aberrant behavior possibly is give by the mutation in a gene that it controls the formation of the spindle, since there are a normal formation of bivalents. On the other hand, Risso-Pascotto (2005) has described multiple spindles in interspecific hybrids in *Brachiaria*, and this hybrids present problems in the segregation although the number of univalents is not as high as in the hybrids between *O. sativa* and *O. latifolia*. Would be possible to be thought that each spindle is involved with each genome of the parents, but the relatively low rate of this aberration in some meiocytes in metaphase I (8%), and the several accumulations of chromosomes in others as micronuclei in others cells, take to think to us that the organization of the chromosomes in groups in the cytoplasm is an event completely random.

Each event of chromosome accumulation creates a different system of microtubules generates from the chromatin in each point of the cytoplasm. In normal later anaphase I, is begin a reassembly of the microtubules of the spindles, followed by the synthesis of polysaccharids how a perpendicular line to the spindle line for the formation of fragmoplast and the later citokinesis (for review see Verma, 2001). In the F_1 hybrids this happens around of the several micronuclei generating a variable number of microcytes (figure 2F). However, this does not happens with all the micronuclei, since sometimes several micronuclei are remains in a unique daughter cell (figure 2E). Therefore is possible that some specific chromosomes inside the groups formed be involved with the cytokinesis. The cytokinesis and morphology of the tetrads is affected also by

the orientation pattern of the spindle lines, this abnormality does not only was seen in F_1 individuals but also in BC_2 and BC_3 individuals with additional chromosomes.

Up to here is clear that the sterility in hybrids F_1 is caused by the high amount of aberrations in the meiotic process, but the really surprising is that from the pollen cells generates in a aberrant meiosis such as this, be possible grow new plants by means backcrossing and even be possible obtain some few seed F_2. But this can be explain by the high presence of random events of chromosomal distribution in the cytoplasm of hybrids meiocytes, each cell have a genome component that will be distributed of different manners in the daughter cells, thus, in the case of one backcross the sexual cell generate of this meiotic events that have major similarity in its genome component with the one of *O. sativa*, will have more probability of pairing with the gametes of this. On the other hand, the few events in which two cells, generates one by microsporogenesis and other by megasporogenesis in a same flower in F_1 individuals, have genomic components seemed also could give rise the seed F_2 that were obtained. However, in embryos generate by backcross is possible that the genome component extra (chromosomes of *O. latifolia*) continue affecting the development of the endosperm being required again for its development the embryo rescue in culture medium. Therefore, the elimination process that take place in the first steps is giving by the formation of micronuclei and later elimination of microcytes is very important for fertility restoration, as also is described recently in others interspecific hybrids by Qian *et al*. (2005) and Mendes-Bonato *et al*. (2006). Uniparental chromosome elimination also has been seen in somatic cells at mitosis in interspecific hybrids (Gernand *et al*, 2005). However, the evaluations achieved in root tips of F_1 individuals has not showed somatic elimination, as well as the fact of that the meiocytes always present 36 chromosomes distributed among univalents and bivalents, reinforce this observation. Although for recovery the diploidy in backcross is necessary the elimination of wild chromosomes, this does not mean that all elimination be uniparental, but only the cell with chromosome component similar to *O. sativa* can pairing for grow a new generation. Thus, in the individuals originated by backcrosses only show a few additional chromosomes, that although affect the fertility of the plants, are eliminated by segregation in one or two generations. The results also has suggest that the fertility in BC_2 and BC_3 individuals, may depend of the additional chromosome present, since some plants with additional chromosomes present production of seed, but not others and the pollen viability is very variable, thus as the presence of some plants with polymorphic pollen. Some regulatory pathway may be controlled by genes located in specific chromosomes, presence of one or several additional chromosomes with genes involved in this regulatory pathway produce dosage effect that could inhibit them (Birchler *et al*., 2005), in this cases pathways or genes involved with the plant reproduction.

Finally, is possible obtain in BC_2 and BC_3, plants with a normal meiotic behavior and a high seed production as well as with interest traits of the wild rice, *O. latifolia*. After of a process of aberrant meiotic and chromosome elimination by mean of backcrossing, the generation of plants with wild introgressions of species of the *Oryza* genus with different genomes at A of *O. sativa* is possible, and is a important step to broad the rice gene pool using all the species pertaining to *Oryza* genus. However, is necessary understand better the process involved in the homeologous recombination, therefore, works in molecular cytogenetic and function and evolution of protein are required for this purpose.

References

Adamowski, E.V., Pagliarini, M.S., Rocha, L.A. Chromosome elimination in *Paspalum subciliatum* (Notata group). 1998. Sex Plant Reprod. **11**: 272-276.

Baptista-Giacomeli, F.R., Pagliarini, M.S., and de Almeida, J.L. 2000. Elimination of micronuclei from microspores in a Brazilian oat (*Avena sativa* L.) variety. Genetics and Molecular Biology, **23**: 681-684.

Birchler, J.A., Riddle, N.C., Auger, D.L., and Veitia, R.A. 2005. Dosage balance in gene regulation: biological implications. Trends in Genetics. **21**: 219-226.

Blanc, G., and Wolfe, K.H. 2004. Functional divergence of duplicated genes formed by polyploidy during Arabidopsis evolution. The Plant Cell, **16**: 1679-1691.

Bowers, J.E., Arias, M.A., Asher, R. Avise, J.A., Ball, R.T., Brewer, G.A., *et al.* 2005. Comparative physical mapping links conservation of microsynteny to chromosome structure and recombination in grasses. Proc. Natl. Acad. Sci. **102**: 13206-13211.

Brar, D.S., and Khush, G.S. 1997. Alien introgression in rice. Plant Molecular Biology, **35**: 35-47.

Caetano-Pereira, C.M., and Pagliarini, M.S. 2001. A new meiotic abnormality in *Zea mays*: multiple spindles associated with abnormal cytokinesis in both divisions. Genome. **44**: 865-871.

Chan, A., and Cande, Z. 1998. Maize meiotic spindles assemble around chromatin and do not require paired chromosomes. Journal of Cell Science, **111**: 3507-3515.

Chen, C.B., Xu, Y.Y., Ma, H., and Chong, K. 2005. Cell biological characterization of male meiosis and pollen development in rice. Journal of Integrative Plant Biology, **47**: 734-744.

Chen, Q., Conner, R.L., Laroche, A., and Ahmad, F. 2001. Molecular cytogenetic evidence for high level of chromosome pairing among different genomes in *Triticum aestivum – Thinopyrum intermedium* hybrids. Theor. Appl. Genet. **102**: 847-852.

Cheng, B.F., Séguin-Swartz, G., and Somers, D.J. 2002. Cytogenetic and molecular characterization of intergeneric hybrids between *Brassica napus* and *Orychophragmus violaceus*. Genome. **45**: 110-115.

Dernburg, A.F., McDonald, K., Moulder, G., Barstead, R., Dresser, M., and Villeneuve, A.M. 1998. Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosomes synapsis. Cell, **94**: 387-398.

Gernand, D., Rutten, T., Varshney, A., Rubtsova, M., Prodanovic, S., BrüB, C., Kumlehn, J., Matzk, F., and Houben, A. 2005. Uniparental chromosome elimination at mitosis

and interphase in wheat and pearl millet crosses involves micronucleus formation, progressive heterochromatinization, and DNA fragmentation. The Plant Cell. **17**: 2431-2438.

Gopalakrishnan, R., and Shastry, S.V. 1966. Cytogenetics of *Oryza latifolia* X *O. australiensis*. Ind. J. Genet. Plant. Breed. **26**: 237-245.

Huang, H., and Kochert, G. Comparative RFLP mapping of an allotetraploid wild rice species (*Oryza latifolia*) and cultivated rice (*O. sativa*). 1994. Plant Molecular Biology. **25**: 633-648.

Jauhar, P.P., Dogramaci-Altuntepe, M., Peterson, T.S., and Almouslem, A.B. 2000. Seedset on synthetic haploids of durum wheat: Cytological and molecular investigations. Crop Science, **40**: 1742-1749.

Jena, K.K., and Khush, G.S. 1989. Monosomic alien addition lines of rice: production, morphology, cytology, and breeding behavior. Genome, **32**: 449-455.

Kapoor, T.M., Lampson, M.A., Hergert, P., Cameron, L., Cimini, D., Salmon, E.D., McEwen, B.F., and Khodjakov, A. 2006. Chromosomes can congress to metaphase plate before biorientation. Science, **311**: 388-391.

Kashkush, K., Feldman, M., and Levy, A.A. 2002. Loss, silencing and activation in a newly synthesized wheat allotetraploid. Genetics, **160**: 1651-1659.

Katayama, T. 1995. Cytogenetical studies on the genus *oryza*. XIV. Intergeneric hybridizations between tetraploid *Oryza* species and diploid *Leersia* species. Jpn. J. Genet. **70**: 47-55.

Kaushal, P., and Ravi. 1998. Crossability of wild species of *Oryza* with *O. sativa* cvs PR 106 and Pusa Basmati 1 for transfer of bacterial leaf blight resistance through interspecific hybridization. Journal of Agricultural Science, **130**: 423-430.

Kim, H.J., Kwon, S.J., Suh, J.P., Kang, K.H., Song, M.T., Hwang, H.G., Moon, H.P., and Ahn, S.N. 2003. Genomic regions affecting heading date and culm length in a backcross line from a cross between *oryza sativa* and *O. grandiglumis*. Korean J. Breed. **35**: 26-31.

Li, H.W., Weng, T.S., Chen, C.C. and Wang, W.H. 1962. Cytogenetical studies of *Oryza sativa* L. and its related species-2. Bot. Bull. Acad. Sinica. **2**: 209-219.

Linde-Laursen, I., and Von Bothmer, R. 1993. Aberrant meiotic divisions in an *Hordeum lechleri* X *H. vulgare* hybrid. Hereditas. **118**: 145-153.

Ma, X.F., and Gutafson, J.P. 2005. Genome evolution of allopolyploids: a process of cytological and genetic diploidization. Cytogenet. Genome Res. **109**: 236-249.

Mariam, A.L., Zakri, A.H., Mahani, M.C., and Normah, M.N. 1996. Interspecific Hybridization of cultivated rice, *Oryza sativa* L. with the wild rice, *O. minuta* Presl. Theor. Appl. Genet. **93**: 664-671.

McKim, S.K. and Hawley, R.S. 1995. Chromosomal control of meiotic cell division. Science, **270**: 1595-1601.

Mendes-Bonato, A.B., Pagliarini, M.S., and Borges do Valle, C. 2006. Abnormal spindle orientation during microsporogenesis in an interspecific *Brachiaria* (Gramineae) hybrid. Genet. Mol. Biol. **29**: 122-125.

Multani, D.S., Jena, K.K., Brar, D.S., de los Reyes, B.G, Angeles, E.R., and Khush, G.S. 1994. Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Domin. to cultivated rice *O. sativa* L. Theor. Appl. Genet. **88**: 102-109.

Multani, D.S., Khush, G.S., de los Reyes B.G., and Brar, D.S. 2003. Alien introgression and development of monosomic alien addition lines from *Oryza latifolia* Desv. to rice *Oryza sativa* L. Theor. Appl. Genet. **107**: 395-405.

Nag, D.K., Scherthan, H., Rockmill, B., Bhargava, J., and Roeder, G.S. 1995. Heteroduplex DNA formation and homolog pairing in yeast meiotic mutants. Genetics, **141**: 75-86.

Nonomura, K.I., Nakano, M., Fukuda, T., Eiguchi, M., Miyao, A., Hirochika, H., and Kurata, N. 2004. The novel gene *HOMOLOGOUS PAIRIN ABERRATION IN RICE MEIOSIS1* of rice encodes a putative coiled-coil protein required for homologous chromosome pairing in meiosis. The Plant Cell, **16**: 1008-1020.

Nonomura, K.I., Nakano, M., Eiguchi, M., Suzuki, T., and Kurata, N. 2006. PAIR2 is essential for homologous chromosome synapsis in rice meiosis I. Journal of Cell Science, **119**: 217-225.

Qian, C.T., Jahn, M.M., Staub, J.E., Luo, X.D. and Chen, J.F. 2005. Meiotic chromosome behavior in an allotriploid derived from an amphidiploid X diploid mating in *Cucumis*. Plant Breed. **124**: 272-276

Risso-Pascotto, C., Pagliarini, M.S., and Borgues do Valle, C. 2005. Meiotic behavior in interspecific hybrids between *Brachiaria* ruziziensis and *Brachiaria brizantha* (Poaceae). Euphytica. **145**: 155-159.

Shishido, R., Apisitwanich, S., Ohmido, N., Okinaka, Y., Mori, K., and Fukui, K. 1998. Detection of specific chromosome reduction in rice somatic hybrids with the A, B and C genomes by multi-color genomic *in situ* hybridization. Theor. Appl. Genet. **97**: 1013-1018.

Vaughan, D., Morishima, H., and Kadowaki, K. 2003. Diversity in the *Oryza* genus. Current Opinion in Plant Biology, **6**: 139-146.

Verma, D.P. 2001. Cytokinesis and building of the cell plate in plants. Annu. Rev. Plant Physio. Plant Mol. Biol. **52**: 751-784.

Vinkenoog, R., Bushell, C., Spielman, M., Adams, S., Dickinson, H.G., and Scott, R.J. 2003. Genomic imprinting and endosperm development in flowering plants. Molecular Biotechnology, **25**: 149-184.

Voss, A., Snowdon, R.J., Lühs, W., and Friedt, W. 2000. Intergeneric transfer of nematode resistance from *Raphanus sativus* into the *Brassica napus* genome. Acta Hortic. **539**: 129-134.

Wing, R.A., Ammiraju, J.S., Luo, M., Kim, H.R., Yu, Y.S, Kudrna, D., Goicoechea, J.L. *et al*. 2005. The *Oryza* Map Alignment Project: the golden path to unlocking the genetic potential of wild rice species. Plant Molecular Biology, **59**: 53-62.

Zhang, L., Tao, J., Wang, S., Chong, K., and Tai, W. 2006. The rice OsRad21-4, an orthologue of yeast Rec8 protein, is required for efficient meiosis. Plant Molecular Biology, **60**: 533-554.

- **Utilization of Wild Rice Species at CIAT to Broaden the Genetic Base of Cultivated Rice in Latin America**

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Abstract

Nearly 13,000 breeding lines in different stages of development were evaluated in Santa Rosa and Palmira; percentage of selected material varied depending on the type of cross combinations. About 1224 advanced lines were selected by participants from diverse NARs in a Breeder's Workshop held in Santa Rosa . Wide segregation for diserable traits including grain quality and a good number of plant selections were made for further testing in crosses involving *O.latifolia*. Elite lines derived from crosses with *O.glaberrima*, *O.barthii*, and *O.rufigon* showed good field performance and high yield potential in replicated trials run by Fedearroz in high yield-environments.

Background

Wild species are valued as a unique source of genetic variation; however, they have rarely being used for the genetic improvement of quantitative traits. Since 1994 the CIAT Rice Project in close partnership with CIAT Biotechnology Unit has been characterizing and utilizing wild rice species to broaden the genetic base of cultivated rice in Latin America. The strategy in place make 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 QTLs associated with traits of agronomic importance to cultivated rice. Recent progress in this area will be reviewed and presented in this paper.

Data that will be presented suggest that several traits of agronomic importance, including yield and yield components, and tolerance to biotic and abiotic stresses, have been transferred from *O.rufipogon*(IRGC105491), *O.glaberrima* (IRGC103544), and *O.barthii* (IRGC104119) to improved cultivars. Breeding implications will be discussed.

On the other hand, samples of wild rice populations collected in two sites in Colombia were classified as *O.latifolia*, a tetraploid species carrying the CCDD genome. Preliminary evaluations suggest that these accessions carry resistance to all rice blast lineages found in our "hot spot" Santa Rosa, as well as resistance to rice hoja blanca virus and *Tagosodes orysicola*.

Materials and Methods

Breeding lines from different crosses (Table 1) were planted and evaluted under different biotic and abiotic stresses in Santa Rosa, Villavicencio and Palmira Experiment Stations. Data on reaction to main diseases and insect pests, and main agronomic traits(including grain quality) were taken and used to identify and select promissing lines for further testing in 2007 or to be distributed to NARs via CIAT-ION nurseries.
On the other hand, selected elite lines were evaluated in a high yielding environment(Aipe, Huila) in collaboration with Fedearroz.

Results

Data in Table 1 shows that percentage of selected material was different depending on the type of genetic material. There was a high disease pressure in Santa Rosa , specially in terms of rice blast ; high panicle sterility was present in cross combinations including *O.glaberrima* and *O.barthii* accessions. However, promising interespecific breeding lines with high yield potential, tolerance to main diseases and good grain quality were identified and included in the CIAT-ION nursery made available to NARs in 2006. Out of the 194 lines, 65 were from interspecific crosses.

About 1224 lines from different cross combinations were selected by breeders that attended a Breeder's Workshop held in Santa Rosa in August 2006 with funding from FAO. Breeders from both private and public institutions from Colombia, Cuba, Bolivia, Dominican Republic, Nicaragua, Panama, Guatemala , Argentina, and Ecuador made selections, which are being seed-increased in CIAT-Palmira for distribution in mid-2007.

About 1370 plant selections for seed multiplication and molecular testing in CIAT Palmira were made out of a collaborative breeding activity initiated in 2003 aimed at the introgression into susceptible rice varieties of Pi1, Pi2, and Pi33 genes shown to confer stable resistance to rice blast(Rice Annual Report 2004,2005). These backcrossed lines have undergone several cycles of phenotypic selection for both leaf and neck blast infection in Santa Rosa and there is a need to use molecular markers associated with the Pi1, Pi2, and Pi33 genes in order to identify which lines are carrying these genes. Best lines will be distributed to our partners in 2007.

Table 2 shows the breeding behavior of F5 lines derived from crosses with *O.latifolia*, which has been showed by our Rice Pathology and Virology groups to be resistant to rice blast, rice hoja blanca virus and its vector *Tagosodes oryzicola*. This finding is very relevant to our breeding work since these biotic stresses have been the most important and difficult production constraint afecting rice production in Latin America and the Caribbean. Data shows a wide segregation for diserable traits including grain quality and a good number of plant selections were made for further testing in Palmira.

Finally, Tables 3, 4, and 5 show field performance of interspecific lines derived from different cross combinations in contrasting environments. Lines from the cross Lemont/O.barthii// Improved line are about 10 days early compared to local checks but with lower yield potential and milling yield. Preliminary data suggest that lines from the cross CT17237 have higher iron content(6-7 mg/kg) than Fedearroz 50(4-5 mg/kg) in milled rice. Some lines from the cross Bg90-2/5980// Fedearroz 50 had better yield potential than local checks; a similar case was observed in lines from the cross Perla2/ *O.rufipogon*. All these lines are the result of the shuttle breeding program carried out between Santa Rosa and Palmira, where diverse climatic, soil, and biotic stresses are found.

Future Activities

1. Use of additional wild rice species(*O.meridionalis*, *O.glumaepatula*, other accessions of *O. latifolia*) in our breeding program .
2. Continue the evaluation of segregating populations in collaboration with our partners .
3. Distribution of CIAT-ION nurseries to NARs and collaborators.
4. Attendance to international conferences and meetings to present our data .

Table 1. Summary of breeding lines evaluated and selected in Santa Rosa and CIAT Experiment Stations in 2006-2007.

		2006		2007	%
Material	**Generation**	**CIAT**	**S. Rosa**	**CIAT**	**Selections**
O. l*atifolia*/*O.sativa*	F_4-F_5	**3315**	**3315**	**4070**	**61.4**
O. *latifolia* /O. s*ativa*	F_2BC_2	**16**		**64***	**100**
O. rufipogon / Fedearroz 50	F_4BC_2 - F_5BC_2		**611**	**651**	**100**
O.*glaberrima* / Fedearroz 50	F_5BC_2, F_5BC_4, F_6BC_2	**377**			To be planted
Lemont/ O. *barthii* // WC	F_7,F_8, F_9	**336**			For CIAT-ION
Lemont / O.*barthii*	R_6	**15**			Germplasm Bank
Advanced interspecific lines	F_{11}, F_{12}		**123**	**45**	**36.6**
Elite breeding lines	F_8,F_9	**691**			NARS
Introgressions in elite lines	F_4		**964**	**675**	**70**
Purification of breeding lines	F_4-F_7		**126**	**182**	**100**
Pyramiding of genes Pi1, Pi2 y Pi33	BC_2F_6 - F_7BC_1		**722**	**1370**	**100**
Biofortification (IRRI)	F_4-F_5	**1445***			**-**
Biofortification	F_3	**1693***			**-**
CIAT-ION-Biofortificación	F_8,F_9		**288**	**71**	**24.7**
Biofortificacion elite lines	F_8,F_9			**50**	**-**
Material introduced from NARs		**100**	**177**		Fe and zn analysis
Multiplication high *Fe lines*	F_2BC_4	**28**		**28**	**100**
Recurrent selection (PCT 8, 19,21,22)	S_3		**209**	**200**	**95.7**
Recurrent selection populations				**4**	**-**
Breeder's Workshop FAO			**1645**	**1224**	**74.4**
Total		**4878**	**8180**	**8542**	**78.4**

Table 2. Breeding behavior of F5 lines from *O. latifolia* / *O .sativa* crosses

	Resistant %	Intermediate %	Susceptible %
Rice blast(P.oryzae)	**70.03**	**21.3**	**8.6**
Rice Hoja Blanca Virus	**12.6**	**27**	**60.3**
	(< 0.8) %		(>0.8) %
White belly/center *	**88.3**		**11.7**
	Extra Long %	Long %	Medium %
Grain Lenght (LG)*	**4.69**	**92.93**	**2.07**
% lines combining all desirable traits	**7.58**	**25.42**	**66.98**

* Data from Santa Rosa 2006

Table 3.Performance of F8 Lines from the Lemont / O.barthii // WC Cross In AIPE-Huila .Fedearroz and CIAT 2006.

	Piedrapintada-Aipe-Huila					CIAT-Palmira -Valle						
		Flower	Height	Exs	Yield	Flower					Milling	Yield
PEDIGREE	Vg	days	cm	(cm)	(Kg/ha)	days	Clk	Len	Amy	Hb	%	(Kg/ha)
CT17237-1-5-7-2-2-1-M	2	86	94.7	2.3	7737	95	1.0	L	27.7	9	46.9	4813
CT17237-1-5-7-2-2-2-M	2	83	99.3	4.0	7326	95	1.6	L	27	5	44.7	3505
CT17237-1-5-7-2-2-3-M	3	85	103.0	3.7	7173	98	3.4	L	26.4	7	43.5	4581
CT17238-1-1-1-2-1-3-M	2	85	107.0	2.7	6609	95	0.8	E.L	27.2	5	39.0	3918
CT17238-1-1-1-2-1-4-M	2	85	110.0	2.0	7029	100	1.4	E.L	25.2	7	48.5	3590
CT17238-1-1-1-2-1-6-M	1	83	105.0	2.3	5647	98	1.8	E.L	25	7	49.4	3922
CT17238-1-1-1-2-2-5-M	2	84	107.0	2.7	6830	100	1.6	E.L	25.8	5	50.3	4146
CT17238-1-1-1-2-3-1-M	2	83	108.3	2.7	6067	103	0.4	E.L	26.8	5	55.6	3563
CT17238-1-1-1-2-3-4-M	2	84	113.0	2.7	5871	100	0.6	E.L	26.6	5	51.4	3797
CT17238-1-1-1-2-3-6-M	1	83	111.3	2.7	6754	100	0.6	E.L	26.1	5	53.5	3630
CT17238-1-1-1-2-4-4-M	2	85	108.3	3.0	7035	100	1.0	E.L	27.1	5	44.3	3958
CT17238-1-1-1-2-4-5-M	2	84	108.7	1.3	6060	100	2.0	E.L	26.2	5	51.4	4131
FEDEARROZ 50	1	96	104.3	2.3	8539	111	0.2	L	29.5		59.9	3549
Oryzica 1	2	90	94.3	3.3	7969	100	0.2	L	32		57.4	4631
Lemont	2	76	81.3	1.3	5963							4508
T.L. FED 473	2	92	93.3	3.0	8903							
Fedearroz 275												4799

Table 4. Performance of F8 lines from the cross Bg90-2 / 5980 // Fedearroz 50 in Piedrapintada-Aipe-Huila.Fedearroz 2006

PEDIGREE	Vg	Flower days	Height cm	Exs (cm)	Yield (Kg/ha)
CT17334-2-1-6-3-1-1-M-M	2	92	111	4	8552
CT17334-2-1-8-3-1-1-M-M	2	92	122	7	8489
CT17334-2-1-6-2-5-3-M-M	2	93	120	6	8318
CT17334-13-7-1-5-M-1-M-M	1	93	122	4	7682
CT17334-13-7-2-1-4-6-M-M	2	95	112	3	7541
CT17334-13-7-2-1-2-5-M-M	2	94	119	3	7538
CT17334-13-7-2-1-4-3-M-M	2	94	118	4	7321
CT17334-13-7-2-1-4-5-M-M	2	96	116	3	7179
CT17334-13-7-2-1-4-1-M-M	2	95	115	3	7021
CT17334-13-3-1-2-3-5-M-M	2	94	110	3	6821
CT17334-13-3-1-5-6-2-M-M	1	89	110	3	6751
CT17334-13-3-1-3-1-M-M	2	93	107	4	6625
FEDEARROZ 50(Local ckeck)	2	98	110	4	8171
FEDEARROZ 369(Local ckeck)	3	81	108	6	7275
FEDEARROZ 473(Local ckeck)	3	91	103	4	8156

Table 5. Performance of F9 Lines from the cross Perla 2/ O. rufipogon // WC in Piedrapintada-Aipe-Huila. Fedearroz 2006

PEDIGREE	Vg	Flower days	Height cm	Exs (cm)	Yield (Kg/ha)
CT16658-4-1-1SR-3-2-3-4-1-M-M	1	88	118	4	8535
CT16658-4-1-1SR-3-2-1-1-1-M-M	1	85	109	6	8518
CT16658-4-1-1SR-3-2-3-4-2-M-M	1	91	108	2	8140
CT16658-4-1-1SR-3-2-3-2-1-M-M	1	87	112	3	7706
CT16659-8-2CT-1-3-5-1-2-M-M	3	93	109	3	7391
CT16659-8-2CT-1-3-5-5-2-M-M	1	92	113	3	7297
FEDEARROZ 50(Local check)	2	98	110	4	8171
FEDEARROZ 369(Local check)	3	81	108	6	7275
FEDEARROZ 473(Local check)	3	91	103	4	8156

- **Development of high iron and zinc rice lines to Combat Malnutrition in Latin America and the Caribbean.**

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Abstract

A fast-track approach is under way to screen rice lines in our germplasm banks. About 13,000 breeding lines, mainly insterespecific crosses, originated from CIAT rice project and 1445 F4/F5 lines from IRRI were evaluated under biotic and abiotic stresses in CIAT-Palmira and Santa Rosa, Villavicencio. Based on preliminary data 28 cultivars were selected out of 533 screened for fe/zn content, and distributed to NARs for evaluation under local conditions. Fedearroz 50, a successful rice variety grown commercially in several countries in Latin America, showed 2-3 times the amount of iron found in rice bought by consumers(2-3 ppm). Main achievements have to do with the near-completion of a clean lab for handling and preparing rice samples for iron and zinc analysis, establisment of a methodology for running iron and zinc analysis in rice at CIAT, and establisment of base lines for iron and zinc. Five breeding lines with intermediate iron and zinc content were introduced from IRRI(HP+). Recurrent selection and mutation breeding activities were started to increase levels of iron and zinc in our populations.

Background

Micronutrient malnutrition, the result of diets poor in vitamins and minerals, affects more than half of the world's population. 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. In Latin America and the Caribbean (LAC) economic and health indicators have been deteriorating. To meet this challenge, the CGIAR is implementing a new paradigm that views agriculture as an instrument for improving human health and nutrition, as well as for increasing productivity. Nutritionally improved staple food provides an inexpensive, cost-effective, sustainable, long-term means of delivering micronutrients to the poor. The goal of the Biofortification Challenge Program (BCP) is to improve the health of the poor by breeding staple foods that are rich in iron, zinc and vitamin A, for poor consumers with priority on Africa and Asia. This program gets funding from diverse sources, including among others, The Melinda and Bill Gates Foundation.

A project funded by CIDA-Canada complements the Biofortification Challenge Program and extends its benefits to Latin America and the Caribbean, through the development of and deployment of high iron and zinc rice lines. Rice has become the most important food grain in LAC, supplying consumers with more calories than other staple crops. Rice has become particularly important in the diets of poor people, who make up about 40% of LAC's total population. Food purchases account for more than half of all expenditures by the poor, and rice accounts for about 15% of their food purchases. Among the poorest 20% of the population, rice supplies more protein to the diet than any other food source, including beef and milk. However, people living in several areas where rice consumption is high have been suffering from a number of major nutritional problems. This is the result of vitamins and/or minerals naturally present in

the rice grain but otherwise removed during the milling process or that naturally are not present in sufficient amounts. Preliminary data obtained at CIAT from 11 cultivars planted under irrigated conditions indicated that on the average59 and 26% of the total iron and zinc present in brown rice is lost after milling, respectively. There were significant differences at the 5% level among genotypes tested.

Research carried out at IRRI in close collaboration with NARS suggests that there is genetic variability in the rice genome to increase Fe and Zinc in the rice grain. More recently, Haas et al (J.Nutr. 135:2823-2830,2005) reported that consumption of biofortified rice, without any other changes in diet, is efficacious in improving iron stores in women with iron-poor diets in the developing world.

In this project for LAC we plan 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 are screened for mineral content to identify products that could have immediate utility, as potential varieties or donors. Meanwhile, a crossing program was also started to combine high-iron and zinc with high yield potential, tolerance to main biotic and abiotic stresses, and good grain quality. This project is carried out in close partnership with research institutions in Colombia, Bolivia, Cuba, Brazil, Dominican Republic, Nicaragua and more recently Panamá. Preliminary results from the screening process and breeding activities will be presented.

Materials and Methods

Several breeding strategies are being used for the development and deployment of high iron(6-8 ppm) and zinc(22-25 ppm) rice lines for micro-nutrient deficiency areas in Colombia, Cuba, Bolivia, Brazil, Nicaragua, Panama, and Dominican Republic. Breeding activities include: 1.Test existing rice germplasm and breeding lines for increased Fe and Zinc content in milled rice; 2. Create segregating populations to increase Fe and Zinc in the rice grain in combination with other desirable traits(high yield potential, tolerance to main diseases and insect pests, good grain quality,etc); 3.GxE studies to determine the influence of climatic and soil factors in the expresion of iron and zinc in the rice grain; 4. Initiate some work on marker assisted selection.and 5. Visits and coordination of collaborative activities carried out by participating NARs.

Results

1.Test of existing rice germplasm and breeding lines for increased iron and zinc content

Seed of 533 traditional, commercial varieties and elite lines from the CIAT breeding program (including interspecific crosses) were planted in two contrasting environments (Ciat-Palmira and Jamundi) and10-grms sample of paddy rice/entry was sent to IRRI for iron and zinc evaluation on milled rice.There were indications of iron contamination in the samples, but after some statistical analysis and a careful selection for both iron and zinc, a set of 28 genotypes(Table 1) was selected for further evaluation and distribution to our Agrosalud partners. IR68552-100-1-2-

2 had the highest value for both iron (8.12mg/kg) and zinc(23.2mg/kg); Tox 1859-102-4M-4 and Norin 22 had 7.3 mg/kg of iron.

An experiment was set up to verify iron and zinc data reported by IRRI. Milled and brown rice samples of 10 genotypes(Table 2), 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 "blanc controls"between samples of selected genotypes during the milling process; 12 grams of paddy rice were used per sample. A Suzuki rice mill(Figure 1) was used to de-husk and milled the rice samples whilst a locally made mill (Figure2) already tested and validated by the bean AgroSalud project, was used to produce rice flour for iron and zinc analysis. Data shown in Table 2 indicate that iron and zinc values obtained at both WAS and CIAT were significantly lower than IRRI, and no indication of contamination was found. Results indicate that higher values reported by IRRI were probably due to contamination. There was high and positive correlations between WAS and CIAT values(Figures 3,4). Data shows 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 are positive and very high;c) Fedearroz 50, a successful rice variety grown commercially in several countries in Latin America, has about 2-3 times the amount of iron found in rice bought by consumers(2-3 ppm).

We are now in the final process of finishing up the construction of a clean room and milling /polishing facilities at CIAT to assure good quality data and a fast turn over for our breeding program. It is expected that this new facility will be up and running by February/07.

We also got some information to stablish 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 represent the kind of rice bought by rice consumers for consumption and come 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 rep./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 ricehad 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(Figure 7). No significant diferences were found in iron and zinc content in both brown and milled rice across countries, indicating 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.

2. Development of segregating populations to increase iron and zinc in the rice grain in addition to other desirable traits, and germplasm exchange.

Based on HP data Azucena, Madhukar, Ketan Lumbu, Gundil Kuning, Perurutong, IR685552-100-1-2-2, 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, specially 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; based on agronomic traits, fertility and yield potential, a total of 1445 F4-F5 plants were selected for further evaluation in 2007. Best lines will be evaluated for iron and zinc content as well.

It has been shown by HP+ that land races and wild rice species contain more iron and zinc than modern cultivated rice. Based on this aprox. 13,000 breding lines derived mainly fom crosses between elite lines and *O.rufipogon*, *O.glaberrima*, *O.barthii* and *O.latifolia* were evaluated under biotic and abiotic stresses in Santa Rosa, Villavicencio and CIAT-Palmira. Best lines have been identified in terms of agronomic traits, tolerance to main diseases and insect, yield potential and grain quality. These lines will be evaluated for iron and zinc in 2007.

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 conten in maize from 10.0% to 26,6%. Male -sterile plants were selected in each population and crossed to the seven high iron/zinc cultivars mentioned early on. This activity is 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;these populations are undergoing the first recombination cycle and single plants selections will be made in 2007 for iron and zinc analysis. Best plants will be used for the next recombination cycle. The breeding squeme used is shown in Figure 8.

On the other hand, five elite lines from the CIAT and FLAR breeding programs were selected and 3 kg. of 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. One kilogram each of seed of lines BG 90, CT11275-4-M-1-M , FL03191 5P-10-1P-3P-M-M-M , FL04577-3P-11-4P-1P-M , and FL03188-7P-5-4P-1P-M was used for mutagenic treatment following two radiation treatments (150 and 250 GY) using Cobalt 60 as the mutagenic source. Aprox 27000 seeds were planted in CIAT as M1 populations. A total of 8000 plants were selected at random and harvested individually. So far 2000 plants were planted in the greenhouse(1 seed per plant)and leaf tissue was harvested for DNA extraction and detection of mutants using the methodology described by Till et al, 2003; these plants were subsequently transplanted to the field for phenotipic evaluation and seed production. Molecular and mutation evaluation in several genes involved in iron homeostasis will be carried out in 2007. For each gene, three or four primer pairs were designed in order to

make screening on the whole sequence of the gene with special attention in the expressed sequences reported in Gramene.
An active germplasm exchange between AgroSalud and HP+ took place; seed of 20 rice lines was sent to Dr. Parmender Virk for field evaluation and iron/zinc analysis at IRRI. This germplasm included several advanced interspecific lines having high yield potential, tolerance to main diseases and insect pests, and good grain quality under our local conditions. On the other hand, 10 breeding lines, including five lines with intermediate iron content(6-8 ppm iron) were sent to us by Dr. Virk for evaluation. This material will be distributed to our AgroSalud partners and used as progenitors in crosses.Seed of this material was also sent to Dr. Janette Palma, one of our collaborators in Brazil.

3. Visits and coordination of collaborative activities carried out by NARs

Colombia(Alejandro Vargas): A collaborative recurrent selection program is being carried out with Fedearroz; four populations have been developed and are in the first cycle of recombination in two contrasting places. A nursery of about 2,000 F3-F4 interspecific lines were sent to Fedearroz for evaluation and selection under adverse climatic conditions(high temperature and humidity).
Bolivia(Roger Taboada): Out of 45 segregating lines from interspecific crosses sent in 2005, 19 lines were selected for further testing in 2006-2007; selected lines performed better than local varieties Epagri 109, Amboro and Paititi. Seed of 100 lines including local varieties grown in Bolivia, were sent to CIAT for iron and zinc evaluation in 2007. Some preliminary evaluations done by Ciat/Aspar in collaboration with small-traditional farmers indicate that Azucena is a good option for small-poor farmers. Preliminary cooking and eating tests done in local restaurants in rural areas suggested that Azucena is acceptable to consumers ; some people specially liked Azucena because of its aroma, taste and flavor. More cooking and eating tests will be conducted in 2006-07. A new set of 136 segregating lines were sent to Bolivia for planting and evalaution in 2006-2007.
Brazil(Jose Luis Viana): The methodology for sample preparation and mineral analysis for polished rice and whole rice was optimized and implemented;97 polished and brown rice samples were analyzed and selected among the Embrapa recommended cultivars, lines introduced from CIAT and breeding lines from Embrapa Rice and Beans.Results indicate that cultivars BRA 02598 and BRA 01506 had 4.4 to 4.8 ppm of iron in milled rice whilst cultivars IAC435, IAC120, Cateto Seda and Vens de Abril had 19 to 20,6 ppm of zinc in milled rice. Besides, 150 local varieties (landraces) collected in Maranhao, Pernanbuco and Ceará States were planted in a experimental field at Embrapa Mid-North. These varieties were harvested in December for iron /zinc analysis in 2007.
Nicaragua(Lázaro Narvaez): A total of 25 advanced lines were evaluated under irrigated and favored upland conditions in two locations(Horno-Sébaco and Posoltega) and six lines were selected for further evaluation , seed multiplication and iron/zinc analysis in 2006-2007. Rice producers were invited to evaluate this material and their observations and suggestions were considered for the final selection . On the other hand, a set of 203 lines was evaluated in a un-replicated yield trial in Posoltega and best lines selected for further evaluation in 2006-07. A regional workshop was carried out in Octubre 2005 in Posoltega with the objective of re-inforcing the breeding capacity of NARs in the region(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. Finally a new set of 231 breeding lines were sent to INTA for evaluation in 2006-07. We received a set of 100 lines for iron and zinc analysis in 2007.

Dominican Republic(Angel Adames): A set of 100 lines, including traditional varieties, was sent to CIAT for iron/zinc analysis in 2007. Our collaborator (Angel Adames) visited our breeding site in Villavicencio in August-September/07 to evaluate and select breeding material for his program.About 248 lines were selected.

Cuba(Violeta Puldon): There was a considerable delay in signing the research contract with our counterpart(IIA) and activities are just getting under way.

4: 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 is 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.

Acording to Angel Adames,ratooning is a very important management practice in Dominican Republic, very profitable and used specially by small-poor farmers. This practice has spread to other countries like USA, Colombia, Venezuela and Central America. No information is available on the effect of ratooning in the content of iron and zinc in the rice grain. Two experiments were planted in two sites using 28 genotypes to assess how much iron/zinc is left in the rice grain affter ratooning compared to iron and zinc in the rice grain harvested from main crop. These experiments are under way and rice samples from each treatment will be sent to CIAT for iron/zinc analysis in 2007.

It has been reported(Caballero et al,2006.UNAM-Mexico, and Roger et al,1992) that the application of N-fixing bacteria such as Azospirillum, increases the grain yield potential in maize and at the same time reduces the negative impact of fertilizers on the ecosystem.It is suggested that these bacteria has the capacity to provide readily available nitrogen to the crop, promotes root growth, and water/ nutrient absorption from the soil. Several experiments were conducted in 2005-2006 by Dr. Luis Armando Castilla (Fedearroz) to study the effect of climatic and soil conditions on the iron/zinc content in the rice grain. In order to determine the effect of the application of iron and zinc, two rice lines(CT11275-4-M-1-M and IR 68552-100-1-2-1) were planted during the rainy and dry season in Tolima ; iron and zinc was either added to the seed or applied as soil treatment or as foliar spray . There was a control treatment with no iron and zinc in a factorial design. Results indicates that under favorable wheather conditions(high solar radiation) lines CT 11275 and IR 68552 yielded 36 and 22% more than under low solar radiation. Seed samples were sent to CIAT for fe/zn analysis in 2007. Two other experiments were carried out during the rainy and dry season to study the effect of three nitrogen dosages(0,1/2, and 2X) and 4 potassium dosis(0,1/2,1 and 2X) on the Fe/Zn content in the rice grain. A factorial design was used with 3 reps. No NxK interaction was observed whislt 250Kg/ha of nitrogen are needed for high rice yield. Seed samples were taken for iron/zinc analysis.

Lastly, 10 genotypes(*O.glaberrima*, *O.rufipogon*, Fedearroz 50, BG90-2, Linea 30, and five interspecific lines) were planted to determine the effect of the application of N-fixing bacteria (*Azotobacter chroococum* and *Azospirillum amazonense*) on the iron/zinc content in the rice grain.Three nitrogen dosis(0,125 and 250 Kg/ha) were used in a split-plot design. There were highly significant differences in grain yield among genotypes and significant differences in the GxN interaction and Nitrogen x inoculation. No significant differences were found due to the inoculation per se. Lines CT13941 and CT13943(Bg90-2/*O.rufipogon*) yielded significantly more when 50% of total nitrogen was applied in addition to the inoculation with the N-fixing bacteria. Seed samples were taken for fe/zn evaluation. This finding suggest that high yield can be obtain with less nitrogen (50%) when N-fixing bacteria is also applied to the crop. This will lower production cost for the farmer.

5. Marker assisted selection for iron and zinc.

Molecular markers tightly linked to desired genes are being used in the development of varieties having the desired gene combination. Recent developments in SNP technology has made it possible to identify SNP associated with particular genes controlling specific traits (Fe and Zn), which can be used to select desired genotypes in breeding populations

Some collaboration was started with Dr. Janette Palma, in Brazil in a Harvest Plus funded project entitled" Identification and expression of genes important for iron translocation to the rice grain " This activity was started to look into iron transporters to the rice grain. New rice varieties with high iron content in the grain (specially in the polished grain) would have a great positive impact in human nutrition. The development of these lines could be achieved by breeding, using available variability, or by genetic engineering. Both approaches would benefit from a better understanding of the physiological processes involved in iron uptake by the plant, translocation into the shoots and proper allocation into the grain. In its way towards the rice grain, iron flux may be limited in several steps, which we assume to be more efficient in rice genotypes with higher iron levels in the grain. We intend to identify these "bottle neck" steps and the genes able to overcome their limitations in "high iron in the grain" genotypes. The Yellow Stripe gene family has obvious candidates to play such important roles, since different Yellow Stripe Like (YSL) genes are considered responsible for uptake and long-distance transport of iron-chelates in different grass species. We have identified eighteen YSL genes in the rice genome, and we propose to determine which ones play major roles towards higher iron allocation into the grain. We will compare their expression in rice lines with high and low iron in the grain, as well as specifically in the polished grain (with higher allocation of iron into the endosperm as opposed to the aleurone layer). In a complementary approach, we will evaluate rice mutant lines with insertions (of the endogenous retrotransposon Tos17 and/or T-DNA) in different YSL genes, looking for the genes that, when mutated, result in altered flux of iron into the grain. We will also search for other genes, presently unforeseen, but potentially also playing major roles in determining iron content in the rice grain. For that, we will use the RDA (representational difference analysis) technique to identify genes with altered expression in "high iron" rice lines. Promising genes identified in this project (YSL and others) will be available to be used as new tools in HarvestPlus breeding programs and/or in transformation of high yielding rice lines aiming at higher iron content in the grain.

As a complement to this approach a thesis project(Olga Ximena Giraldo) was initiated to design SNPS markers that will be evaluated in rice lines with high and low iron content in milled rice.

This SNPS could be associated with genes pertaining to the YSL family and/or other genes and would be very valuable in a MAS program.

Future Activities

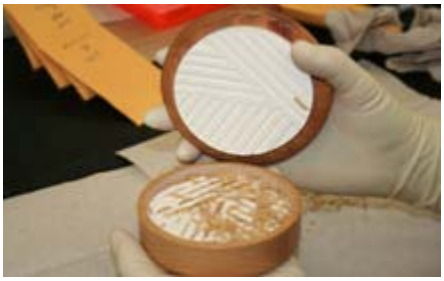
1. Continue the evaluation of cultivars and breeding lines from the germplasm banks and conventional breeding programs for increased iron and zinc content.
2. Evaluation of segregating populations obtained trough the conventional and recurrent selection methods, as well as trough mutation breeding.
3. Continue the work on identification of SNIPs for iron and zinc.
4. Conduct more field experiments to understand GxE factors affecting the expression of iron and zinc in the rice grain.
5. Field visits to Agrosalud partners and assistance to confrences and meetings related to biofortification.
6. Distribution of data and nurseries to our collaborators.

Protocol for preparing rice samples for Fe and Zn analysis

Clean lab (Outside and inside areas)

Paddy rice samples(5grm)

Manual de-huller



Brown rice sample

Protocol for preparing rice samples for Fe and Zn analysis

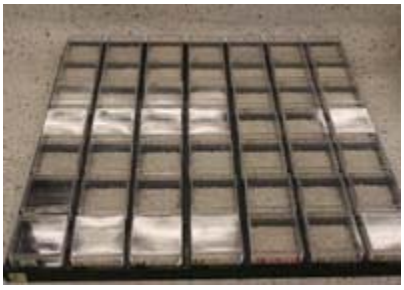
Zuzuki rice mill

De-hulling and polishing rice samples

25 seconds operation

Polished rice sample

Protocol for preparing rice samples for Fe and Zn analysis



Tray with milled rice samples

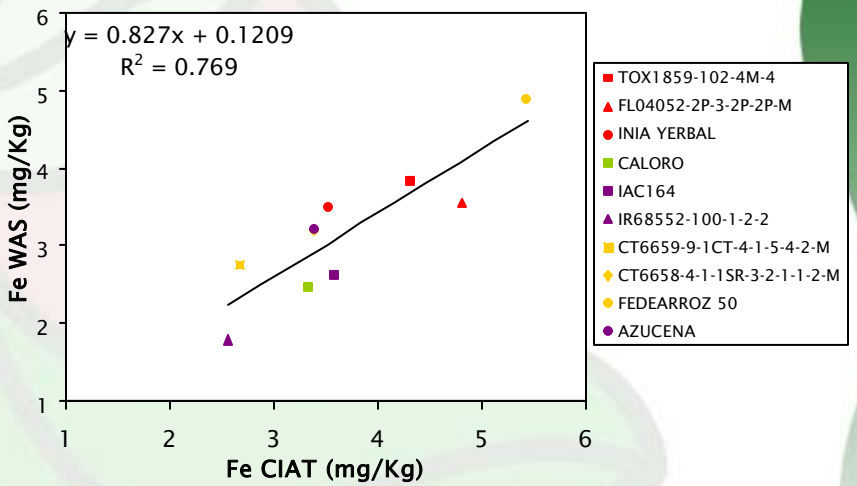
Teflon chambers and zirconium balls

Tray holding 16 teflon chambers

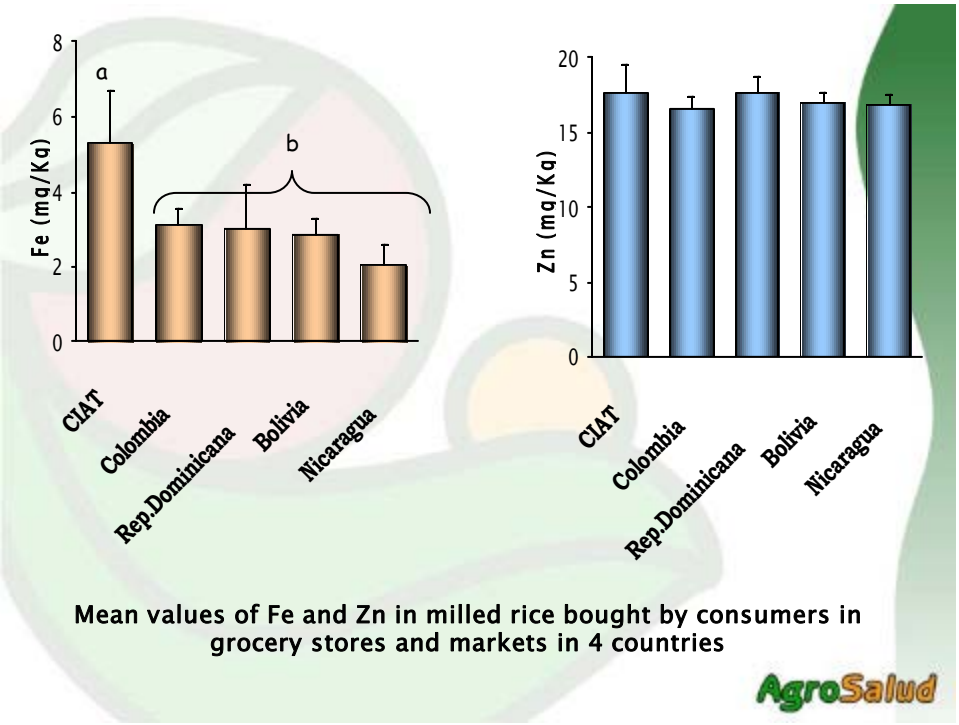
Rice mill to get flour(3-5 minutes)

Analysis of Fe and Zn by Atomic Absorption Method

Muestra	Descripcion	Fe (mg/kg)	Zn (mg/kg)
1	201	2.62	13.08
2	202	2.05	15.28
3	203	2.22	17.16
4	204	2.13	17.69
5	205	1.78	14.42
6	206	2.21	12.79
7	207	2.80	16.39
8	208	1.90	11.20
9	209	2.57	16.83
10	210	2.67	10.90
11	211	3.88	13.49
12	212	3.13	11.90
13	213	1.79	14.06
14	214	7.83	8.37
15	215	1.99	13.33
16	216	2.36	13.27
17	217	2.82	12.71



Correlation between mean values of Fe found in milled rice of 10 rice cultivars determined at CIAT and WAS-Australia.2006



- **Validation of SNP markers located in rice iron homeostasis candidate genes**

Contributors
C. Quintero[1], F. Rodríguez[1], G. Plata[1], O.X. Giraldo[1], C.P. Martínez,[2] and J. Tohme[1].
[1]SB-2 Project; [2]IP-4 Project.

Abstract

This activity was conducted as a proof of concept; 32 SNP-containing sequences (100bp length) were selected to test the multiplex capacity of PCR and single base extension reactions. Results across the different assays were consistent and no ambiguous detection was observed. Percentage validated SNPs ranged between 50% and 72%. The most desirable assay, that assemble high multiplex capacity and large amount of results was the one that involved two multiplex PCR reactions of 16 amplicons and one single SBE reaction of 32 SNP alleles. To date, 56 SNPs are ready to be used for the screening of rice genotypes having contrasting levels of iron content in the polished grains.

Introduction

New rice varieties with high iron content in the polished grain, would have a great positive impact in human nutrition. The development of these lines could be achieved by breeding, using the available variability, or by genetic engineering. Both approaches would benefit from a better understanding of the physiological processes involved in iron uptake by the plant, translocation

and proper allocation into the grain. In its way towards the rice grain, iron flux may be limited in several steps; in previous studies scientists tried to identify the "bottle neck" and the genes able to overcome their limitations in "high iron in the grain" genotypes. Several proteins are involved with iron mobilization, transport and storage in plants. Gross *et al.* (2003) identified 43 genes potentially encoding such proteins, comprising five distinct gene families: eighteen YSL (Yellow Stripe Like), two FRO (Fe^{3+} -chelate reductase oxidase), thirteen ZIP (Zinc Regulated Transporter/Iron Regulated Transporter Protein), eight NRAMP (Natural Resistance – Associated Macrophage Protein) and two Ferritin genes. Some of the objectives of this project are the identification and validation of single nucleotide polymorphisms located in these candidate genes. Then, rice lines with high and low iron content in the polished grain will be genotyped and tagged SNPs associated with high iron content could be used in breeding programs for marker-assisted selection purposes.

Materials and methods

Plant material

Genomic DNA from eight rice genotypes was extracted following Dellaporta (1983) protocol with some modifications. Three belonged to *Oryza sativa* subsp. *japonica* (Koshihikari, Nipponbare and Caiapo), three to *indica* subsp. (93-11, BG90 and Oryzica Llanos 5) and the remaining to the African rice species *Oryza glaberrima* and *Oryza barthii*.

SNP identification and primer design

Plata, Rodríguez and Tohme (this issue), designed a database to identify putative SNPs (*indica/japonica*) in 43 genes related to iron metabolism (Gross. *et al.*, 2003). SNP genotyping was carried out with the single base extension method using the flow cytometer Luminex^{100} as platform (Quintero *et al*, 2005) following the these steps:

- PCR amplification of SNP-containing DNA fragments.
- Enzymatic removal of excess dNTPs and primers.
- Single base extension (SBE) or minisequencing.
- Hybridization of extended SBE primers with the beads.
- SNP detection as Mean Fluorescent Intensity with the Luminex^{100}
- Allele calling with Masterplex GT (Miraibio, Inc).

As a proof of concept, 32 SNP-containing sequences (100bp length) were selected to test the multiplex capacity of PCR and single base extension reactions. PCR primer pairs were designed under multiplex conditions using the FastPCR software (Kalendar, 2007). Single base extension primers were designed through SBEprimer software (Kaderali *et al.*, 2003).

Once the accuracy of the methodology was demonstrated, either for primer design and SNP detection, Plata and Rodríguez designed a set of scripts for the construction of multiplex PCR sets in an automated way, since the FastPCR design was found to be time-consuming. Then two sets of 27 multiplex PCR based SNPs were assayed in this second stage of the validation process.

Results

SNP Validation proof of concept

Among the three primer combinations (forward and reverse) reported by the FastPCR software for each SNP-containing region, the least interfering with each other were selected for the assay. Individual fragment amplification was carried out using conventional *Taq* polymerase to confirm the presence of single and bright bands. The AccuPrime™ *Taq* Polymerase System (Invitrogen™) was used to perform multiplex PCR reactions. Single base extensions were carried out in multiplex of 8, 16 and 32 SBE primers. No fluorescent signal was observed for 7 SNPs in all three assays, which meant that they were probably arising from sequencing errors.

Results across the different assays were consistent and no ambiguous detection was observed. Percentage validated SNPs ranged between 50% and 72% (Table 1). The most desirable assay, that assemble high multiplex capacity and large amount of results was the one that involved two multiplex PCR reactions of 16 amplicons and one single SBE reaction of 32 SNP alleles.

Table 1. Comparison between the assays performed for the validation of the first set of SNPs located on iron metabolism genes.

PCR amplification	Number of SNP alleles per SBE reaction	Validated SNPs	
		Number/total	%
Individual	8	23/32	72
	32	21/32	66
Multiplex (16 amplicons)	16	22/32	69
Multiplex (32 amplicons)	32	16/32	50

Every time a scorable signal was detected, the predicted polymorphisms of Nipponbare and 93-11 were reproduced unequivocally. For 20 markers, SNPs alleles could be detected in the *O. sativa* relatives, *O. barthii* and *O. glaberrima*.

Enhancing multiplex capacity

After automated primer design performed by Plata, 54 SNPs were assayed. Multiplex PCR was done in sets of 13, 14 and 27 primer pairs.

Eighteen did not yield any fluorescence, three were monomorphic for all the rice genotypes tested, two were monomorphic between Nipponbare and 93-11, and unexpectedly, three A/T SNPs showed opposite alleles when compared with the predicted ones. Finally, 28 SNPs genotyped correctly the two sequenced rice varieties, with high mean fluorescent intensity values observed. No differences were found between the results obtained from PCR multiplex of 13, 14 and 27 primer pairs.

To date, 56 SNPs are ready to be used for the screening of rice genotypes having contrasting levels of iron content in the polished grains.

On-going activities:

- Continue the validation of the remaining 111 SNPs from which PCR and SBE primers have been already designed.
- Initiate the genotyping of 536 rice lines with high iron content and checks.
- Scale single base extensions up to 50 SNP alleles per reaction.

References

Dellaporta S. L., Wood, and J. B. Hicks. 1983. A plant DNA minipreparation: version II. Plant Molecular Biology Reporter 1(14):19-21.

Gross J, R.J. Stein, A.G. Fett-Neto and J. Palma Fett. 2003. Iron homeostasis related genes in rice. Genetics and Molecular Biology, 26, 4, 477-497.

Kaderali, L., Deshpande, A., Nolan, J.P. and White, P.S. (2003) Primer-design for multiplexed genotyping, *Nucleic Acids Res*, 31, 1796-1802.

Kalendar R. 2007. FastPCR: a PCR primer design and repeat sequence searching software with additional tools for the manipulation and analysis of DNA and protein. (www.biocenter.helsinki.fi/bi/programs/fastpcr.htm).

Quintero C., E. Gaitán-Solís, C. Quigley, P. Cregan and J. Tohme. 2006. Genetic mapping and use of single nucleotide polymorphisms (SNPs) for the characterization of common bean (*Phaseolus vulgaris* L.) germplasm. Annual Report 2005, SB-02 project. 107-113p.

1C. The Use of Anther Culture and Embryo Rescue for Enhancement of Gene Pools

M. Quintero and Z. Lentini. IP4. Funding CIAT

Abstract

The Anther Culture Laboratory (ACL) gives an active support to the various rice breeding efforts at CIAT by aiding the production of fixed lines through the generation of doubled haploids and embryo rescue from inter-specific hybrids. This task is accomplished by the coordinated planning and evaluation between the cell tissue culture specialists and the breeders. This year report summarizes the recovery of inter-specific hybrids and the production of doubled haploids lines from various crosses of the CIAT and FLAR breeding programs with their corresponding selection in the field at early and advanced generations in Colombia and abroad.

Key Words: doubled haploids, anther culture

Background

Homozygous doubled haploids (DH) lines derived from spontaneous chromosome doubling of the microspore haploid genome of rice can be obtained through anther culture (AC) in less than one year, saving time in evaluation trials (DH vs. F_6) and in building up pure stocks. It is also possible to gain efficiency with DH populations when selecting for qualitative traits because of the absence of dominance, and for quantitative traits due to a greater additive variance, no intra-family segregation, and no interplant competition (Snape and Simpson, 1981; and Snape, 1989). At CIAT, AC has proved to be useful in accelerating the development of germplasm tolerant to low temperatures and having excellent grain quality, increasing the recovery of useful recombinants from wide crosses for disease and pest resistance, drought tolerance; and facilitating the production of materials suitable for molecular markers gene tagging. The CIAT rice anther culture laboratory (ACL) currently focus on developing doubled haploid lines for the various breeding efforts stationed at CIAT. In the case of CIAT, the work has been mainly directed to advanced populations adapted to the irrigated and upland savanna ecosystems, as well as backcross populations derived from crosses between cultivated rice and wild rice species (Castaño, 2002). In the case of FLAR, CIAT has given a support service. The laboratory has generated lines from FLAR crosses targeting the sub-tropical and cold tolerant breeding lines pools for the Southern cone, and produced somaclone lines for Tropical Latin America. In addition to this support, the laboratory also aids the generation of broad crosses through the rescue of immature embryos from inter-specific hybridizations that otherwise abort few days after pollination.

Materials and Methods

For anther culture, plants are planted in the field, panicles harvested and cold pre-treated, and anthers dissected and cultured *in vitro* according to Lentini *et al* (1995). Regenerated plants are delivered to the breeders to continue with the selection process under field conditions. In the case of embryo rescue, embryos are cultured *in vitro* few days after pollination when the endosperm is still in milky stage (liquid). Rooted and elongated seedlings gown *in vitro* are transferred to the

greenhouse and 25-day-old plants are grown in the field. Selected plants are then processed through anther culture.

Results and Discussion

Embryo rescue from inter-specific hybrids
M. Quintero (IP_4), G. Delgado (IP_4), S. Sánchez (IP_4), *S.J. Carabalí (IP_4),* J. Borrero (IP_4), C. Martínez (IP_4, SB_2), Z. Lentini (IP_4, SB_2)

Last year, plants were recovered via embryo rescue from inter-specific crosses between wild *Oryza* species and varieties Fedearroz 50 and *Wab 788, 54-1-1-2-B-4*. These plants were then backcrossed with advanced lines BCF1720 and BCF1658, and the varieties Fedearroz 50, Pi9 and Fanny, with the objective of improving their fertility. At present, these backcrosses are in F5 generation and represented by 4000 lines for selection. These lines will be evaluated for resistance to RHBV and *Tagosodes* mechanical damage, in addition to other agronomic characteristics.

Doubled haploids lines aiding gene mapping
M. Quintero (IP_4), *G. Delgado (IP_4), T. Agrono (IP_4), C. Ordóñez (IP_4), J. Borrero (IP_4), C. Martínez (IP_4, SB_2), Z. Lentini (IP_4, SB_2)*

Triple crosses from (*japonica rice*/ wild species *O. barthii)* / *indica* rice were processed through anther culture. R_2 lines were then evaluated for RHBV and *Pyricularia* resistance, yield potential and grain quality. Of the R_4 lines, a total of 23 lines were selected for *Pyricularia* resistance in Santa Rosa experimental station. Three of these lines were evaluated in replicated yield trials during the first semester of 2006 at the experimental station in Villavicencio, from these a total of 15 individual plants were selected for a subsequent evaluation for agronomic traits at CIAT Experimental Station in Palmira finally selecting 7 lines.

Genetic fixation through the doubled haploids generation
M. Quintero (IP_4), G. Delgado (IP_4), J.D. Arbelaez (Fedearroz), M. Lorieaux (SB_2), C. Martinez (IP_4, SB_2), Z. Lentini (IP_4, SB_2)

Curinga (Brazilian elite variety) is a tropical japonica rice chosen as recurrent parent for the development of 4 populations of introgression lines with 4 wild *Oryza* species AA genome (*O. rufipogon, O. bartii, O meridionalis and O. glumaepatula*). This year a preliminary text was conducted to evaluate the anther culture response for future work with these four populations. A total 211 plants were regenerated from anther culture of which the 36% were DH. This results suggest that crosses made with Curinga as recurrent parent are highly likely to response to anther culture, facilitating the development of fixed lines for molecular introgression analysis.

Anther culture for advancing breeding populations of FLAR

M. Quintero (IP_4), G. Delgado (IP_4), T. Agrono (IP_4), C. Ordóñez (IP_4), J. Ortega (FLAR), E. Torres (FLAR), M. Cruz (FLAR), E. Pulver (FLAR), Z. Lentini (IP_4, SB_2)

Doubled haploids are used as a tool to ease the development of lines high yielding with cold tolerance and commercial grain quality traits. Crosses processed by CA in 2001 had been subjected to pedigree method breeding selection process in various locations across South America. In 2006, 6 lines were selected in Santa Victoria do Palmar (Brazil) and 4 lines in Uruguay. Of the 889 F3 advanced lines selected from triple crosses, one of the parents was obtained through anther culture. Additionally, the genetic base of hybrid vigor is being explored by FLAR. Last year, DH plants were generated from 5 FLAR crosses obtaining 157, 202, 51, 59 and 168 doubled haploids plants from FLO6941, FL06963, FLO6976, FLO6956 and FLOS6952 respectively. This year, ten DH lines from FL06963 that combined good plant type, panicle length (extra-large) and cold tolerance were selected. Of these lines, two were selected as parent for future crossing program for Southern Cone. Populations generated are at F_2 generation.

Conclusions

The Anther Culture Laboratory had demonstrated a solid record of service generating doubled haploids and/or embryo rescue from broad crosses, to the breeding programs of CIAT and FLAR, as well as for developing populations for strategic research, including the development of population for molecular genome introgression analysis, at Headquarters by various partners (CIAT, IRD, and CIRAD) and elsewhere NARS from Latin America, IRRI, Texas A&M, Dupont, Cornell University, among others. The proof of concept of this efficiency was transferred to WARDA during the 90s, capacity that was used to generate the NERICA lines currently evaluated throughout Africa. The efficiency at CIAT has been maintained not only due to the earlier research investment improving a protocol adapted to Latin American indica and japonica germplasm, but also by incorporating new knowledge in tissue culture procedures such temporary immersion systems and for plant regeneration.

Future Activities

Future activities are uncertain. Activities will depend on third party financial support since due to the current restructure scenario of CIAT this laboratory seems likely to be phased out.

References

Castaño, R. C. 2002. Identification of QTLs for yield and yield components using microsatellites in a doubled haploid population derived from advanced back-cross between the variety Caiapo and the wild species *Oryza glaberrima*. B.Sc. Thesis. Universidad de Los Andes. Department of Biological Sciences.

Lentini Z., P. Reyes, C.P. Martínez, and W.M. Roca. 1995. Androgenesis of highly recalcitrant

Snape, J.W. 1989. Doubled haploid breeding: theoretical basis and practical applications. p.19-33. *In* A. Mujeeb-Kazi & L.A. Stich (eds.). Review of Advances in Plant Biotechnology. 2nd. International Symposium on Genetic Manipulation in Crops. CIMMYT and IRRI.

Snape, J.W., and E. Simpson. 1981. The genetic expectations of doubled haploid lines derived from different filial generations. Theory. Apple. Genet. 60:123-128.

- **Increased frequency plant regeneration for various pretreatment combinations to callus derived from anther culture of indica rice (Oryza sativa L.) varieties**

M. Quintero[2], E. Tabares [1], G. Delgado [2], Z. Lentini[1, 2]. [1]SB2, [2]IP4. Funding CIAT

Abstract

Previous year report indicated that three fold increased callus induction in *indica* rice is obtained when PAA 10mg/l is added to the induction medium and maintained in constant agitation in a shaker at 80rpm, and that improved in green plant regeneration efficiency is reached when callus were subjected to desiccation or subculture prior plant dedifferentiation induction (Quintero et al., 2005 in SB2 annual report 2005). This year, the work focused in three aspects to improve embryogenesis and regeneration from recalcitrant indica genotypes: 1) to evaluate of the interaction between PAA concentration in the induction medium and the aeration treatments using low cost procedures (shaker); 2) to test different desiccation period treatments and its interaction with various combinations of growth regulator compositions in the medium and/or sub-culture to induce green plant regeneration of *indica* rice; 3) to determine the effect various culture conditions to induce cell division from microspore cultures.

Key Words: anther culture, callus induction, plant regeneration

Background

Production of callus and its subsequent regeneration are the prime steps for the generation of doubled haploid (DH) plant to be manipulated by conventional breeding programs or by biotechnological means. The response to callus induction and plant regeneration are genotype (variety) dependant, and a highly efficient regeneration from *indica* rice still poses a major bottleneck for genetic manipulation through innovative approaches (Toki, 1997). However some *indica* rice genotypes are more amenable than others for callus induction, but plant regeneration of green plants still remains a major problem (Lentini et al., 1995). Strategies to improve plant regeneration frequency in cereals, including rice, have been steadily evolving during the last decade (Datta *et al*., 1992; Raman *et al*., 1994). The factors affecting callus formation and plant regeneration from *in vitro* cultured anthers of rice were initially studies by Niizeki and Oono (1968), Iyer and Raina (1972), Chand et al. (1977), Datta et al. (1992), and more recently by Saharan et al. (2004). Studies on green plant regeneration were centered in the medium composition and environmental conditions, nowadays it is focused on various types of pretreatment to the callus prior the induction of plant differentiation.

Materials and methods

Anther Culture. Anther culture of *indica* rice Cica 8, Fedearroz 2000, Fedearroz 50 and CT 11275 were used. Anther donor plants were grown in the field, and then rice panicles were collected, treated and cultured according to Lentini *et al*. (1995). Anther were cultured in liquid medium M1 (Quintero et al., 2003) or NL (Lentini et al., 1995) contained in baby food jars

closed with perforated plastic caps with a foam plug in a hole for aeration, and placed either in shelves (stationary) or with in a shaker (agitation).

Plant regeneration. Three experiments were conducted to evaluate various treatments for plant regeneration of anther-derived callus of *indica* rice Cica 8, Fedearroz 2000, Fedearroz 50 and CT 11275. Control consisted of callus induced in M1 medium containing PAA 10mg/L, and cultured in a shaker at 80 rpm, then callus were transferred onto MS (Murashige and Skoog, 1962) solid medium to induce plant regeneration according to in Lentini et al. (1995). The treatments consisted of: *Experiment 1, Partial desiccation,* where different desiccations times were tested (0, 12, 24, 48 and 72 hours) prior transfer to plant regeneration induction medium. Desire extent of desiccation was obtained by transferring 4 weeks old callus to sterile empty petri dishes containing two sterile Whatman-5 filter papers. The petri dishes were sealed with parafilm and kept at 25 ± 1°C in the dark for different periods of time to induce the callus desiccation. After pretreatments, stressed callus were transferred to MS medium for regeneration and incubated in light according to Lentini *et al*. (1995). *Experiment 2, Subculture calluses*: Induced callus were directly transferred to solid MS medium and after two weeks of culture, only those callus with green meristematic zones or buds were sub-cultured on the same medium. Experiment 3, *Combinations of experiments 1 and 2*: Callus were pretreated by desiccation for 48hr in Whatman paper and then cultured on solid medium MS; after eighth days, only those callus with green meristematic zones or buds were sub-cultured on the same medium. A factorial completely randomized experimental design was used. At least 10 replicates of 20 calluses each was evaluated per treatment.

Microspore isolation. Microspore donor plants from *indica* rice Cica 8, Fedearroz 2000, Fedearroz 50 and CT 11275 were grown at CIAT's experimental field. These indica rice genotypes were selected because their response to embryogenesis and green plant regeneration from anther culture. Rice tillers were harvested, sterilized and the microspores were isolated by a method similar to the described by Weiguo, et al. (2002). The boot were sterilized and then the spikes were aseptically cut in sections of 2 to 3 cm of length and placed on sterile flasks that contained 50 ml of pre-treatment solution 2HNA (0.18mM). The flasks were placed in a shaker at 80 rpm and incubated at 27±1ºC for 48h in the dark. Florets obtained from spikes section were blended in 50ml of autoclaved 0,3*M* mannitol solution for 20s. To eliminate the large debris, the resulting slurry was filtered through mesh of different pore sizes and recovered in a capped tube containing 5 ml solution of 21% maltose and centrifuged at 450g for 3 min. The microspores were rinsed with M1 medium and finally cultured in the same medium at a density of 4 x 10^3/ml, in 60 mm plastic Petri dishes, each Petri dish contained 3 ml of MI medium.

Results and discussion

Anther Culture. An increase of two to three folds in callus induction was obtained when PAA was added to the induction medium. Interaction PAA and different culture conditions (stationary Vs shaker) showed that Shaker significantly increase callus induction and embryogenesis to the different genotypes as in previous experiments (Quintero et al., 2005 in SB2 annual report 2005).

Partial desiccation. Plant regeneration was lower in controls (without desiccation) than with desiccation treatments. Significant higher green plant regeneration was obtained when

desiccation was applied for 48 hr. Twice as many green plants were obtained with this treatment respect to the control (Figure 1A). Similar results was reported by Saharan et al. (2004), who reported that shoot regeneration frequency was also higher by 1.2 to 5.6 fold in both cultivars in 48 h desiccation whereas in 72 h desiccation treatment regeneration frequency declined. These results were in conformation with the reported by Diah and Bhalla (2000) and Chand and Sahrawat (2001).

Subcultured calluses. Increase plant regeneration was seen after subculture of callus with meristematic green plant primordia. During subculture the shoot buds elongated further and multiplied vigorously. The regeneration frequency of 75.5%and 92.5% was obtained when callus were sub-cultured on MS without or with growth regulators, respectively. An increase of 3 to 4 fold in shoot regeneration frequency was obtained with callus sub-cultured as compared to control (Figures 1B). The results of these experiments indicate that the retransfer of callus was optimal for improvement of green plant regeneration in *indica* rice varieties.

Combination experiments 1 and 2. Partial desiccation and subculture showed to be the best combination to improve plant regeneration efficiency from callus of the different *indica* genotypes. Response of 53.8%, 77.2% and 90% on the average were obtained with partial desiccation, subculture or a combination of both pretreatments respectively. Significant higher increase in plant regeneration efficiency of 6 fold is achieved when the two pretreatments are combined for the *indica* rice Cica 8 recalcitrant to tissue culture (Figure 2). Results indicated that desiccation increased the number of callus with green buds primordial and that subculture promotes these buds to differentiate into plants. Results suggest that depletion of nutrient in the medium and /or accumulation of inhibitory substances in the medium such as phenolic compounds exuded by the callus may affect the plant differentiation process. Regenerated plants were transplanted into pots in the greenhouse. About 50 – 60% of the plants were doubled haploids, average which is in the range previously reported by Lentini et al. (1995).

Microspore isolation and culture. After isolation and culture, embryogenic microspores have typically eight or more small vacuoles immediately enclosed by the cell wall (Figure 3.). These vacuoles surround the condensed cytoplasm in the center, forming a fibrillar structure. This protocol allowed inducing initial cell divisions at 10 – 12 days after culture from *indica* rice Fedearroz 2000 and CT 11275. The chemical, 2-HNA has been previously reported to increase the efficiency of androgenesis in anther culture when applied to wheat spikes at a critical developmental stage (Konzak et al., 2000). The 2-HNA can be effectively and conveniently delivered to act on microspores by the described method. Results indicate that is possible to induce embryogenesis from micropore culture of indica Latin American genotypes. Microspore culture maybe an useful biological cell system to identify factors for promoting cell division response at early stage of culture, but this system to be efficient it is necessary to improve the protocol by increasing the yield of clean microspore suspensions and testing various modifications of the culture medium including different osmolality and co-culture treatments with ovaries for providing the nurse factors, which the embryogenic microspores apparently cannot efficiently synthesize.

Future Activities

- To test different concentrations of 2-HNA in the pretreatment of spikes sections and evaluate its effects on callus induction.
- To test the effect of cool-pretreatment of panicles on the isolated microspore culture and the changes in amino acid ratio in anther cultures of japonica and indica rice, and the relationship between free amino acids change and culture response of isolated microspores.
- To test the shed pollen culture system, in which anthers are cultured on the liquid /solid inter-phase medium

References

Chand S, Sahrawat AK (2001). Stimulatory effect of partial desiccation on plant regeneration in *indica* rice (*Oryza sativa L.*). Journal Plant Biochemical & Biotecnology 10:43-47.

Diah P, Bhalla PL (2000). Plant regeneration from mature embryo derived callus Australian rice (*Oryza sativa L.*) varieties. Australian Journal Agriculture Research 51:305-312.

Lentini, Z.; Reyes, P.; Martínez, C.; y Roca, W. (1995). Androgenesis of highly recalcitrant rice genotypes with maltose and silver nitrate. Plant Science 110: 127-138

Murashige T., and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473 – 497.

Quintero M., Tabares E., Delgado G., Escobar R., Lentini Z. (2003). Ajuste del sistema RITA® para la inducción de callo embriogénico y regeneración de plantas a partir del cultivo de anteras de arroz. En: Tesis de pregrado, Universidad Nacional de Colombia sede Palmira. 107p.

Quintero M., Tabares E., Delgado G., Lentini Z. (2005). Improvement of callus induction and dobled haploid plant regeneration from rice anther and microspore cultures. Annual report 2005 CIAT, Cali-Colombia. pp. 29 - 35

Raman R, Chahal, G.S., Dhaliwal, H.S. (1994). Screening of genotypes for callus induction and plant regeneration in rice. Crop Improvement 21:1-2.

SaharanV, Yadav RC, Yadav NR, Chapagain BP (2004). High frequency plant regeneration from desiccated calli of *indica* rice (*Oryza sativa L.*). African Journal of Biotechnology 3(5): 256-259.

Tsukahara M, Hirosawa T (1992). Simple dehydration treatment promotes plantlets regeneration of rice (*Oryza sativa L.*) callus. Plant Cell Report 13:647-665.

Toki, S. (1997). Rapid and efficient Agrobacterium-mediated transformation in rice. Plant Molecular Biology 15:16-21.

Weiguo L., Ming Y.Z., Polle E.A., and Konzak C.F. (2002). Highly efficient dobled-haploid production in Wheat (*Triticum aestivum* L.) via induced microspore embryogenesis. Crop Science 42:686 – 692.

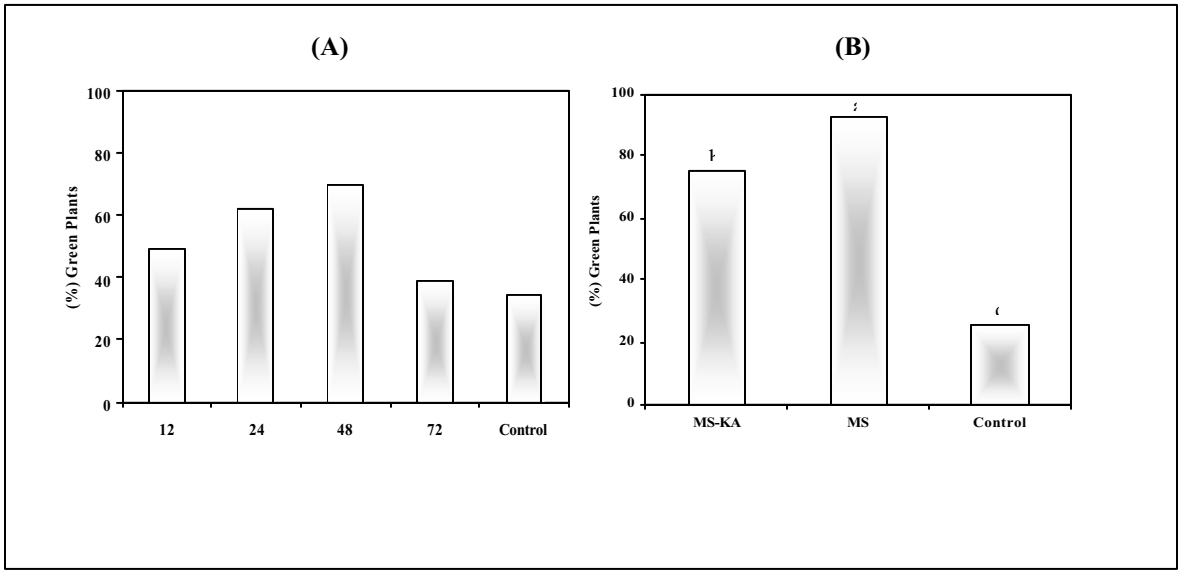


Figure 1.- Effect of desiccation (A) or callus subculture (B) pretreatments on the green plants regeneration of *indica* rice callus.

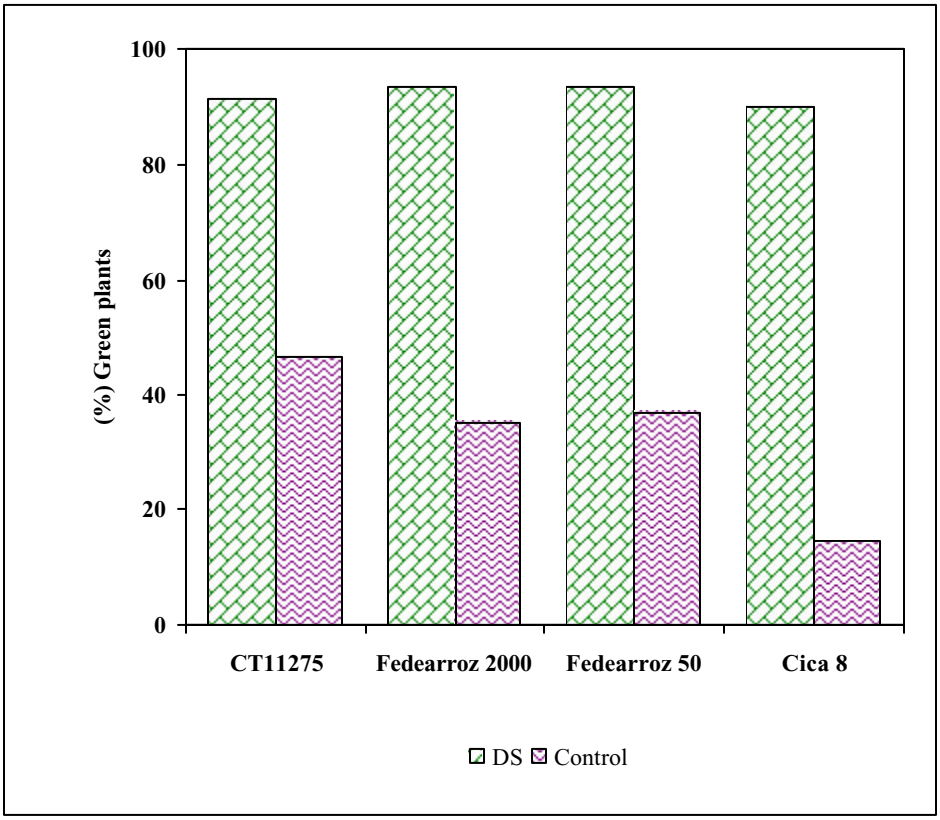


Figure 2.- Effect of combining desiccation and callus subculture on green plant regeneration of *indica* rice callus

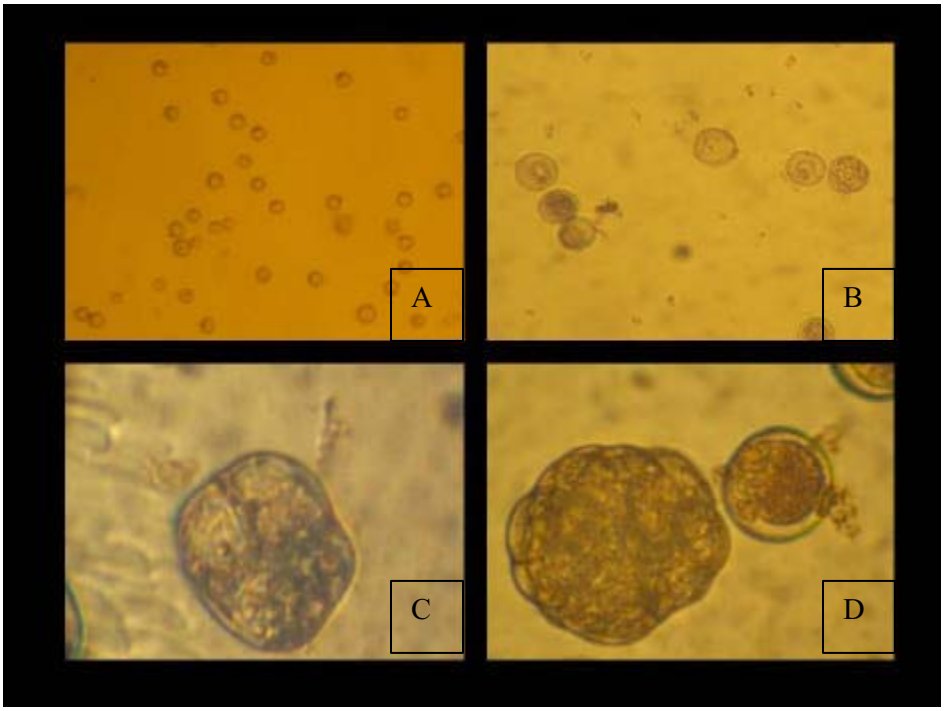


Figure 3.- Embryogenesis of Fedearroz 2000 microspores isolated, and then treated with 0.18 mM 2-HNA. (A) Microspores immediately after isolation; (B) Embryogenic microspores induced by 2-HNA after 48h; (C) Multi-cellular structures 7 days after culture; (D) Proembryoid structures at 14 days in induction medium.

1D. Gene Flow Analysis and Genetic Diversity in Rice

- **Characterization of Genetic Diversity: Relationships and potential origin of the weedy rice complex in Colombia**

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Abstract
The Colombian weedy rice has been characterized in preliminary works conducted by our research group (Gonzalez *et al*., 2003. SB2 Annual Report 2003) using 148 Colombian weedy rice accessions collected in farmer fields evaluated with 19 microsatellites markers. In this report we add up 7 SSR and 13 *Oryza rufipogon* accessions with the objective to evaluate the genetic relationships between weedy rice and *O. sativa* japonica and indica type varieties, landraces, wild species and hybrids between red and cultivated rice to have a better knowledge of the origin of weedy rice and correlate diversity analysis to find strategies to control weedy rice.

Key Words: weedy rice, genetic diversity, molecular markers, SSR

Background

Weedy rice is a common weed in most irrigated rice production areas in the Americas: Bolivia (Llanos et al. 1993), Brazil (Noldin 1988), Chile (Pedreiros and Alvarado 1990), Colombia (Montealegre and Vargas 1989), Guyana (Rai 1973), The United States (Dunand 1988), and Venezuela (Ortiz et al 1997). The only areas where weedy rice is not considered a major problem are California (USA) and Uruguay, but it may still be found infesting some fields (Noldin 2000). This weed is a superior competitor for crop cultivars due to early vigor, greater tillering, and greater height of plants, furthermore has colored pericarps that results in lowered grain quality in most rice markets, and early shattering that reduces harvestable yield (Mortimer et al. 2000). This weed shows a high similarity with cultivated rice varieties in the early growth stages. At maturity it has wide-ranging characteristics, including competitive ability, tillering capacity, flowering date, seed shattering and dormancy, pigmentation of several plant parts in particular of the pericarp (Diarra *et al*., 1985). Different reports including our work (Lentini and Espinoza, 2005) show that some weedy rice can also have intermediate characteristics between wild *O. rufipogon* and cultivated *indica* or *japonica* varieties of *Oryza sativa* (Bres-Patry *et al*., 2001) or could be divided into *O. sativa* ssp. japonica and *O. sativa* ssp. indica-like groups, with some intermediate accessions having characteristics from both groups (Cho *et al*., 1995; Lentini and Espinoza, 2005). In addition, the high rate of fertility in crosses supports the traditional argument that red rice and cultivated rice are both *O. sativa.* However not everyone agrees with this traditional classification. It has been argued that in the tropics, weedy rice complex may also include other annual *Oryza* species such as *Oryza barthii*, *Oryza longistaminata*, *Oryza rufipogon*, *Oryza perennis*, or *O. punctata* (Kwon *et al*. 1991). These arguments are based on morphological characteristics, but the high degree of variation and lack of a clear classification system make it difficult to definitively categorize red rice. Another hypothesis is that weedy rice may have endo-ferally evolved through the dedomestication of cultivated rice to weedy types,

where wild rice is not present (Vaughan *et al*., 2003). In despite of the different hypotesis to solve the origin of weedy rice, its knowledge must begin understanding the variation in the several populations considering that very different very different processes are involved both within and between countries and regions (Mortimer et al. 2000). The Colombian weedy rice has been characterized in works conducted by our research group (Gonzalez *et al*., 2002, Ruiz et al., 2002; Vasquez, 2002; Ruiz, 2003) using 148 Colombian weedy rice accessions collected in farmer fields evaluated with 19 microsatellites markers. This analysis generated five groups three of which grouped all weedy rice accessions. One of these groups clustered closely to *O. rufipogon*. The wild species *O. barthii* and *O. glaberrima* clustered together in the fourth group, and *O. glumaepatula* in the fifth group. Subsequently, a following study reported last year evaluated the genetic variation among weedy rice types, hybrids between weedy rice and cultivated rice, some rice *O. sativa* cultivars japonica and indica types, Colombian landraces and wild species using the same 19 microsatellites markers used before to have a better understanding of the relationships and the potential origin of the weedy rice complex in Colombia. In this report we add up 7 SSR and 13 *Oryza rufipogon* accessions in addition of the materials and markers reported earlier.

Materials and Methods

Plant Material and Genetic Analysis using Microsatellites Markers: Materials used in this study consisted of 148 weedy rice accessions collected in Colombia; 19 *O. sativa* indica rice commercial varieties and 12 *japonica* type; 16 hand-made manual crosses between the RHBV-resistant transgenic Cica 8 line and non-transgenic variety purple or selected weedy rice accessions; 20 accessions of wild *Oryza* species AA genome, and 15 Colombian landraces. Twenty-six SSR primers derived from rice were amplified in all samples. The PCR products were resolved on silver-stained polyacrylamide gels and microsatellites alleles were sized by comparison to 10 bp molecular weight standard (Promega).

Statistical Anaysis: Allelic frequencies were calculated for all materials analyzed. Pearson chi square test was used to evaluate the association between the microsatellites alleles with seed morphological traits. Two multiple correspondence analyses (MCA) were conducted. The first analysis only included the molecular markers data (MCA-M), and the second analysis included both the molecular and seed morphological data (MCA-MSM). The Pearson chi-square and MCA are tests applied to establish the significance of association between categorical variables. The Pearson chi-square test is based on expected frequencies in a two-entry data set, whereas MCA is a modeling technique to analyze associations in multi-entry data set. All analyses were conducted using SAS software (SAS, 1989).

Results and Discussion

A total of 372 alleles were scored from the 230 accessions using 26 polymorphic microsatellite markers. The allelic size ranged from 89 to 276 bp and the number of alleles per locus oscillates from 10 to 24 (average of 14.3 alleles per locus). A total of 194 specific alleles were identified from the total number of 372 alleles, thus 52% of alleles are specific and only 48% are shared among different rice types. At least one specific allele is found per each SSR tested in the population analyzed. The large number and origin diversity of wild *Oryza* accessions used, in

particular of *O. rufipogon*, account for the largest number of specific alleles (123 specific alleles) found in this study, followed by the weedy rice accessions with 19 specific alleles, indica rice with 16 specific alleles, rice landraces with 10 specific alleles and japonica rice with the lowest number of 6 specific alleles. On the other hand, despite the weedy rice group was represented by the largest number of accessions (148), this population displayed no more than 19 specific alleles of the total 162 alleles detected in the population.

Multiple correspondence analyses using 26 SSRs (MCA-M) indicated that 93.6% of the variability is represented by 12 groups (Figure 1). The largest group (Group 1) included all accessions of weedy rice, *O. sativa* indica type varieties and manual crosses between the rice and selected weedy rice accessions, 40% of landraces and one accession of *O.nivara* (China), one cross *O.nivara/O.rufipogon* (China), *O.rufipogon* (Taiwan), *O.rufipogon* (Myanmar) and *O rufipogon* (Malaysia). Group 2 is composed by 60% of landraces and all accessions of *O. sativa* japonica type varieties. Group 3 contained six accessions of wild species: *O. nivara* (China), five accessions of *O.rufipogon* from China (2), India(1), Cambodia(1), New Guinea(1). The other nine groups are composed by individual accessions (one per each group) of *O. glumaepatula* (Costa Rica), *O.rufipogon* (two different accessions from China, one from India and one from Bangaldesh), one cross *O. nivara/O. rufipogon* (China), O. *rufipogon* (China), *O. rufipogon* (India), *O. barthii* (Chad), *O. glaberrima* (Africa). The variability obtained in the MCA-M analyses, could be explained by 12 SSRs alleles, all of them which are specific to wild species. It is important to highlight that the weedy rice accessions clustered with all the indica rice varieties and with accessions of *O. nivara*, *O. rufipogon* and one cross *O. nivara/ O. rufipogon*, but with none of the japonica rice accessions. A better resolution of the composition of these groups within the weedy rice and landraces population is obtained when the analysis is expanded to include 99% of the variability (generating 28 groups, Figure 2). The manual crosses between rice and weedy are separated from the main weedy rice group probably due to the high presence of heterozygote individual patterns. Thus, Colombian weedy rice appears to be genetically closer to *O. sativa* indica type varieties. Vaughan *et al.* (2001) also reported that all of the weedy rice accessions were genetically related with indica type varieties, except the accession MS5 which was closer to japonica varieties. Additionally, the analysis showed clear similarity between some accessions (60%) of the Colombian landraces and japonica varieties suggesting a possible origin from *O. sativa* japonica type for these Colombian landraces. The addition of morphological traits to the multiple correspondence analyses did not add more discrimination among the groups analyzed.

Conclusions

Multiple correspondence analyses using the selected polymorphic 26 SSRs markers suggest that the Colombian weedy rice are more genetically related to indica type varieties. Additionally, Colombian landraces appear to have a japonica rice type origin.

References

Bres-Patry, C., Lorieux, M., Clément, G., Bangratz, M. and Ghesquière, A. 2001. Heredity and genetic mapping of domestication-related traits in a temperate *japonica* weedy rice. Theor. Appl. Genet. 102: 118-126.

Dunand RT. 1988. Red rice—its impact on grain quality and its cultural control: a review of research in Louisiana, 1960-1982. Baton Rouge, LA: Louisiana Agricultural Experiment Station. 18 p.

Cho, Y-C., T-Y, Chung and H.-S. Suh. 1995. Genetics characteristics of Korean weedy rice (*Oryza sativa* L.)by RFLP analysis. Euphitica 86: 103-110.

Diarra, A., R. Smith. and R. Talbert. 1985. Growth and morphological characteristics of red rice (Oryza sativa) biotypes. Weed Science 33: 310-314

González, E. , J. J. Vásquez, , P. Ruiz, E. Corredor, L. F. Fory, A. Mora, J. Silva, M. C. Duque, and Z. Lentini. 2002. Molecular characterization of rice and rice wild/ weedy relatives using microsatellites and their use to assess gene flow in the Neo-Tropics. Poster. The 7th International Symposium on the Biosafety of Genetically Modified Organisms. Beijing, China. October 10-16, 2002.

Lentini Z and A.M. Espinoza. 2005. Coexistence of Weedy Rice and Rice in Tropical America: Gene Flow and Genetic Diversity. Chapter 19. p: 303-319. *In:* J. Gressel (Ed.). "Crop Ferality and Volunteerism: A Threat to Food Security in the Transgenic Era?". CRC Press. Boca Raton, FL

Llanos R, Mejia E, Ojeda N. 1993. El arroz rojo (*Oryza sativa* L.) en Santa Cruz: un diagnostico multidisciplinario. Estudio de Campo no. 6. Santa Cruz, Bolivia: CIAT/Banco Mundial. 45 p.

McCouch, S. R., G. Kochert, Z. Y. Wang G. S., Khush, W. R. Coffman & S. D. Tanksley. 1988. Molecular mapping of rice chromosomes. Theoretical Applied of Genetics 76: 815-829.

Montealegre FA, Vargas JP. 1989. Efecto de algunas praticas culturales sobre la poblacion de arroz rojo y los rendimientos del arroz comercial. Arroz 38(359):19-24

Mortimer M, Pandey S, and Piggin C. 2000. Weedy rice: approaches to ecological appraisal and implications for research priorities. Wild and weedy rice in rice ecosystems in Asia - A review. p:97-105.

Noldin JA. 1988. Controle de arroz vermelho no sistema de semeadura em solo inundado Lavoura Arrozeira 41(377):11-13.

Noldin JA. 2000. Red rice status and management in the Americas: Wild and weedy rice in rice ecosystems in Asia - A review. p:21 - 25.

Ortiz A, Lizaro J, Lopez L. 1997. Caracterizacao morfologica de ecotipos de arroz vermelho e variedades de arroz na Venezuela. Anais da Reuniao da Cultura do Arroz Irrigado 22:475-477.

Pedreiros L, Alvarado A. 1990. Arroz rojo en Chile. Investigacion y progresso agropecuario Quilamapu 46:3-7.

Rai BK. 1973. The red rice problem in Guyana. PANS 19:557-559.

Ruiz, P., J. J. Vásquez, E. Corredor, E. González, L. F. Fory, A. Mora, J. Silva, M. C. Duque, and Z. Lentini. 2002. Gene Flow Analysis from rice into wild/weedy relatives in the Neo-Tropics: Morphological and phenological characterization of red rice. Poster. The 7th International Symposium on the Biosafety of Genetically Modified Organisms. Beijing, China. October 10-16, 2002.

Ruiz, P. 2003. Morphological, phenological and genetic characterization of weedy rice collected in rice farmers fields of Colombia. Part II. Fields planted with Fedearroz 50, Coprosem and Cimarrón rice commercial varieties. B.Sc Thesis. Universidad Javeriana, Bogota. 186 p

SAS, I.I. 1989. SAS/STAT. User Guide, Version 6, Fourth edition, Volumen 2. Cary, N.C: S.A.S. Institute Inc. p 864.

Vasquez, J.J. 2002. Morphological, phenological and genetic characterization of weedy rice collected in rice farmers fields of Colombia. Part I: Field planted with Oryzica 1 rice commercial variety. B.Sc Thesis. Universidad de Los Andes, Bogota. 210 p

Vaughan L.K., Ottis B.V., Prazak-Havey A.M., Bormans C.A., Sneller C., Chandler J.M and Park W.D. 2001. Is all red rice found in commercial rice rally *Oryza sativa*?. Weed Science 49: 468-476.

Vaughan, D., Morishima, H. and Kadowaki, K. 2003. Diversity in the *Oryza* genus. Current Opinion in Plant Biology. 6: 139-146.

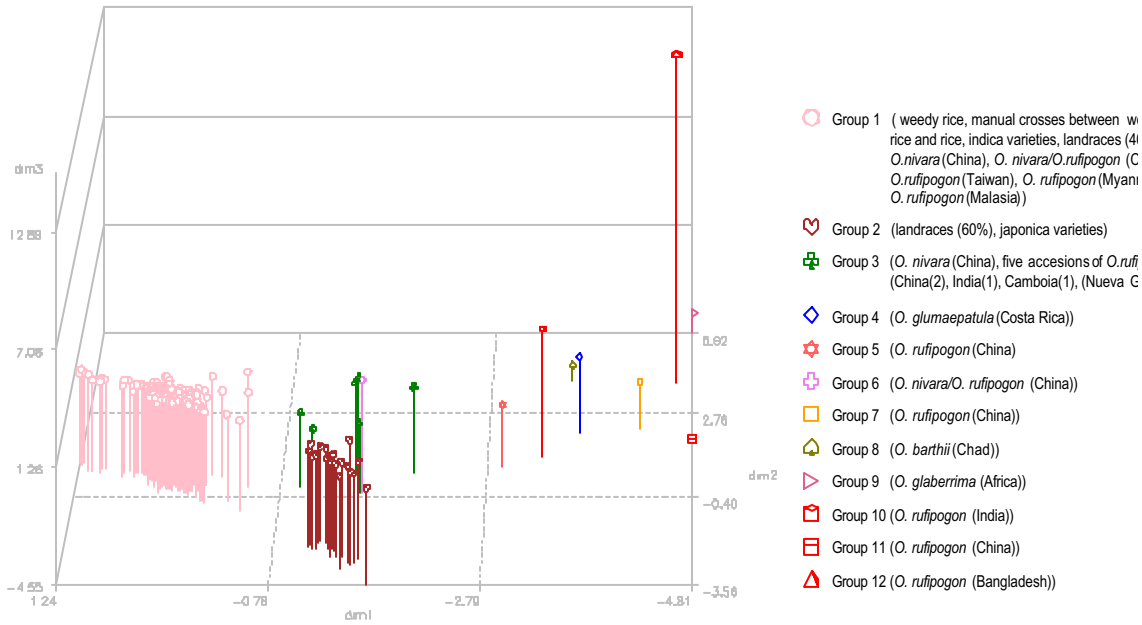

Figure 1.- Multiple correspondence analysis (MAC) using 26 SSRs markers. Group 1 (weedy rice, manual crosses between weedy rice and rice, inc varieties, landraces (40%), *O.nivara* (China), *O. nivara/O.rufipogon* (China), *O.rufipogon* (Taiwan), *O. rufipogon* (Myanmar), *O. rufipogon* (Malasia)). Group 2 (landraces (60%), japonica varieties). Group 3 (*O. nivara* (China), five accesions of *O.rufipogon* (China(2), India(1), Camboia(1), (Nueva Guinea(1)). Group 4 (*O. glumaepatula* (Costa Rica)). Group 5 (*O. rufipogon* (China). Group 6 (*O. nivara/O. rufipogon* (China)). Group 7 (*O. rufipogon* (China)). Group 8 (*O. barthii* (Chad)). Group 9 (*O. glaberrima* (Africa)). Group 10 (*O. rufipogon* (India)). Group 11 (*O. rufipogon* (China)). Group 12 (*O. rufipogon* (Bangladesh)).

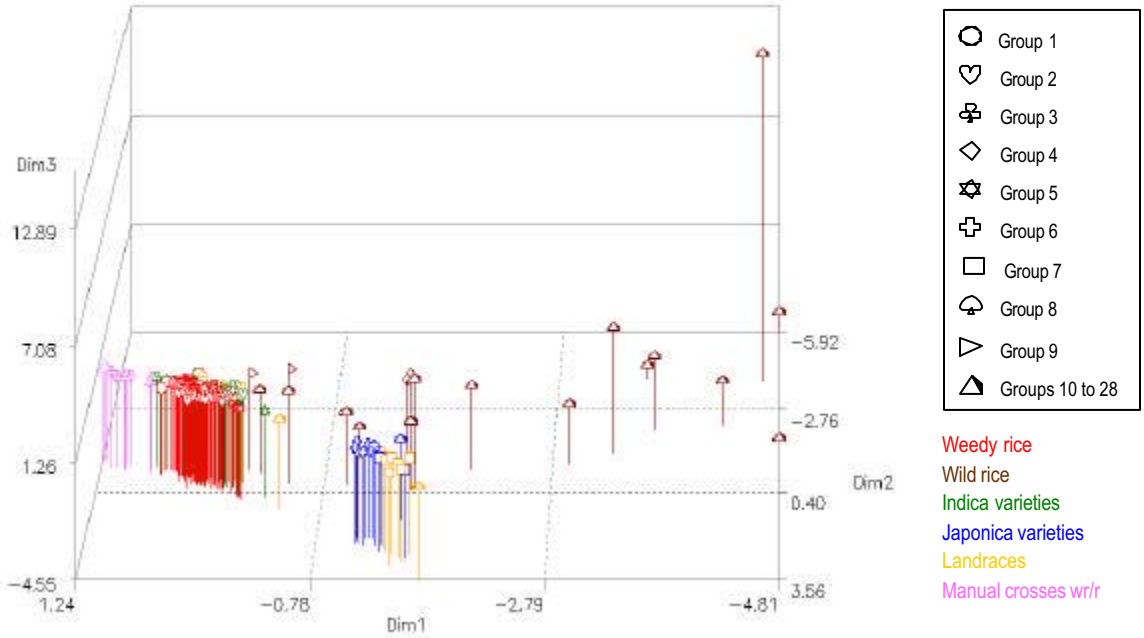

Figure 2.- Multiple correspondence analysis (MAC) using 26 SSRs markers with 230 individuals in 28 groups. Group 1 (weedy rice (48%), one accesion of manual crosses between weedy rice and rice, indica varieties (28%), landraces (13%)). Group 2 (weedy rice (47%), indica varieties (50%), landraces (20%), *O. nivara/O.rufipogon* (China). Group 3 (manual crosses between weedy rice and rice (63%) and Cica 8. Group 4 (manual crosses between weedy rice and rice (31%). Group 5 (weedy rice (4%), indica varieties (19%). Group 6 (japonica varieties (82%), landraces (13%)). Group 7 (landraces (40%), (japonica varieties (18%)). Group 8 (two accesions of *O.rufipogon* (China and Camboia). Group 9 (two accesions of *O.rufipogon* (Taiwan and Myanmar). Group 10 to group 28 are composed by one accesion: one accesion of landraces, *O.rufipogon* (Nueva Guinea), O.nivara (China), *O. rufipogon* (Malasia), *O.nivara* (China), *O. rufipogon* (India), *O. rufipogon* (China), one accesion of weedy rice, one accesion of japonica variety, one accesion of landraces, *O. glumaepatula* (Costa Rica), (*O. rufipogon* (China), *O. nivara/ O.rufipogon* (China), *O. rufipogon* (China), *O. barthii* (Chad), *O. glaberrima* (Africa), *O. rufipogon* (India), *O. rufipogon* (China), *O. rufipogon* (Bangladesh).

- **Scaling up analysis gene flow analysis from rice into weedy rice at landscape under farmers' commercial conditions**

Part I.
SSR, an alternative for the gene flow evaluation from Clearfield CF205 ® to weedy rice.

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Abstract

This report shows the SSRs molecular characterization of 114 accessions of weedy rice collected from commercial rice fields after planting 2-3 cycles with the variety Clearfield CF205 ® tolerant to the herbicide Imazapic (IMI) in Tolima (Colombia), and the progress of the corresponding characterization of 187 accessions of weedy rice collected from farmers fields prior and after planting 1 cycle with the same herbicide tolerant variety in Jamundi (Valle del Cauca). This information attempts to standardize a methodology for large scale trace of gene flow from rice into red rice and anticipate the emergence of herbicide tolerant weedy rice.

Key Words: weedy rice, herbicide tolerance, IMI, CF205 ®, SSR, molecular analysis

Background

This report is a part of a series of total of four documents presented in this Annual Report associated with the 2nd phase of a project entitled "Gene Flow Analysis for Environmental Safety In the Tropics", which main goal is to generate baseline genetic information for the development of guidelines on the safe introduction and use of novel agriculture traits (biotechnology derived or not native from the place of introduction), while reducing potential environmental impact on native biodiversity in the Neotropics, using two staple crops, bean and rice, as models. The objective of this second phase is to assess the impact of specific non-transgenic traits on biodiversity (genetic structure of recipient population) due to gene flow over time at landscape in countries that harbor land races, weedy/wild species of these two crops. In the case of rice, herbicide is among the current methods preferred by farmers to control weedy rice, a major bottleneck for rice production in this region. Herbicide resistant rice varieties had been released in several productions sympatric to natural environments harboring native wild relatives of rice. Herbicide resistance in rice here derived from mutagenesis (imidazolinone resistance, Clearfield®) had been bred into elite local materials and released as improved varieties in Central America and Colombia. Because of its easy tracing, herbicide resistance provides an excellent model to evaluate the unintended transfer of traits deployed in the crop by cross-pollination to the sexually compatible weedy rice complex for which the herbicide is used as a form of chemical control (positive selection), and in the wild *Oryza* compatible relatives that are found in natural environments in the crop contact zones (neutral selection).In addition, the use of non-transgenic herbicide resistance source is an ideal case study for the comparison of the same trait in transgenic vs. non-transgenic allowing to elucidate the effects due to the trait itself independently from the gene source. This model will give information on impact of introgress non-transgenic resistance genes that may affect fitness of derived hybrids, invasiveness,

population dynamics and genetic structure of the corresponding wild/weedy. It will also serve for anticipating a potential impact from a transgenic situation. In this report, the genetic profile of the herbicide resistance donor (CF205 ® variety) and the potential herbicide resistance recipient is presented, as well as progress attained to trace the mutated ALS (Acetolactate synthase) gene sequence involved in the resistance to IMI. These mutations can be determined by means of targeting induced local lesion in genomes (Tilling), a methodology utilized to determine mutants in other species as *Arabidopis*, and or identifying SNPs. It is also necessary to standardize the methodologies used in field and laboratory for the weedy rice selection resistant to the herbicide Imazapic, which belongs to the imidazolinone group.

Materials and Methods

Plant Material. The materials used in this study consisted of: 290 accessions of weedy rice and 188 accessions Clearfield CF205 ® variety collected in Tolima and Valle del Cauca. Six biotype (1-3-4, 5-48-2, 5-38-5, 4-12-2, 1-21-3 and 5-36-4) collected in Tolima in 2001, four commercial *indica* type rice varieties (Fedearroz-50, Oryzica I, Cimarrón and Coprosem II), four accessions of wild Oryza species (*O.rufipogon* IRGC-105491 Malasia, *O.rufipogon* IRGC-100916 China, *O. barthii* IRGC-104119 Chad, *O.glumaepatula* Costa Rica) and the cultivated rice *O. glaberrima* IRGC-103544 were used as reference.

Characterization of the weedy rice accessions from Jamundi-Alsacea. In order to identify the alleles of weedy rice, we collected weedy rice samples prior planting for the first time in the field the CF205 variety in the *Jamundi-Alsacea*. The plants were labeled and transplanted under greenhouse conditions. The molecular and morphological characterization of the collected seeds are in progress following similar methodologies previously used to characterized weedy rice accessions collected from the Tolima region in year 2001 prior the commercial introduction of CF205 in Tolima in 2003.

Plot selection, localization of plants and seed harvest. *Four plots planted with CF 205 were identified in Tolima and Valle del Cauca departments. The parameters used for sampling the weedy rice population included: 1) plots with known agronomic history and with high weedy rice infestation up to 60 %; 2) synchronization of flowering between CF205 variety and weedy rice; 3) sampling area larger than 6 ha; 4) in the Tolima, the two plots selected (7 and 20 ha) had two consecutive cropping cycles of CF205. In Tolima (Saldaña and Espinal), weedy samples were collected at random through each plot from weedy rice spread in patches and intermingled and in contact with CF205. In Jamundi, samples were collected at random from an area between 200 and 250 $m^{2\,from}$ each of the two plots. Each plot was sub-divided into squares of 52 m^2 (Figure 1). The samples were collected and located by GPS, and the topographic map constructed in each case. In all cases (Tolima and Jamundi) weedy samples collected flowering panicles were in physical contact with flowering panicles from CF205. Each mother plant was labeled; progeny seeds harvested, leaf tissue samples collected for molecular analyses, and the original plant transplanted from the field to greenhouse conditions.*

DNA extraction and bulk analysis by SSRs The genomic DNA of parental material was extracted from rice leaves according to McCouch (1988). Six or eight polymorphic microsatellite markers

clearly distinguishing weedy rice and Clearfield CF 205 were used to determine the profile of the materials collected.

Statistical analysis. A multiple correspondence analysis (MCA) was conducted. This analysis was conducted using SAS software (SAS, 1989).

Results and Discussion

Multiple correspondence analysis (MAC) using SSRs markers generated eight groups and a clear separation between the weedy rice and the wild species, *O. barthii*, *O. glumaepatula* and *O. rufipogon,* and the African cultivated species *O. glaberrima* (Figure 1). These results are in accordance to the previous report presented herein by González et al. 2006 suggesting that Colombian weedy rice accessions are genetically close related to *O. sativa* species. Weedy rice formed four distinguishable groups (Figure 1). Group 1 included 59 weedy rice (73 %) accessions from Saldaña of which 77 % (62) have awns. Group 2 enclosed all Clearfield CF205 ® individual plants collected from the Saldaña and Espinal rice field; 30 % (14) of the weedy rice accessions from Espinal and three (3) from Saldaña. This group included 98 % of the awn-less weedy rice accessions. Group 3 was composed by 95% accessions from Espinal and awn-less (80 %). Group 4, included accessions No. 10-32 and 11-56 with brown-black hull and awns clustering together with *O. rufipogon* IRGC-105491 (Malaysia) and weedy rice 5-48-2 (clustering with *O.rufipogon* in earlier reports as well) used herein as control representing a weedy rice *O. rufipogon* like. These results support the hypothesis that the weedy rice is a complex of several species of the *Oryza* genus. In the case of population from Jamundí, 149 Clearfield CF205 ® accessions and 180 accessions weedy rice are being evaluated using eight SSRs. The analysis is in progress.

References

Fory L, Corredor E, Agrono T, Gonzalez E, Ordoñez C, Duque M. and Z. Lentini. 2005. Scaling up analysis gene flow analysis from rice into weedy rice at landscape under farmers' commercial conditions. SB-02 Project. International Center for Tropical Agriculture.

González E, Fory L. Vásquez J, Ruiz P, Silva J. Duque M. and Z. Lentini. 2003-2005. Relationships and potential origin of the weedy rice complex in Colombia. SB-2003-2005. Annual Report.

McCouch S, Kochert G, Yu H, Wan Z, Khush G, Coffman W. and Tanksley T. 1988. Molecular mapping of rice chromosomes. Theoretical and Applied Genetics 76: 815-829.

SAS. 1989. SAS/STAT. User Guide, Version 6, Fourth edition, Volumen 2. Cary, N.C: S.A.S. Institute Inc. p864.

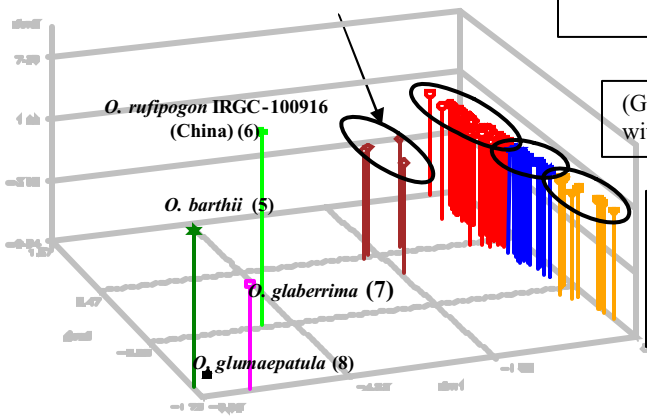

Figure 1. MAC using SSR for 110 accessions of weedy rice collected in Tolima. The numbers in parenthesis correspond to the groups generated.

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- **Scaling up analysis gene flow analysis from rice into weedy rice at landscape under farmers' commercial conditions**

Part II.
Molecular detection of IMI herbicide resistance gene in the Clearfield CF205 ® variety.

L. Fory[1] and Z. Lentini[1, 2]. [1]*SB2,* [2]*IP4.Funding from GTZ, Germany and CIAT*

Abstract

Main objectivity of this work is to trace gene flow from the imazapic herbicide resistant Clearfield CF 205 variety into weedy rice population in farmer's fields in main rice crop areas of Colombia, as a first step to standardize methodologies applicable to environmental biosafety in the tropics. DNA primer sequences were tested and used to detect the herbicide resistant gene. Herein we report the partial sequence of the ALS gene found in CF205 variety, since there is no clear information on the mutant used as progenitor donor of the herbicide resistance.

Key Words: weedy rice, herbicide tolerance, IMI, CF205 ®, ALS sequence analysis

Background

Acetohydroxy acid synthase, also known as acetolactate synthase (ALS), is a key enzyme in the biosynthesis of the branched chain amino acids valine, leucine and isoleucine which are fundamental for the normal development of the plants. The ALS is the target of several classes of herbicides such as sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidyloxybenzoates and sulfonylaminocarbonyl-triazolinones. ALS herbicide resistance is conferred by a single mutation dominant gene. The nuclear gene is inherited via seed and pollen (Tan et al., 2005). The resistance occurs as a result of reduced sensitivity of the target ALS enzyme to inhibition by the herbicide or the resistance can be the result in rapid detoxification of the herbicide. In plants, five highly conserved amino acids (Ala 122, Pro197, Ala205, Trp574, and Ser653) have been identified that when mutated, confer resistance or cross resistance to one or more ALS herbicides (Tranel and Wright, 2002). Two SNPs mutations in the rice ALS gene conferring resistance to the imazethapyr herbicide were also reported in Clearfield 161 variety (CL 161). Of these two mutations, the substitution for amino acids serine to asparagine (codon 653), prevents binding of imidazolinone herbicides to its catalytic site and confers resistance to herbicide (Tang et al., 2005; Rajguru et al., 2005). Up to these investigations, a DNA –based method that involves design and application of allele specific primer using PCR assay to distinguish herbicides susceptible and resistant alleles in homozygous or heterozygous genotypes produced between CL161 and weedy rice have been reported (Kadaru et al., 2006).

Materials and Methods

DNA sequencing and alignment. In order to sequence the plants resistant to the imazapic herbicide, CF205 seeds were placed in a solution of herbicide (25 ppm) during 8 days. Following the methodology described in this annual report (Fory et al., 2006. SB-2 Annual Report 2006),

the plants that survived the application of herbicide were used for the ALS gene sequence analysis. DNA was extracted from young leaves according to McCouch et al. (1998). A polymerase chain reaction (PCR) based approach was used to identify the gene coding ALS sequence in rice. Five primers reported by Rajguru et al. (2005) were used. These primers amplify and locate 2047 pb rice ALS according to Figure 1. The primers were designed on the basis of ALS sequence of rice in the GenBank database (AB049822) (http://www.ncbinlm.nih.gov/). PCR was performed in 25 µL reactions consisting of 1X PCR buffer, 0.2 µM of each dNTPS, 1.5 mM, dNTP's 0.2 mM, primer forward y reverse and 0.3 µL de Taq polymerase. The cycling conditions of 95°C for 2 min, 40 cycles of (94°C for 30s; 55°C or 63°C for 1 min, depending annealing temperature, 72°C for 30 s) and 72°C for 5 min. The amplicons were separated on 1.5 % agarose and all fragments of correct length were purified with a wizard® PCR Clean up system (Promega) The PCR fragments were cloned into the PCR®2.1 (TA Cloning. Invitrogen Life Technologies. USA) following the instructions of the manufacturer. TOPO10 Coli cells were plated on selective LB Medium [1 % (w/v) Tryptone, 0.5 % (w/v) Yeast Extract, 1 % (w/v) NaCl, 1.5 % (w/v) Agar] that contain 50 µg/mL ampicillin, X-Gal and IPTG and grown overnight at 37 °C. One to five recombinant clones were selected and grown over night a 37 °C in liquid LB medium [1 % (w/v) Tryptone, 0.5 % (w/v) Yeast Extract, 1 % (w/v) NaCl]. Plasmid DNA was extracted from each of the clones with a Wizard® minipred DNA purification system. The insertion of the PCR fragment into the plasmid vector was confirmed with Eco RI digestion. This enzyme restriction released the fragment cloned. DNA fragment was sequenced using T7 and M13 reverse flanking the multiple cloning sites. The ALS sequences were compared with sequences in the GenBank using the BLASTX algorithm to confirm similarity. The sequences were aligned using NT-Vector 10.

Allele specific polymerase chain reaction (AS-PCR). Seven weedy rice biotypes collected in Tolima (11-41, 11-73, 11-99, 11-113, 11-121, 11-123 and 10-123) and two Clearfield CF205 ® accessions were used. DNA was extracted from young leaves according to McCouch et al. (1998). The cycling conditions were 95°C for 2 min, 27 cycles of (95°C for 15 s, 60°C for 15 s, 72°C for 15 s) and 72°C for 5 min (Kadaru et al., 2006). For the standardization, we changed the temperature and number of cycles. The products were observed by electrophoresis on a 2 % agarose gel and non-denaturing 6.0 % acrylamide bis acrylamide (19:1) polyacrilamide gel stained with silver.

Results and Discussion

Three of five combinations, with specific homology at ALS gene were cloned in vector TA cloning. The clones between 320 to 530 pb corresponding to PCR product size obtained from several plants were sequenced (Figure 2). The sequences were edited and cleaned of the vector and an assemblage by the Secuencher v 3.0 programs was carried out. A partial sequence of the gene (1164 pb) was obtained after joining all individual sequences. The similarity of the sequence of the Clearfield CF205 ® ALS gene was verified through BLAST-N and BLAST-X using the information displayed on the NCBI web site. The sequences from CF205 were aligned and compared to japonica rice type resistant to herbicide (AB049823) and to japonica rice type susceptible to the herbicide (AB049822). The ALS partial sequence of Clearfield CF205® showed 97% similarity, Score of 2116 and E-value 0.0 with the gene resistance japonica (AB049823) and 98 % similarity and score of 2139 and E-value 0.0 with susceptible japonica

(AB049822). This indicates that the ALS sequence CF205 is highly conserved among cultivated rice. However, change in the sequence may occur as result of rearrangements, insertions and deletions, which may result in intra-specific variations.

Results showed that when comparing the ALS gene terminal region at the aminoacid level the two sequences reported by the GenBank, japonica rice type resistant to imazathepyr (AB049823) and to japonica rice type susceptible to imazathepyr (AB049822) a change of serine for asparagine (codon 653 *A. thaliana* reference) reported by Tranel and Wright (2002) and Tang Rajguru et al. (2005) was observed. But when comparing the CF205 sequence with accession resistant japonica (AB049823), three changes at the aminoacid level are registered on the sequence but the mutation in the codon (654) is the most important where glycine was substituted by glutamic acid. These two changes in the codon 653 and 654 have precisely been associated specifically with the resistant to imidazolinones. The sequences of the progenitor 93AS3510 and PW16, PWC23, CMC29, WDC33 and WDC38 haven been reported. The encoded ALS protein sequence showed that the position of the mutation for 93AS3510 in codon 654 where glycine was substituted by glutamic acid and target site mutation for the PW16, PWC23, CMC29, WDC33 and WDC38 are at codon 653 where serine is substituted by aspargine. The mutation in the (codon 653) position of ALS gene has been reported for X112 maize mutant and the PM1 oilseed rape mutant (Tan et al., 2005). It is important to take into account that the development of rice tolererant to imidazolinones was accomplished by chemically induce mutagenesis of the seed with EMS using rice line 93-AS-3510 and Cypress. Since then, several rice cultivated tolerant to imidazolinones have been developed through breeding programs using 93-AS-3510 and Cypress as the male parent line. CL121 and CL141 were developed from 93-AS 3510 and these lines were the first commercially released in 2001 (Crouhgan 1994). CL 161 and XL8 are mutants directly developed up to the seven lines (PWC16, PWC23, CMC29, CMC31, WDC33, WDC37 and WDC38) with tolerance to IMI. These lines were developed by using a mutated cypress source (Tan et al., 2005; Wenefrida et al., 2004 cited by Leavy, 2004).

There was no discrimination between the susceptible and resistant accessions to the imazapic herbicide (Figure 3). Although a 134 pb fragment was visualized in agarose gel, the amplification was not specific when using the two sets of primers reported by Kadaru et al. (2006). These results were confirmed when visualizing the fragment in polyacrilamide gel and at a higher level of resolution. Some unspecific bands were observed in the polyacrilamide gel. The temperature of aniling and the number of cycles in the PCR was modified. The ALS gene is variable when comparing japonica and indica varieties. Two hundred SNPs between these two types of genome for the ALS gene have been reported. These results indicate that the sequence at the nucleotide level Cl 161 (japonica type) is different from the CF205 variety (indica type), affecting the design of primers. The mutation in CF205 is in the codon 654 whereas the designed SNPs for CL161 japonica variety is in codon 653.

Future Activities

Test the primers designed to detect the herbicide resistance gene in Clearfield CF205 ® , in its progenitors (Yacu 9, herbicide susceptible; and Cypress, herbicide tolerant) in order to confirm its utility for tracing the gene in potential hybrids with weedy rice.

References

Crouhgan, T. 1994. Application of tissue culture techniques to the development of herbicide resistant rice. Louis. Agric. 37 (3): 25-26.

Kadaru S, Yadav A, and Oard J. 2006. Development and Application of PCR-Based Assays for High-Throughput Screening of Aromatic and Herbicide Resistant Rice Department of Agronomy and Environmental Management, LSU Agricultural Center, Louisiana State University, Baton Rouge, LA 70803.

Leavy, 2004. Imidazolinone –Tolerant rice: weed control, crop response, and environmental impact. Thesis PhD. Department of Aronomy and Envionmental Management. Lousiana State University. 60 p.

Madsen, K. H., Valverde, B. E., & Jensen , J. E. 2002. Risk assessment of herbicide resistant crops: A Latin American perspectiva using rice (*Oryza sativa)* as a model. Weed tech. 16 (1): 215-223.

McCouch SR, Kochert G, Yu H, Wan Z, Khush G, Coffman W and Tanksley. 1988. Molecular mapping of rice chromosomes. Theoretical and Applied Genetics 76: 815-829

Rajguru, S., Burgos, N., Shivrain, V. & Stewart, J. 2005. Mutations in the red rice ALS gene associated with resistance to imazethapyr. *Weed Science*. 53: 567-577.

Tan, S., Evans, R., Dahmer, M., Singh, B. & Shaner, D. 2005. Imidazolinone-tolerant crops: history, current status and future. *Pest Manag. Sci*. 61: 246-57.

Tranel, P and Wright, T. 2002. Resistance of weeds to ALS-hinhibiting herbicide: what have we learned? Weed Sci: 50 700-712.

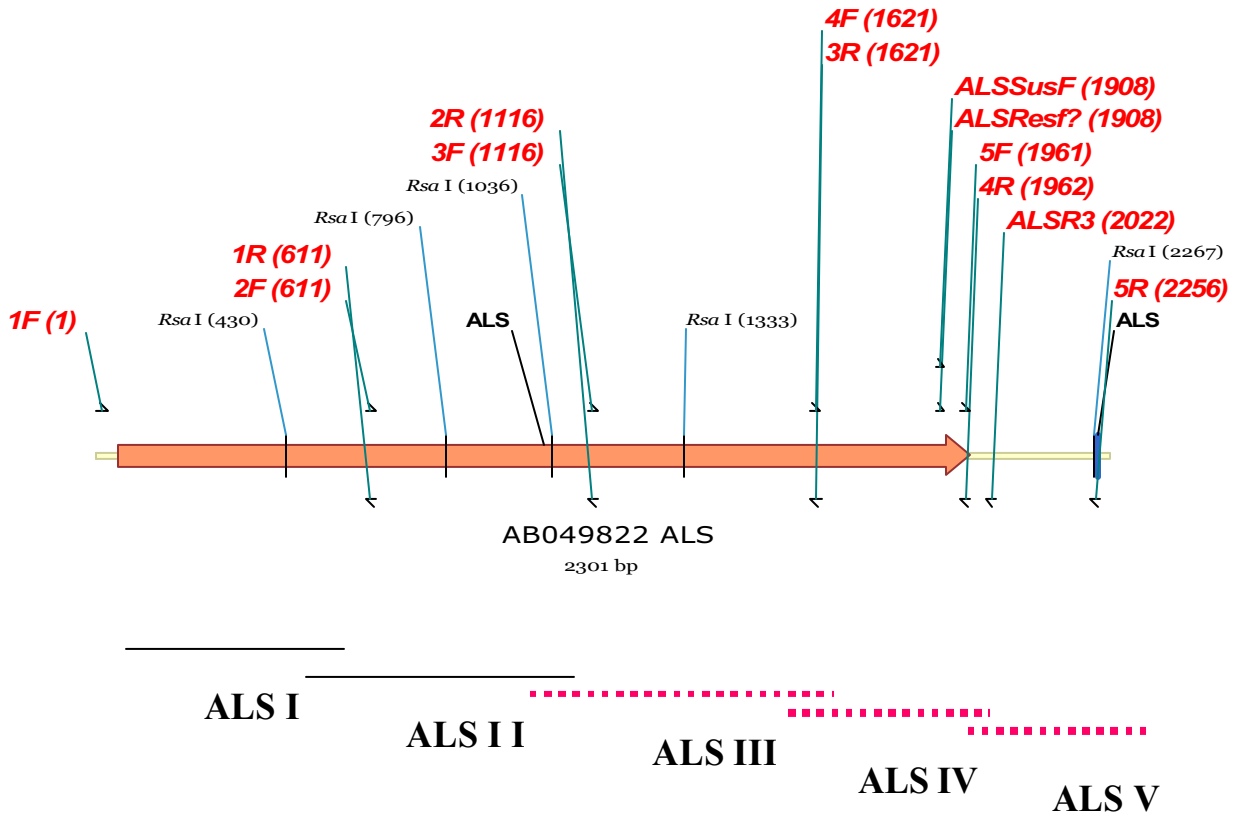

Figure 1. Schematic representation of the ALS gene (AB049822) indicating region amplified by different primers. Red lines indicate the sequenced region. Red letter indicate the primers.

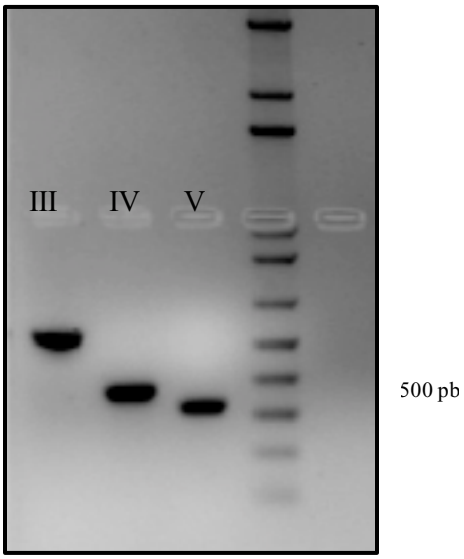

Figure 2. PCR of the *ALS* gene for the regions III, IV and V.

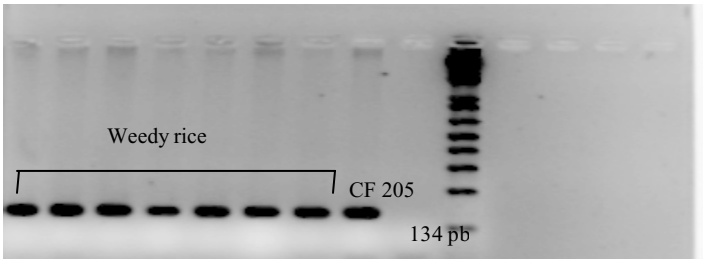

Figure 3. AS-PCR results for *ALS* gene for seven representative weedy rice and Clearfied CF205 ® variety on the 2%

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Part III.
Tilling as an alternative method to evaluate gene flow.

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Abstract

Herein it is reported the use of tilling as an alternative method to trace gene flow from rice into weedy rice by large scale analysis applicable to farmer fields, in this case using IMI herbicide resistance as a model

Key Words: weedy rice, herbicide tolerance, IMI, CF205 ®, SSR, tilling analysis

Background

Targeting induced local lesion in genomes (TILLING) is a reverse genetic method that combines random chemical mutagenesis with Polymerase Chain Reaction (PCR) –based screening of gene regions of interest associated with the hetero-duplex analysis or mismatch cleavage to detect mutations in specified target region. Chemical mutagens generate an allelic series at any target locus, resulting in a change of function, reduced activity or specificity or a knockout mutation (McCallum et al., 2000a; McCallum et al., 200b). Tilling protocols were first implemented by Seattle TILLING Project (STP) for screening ethyl mehanesulfonate (EMS) mutagenized *Arabidopis* populations (Till et al., 2004, McCallum et al., 2000a). TILLING have also been used in *Drosophila melanogaster* (Bentley et al., 2000 cited by Colbert et al., 2001), and maize (Purdue University, Lafayette, IN, USA; http:// genome.purdue.edu/maisetilling/). TILLING is currently being used at CIAT in crops such as rice where gene mutations associated with iron transport are identified. In beans, mutant populations (BAT 93) is being generated with EMS, and in the case of the cassava, it is being used to identify natural mutants involved in starch synthesis. TILLING is a simple methodology and the advantage is that it is not necessary to sequence each individual, DNA from various individual can be pooled together (bulk analysis) to increase throughput. The PCR product is heated and re-annealed allowing forming hetero-duplex between mutant and non-mutant DNA. Hetero-duplex are identified through cleavage of mismatched sites by the Cel I endonuleases (Slade et al., 2005). This plant enzyme has been purified from celery (*Apium graveolens*), and the raw extract is used to cleavage the full length PCR products that then can be visualized by size on agarose or polyacrilamide gel (Raghawan et al., 2006). Since the IMI herbicide resistance gene contained in Clearfield CF205 ® is a variety generated by direct mutagenesis, it is possible to use TILLING for mass analysis of weedy rice populations collected from rice fields and determine if the weed had acquired the imazapic resistant gene from Clearfield CF205 ® through outcrossing.

Materials and Methods

DNA extraction and PCR. DNA was extracted from 100 mg leaf tissue (McCouch et al., 1988). DNA was quantified and diluted to a concentration of 20 ng/µL. To test the optimum pool size for TILLING, tissues of Clearfield CF205 and the variety Cica 8 (herbicide susceptible) were combined at ratios of 1:1, 1:3, 1:5, 1:7, 1:10, 1:20. Alternatively for comparison, Clearfield CF205 DNA was diluted with DNA from the variety IR64, also herbicide susceptible, using the same ration as for Cica 8. Six highly polymorphic SSRs markers distinguishing weedy rice from Clearfield CF 205 were used to determine the optimal DNA pool size for bulk analysis. The ALS gene located in chromosome 2 was the target in this study. The primers used to detect the ALS gene were those designed by Rajguru et al. (2005). The PCR was performed as follows: one cycle at 95°C for 2 min, 40 cycles following the sequence of 94°C for 30s; 55°C or 63°C for 1 min (depending annealing temperature) ending with 72°C for 30 s; and then 72°C for 5 min. The PCR reaction was carried out at a final volume of 25 µL (DNA 50 ng, PCR buffer $MgCl_2$ 1.5 mM, dNTP's 0.2 mM, concentration of primers forward and reverse of 0.2 µM, and 0.3 µL Taq polymerase.

Heteroduplex. The PCR products were denatured at 95°C for 10 minutes and re-natured initially at 85°C for 20 seconds followed by 69 cycles to decrease the temperature up to 25°C step-wise by intervals of 0.1°C per cycle.

Digestion. The celery extract juice produced by CIAT Bean laboratory was used as enzyme source: 10 µl PCR amplified DNA was mixed with 0.4 µL of celery enzyme, 2 µl CEJ buffer (10 mM de HEPES pH 7.5, 10 mM $MgSO_4$, 0.002% Triton X-100, and BSA 20 ng/mL) and 7,6 µL of water per reaction. The digestion was carried out at 45°C for 35 minutes. Enzyme activity was stopped by adding 5 µL EDTA, and incubated at 45 °C for 30 minutes. Digestion products were resolved in 1.5 % (p/v) agarose gel by staining with ethidium bromide.

Results and Discussion

ALS gene sequence of Clearfield CF205 ® variety (reported in this Annual Report by Fory et al. 2006) was compared with those of reference genotypes, IR64 indica rice and Nipponbare japonica rice, genotypes for which complete genome sequences are available (International Rice Genome Sequencing Project 2005). These sequences showed the high level of variation present in the ALS gene between different genotypes.

Six and seven mutations are identified when comparing the ALS gene partial sequence of Clearfield CF205® with those of IR-64 and Nipponbare respectively. The mutations are not located in the same DNA bp supporting the finding of high ALS gene variability between indica and japonica genomes. Although a mutation is associated with the imazapic herbicide resistance in Clearfield CF205®, it may also be possible that other specific mutations could had been generated by the radiation and be present in the variety, thus those Clearfield CF205® specific mutations could also be useful for identifying potential hybrids between Clearfield CF205® and weedy rice as product of outcross in the field..

Once the ALS gene is sequenced, it is possible to determine and confirm the expected fragments by digestion with the Cel I enzyme. SNP detection analysis of Clearfield CF205 ®, IR 64 variety and two weedy rice accessions is shown in Figure 1. Un-digested DNA is shown in the first four rows of each panel (ALS-V and ALS-III PCR amplified regions) indicating the absence of small size fragments as expected corroborating the specificity of Cel I enzyme for cutting hetero-duplex. When the ALS-V PCR amplified region of the ALS gene [region reported by Rajguru et al. (2005)] was digested with Cel I enzyme, two hetero-duplex fragments of 320 pb and 149 pb are observed (Figure 1, tracks 5 and 7) which are not present in the undigested DNA samples of Clearfield CF205® variety and the IR64 variety (Figure 1, left panel tracks 1 and 2), and are specific to Clearfield CF205®. Similarly when digesting the ALS-III PCR amplified region of the ALS gene, in addition to the reference 530 pb fragment, two additional fragments product from the Cel I digestion are resolved corresponding to the hetero-duplex fragments 360 bp and 170 pb, which correspond to the ALS specific mutation present in Clearfiled CF205 ® (Figure 1, right panel tracks 7 and 8). In both cases, the hetero-duplex fragments detected in ALS-V and ALS-III regions are specific Clearfield CF205® and those fragments are present in neither IR64 nor the weedy rice 1-21-3 and 4-12-2. By combining the analysis of ALS-III and ALS-V, three possible specific markers for CF205® are identified, which may or not be associated with the herbicide resistance trait, association that needs to be determine. The CF205® specificity of these hetero-duplex fragments needs to be corroborated in larger populations including increased number of weedy rice accessions.

The CF205® hetero-duplex specific fragments were resolved up to dilution of 1:20 ratio when mixing hetero-duplex DNA with Cica8 DNA. The reproducibility of this result is being tested. Other reports showed that SNP detection in agarose gel can be an efficient method to map gene from parental lines that show low levels of polymorphisms and it is possible to detect the SNPs in pools of 15 samples (1:15 ratio) [Raghawan et al., 2006]. At CIAT, assays in rice to genes associated with iron metabolism indicate that the specific iron SNPs may be efficiently detected in ratios of 1: 16 (Sanabria et al., 2006 SB-02 annual report). In addition to TILLING, the analysis can be complemented with bulk analysis using specific polymorphic SSRs, which already had been used by our research group and demonstrated an efficiency detection at 1: 20 ratio.

Future Activities

To design and evaluate other primers that may detect ALS other gene mutation located in the ALS-IV region and its association with the imazapic herbicide resistant. TILLING protocol needs to be standardized and its reproducibility needs to be confirmed using older leaf tissues from filed samples.

Reference

Caldwell D, McCallum N, Shaw P, Muehlbauer G, Marshall D. and Waugh R. 2004. A structured mutant population for forward and reverse genetics in Barley (*Hordeum vulgare* L.). The Plant Journal 40: 143-150.

Colbert T, Bradley J, Tull J, Tompa R, Reynolds S, Steine M, Teung A, McCallum C, Comai L. and Henikoff S. 2001. High throughput screening for induce point mutations. Plant Physiology. 126: 480-494.

McCallum C, Comai L, Greene E. and Henikoff S. 2000a. Targeted screening for induced mutations. Nat. Biotechnol. 18: 455-457.

McCallum M, Comai L, Greene E, and Henikoff S. 2000b. Targeted induced local lesions in genomes (Tilling) for plant functional genomics. Plants physiol. 123: 39-442.

McCouch S, Kochert G, Yu H, Wan Z, Khush G, Coffman W. and Tanksley T. 1988. Molecular mapping of rice chromosomes. Theoretical and Applied Genetics. 76: 815-829.

Slade A, Fuerstenberg I, Loeffler D, Steine M. and Facciotti D. 2005. A reverse genetic nonotrangic approach to wheat crop improvement by tilling. Nature Biotecnhology. 23: 75-81.

Raghawan C, Naredo E, Wang H, Atienza G, Lui B-. Qiu F, McNally K. and Leung H. 2006. Rapid method for detecting SNPs on agarose gels and its application in candidate gene mapping (in press).

Till BJ, Burtner, C Comai, L. and Henikoff S. 2004. Mismatch cleavage by single-strand specific nucleases. Nucleic Acids Res. 32: 2632-2641.

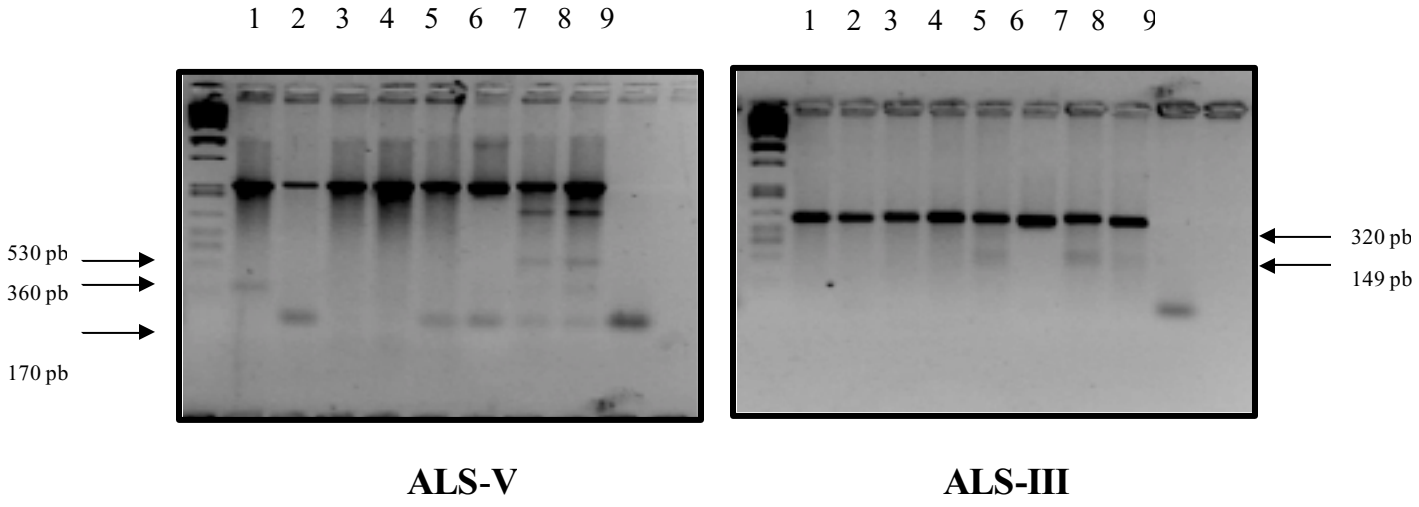

Figure 1.- Match of SNP detection between Clearfield CF205 (CF205) and IR 64 at ALS-V and ALS-III PCR amplified regions of ALS gene. Row 1: CF 205; row 2: IR 64 ; row 3: weedy rice 1-21-3; row 4: weedy rice 4-12-2; from row 1 to 4 undigested DNA. From row 5 to 9, Cel I digestion. Row 5: IR64 + CF205; row 6: IR64 + CF205 not digestion with Cel I; row 7: weedy rice 1-21-3 + CF205; row 8: weedy rice 4-12-2 + CF205; row 9: Control, PCR without ADN. The arrows are indicating the CEL 1 cleaved products SNPs between CF205 and IR64.

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Part IV.
Standardization of herbicide resistance evaluation under field and laboratory conditions using Masterkey DG®.

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Abstract

Following it is reported the standardization of IMI herbicide tolerance evaluation in rice under field and laboratory conditions using the Masterkey DG® system recommended by BASF. The methodology has been adapted to assess at large scale the detection of possible hybrids between the herbicide resistant Clearfield CF205® variety and weedy rice.

Key Words: weedy rice, herbicide tolerance, IMI, CF205 ®, weedy rice, Masterkey DG®, field, laboratory

Background

Three systems of rice herbicide tolerance had been developed in the past decade. Transgenic rice tolerant to glyphosate or glufosinate, and non-transgenic rice derived from mutagenesis with tolerance to imidazolinones (IMI) herbicide had been generated, which IMI rice is the only one commercially available since 2002. Current evidence suggests that out-crossing has led to the formation of IMI resistant weedy rice hybrids in the USA (Gealy, 2005). The IMI herbicide is absorbed by the leaves and roots, and has residual activity in the ground. This herbicide controls different types of weeds, including weedy rice, by inhibition of the acetohydroxyacid synthase enzyme (AHAS, also called ALS) (Tan *et al.,* 2005). In Colombia, the Clearfield System created by BASF and the Louisiana University was used by Fedearroz to develop Clearfield CF205® variety tolerant to the Masterkey DG® herbicide system. Clearfield CF205 ® is derived from crosses made between USA IMI rice variety (herbicide tolerant donor) and Colombia (herbicide tolerance recipient). Clearfield CF205 ® has been grown commercially in Colombia since 2002 in the Tolima and Huila region, and first sown in Jamundi (Valle del Cauca) in 2005. Herbicide tolerance provides an excellent model to evaluate the unintended transfer of crop traits from gene flow by cross-pollination to the sexually compatible weedy rice complex for which the herbicide is used as a form of chemical control (positive selection), and in the wild *Oryza* compatible relatives that are found in natural environments in the crop contact zones (neutral selection). In addition, the use of non-transgenic herbicide tolerance is an ideal case study for the comparison of the same trait in transgenic vs. non-transgenic allowing to elucidate the effects due to the trait itself independently from the gene source. In order to assess the impact of specific non-transgenic traits on genetic structure of recipient population due to gene flow over time at landscape between CF205 variety and weedy rice, a total of 1083 accessions of weedy rice were collected from Clearfield fields in Tolima and the Valle del Cauca in 2005 (Fory et al, 2005. SB2

Annual Report). Here it is reported the evaluation and standardization of IMI herbicide tolerance for rice under field and laboratory conditions using the Masterkey DG® system recommended by BASF, in order to assess gene flow in the F1 generation of self-progeny seeds derived from the original weedy rice collected in the field. Herbicide tolerance evaluation in the field requires more labor than in the laboratory, reason why in addition of the proposed field evaluation attempts were also conducted to establish a laboratory protocol.

Materials and Methods

Standardization of the evaluation system of the Masterkey DG® herbicide under field conditions. The experimental materials used in this preliminary study consisted of 28 weedy rice accessions that were collected in Tolima and Huila (15 accessions) in 2001, and in Jamundí (13 accessions) in 2005, in both cases prior the release and cultivation of Clearfield CF205 ® put under the action of the herbicide. These materials were chosen according to their genetic and morphologic diversity (Lentini and Espinoza, 2005; Fory et al. 2005; Hernandez, 2006). Six commercial rice varieties (Cica 8, Fedearroz 50, Oryzica 1, Cimarron and Coprosem II) and the variety Clearfield 205 were used as controls.

Experimental design and treatments. Replicated randomized split-plot design was used with a total of four treatments and three repetitions by treatment. Each repetition was subdivided in four seed beds (6 m long by 1,2 ms wide, row size). Fifty (50) seeds per each accession were sown per rows and 10 cm between rows. Treatment consisted of various number of herbicide applications at different times of crop cycle: 1) One application of imazapic at pre-emergence (PRE) followed by one at post-emergence (POST) 15 days after seed sown (DAS). 2) One POST at DAS. 3) Two POST applications at 15 and 25 DAS. 4) No herbicide application, control. Imazapic herbicide was applied using a concentration of 115g/ha according to the manufacturer recommendation (BASF, 2003). Herbicide was diluted in water, and a nonionic surfactant was added to solution at 0.5 %. The herbicide was applied by aspersion with a backpack sprayer A-Z at a height of 1,20 m. Clearfield CF205 ® and the susceptible rice commercial varieties were included three timed in each repletion to assess the efficiency of the herbicide application. The number of alive and dead plants was recorded at 15 days, 25 days and 35 days after the herbicide final application (DAA). Plant toxicity was evaluated at 15 and 25 DAA, following the ordinal herbicide susceptibility scale established by Finol et al. (1999). Score 1 refers to zero damage, plants similar to non-herbicide application control. Score 3, refers to moderate damage, characterized by general plant chlorosis; and score 5 refer to severe damage indicated stunted plants or death.

Evaluation of herbicide tolerance under laboratory conditions. The variety Cica 8 (susceptible control) and Clearfield CF205® (tolerant control) were used. Surface sterilized seeds were sown on either germination paper or standard paper towel. Four doses of herbicide (0, 5, 15 and 25 ppm) were evaluated in two treatments: 1) Seeds were imbedded in the herbicide solution (50 mL) by 1 hour followed by two washes with distilled water, then incubated for germination in the dark on either germination paper or standard paper towel damped with water. 2) Seeds were sown on either germination paper or standard paper towel and then damped with the herbicide solution by 16 hours or 8 days, after which were transferred and incubated for germination in the

dark on either germination paper or standard paper towel damped with water. Treatments were arranged using a completely randomized block design with three repetitions of 20 seeds per treatment. The percentage of plant emergence and survival were recorded at 7 and 15 DAS. Plants that survived the treatment were transplanted to pots in the greenhouse and their survival (%) was recorded at 21 DAS. The percentage of absolute reduction in survival was calculated and referred as %R = [(T - X) x 100]/T, where X refers to the survival of the control (without herbicide) and T the survival of the treatment.

Results and Discussion

Clear cut visible damage caused by the herbicide in susceptible plants (including susceptible controls) was observed 10 to 15 DAS. Symptoms included chlorosis, withering and some cases plant death. As expected, the resistant CF205 variety did not show any damage symptoms, although seed germination without herbicide treatment was lowered than the other commercial verities. No significant differences were observed between the different treatments in the field. In all the cases, 80% mortality was noted (Figure 1). Similar damaged were observed at 15, 25 and 35 DAA, which indicates that the herbicide is highly effective on weedy rice and susceptible varieties Cica 8, Fedearroz 50, Oryzica 1, Cimarron and Coprosem. Other reports had indicated that weedy rice (97 %) is controlled with another IMI herbicide (imazathapyr) and no differences are observed between the imazethapyr PRE y POST applications (Leavy, 2004). It is important to highlight that in the control treatment (without application) the natural mortality of the weedy rice and commercial varieties including the resistant variety did not surpass the 16%, which indicates that the test was not affected by external factors that may influence the normal development and growth of in the field. It is also important to highlight that one weedy rice accession (No 9-11) collected from Jamundi showed some tolerance to the herbicide about 40 % survival indicated by the recovery of plants after treatments. This tolerance should be subject of future studies, since in this case a cross-herbicide resistance may be present since this accession was collected prior IMI herbicide had been used for the first time in that location and other reports suggest the possibility that other herbicides may have the same mode of action.

In the case of the laboratory treatment, higher germination was obtained when using paper towel than germination paper without herbicide. It appears that the germination paper has some toxic inhibitory substance that is released after watering the paper. Herbicide treatment did not affect the percentage of germinating seeds in neither susceptible variety Cica 8 nor the tolerant Clearfield CF205 ® . From 90% to 100% reduction in plant survival of the susceptible control was obtained by sowing the seeds on standard paper towel, damped it with the herbicide (5, 15 and 25 ppm) solution for 8 days, and then transferred the seeds onto fresh paper damped with water and incubated in the dark. Similarly studies made in Brazil using transgenic seeds of resistant rice to ammonium gluphosinate have demonstrated that both methods when dampening the paper with the herbicide in a concentration 0,004% and submerging the seeds in the solution of herbicide 0,4% showed a good control for the selection of resistant seeds to the herbicide (Gilneililge et al., 2003). It has been possible to implement the fast test in the selection of sorghum seeds resistant to imidazolinones, exposing the seeds to a 4.6 p.p.m dose of imazathapyr during five or six days (Beadle, 1998 cited by Gilneililge et al., 2003). The simple, efficient and not expensive methodology described in this report for assessment of tolerance of imazapic offers an alternative when field evaluations cannot be done, and may be more practical since IMI

herbicide has long residual effect in the soil impeding the cultivation of rice in the same plot for about 1 semester.

Future Activities

The herbicide tolerance of F1 generation of self-progeny seeds (41.947 plants) derived from the original weedy rice accessions collected in Tolima and Huila from farmer fields planted with Clearfield CF205 ® will be tested on December 2006 , and the tolerant plants will be assayed molecularly to determine weather or not these tolerant plants contain the IMI resistance gene from Clearfield CF205 ® .

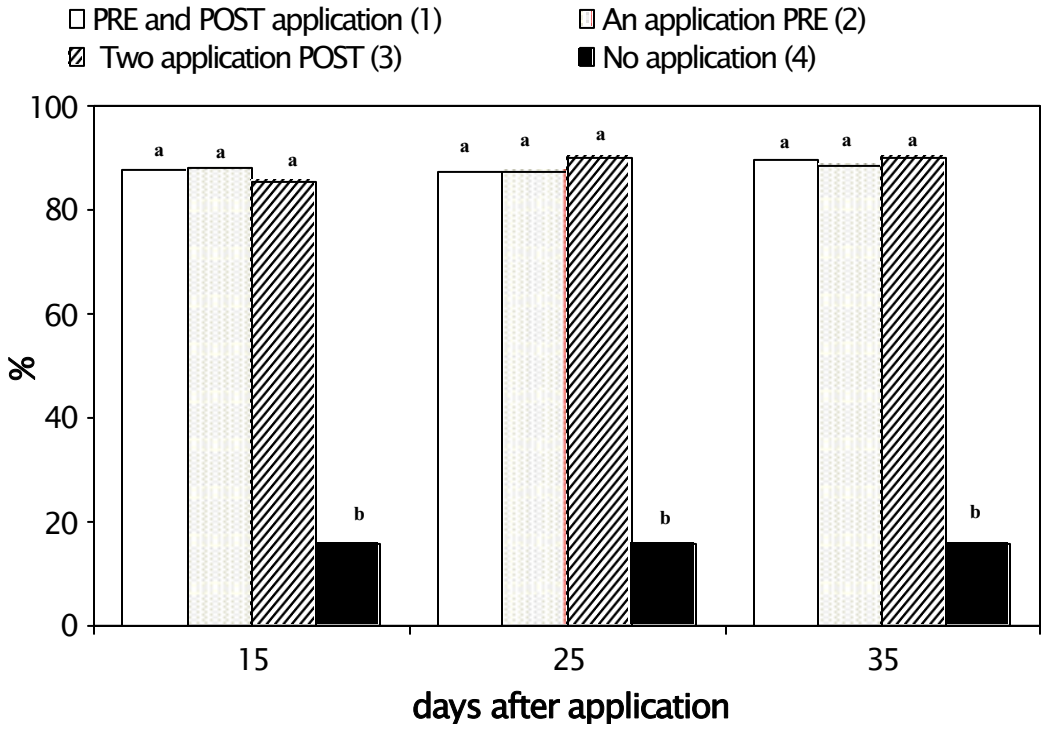

Figure 1. Mortality percentage of plants 15, 25 and 35days after application of the herbicide Masterkey ® under field conditions. Values followed by the same letter are not significantly different (*p=0.05*) Ryan-Einot-Gabriel-Welsch multiple range test.

References

BASF. 2003. Folleto técnico del sistema Clearfield CF 205 ®. Sistema de Producción de arroz que optimiza sus recursos. Pp30

Finol L, Medrano C, Gutierrez W, Gonzalez G, Martinez W, Báez J, Bracho B. and Medina B. 1999. Evaluación de la eficacia del herbicida halosulfuron metil, aplicado solo y en mezcla con acetocloro en tomate *Lycopersicon esculentum* Mill. Rev. Fac. Agron. 16: 266-275.

Fory L, Corredor E, Agrono T, Gonzalez E, Ordoñez C, Duque M. and Z. Lentini. 2005. Scaling up analysis gene flow analysis from rice into weedy rice at landscape under farmers' commercial conditions. SB-02 Project. International Center for Tropical Agriculture.

Gealy, 2005. Gene Movement between rice (*Oryza sativa*) and weedy rice (*Oryza sativa*-a U. S Temperate rice perspective. Edited by Jonathan Gressel, USA. In : Crop ferality and volunteerism. In: Chapter 20: 323-354.

Gilneililge C, Tillmann A, Villela F, Biccadode L. 2003. Identificaco de sementes de arroz tranformado geneticamente resistentes ao herbicida glufosinato de anonio. Revista Brasileira de sementes. 25: 87-94.

Hernández, 2006. Caracterización morfológica y molecular de accesiones de arroz maleza procedentes de campos cultivados con la variedad CF-205 resistente al herbicida IMI. Universidad Francisco de Paula Santander.

Leavy, R. 2004. Imidazolinone –Tolerant rice: weed control, crop response, and environmental impact. Thesis PhD. Department of Aronomy and Envionmental Management. Lousiana State University. 60 p.

Lentini Z. and Espinoza A. 2005. Coexistence of weedy rice in tropical America-gene flow and genetic diversity. Edited by Jonathan Gressel, USA. In : Crop ferality and volunteerism. Chapter 19: 306-307.

Tan S, Evans R, Dahmer M, Singh B. and Shaner D. 2005. Imidazolinone-tolerant crops: history, current status and future. *Pest Manag. Sci.* 61: 246-57.

- **Use of chloroplast DNA polymorphisms for gene flow analysis in rice.**

Part I.
Characterization of wild rice species collected in Colombia and Venezuela.

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Asbstract

This work is part of the project **"**Gene Flow Analysis for Assessing the Safety of GMOs in the Neo-Tropics" directed to analyze the gene flow from non-transgenic or transgenic rice into wild/weedy relatives in the Neotropics, and its effect(s) on the population genetic structure of the recipient species. The objective of this part of the work is to evaluate cp and nuclear DNA regions to quantify gene flow and direction from rice into weedy rice and wild *Oryza* species. This report describes preliminary work of the use of this markers in the characterization of samples collected in Colombia and Venezuela.

Key Words: cpDNA, nuclear molecular markers, gene flow, weedy rice, *Oryza* species

Background

The genus *Oryza* is composed of two cultivated and 21 wild species with 10 recognized genome types (A, B, C, BC, CD, E, F, G, HJ and HJ) (Vaughan et al., 2003). The cultivated species are *Oryza sativa* and *Oryza glaberrima* of Asian and African origin, respectively. Four wild *Oryza* species have been reported in Latin America: four tetraploids (CCDD genome) *O. alta*, *O. grandiglumis* and *O. latifolia*, (Vaughan, 1994), and one dploid (AA genome) *O. glumaepatula*, sometimes described as *O. rufipogon* americana (Vaughan, 1994; Lentini and Espinoza, 2005). All the described diploids species for the American continent have the genome AA likewise *O. glumaepatula*; whereas the *O alta*, *O. latifolia* and *O. grandiglumis* species have the CD genome. *O alta*, *O. latifolia* and *O. grandiglumis,* are tetraploids species very closely related and the key characteristics that distinguish them are not clear. In the Americas, only Costa Rica and Brazil have conducted complete collection and characterization of the wild *Oryza* present thoughout each country. Different molecular methods are available and reported as tools for identifying rice genomes or species. Maternal inherited genome analysis using either mitochondrial DNA (mtDNA) or chloroplast DNA (cpDNA) can be accomplished and complemented by RFLP analysis of PCR amplified nuclear DNA regions (Ge et al., 1999; Buso et al., 2001). One of these markers is the restriction site-polymorphism of PCR-amplified alcohol dehydroenase (Adh) genes and internal transcribed spaces (ITS) (Ge et al., 2001; Ying and Ge, 2003). These molecular tools can be used to facilitate the identification of collected wild rice germplasm, and and also to clarify phylogenetic relationships and provide a rationale for choosing strategies for breeding, and used of genetic resources (Ge *et a*l., 2001). This report describes the use of cpDNA polymorphisms (maternal inheritance) to give a comprehensive understanding of the hybridization and dynamic introgression under field conditions.

Material and methods

The materials used in this study consisted of weedy rice accessions (3) selected as indicators of genetic diversity collected in Colombia based on previous work, the commercial rice varietie CF205, 4 accessions of *O. rufipogon*, *one accession each of O. glaberrima* and *O. barthii* (1), 4 accessions of *O. glumaepatula* (4), accessions of *O. alta*, (1), *O. grandiglumis* and (3) *O. latifolia* (7). Total DNA was isolated from young leaves according to the method described by McCouch et al. (1988). The PCR was carried out in 50 µL total volume containing the following components: 80 ng of genomic DNA, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 2.0 U Taq polymerase, 1X PCR buffer 0.5 µM of each primers was used. Some primers were redesigned on the chloroplast sequence NC001320 (www.ncbi.nlm.nih.gov/entrez). These primers had been designed to amplify non-coding regions of chloroplast sequences of *Oryza sativa* and *Nicotiana tabaccum* (Demessure et al., 1995; Chacon, 2001). Thirty one endo-nucleases were tested and some enzymes have been reported by Busso et al. (2001) due to their ability to restrict and identify polymorphic fragments. The amplification was carried out using 1 cycle of 2 min at 94°C, 40 cycles of 30 s at 94°C, 30 s at 58°C, at 72°C for 5 min and one cycle of 10 min at 72°C. The PCR products were separated on agarose gel (1.4 %), stained with ethidium bromide. The regions including the ITS were amplified using universal forward and reverse primers (ITS1 and ITS4). The PCRs included the following cycles 1 cycle of 2 min at 94°C, 30-40 cycles of 30 s at 94°C, 1 min at 52°C or 62°C, depending on the annealing temperature and one cycle of 10 min at 72°C. These PCR products were then digested by incubation with 10µL of PCR product with different enzymes at 37°C, for 3 h. The digested amplifications were then separated on 1.5% agarose gel.

Results and Discusion

Fragments between 300 and 2800 bp were amplified using 13 primer combinations. As expected, more polymorphism was found between the AA and CCDD genomes, followed by different species with the same genome. A 400 bp fragment of TrnL-Trnf region sequence was identified in the cpDNA of all seven accessions of CCDD genome species represented by 6 acessions of *O. latifolia,* (including one from Venezuela and two from Colombia), one accession *O. alta* and three accessions *O. grandiglumis*. This fragment was not noted in AA genome diploids in which a fragment of 344 pb was observed (Figure 1). This result indicates that non-restriction PCR amplified fragment of TrnL-Trnf sequence can be used to easily distinguish tetraploid (CD) from diploid (AA). These results were confirmed by means of the amplification and restriction of three regions of chloroplast PsbC [pstI 44 kd protein] and trnS [tRNA-Ser-(UGA)], trnS [tRNA-Ser- (UGA)] and trnfM [tRNA-fMet (CAU)], TrnC [RNA-Cys (GCA)] and trnD [tRNA-Thr-(GGC)] with seven restriction enzymes. These combinations also allowed differentiating the species CD from the AA (data not showed). Three of the four accessions of the *O. glumaepatula* revealed cpDNA polymorphisms in the region CP8 corresponding to the non-coding regions between the amino acid trnS [TRNA-Ser- (GGA)] and trnT [tRNA-Thr (UGU)]. Two polymorphic fragments of 298 bp and 98 pb were observed in *O. glumaepatula* accessions from Costa Rica, Colombia and Venezuela, after digestion with DraI (Figure 2). These fragments also were observed in the sample of *Oryza sp* collected from the "Estero de Camaguan" in Venezuela, while the other species of the genome CD and AA also including *O. glumaepatula* (Brazil) showed a 396 pb fragment. It is important to include more species of *O. glumaepatula* since

three or three ecotypes of this species has been described using genotypic, biochemical and molecular analyses recognizing ecotypes groups for Central America and the Caribbean, the Amazon and the Pentanal region in the Southeastern of Brazil (Akimoto et al., 1998, Vaughan et al., 2003). Only one polymorphic combination in region CP3/ RSA for *O. rufipogon* and the weedy rice was found. This combination allows separating the weedy rice accession 5-48-2 from the other two weedy rice used.

The analysis of similarity using the cpDNA regions allowed a clear separation between the tetraploids and diploids genomes (Figure 3). Nevertheless, although the wild species of *Oryza* and rice are closed related (92 % similarity), with these sequences is possible to identify four groups: the first group contains CD species, *O. alta*, *O. grandiglumis* and *O. latifolia*. Two *O. latifolia* samples collected in Colombia (Meta and Pacifico) and one sample from Venezuela (Portuguesa) are found in this group. The cpDNA sequences analyzed do not allow differentiation among the three CCDD genome species. Nevertheless, studies at molecular level using nuclear and chloroplasts sequences indicate that *O. grandiglumis* and *O. alta* are more close related and this complex is different from *O. latifolia*. Comparison analysis of the ITS region allow separation of *O. alta* and *O. grandiglumis* from *O. latifolia* (Ying and Ge, 2003) when using digestion with restriction enzymes Dra III and Fok I. The second group contains all accessions *O. glumaeputula* from IRRI , Costa Rica, Colombia and Venezuela and the *Oryza* sp sample collected in the "Estero de Camaguán", Venezuela. These species are clearly different from *O. rufipogon*. An interesting results is that one accession of *O. glumaepatula* from Brazil clustered in the same group as *O. glaberrima*. The fourth group contains the species of *O. rufipogon*, *O. sativa* and *O. barthii* and weedy rice accessions. The weedy rice accession 5-48-2 clustered with *O. rufipogon* and *O. barthii.* The accession 5-48-2 weedy rice has been also been associated at molecular and morphological level with the accession *O. rufipogon* (IRGC 105491) in previous studies (Gonzales *et al*., 2003, SB2 Annual Report 2003).

Future Activities

The evaluation of cpDNA, amplified product using other additional restriction enzymes will be tested in order to identify specific polymorphic patterns between varieties, weedy rice and wild *Oryza* species, and the amplified DNA fragment will be sequenced. Other markers allowing differentiating the weedy rice complex, *O. sativa* and *O. rufipogon* will be tested including V-ATPase B-subunit (p-VATPase) region, and a nuclear pseudogene with low pressure to selection. This region has been used in previous works and shown its utility indicating that the two subspecies of rice (indica and japonica) were domesticated from geographically different wild rice gene pool. The identification of specific haplotypes is in progress.

References

Akimoto M, Shimamoto Y. and Morishima. 1998. Genetic differentiation in *Oryza glumaepatula* and its phylogenetic relationships with other AA genome species. Rice Genet. Newsl 14:37-39.

Buso G, Nakano R. and Ferreira M. 2001. Analysis of random and specific sequences of nuclear and cytoplasmic DNA in diploid and tetraploid American wild rice species (*Oryza* spp.). Genome 44: 476-494.

Chacon, M. I. 2001. Chloroplast DNA polymorphisms and the evolution and domestication of the common bean (*Phaseolus vulgaris L.*). Thesis. Doctor of Philosophy. Department of Agricultural Botany, School of Plant Sciences, The University of Reading.

Demuesure B, Sodzi N. and Petit R. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Molecular Ecology 4: 129-131.

Ge S, Sang T, Hong D. 1999. Phylogeny of rice genomes with emphasis on origin of allotetraploid species. *Proc. Natl. Acad. Sci: 96: 14400-14405.*

Ge S, Sang T, Bao-Rong L, and Hong D. 2001. Rapid and reliable identification of rice genomes by RFLP analysis of PCR-amplified adh genes. Genome 44: 1136-1142.

Lentini Z. and Espinoza A. Coexistence of weedy rice in tropical America- gene flow and genetic diversity. Edited by Jonathan Gressel, U.S.A. 2005. In: Crop ferality and volunteerism, Charter 19. p 306-307.

McCouch S, Kochert G, Yu H, Wan Z, Khush G, Coffman W. and Tanksley. 1988. Molecular mapping of rice chromosomes. Theoretical and Applied Genetics 76: 815-829.

Vaughan D. 1994. The wild relatives of rice — a genetic resources handbook. International Rice Research Institute (IRRI); Philippine.

Vaughan D. Morishima H., Kadowaki K. 2003. Diversity in *Oryza* genus. Curr Opin Plant Biol 6: 139-146.

Ying, B. and Ge S. 2003. Identification of Oryza Species with the CD genome based on RFLP analysis of nuclear Ribosomal ITS Sequences. Acta Botánica Cínica 45 (7): 762-765.

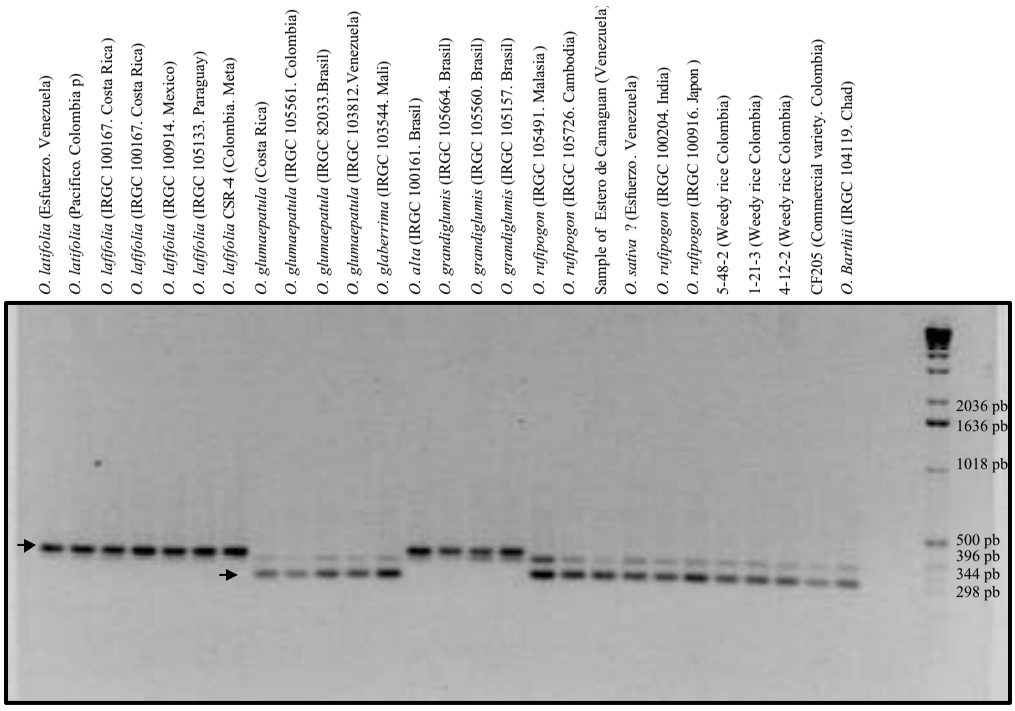

Figure 1. Amplification of cpDNA TrnL-Trnf sequence of several *O. sativa* wild species, weedy rice and rice.

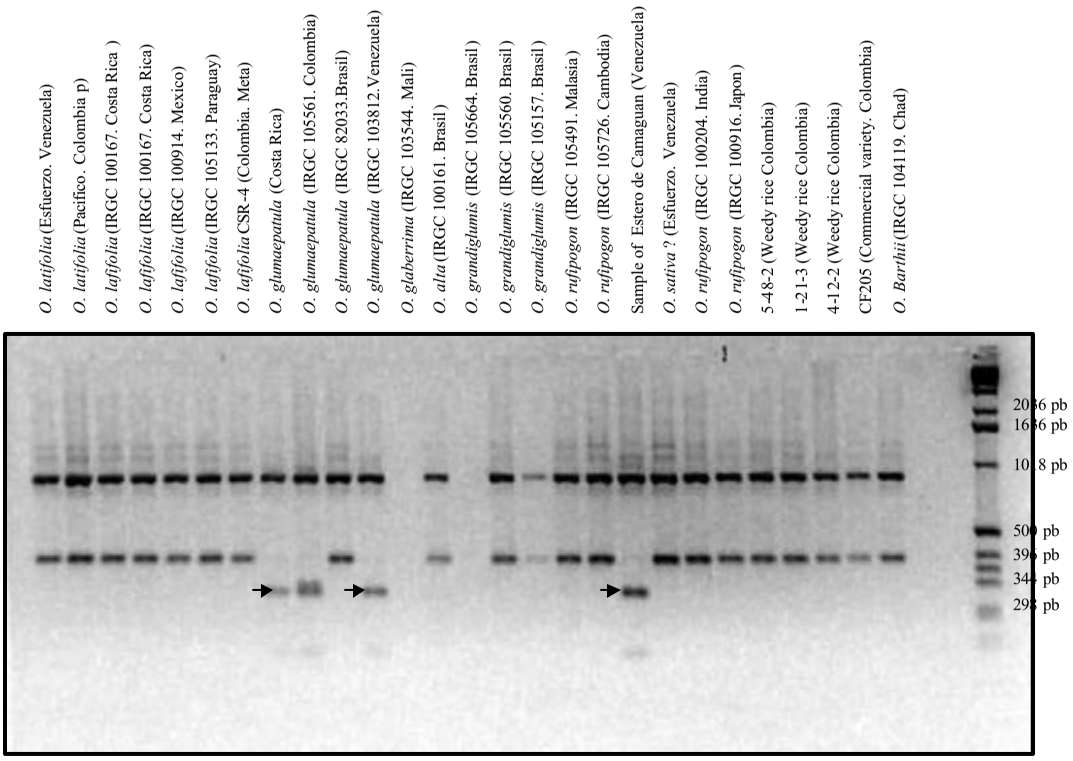

Figure 2. Amplification of trnS [TRNA-Ser- (GGA)] and trnT [tRNA-Thr (UGU)] sequence of several *O. sativa* wild species, weedy rice and rice.

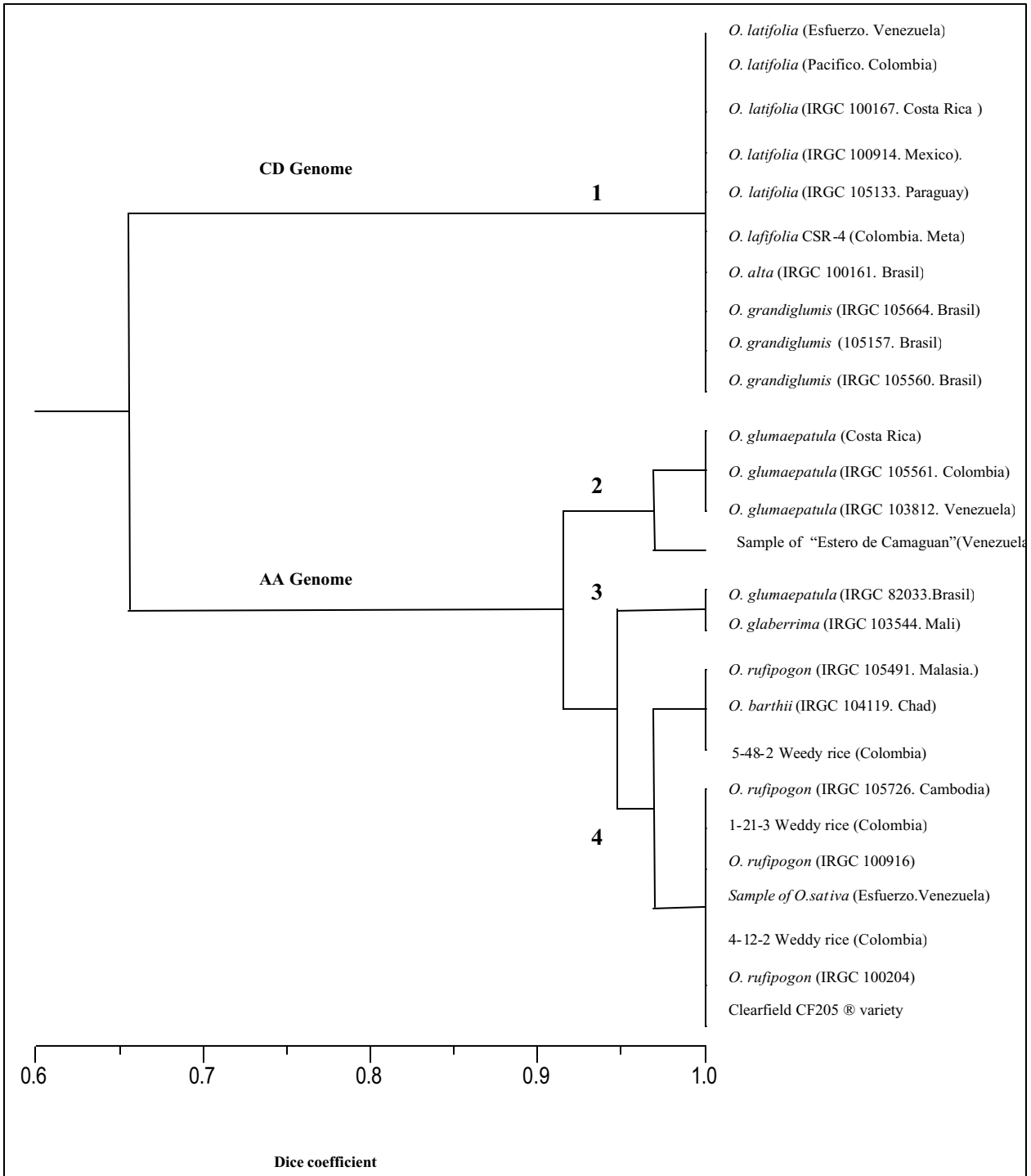

Figure 3. Genetic similarly based on chloroplast polymorphisms using *O. sativa*, weedy rice, *O. latifolia*, *O. grandiglumis*, *O. alta* and *O. glumaepatula.*

- **Use of chloroplast DNA and nuclear sequences for characterization of weedy rice. Part II. characterization of weedy rice species collected in Colombia.**

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Abstract

The objective of this work is to standardize methodology for bulk analysis of large number of field samples allowing discerning weedy rice and wild *Oryza* species, and direction of gene flow with cultivated rice, as pre-requisite for completing environmental safety dossier of potential release of transgenic rice in the Neo-tropics..

Key Words: cpDNA, nuclear molecular markers, gene flow, weedy rice, *Oryza* species

Background

Several hypotheses exist about the origin of the weedy rice complex. One of them suggests it has arisen from hybridations between the subspecies *O. sativa* japonica and indica types, since weedy rice showed intermediate characteristic to both groups. Another theory suggests that weedy rice evolved from wild *Oryza* species or by degeneration of the cultivated rice form present in marginal no cultivated sites (Suh et al., 1997 Bres-Patry et al., 2001, Lentini and Espinoza, 2005). Some markers that had been used by other groups for taxonomy and phylogeny analysis of rice were selected in this study for the characterization and identification of the sample populations. The amplification of the chloroplast sequence ORF100 using the combinations of primers reported by Sun et al (2002) and Garris et al. (2005) was standardized. This sequence has been used to differentiate between indica and japonica type varieties, and also between tropical and temperate japonica cultivars at the cytoplasmic level (Chen et al. 1993). The current report showed the characterization of 251 Colombian weedy rice accessions using cytoplasmic markers that correspond to the sequences of the ORF100 region. The RFLPs of cpDNA and specific nuclear sequences (Ge et al., 2001 and Ying and Ge, 2003) were used to identify the genome type and ploidy level, and discern if either any of the American Oryza species (*O. latifolia, O. grandiglumis, O. alta and O. glumaepatula*) maybe part of the weedy rice complex. Additionally 5 cp-SSRs that show intra- e inter-specific variation in AA genome were used in the characterization of the weedy and wild rice accessions. The combined data of cytoplasm and nuclear genome profiles would help elucidate the potential origin of this weedy rice population.

Material and methods

Plant materials. Accessions of weedy rice (251) collected in Huila and Tolima in 2001 and 2005, Clearfield CF205 ® accession (40) collected in Clearfield field commercial rice also include indica (22) and japonica (12) rice varieties types, *O. rufipogon* (8), *O. glaberrima* (1), *O. barthii* (1), *O. glumaepatula* (1), *O. alta*, (1), *O. grandiglumis* (1), *O. latifolia* (3), two cross of O. nivara /O. rufipogon (2) and O. nivara (2). Total DNA was isolated from young leaves according to the method described by McCouch et al. (1988).

cpSSR. The molecular analysis was conducted with five cp-SSRs chloroplast markers (RCt1, RCt3, RCt5, RCt8 and RCt9) reported by Ishii et al. (2001). PCR was performed in 20 µL containing: 40 ng of genomic DNA, 0.75 mM dNTPs, 1.8 mM $MgCl_2$, 1.U Taq polymerase, 1X PCR buffer 0.5 µM of each primers. The amplification was carried out using 1 cycle of 3 min at 94°C, 34 cycles of 15 s at 94°C, 15 s at 55°C, at 72°C for 15 s and one cycle of 5 min at 72°C. The PCR products were resolved on silver-stained polyacrylamide gels and microsatellites alleles were sized by comparison to the 10 and 25 bp molecular weight standards (Promega). Five cp-SSRs and one nuclear-SSR (RM234) were evaluated using accessions of weedy rice (46) and all wild rice accessions and also commercial rice (15) and wild species (18).

ORF100 region and RFLPs- chloroplast. The plastid sequence, which captures the ORF100 region, was amplified as described in Fory et al., 2005 (SB2 Annual Report 2005). The region CP8 corresponding to the non-coding regions between the amino acid trnS [tRNA-Ser- (GGA)] and trnT [tRNA-Thr (UGU)] and TrnL-Trnf F, were amplified following the protocol describe here in Fory et al., 2006. SB2 Annual Report 2006.

Adh-2. The regions including the Adh-2 gene were amplified using universal forward and reverse primers (Adh F1 and adh RR) reported by Ge et. (2001). PCR was performed in 20 µL volume containing the following components: 200 ng of genomic DNA, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 1.U Taq polymerase, 1X PCR buffer 0.1 µM of each primers The reaction was carried out using the following program; cycles 1 cycle of 70°C 4 min, 94°C 1 min, 52°C 30 s 72°C 1 min 30 s, 35 cycle of 94°C 20 s, 55°C 20s, 72°C 1 min 30 s one cycle of 10 min at 72°C. These PCR products were then digested by incubating 10µL of PCR product with EcoNI at 37°C, for 3 h. The digested amplifications were then separated on 1.4% agarose gel.

Results and Discusion

Multiple correspondence analysis (MAC) using SSRs markers generated seven groups. All wild *Oryza* species were distributed in four different groups (Figure 1A). The weedy rice accessions are separated in two groups (1 y 2). Group 1: included 38 accessions, which 31 are weedy rice. Eighty one percent of the weedy rice showed dark hull. This group also included six commercial varieties (Bonanza, Cica 8, Fedearroz 50, IR64, Taducan and Fedearroz 200). One accession of *O. rufipogon* (IRGC 105726) was included in this group. It is important to remark that the accessions that belong to this group had indica type chloroplast. Group 2: include 11 weedy rice accessions seven of which are black. This group enclosed four japonica varieties Moroberkan, Azucena, Bluebelle and Cypress and one variety indica, Cuba 65. All varieties classified as japonica showed japonica type chloroplast. Cuba 65 was the only indica variety included in this group that showed chloroplast japonica type. Group 3: it included the tetraploids *Oryza latifolia* (Pacifico), *Oryza latifolia* IRGC 100167 (Costa Rica) *Oryza alta* IRGC100161 (Brazil) y *Oryza grandiglumis* IRGC 105664 (Brazil). Group 4: contains five (5) *Oryza rufipogon* (IRGC 100204, IRGC 105349, IRGC 100923, IRGC 103823, IRGC 100916), two *Oryza nivara* (IRGC 103821, IRGC 103824), two (2) crosses *Oryza nivara /Oryza rufipogon* (IRGC 103813 y IRGC 103814). The varieties CO25 and Agostano are included in this group. Group 5. *Oryza glaberrima* and one

weedy rice 4-19-2. Group 7: *O. barthii* (Chad) Group 6: *O. glumaepatula* (accession from Costa Rica).
Multiple correspondence analyses (MAC) were conducted using cp-SSR (MAC-cpSSR) and were complemented with one nuclear SSR markers, and ORF100 chloroplast region and seed morphological traits (cpSSR-SSR-ORF100-MT). This total analysis generated eight groups. Wild *Oryza* species were distributed in six different groups (Figure 1B). Group 1: contained 83 % weedy rice accessions, six indica rice type commercial varieties (Cica 8, Fedearroz 50, Fedearroz 2000, Bonanza Taducan and IR64) and one accession of *O. rufipogon* (IRGC 105726). The 100 % of the accessions showed the indica type chloroplast, and 83% and 68% of the accessions had showed straw hull and straw apiculus, respectively. Group 2: it contains seven (7) weedy rice accessions, which six accessions (85 %) had brown hulls and awns and 100 % of the accessions had japonica type chloroplast. This group enclosed four (4) commercial varieties. They were considered a morphologic level as japonica type cultivated rice (Azucena, Bluebelle and Cypress), and one indica type cultivated rice (Cuba 65). Group 3: *O. latifolia* (Colombia) and *O. alta* IRGC 100161 (Brazil) and *O. grandiglumis* IRGC 105664 (Brazil). Group 6: *O. glumaepatula* (Costa Rica). Group 7: one crosses between *O. nivara* and *O. rufipogon* from China (103813). Group 8: *O. barthii* (Chad). Some wild species were clustered together with weedy rice and commercial varieties. For example, the Group 4: contains four *O. rufipogon* accessions (100204 India, 105349 India; 103823 China; 100923 Myanmar) and two *O. nivara* 5 IRGC 103821 (China), and *O. nivara* 7 IRGC 103824 (China), one cross between *O. nivara* and *O. rufipogon* from China (IRGC 103814). This group also included three (3) commercial varieties Agostano, CO25, Moroberekan (BCF 363), and one weedy rice accession 7-10-2. Group 5: *O. glaberrima* IRGC 103544 (Mali), *O. rufipogon* IRGC 100916 and two weedy rice (4-19-2 and 7-3-2). The weedy rice accessions were principally clustered in two groups.

When comparing the analyses of MAC-cpSSR and MAC-cpSSR-ORF100-MT, it is important to note that both analyses separate the majority of wild Oryza species, O. *glumaepatula, O. barthii* and *O. latifolia, Oryza alta* and *Oryza grandiglumis.* Some *O. rufipogon* accessions were clustered with other *O. rufipogon / O.* nivara accessions or with weedy rice, and *O. sativa.* In both analyses some weedy rice accessions were associated with the japonica type varieties, and the rest of weedy rice accessions were indica type. It was also found that 14% of individuals present japonica type chloroplast with ORF100 region. When the ACM analysis was carried out using nuclear SSR (González et al., 2003-2005), the weedy rice was associated mainly with the indica varieties. However, Vaughan et al. (2001) report that one weedy rice accession (MS5) was similar to the japonica type varieties. These results indicate that in addition to indica type, a small proportion of weedy rice may have a different origin in this case of japonica type including 6 chloroplast regions (RCt1 RCt3 RCt5 RCt8 RCt9, and OR100 region). Similar results were obtained analyzing the ORF100 region. The OR100 region allows a clear separation of *O. sativa* types since X^2 Test (17.01 $p<0.0001$) showed an association chloroplast type with the morphological classification given for the japonica or indica type cultivated *O. sativa.*

So far of the weedy rice accessions analyzed (79) none of them are similar to *O. glumaepatula* by analyzing neither the CP8 [tRNA-Ser- (GGA)] nor the trnT [tRNA-Thr (UGU)]/Dra I specific sequences. Two polymorphic fragments of 298 and 98 were observed in the *O. glumaepatula* accessions from Costa Rica (Figure 2A), whereas the weedy rice accessions showed 1000 pb band which is common in all species. No tetraploid individuals had been detected in population

of 146 weedy rice individuals analyzed using the TrnL-Trnf F spacer sequence. This marker allows having a clear distinction between AA diploids and the CD tetraploid using as reference *O. alta*, *O. grandiglumis* and *O. latifolia* (Figure 2B). Similar results were obtained in the analysis of the nuclear gene Adh-2 and amplified sequence digested with EcoNI. This combination gene/enzyme allows a clear differentiation *Oryza sativa* (AA) from the complex from *Oryza officinalis* (CD), where the CD accession as in the case of *O. grandiglumis* and *O. latifolia* show 3 additional bands which correspond to 0.6, 1.0 kb y 1.6kb whereas the A genome shows an only 1.6 kb band (Figure 2C). Ge et al. (2001) were able to discern 10 genome types conducting restriction-enzyme Adh-2 and Adh-1 gene sequences. Results suggest that the weedy population analyzed is composed by AA genome individuals, including those accessions characterized by tall plants with broad leaves resembling tetraploid *Oryza* species. So far it cannot rule-out the presence of wild species in the weedy rice complex since some accessions are closely related to *O. rufipogon* by SSR analysis.

Future Activities

Detail analysis of weedy rice population collected from Jamundi (Valle del Cauca) and *Oryza* sps from Venezuela is in progress. These population will be analyze using the ORF100 and the CP3 TrnC [tRNA-Cys- (GCA)] and trnD [tRNA-Asp (GUC)] sequences among others. The specific alleles and identification of haplotypes are in progress

References

Bres-Patry C, Lorieux M, Clément G, Bangratz M. and Ghesquière A. 2001. Heredity and genetic mapping of domestication-related traits in a temperate *japonica* weedy rice. Theor. Appl. Genet. 102: 118-126.

Chen W, Nakamura I, Sato Y. and Nakai H. 1993. Distribution of deletion type in cpDNA of cultivated and wild rice. Jpn. J. Genet. 68: 597-603.

Fory L, Morales M. and Lentini Z. 2005. Use of chloroplast DNA polymorphisms for gene flow analysis in rice. SB-2005. Annual Report.

Garris G, Tai T, Oburn J, Kresovich S. and McCouch S. 2005 Genetic Structure and Diversity in *Oryza sativa* L. Genetics, Vol. 169, 1631-1638.

Gealy D, Tai T. and Sneller C. 2002. Identification of red rice, rice, and hybrid populations using microsatellite markers. *Weed Sci.* 50: 333-339.

Ge S, Sang T, Bao-Rong L, and Hong D. 2001. Rapid and reliable identification of rice genomes by RFLP analysis of PCR-amplified adh genes. Genome 44: 1136-1142.

González E, Fory L. Vásquez J, Ruiz P, Silva J. Duque M. and Z. Lentini. 2005. Relationships and potential origin of the weedy rice complex in Colombia. SB-2005. Annual Report.

Suh H., Sato Y. and Morishima H. 1997. Genetic characterization of weedy rice (*Oryza sativa* L.) based on morpho-physiology isozymes and RAPD markers. Theor Appl Genet 94:316-321.

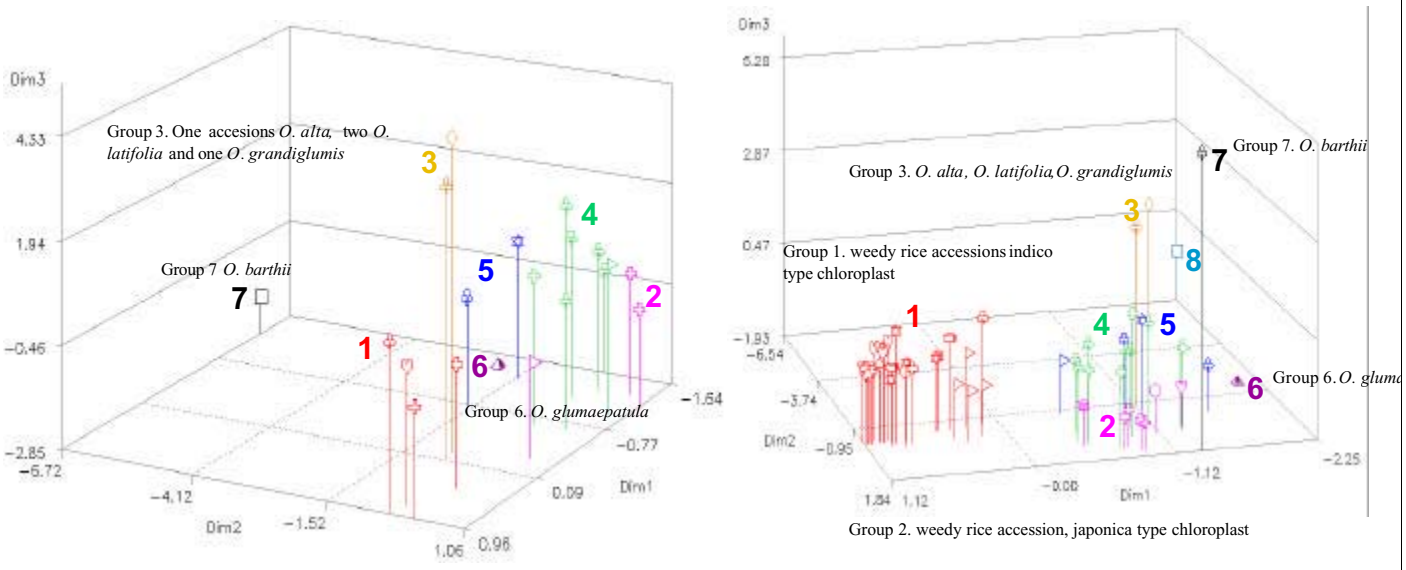
Sun C, Wang X, Yoshimura A. and Doi K. 2002. Genetic differentiation for nuclear, mitochondrial and chloroplast genomes in common wild rice (*Oryza rufipogon* Griff) and Cultivated rice (*Oryza sativa L.*). Theoretical and Applied Genetics.

Ishii T. Xu I. and MacCouch S. 2001. Nuclear and chloroplast microsatellite variation in A-genome species of rice. Genome 44: 658-666.

McCouch S, Kochert G, Yu H, Wan Z, Khush G, Coffman W. and Tanksley. 1988. Molecular mapping of rice chromosomes. Theoretical and Applied Genetics 76: 815-829.

Lentini Z. and Espinoza A. 2005. Coexistence of weedy rice in tropical America- gene flow and genetic diversity. Edited by Jonathan Gressel, U.S.A. 2005. In: Crop ferality and volunteerism, Charter 19. p 306-307.

Ying B. and Ge S. 2003. Identification of Oryza species with the CD genome based on RFLP analysis of nuclear Ribosomal ITS Sequences. Acta Botánica Cínica 45 (7): 762-765.



A. MAC-cpSSR **B. MAC-CpSSR-ORF100-MT**

Figure 1. A) MAC-cpSSR and B) MAC-CpSSR-ORF100-MT. 1A). In the Figure 1 A) The group 4 contain: five *Oryza rufipogon*, two *Oryza nivara*, two crosse of *Oryza nivara /Oryza rufipogon* and two varieties. The Group 5: *Oryza glaberrima* and one weedy rice 4-19-2. In the Figure 1 B. The group 4: contain four *O. rufipogon* accessions and two *O. nivara*, one crosses between *O. nivara* and *O. rufipogon,* three commercial varieties and one weedy rice accession 7-10-2. Group 5 contain *O. glaberrima*, *O. rufipogon* and two weedy rice (4-19-2 and 7-3-2).

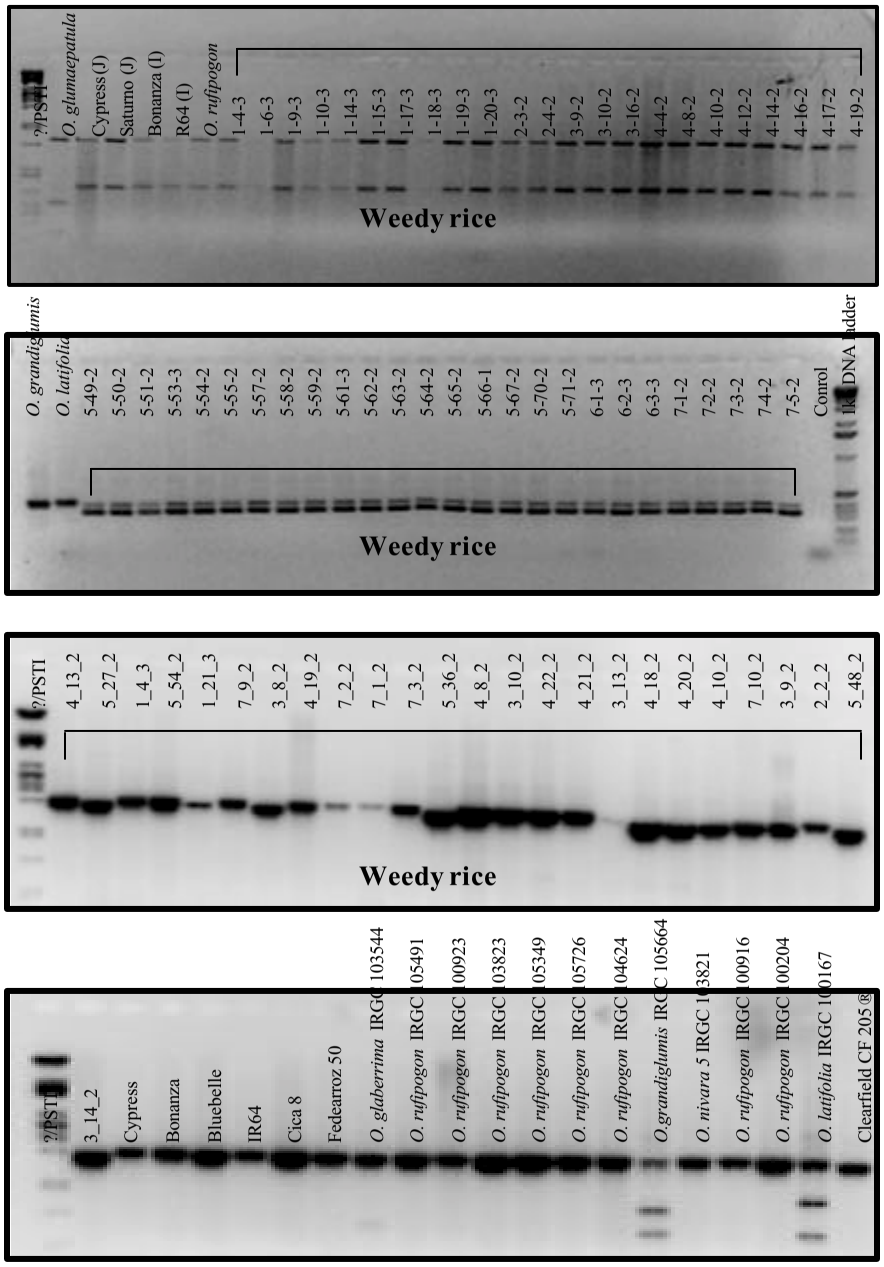


Figure 2. A Amplification of trnS [TRNA-Ser- (GGA)] and trnT [tRNA-Thr (UGU)] sequence and cut with Dra I. B, PCR of the cpDNA TrnL-Trnf sequence. D, Restriction profiles of the PCR amplification of the Adh –2 and cut with EcoNI

OUTPUT 2. CHARACTERIZING RICE PESTS AND GENETICS OF RESISTANCE

2A. Rice Blast Disease (*Pyricularia grisea*)

- **Identification of molecular markers linked to rice blast resistance genes**

Highlights

- The present work evidenced the usefulness of combining near-isogenic progeny analysis with rice genome information available in public databases to identify molecular markers highly linked to blast resistance genes in rice. Although a limited number of polymorphic markers can be expected when near-isogenic lines are used as progenitors, here we found six polymorphic markers in a region of only 13 cM surrounding the blast resistance gene *Pi-1(t)*. Additionally, two of these markers (RM1233*I and RM224) were closely linked to the gene. Our results support the utility of these DNA markers in MAS and gene pyramiding rice breeding programs addressing the improvement of blast resistance in rice cultivars; and eventually to map based cloning of the gene. The speed, simplicity and reliability of PCR based approaches make microsatellite analysis on agarose gels an attractive tool for marker-assisted selection in rice breeding programs aiming at developing durable rice blast resistant cultivars.

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Keywords: Blast (*Pyricularia grisea* Sacc) - marker assisted selection (MAS) - Microsatellite - Resistance gene - Rice (*Oryza sativa* L).

Abstract

The present work was conducted to identify microsatellite markers linked to the rice blast resistance gene *Pi-1(t)* for a marker-assisted selection program. Twenty-four primer pairs corresponding to nineteen microsatellite loci were selected from the Gramene database (www.gramene.org) considering their relative proximity to *Pi-1(t)* gene in the current rice genetic map. Progenitors and DNA bulks of resistant and susceptible families from F_3 segregating populations of a cross between the near-isogenic lines C101LAC (resistant) and C101A51 (susceptible) were used to identify polymorphic microsatellite markers associated to this gene through bulked segregant analysis. Putative molecular markers linked to the blast resistance gene *Pi-1(t)* were then used on the whole progeny for linkage analysis. Additionally, the diagnostic potential of the microsatellite markers associated to the resistance gene was also evaluated on seventeen rice varieties planted in Latin America by amplification of the specific resistant alleles for the gene in each genotype. Comparing with greenhouse phenotypic evaluations for blast resistance, the efficiency of the microsatellite assay was corroborated. As expected, the phenotypic segregation in the F_3 generation agreed to the expected segregation ratio for a single gene model. Of the twenty-four microsatellite sequences tested, six resulted polymorphic and linked to the gene. Two markers (RM1233*I and RM224) mapped in the same position (0.0 cM) with the *Pi-1(t)* gene. Other three markers corresponding to the same genetic locus were located at 18.5 cM above the resistance gene, while another marker was positioned at 23.8 cM below the gene.

Microsatellite analysis on elite rice varieties with different genetic background showed that all known sources of blast resistance included in this study carry the specific *Pi-1(t)* allele. Results are discussed considering the potential utility of the microsatellite markers found, for marker-assisted selection in rice breeding programs aiming at developing rice varieties with durable blast resistance based on a combination of resistance genes.

Background

Rice blast caused by *Pyricularia grisea* (Cooke) Sacc., the anamorphous state of *Magnaporthe grisea*, is the most limiting biotic factor for rice production in the world. The use of resistant cultivars is the most effective and economical way of controlling blast disease, therefore, breeding efforts for developing resistant cultivars continue to be a priority of rice breeding programs. One way to improve the durability of blast resistance is to "pyramid" resistance genes by crossing rice varieties with complementary genes to provide multigenic resistance against a wide spectrum of blast races. Combining these resistance genes broadens the number of races that a variety can resist, and there is evidence that multiple resistance genes make it more difficult for virulent races to evolve (Correa-Victoria et al., 2002). Unfortunately, pyramiding genes is difficult using conventional greenhouse screening procedures because blast races carrying individual avirulence genes to be used in inoculations for the identification of the corresponding resistance gene are normally not present in nature. As a result, accumulation of several resistance genes in a common background cannot be easily distinguished without a test cross. Recent advances in molecular marker technology, such as development of tightly linked molecular markers, has made it possible to pyramid major genes and QTL's into one genotype and to simultaneously select several complex characters.

The blast resistance gene *Pi-1(t)*, originally identified in the cultivar LAC23, an upland cultivar from Liberia confers complete resistance to several blast populations from Latin America when combined with the blast resistance genes *Pi-2(t)* and *Pi-33(t)* (Correa-Victoria et al., 2002). The Pi-1 gene confers resistance to all races present in one of the most predominant genetic lineages (SRL-4) from Colombia, while the other two genes confer resistance to all races within two other predominant lineages (SRL-5 and SRL-6), respectively. Mapping studies showed that the *Pi-1(t)* gene is located near the end of chromosome 11, linked to the Npb181 and RZ536 RFLP markers at a distance of 3.5 and 14.0 cM, respectively. However, RFLP approaches are expensive and laborious limiting their use in applied breeding programs, where a considerably high number of samples need to be analyzed. Convenient and cost-effective microsatellite markers, particularly those that can be scored on agarose gels, seem to be promising for the identification of blast resistance genes and for pyramiding or introgression of these genes into rice commercial varieties and elite lines. Microsatellite markers are hypervariable, abundant and well distributed throughout the rice genome and they are now available through the published high-density linkage map or in the public database (www. gramene.org).

We have designed a molecular marker-assisted breeding program in rice aiming at developing durable blast resistance in elite rice lines and cultivars by pyramiding the resistance genes *Pi-1(t)*, *Pi-2(t)* and *Pi-33(t)*; which are potentially useful to control blast pathogen populations in the Latin American region (Correa-Victoria et al., 2002). Here we report new microsatellite markers that cosegregate with the blast resistance gene *Pi-1(t)*, using sequences available in a public

database. These markers can be potentially used in MAS to introduce this gene into blast susceptible varieties, and provide the basis for map based cloning of this blast resistance gene.

Materials and Methods

The near-isogenic lines C101LAC (resistant line to isolates carrying avr *Pi-1(t)*) and C101A51 (susceptible line) developed at IRRI were crossed (cross CT 13432) and F_1 seeds generated. The F_2 progeny, resulting from self-pollination of F_1 individuals, were self-pollinated to generate 283 CT13432 F_3 lines. Rice varieties from Latin America were obtained from CIAT's rice germplasm bank. Ten rice seedlings 21 days old per pot were sprayed with 2.0 ml of blast inoculum suspension ($5x10^5$ spores/ml of isolate Oryzica Yacu 9-19-1 carrying avr *Pi-1(t)*) and incubated in the greenhouse at a temperature of 24-28^oC and relative humidity above 85 %. Plants were evaluated 15 days (two life cycles of the pathogen) after inoculation and scored for resistance and susceptibility in two replications as described by Correa-Victoria and Zeigler (1993). Resistant genotypes exhibit complete resistance with no lesions or few non-sporulating lesions type 1 or 2, and susceptible genotypes exhibit typical sporulating blast lesions type 3 or 4 covering more than 1 % of leaf area.

DNA concentrations were determined in a TKO 100 minifluorometer with the DNA-specific fluorescent dye. DNA bulks were prepared from 13 resistant and 13 susceptible lines within the CT13432 F_3 families evaluated for their blast reaction using the blast isolate Oryzica Yacu 9-19-1. Polymerase chain reaction (PCR) was conducted in a final volume of 20 µl containing between 25-50 ng of template DNA, 0.5 µM of each primer, 200 µM of each dNTP, 3.1 mM $MgCl_2$ and 1 unit of Taq DNA polymerase. For the majority of microsatellite markers studied the reaction was processed as follow: 94^oC for 1 min, followed by 40 cycles consisting of 94^oC for 30 sec, 50 and/or 55^oC for 30 sec and 72^oC for 30 sec and a final extension step of 72^oC for 10 minutes. After the PCR reaction, 5 µl of blue juice (30 % glycerol, 0.25% bromophenol blue) was added to the amplification product and 20 µl per sample were loaded on high-resolution agarose gels prepared mixing 1.5 % Sinergel (Diversified Biotech) and 0.7 % molecular grade products and containing 0.5mg/mL of ethidium bromide.

Twenty-four primer pairs corresponding to nineteen microsatellite loci (Figure 1B) were selected from the Gramene database (www. gramene.org) considering their relative proximity to the *Pi-1(t)* gene in the current rice genetic map (Figure 1A). The isogenic lines C101LAC and C101A51 and their common genetic background, the susceptible recurrent parent CO39, were used to identify microsatellite polymorphisms associated to the blast resistance genes. Polymorphic markers identified above were assayed by bulked segregant analysis (BSA).

Genetic analysis of the resistance was conducted measuring the goodness-of-fit to the expected ratio for a single gene model using a chi-square test. For this purpose, we used 283 F_3 near-isogenic lines derived from 283 F_2 plants with no selection. Molecular markers that resulted positive in BSA for the *Pi-1(t)* gene were used in linkage progeny analysis using 158 F_3 near-isogenic lines. Associations between markers and the resistance gene were demonstrated using a chi-square test. Linkage analysis was performed using the software MAPMAKER/EXP V 3.0 on the segregation data obtained from markers and blast resistance scoring of the CT13432 F_3 population. Conversion of the recombination fraction into centiMorgans (cM) units was obtained

with the Kosambi's mapping function. The final map was drawn using the software QGene V 3.04.

The diagnostic potential of the markers associated with the *Pi-1(t)* gene was also evaluated on DNA obtained from nineteen rice genotypes including seventeen elite cultivars grown in Latin America. For this purpose, the criteria followed for determining the presence or absence of the resistance gene was the amplification of the specific *Pi-1(t)* microsatellite allele in each rice genotype. Comparing with phenotypic evaluation obtained as indicated above, the veracity of the assay was corroborated.

Results

Genetic analysis of the resistance was conducted using 283 F_3 near-isogenic lines of the cross CT 13432. Expected and observed segregation ratios for this population are shown in Table 1. The population analysis showed a good fit to the expected segregation ratio (1:2:1) for a single gene model confirming the hypothesis of a single dominant gene for *Pi-1(t)* locus.

From the reported position of *Pi-1(t)* on chromosome 11 relative to RZ536 RFLP marker, it was possible to estimate its approximate position on the Rice-Cornell microsatellite genetic map (Figure 1A). Using this information, twenty-four microsatellite sequences were selected from this region of chromosome 11 from the Gramene database (www.gramene.org) as potential markers for *Pi-1(t).* These markers were first tested for polymorphism in the susceptible and resistant parent and later for linkage to *Pi-1(t)* in pooled C101LAC/C101A51 samples. Of the twenty-four primer pairs tested six (corresponding to four microsatellite loci) were polymorphic in agarose gel electrophoresis, all of them showing positive results in bulked segregant analysis; five markers were not polymorphic, and thirteen principally repeats with TA sequences did not show consistent amplification with the different annealing temperatures assayed.

Linkage between these six markers and blast resistance was confirmed by screening 158 F_3 near-isogenic lines from the cross C101LAC/C101A51 segregating for *Pi-1(t).* Chi-square test indicated that these markers were linked to *Pi-1(t).* The genetic distance between the markers and the *Pi-1(t)* locus ranged from 0.0 (no recombination between the markers and the resistance factor) to 23.8 cM (Figure 1C). Among the six makers linked to *Pi-1(t)* gene, two (RM1233*I and RM224) mapped in the same position (0.0 cM) with the *Pi-1(t)* gene. Other three dominant markers corresponding to the same genetic locus (RM7654) were located at 18.5 cM above the *Pi-1(t)* gene, while marker RM6094 was identified at 23.8 cM below the gene.

To examine whether the markers identified would be of general utility on a wider range of rice germplasm used in applied breeding programs in Latin America, the presence of resistant bands for five markers were examined in elite rice cultivars and compared to the reported inheritance of *Pi-1(t)* (Table 2). For this purpose, we used known sources of blast resistance as positive controls and considered as predictive criteria of the resistance event the amplification in each variety of the resistant microsatellite band and therefore the presence of the resistant allele for the *Pi-1 (t)* gene. Comparing with phenotypic data on blast resistance our results showed that our known sources of resistance (C101LAC, Cica 8, Oryzica 2, BR IRGA 409, CR 1113, El Paso 144 and Panama 1048) carry the resistance *Pi-1(t)* allele; on the other hand, the susceptible cultivars

(Colombia XXI, Epagri 108, Capirona and Oryzica 1 and CO-39) had not the resistant allele. In addition, other seven varieties (Jucarito-104, Fedearroz 2000, CR 1821, Primavera, Cimarrón, Bonanza and Fedearroz 50), which were resistant in the pathogenicity assay, did not show the allele characteristic of the *Pi-1(t)* gene.

Discussion

This study demonstrates that approaches combining near isogenic progeny analysis and rice genome information available in a public database constitute a very useful tool for identifying molecular markers closely linked to blast resistance genes. The reported marker most closely linked to blast resistance gene *Pi-1(t)* was the cDNA Npb181, identified at 3.5 cM from the gene. Here, using a segregating population with identical genetic background (CO39) but with a higher number of segregant lines than the one used by these authors, we have identified two new microsatellite markers (RM1233*I and RM224) highly linked to gene *Pi-1(t)* (at 0 cM of the gene). From the reported position of the *Pi-1(t)* gene relative to the RZ536 marker, it was possible to estimate its putative position on the Rice-Cornell microsatellite genetic map flanked by the microsatellite RM254 and the RFLP RZ536 markers between the 110.0 and 125.6 cM at the end of chromosome 11. Here we have reported two microsatellite markers very closely linked to gene *Pi-1(t)*, which is in agreement with the information included in the Rice-Cornell microsatellite genetic map, positioning these markers between 112.9 and 120.1 cM at the end of chromosome 11. However, the two remaining microsatellite loci RM7654 and RM6094 were outside of the mentioned 7.2 cM chromosomal region, mapping to 18.5 and 23.8 cM from the gene *Pi-1(t)*, respectively.

We have shown that the known *Pi-1(t)* resistance sources such as C101LAC, Cica-8, Oryzica 2, BRIRGA409, El Paso 144, Panamá 1048 and CR1113 (Correa-Victoria et al., 2002) exhibited microsatellite alleles associated with this gene of resistance, while susceptible varieties don't. Interestingly, six varieties (Fedearroz 2000, Fedearroz 50, Primavera, Bonanza, Cimarrón and Jucarito-104) that were resistant to the rice blast isolate Oryzica Yacu 9-19-1 did not show the resistant *Pi-1(t)* alleles. One possibility for this resistance reaction in these cultivars could be the presence of different resistance genes interacting with corresponding avirulence genes different from the avr *Pi-1(t)* in the pathogen.

This study is part of a molecular marker-assisted rice breeding program aiming at developing durable blast resistance in rice cultivars by pyramiding the resistance genes *Pi-1(t)*, *Pi-2(t)*, and *Pi-33(t)*, which are potentially useful for controlling blast pathogen populations in the Latin American region (Correa-Victoria et al., 2002). Disease assays to evaluate resistance to rice blast are time-consuming and laborious procedures that also require specialized facilities. PCR analysis can greatly reduce the amount of labor needed for evaluating phenotypes by prescreening with MAS. Cost-effective microsatellite markers linked to the blast resistance *Pi-1(t)* gene and suitable for agarose gel electrophoresis facilitating the introgression and pyramiding of the gene into rice commercial cultivars, were developed here.

The microsatellites reported in this study seem to be suitable for assisting rice breeders in the introduction of the *Pi-1(t)* resistance gene in different rice cultivars, and serve as an indicator for the presence of others. Thus, the *Pi-1(t)* gene markers may serve as indicators for the presence of

resistance gene clusters in the indicated chromosome region and for the selection of breeding parents for developing rice cultivars with a broader-resistance spectrum to blast. Additionally, these microsatellite markers could provide a starting point for efforts eventually aimed at cloning and isolating this gene.

Concluding remarks

The present work evidenced the usefulness of combining near-isogenic progeny analysis with rice genome information available in public databases to identify molecular markers highly linked to blast resistance genes in rice. Although a limited number of polymorphic markers can be expected when near-isogenic lines are used as progenitors, here we found six polymorphic markers in a region of only 13 cM surrounding the blast resistance gene *Pi-1(t)* (Figure 1). Additionally, two of these markers (RM1233*I and RM224) were closely linked to the gene. This finding supports the hypothesis that when polymorphisms are found in near-isogenic derived populations, differing only in the presence or absence of a gene, the probability that these markers be closely linked to the gene is very high. Besides, polymorphic markers linked to resistance genes in near-isogenic populations, can also be expected to detect polymorphism and presence of the linked genes in commercial rice varieties with certain level of inbreeding. Our results support the utility of these DNA markers in MAS and gene pyramiding rice breeding programs addressing the improvement of blast resistance in rice cultivars; and eventually to map based cloning of the gene. However, the use of these markers as a diagnostic tool for determining the presence of the resistance gene *Pi-1(t)* in a wider range of rice germplasm require additional studies for further confirmation of the results reported here. The speed, simplicity and reliability of PCR based approaches make microsatellite analysis on agarose gels an attractive tool for marker-assisted selection in rice breeding programs aiming at developing durable rice blast resistant cultivars.

Table 1. Segregation of F_3 near-isogenic lines of the genetic cross between C101LAC (*Pi-1(t)*)/C101A51 inoculated with the blast isolate Oryzica Yacu 9-19-1 of *Pyricularia grisea*.

Population	Expected ratio[1]	No. of lines expected			No. of lines observed		
		S	SG	R	S	SG	R
F_3 near-isogenic lines	1:2:1 (χ^2 = 1.0, $p<0.05$)	71	141	71	76	133	74

(1) According to a model based on a single dominant gene as indicated in materials and methods; (S): Susceptible, (SG): Segregant; (R): Resistant

Table 2. Analysis of the predictive capacity of six microsatellite markers for blast resistance gene *Pi-1(t)* in 19 commercial rice cultivars.

Variety	Origin	PA	Marker analyzed				
			RM1233*I	RM7654*A	RM7654*H	RM7654-2	RM224
CO-39 [1]	Philippines	S	-	-	-	-	-
C101LAC [2]	Philippines	R	+	+	+	+	+
Cica-8	Colombia	R	+	+	+	+	+
Fedearroz 2000	Colombia	R	-	-	-	-	-
Colombia XX1	Colombia	S	-	-	-	-	-
Oryzica 1	Colombia	S	-	-	-	-	-
Oryzica 2	Colombia	R	+	+	+	+	+
Fedearroz 50	Colombia	R	-	-	-	-	-
Epagri 108	Brazil (irrigated)	S	-	-	-	-	-
BRIRGA409	Brazil (irrigated)	R	+	+	+	+	+
Primavera	Brazil (upland)	R	-	-	-	-	-
Bonanza	Brazil (upland)	R	-	-	-	-	-
El Paso 144	Uruguay, Argentina	R	+	+	+	+	+
Cimarron	Venezuela	R	-	-	-	-	-
Capirona	Peru	S	-	-	-	-	-
Panamá 1048	Panama	R	+	+	+	-	+
CR 1113	Costa Rica	R	+	+	+	+	+
CR 1821	Costa Rica	R	-	-	-	-	-
Jucarito-104	Cuba	R	-	-	-	-	-

(1): Susceptible control; (2): Resistant control; PA: Results of the pathogenicity assay using blast isolate Yacu 9-19-1, R: resistant genotype, S: susceptible genotype, (+) presence of resistant allele, (-) absence of resistant allele.

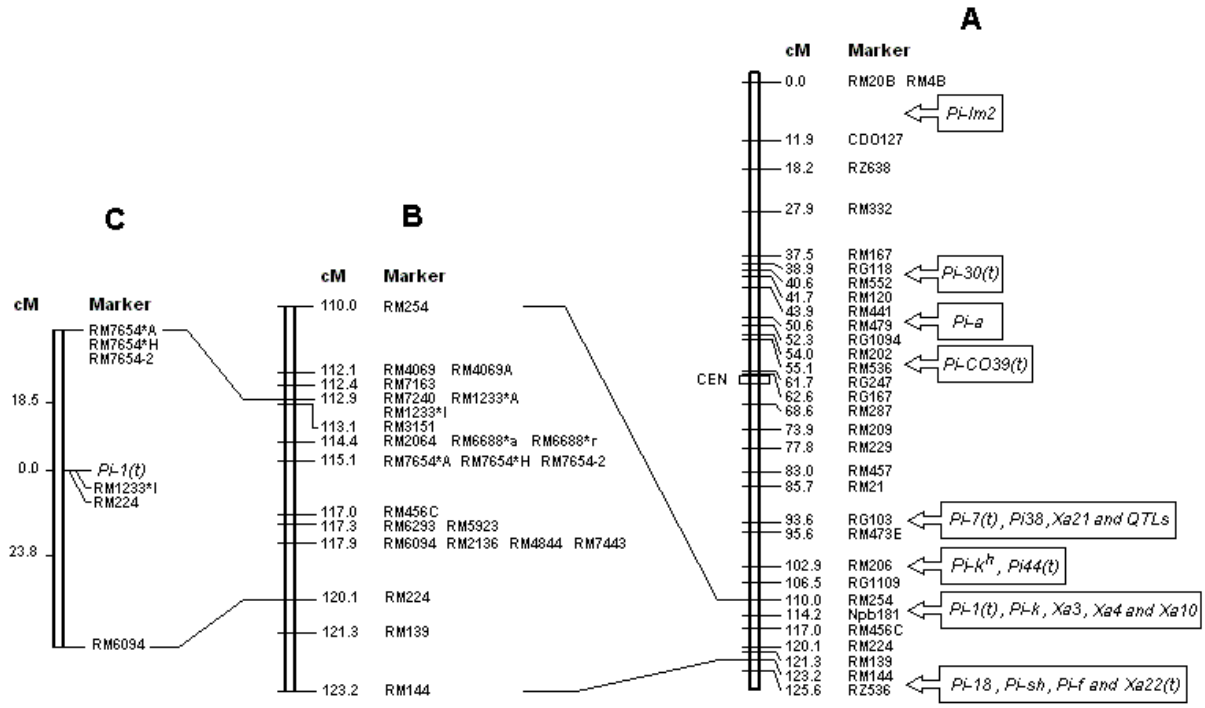

Figure 1. Genetic map of rice chromosome 11 (A) as indicated by Temnykh et al. (2001) and by McCouch et al. (2001). Region between the 110.0 and 123.2 cM (B) was complemented with public information available at Gramene database (www.gramene.org). Information about position of the resistance genes on chromosome 11 was obtained as follow: *Pi-1(t)* (Yu et al. 1996; Hittalmani et al. 2000), *Pi-7(t)* and quantitative trait bcus (QTL) to partial resistance to blast (Wang et al. 2001; Zenbayashi et al. 2002), *Pi-CO39(t)* (Chauhan et al. 2002), *Pi-18(t)* (Ahn et al. 2000), *Pi38* (Gowda et al., 2006), *Pi-44(t)* (Chen et al. 1999), *Pi-a, Pi-k, Pi-sh, Pi-f, Pi-lm2 and Pi-30(t)* (Sallaud et al. 2003), *Pi-k*^{h} (Sharma et al., 2005), *Xa3, Xa4, Xa10, Xa21* and *Xa22(t)* (Causse et al. 1994; Mackill and Ni, 2001). Chromosome 11 generated through linkage analysis (C).

- **Mapping resistance genes associated with the durable blast resistance in the commercial rice cultivar Oryzica Llanos 5**

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Abstract

The genetic basis of the high level of durable resistance to rice blast in the cultivar Oryzica Llanos 5 was characterized in two Recombinant Inbred Lines (RILs) from a cross between the susceptible cultivar Fanny and O. Llanos 5. The number and chromosomal location of quantitative trait loci (QTL) conferring resistance against eight isolates of the blast fungus were tested in these two populations. A linkage map was constructed using 350 molecular markers: SSR, RFLP and RGAs. Twenty-one QTL were detected and associated with the resistance traits, disease leaf area and lesion type, on nine rice chromosomes. Most but not all of the QTL occurred in the same genomic regions as either genes with major race-specific effects or other resistance QTL that had been described in previous experiments. Most of the QTL appeared to be race-specific in their effects but it is possible that some of the QTL with smaller effects were nonspecific. Three QTL affected resistance to one blast isolate, which causes limited disease on O. Llanos 5 and was probably virulent on most or all of the major genes form the cultivar. One of the QTL mapped to a region on chromosome 9 where no blast resistance genes had yet been mapped. An advanced backcross strategy with marker-assisted selection for O. Llanos 5 alleles in QTL regions was used to generate five BC_2F_3 populations carrying five different target regions associated with partial resistance to rice blast disease. These populations were analyzed for segregation for resistance to the blast isolate compatible with O. Llanos 5 and one QTL near the bottom of rice chromosome 11 was found to be significantly associated with partial blast resistance. This QTL accounted for 12.4% and 8.0% of the phenotypic variation in diseased leaf area and lesion type, respectively, observed under greenhouse inoculations. As a whole, the observed durable resistance in Llanos 5 could be the result from a combination of quantitative and qualitative resistance genes. The information from this study will be integrated into the development of improved lines with O. llanos 5 derived QTL for resistance.

Key words: QTL, partial resistance, durable resistance, microsatellite markers

Background

Blast resistance in the Colombian commercial rice varieties has been defeated in periods of 1-3 three years after cultivar release. However, the resistance of the cultivar Oryzica Llanos 5 has been durable and has remained stable under field conditions for more than 14 years in Colombia and other blast nurseries around the world. Blast resistance in rice has been classified into two types, qualitative and quantitative or partial. The first type is controlled by single genes that provide high levels of resistance, but only to specific races of the blast fungus. Partial resistance allows lesions to form but they are typically fewer in number, reduce in size or slower to develop than those produced in highly susceptible lines. Partial resistance is thought to be nonspecific and therefore more promising for long-term blast control. Genetic studies have indicated the presence of at least four major genes controlling the resistance to some blast isolates. Based on the presence

of avirulence genes in our blast populations we have inferred that the cultivar O. Llanos 5 carries at least 8 major genes. Studies retrieving blast isolates from the immediate parents giving origin to this cultivar and characterizing their genetic structure and virulence composition suggest that the durable resistance is associated with the pyramiding of complementary resistance genes to the different lineages of the pathogen present in those parents. It is therefore important to understand the basis of the durable resistance of this rice cultivar in order to establish a breeding strategy based on the same principle. In this study, a blast isolate partially compatible with the cultivar Oryzica Llanos 5 was identified and used in the inoculation of two recombinant inbreed line populations for the dissection of QTLs associated to the partial resistance exhibited by the cultivar. Because the genetics of O. Llanos 5 resistance is thought to be complex and unlikely to segregate in a Mendelian manner, a QTL mapping approach was used to identify genes conferring its partial resistance to several blast isolates. This study was initiated to identify and localize major and minor loci genes controlling the resistance in O. Llanos 5 in collaboration with Kansas State University. The objectives of the study were mainly to estimate the number, genomic position and genetic effects of the O. llanos 5 genes controlling resistance to eight different isolates of the fungus belonging to five different genetic lineages; and to compare the predicted loci with previous QTL and resistance loci reported for the blast pathogen. In summary, it was found that blast resistance in the cultivar O. Llanos 5 is due to the combined effects of multiple loci with major and minor effects. Somme of them mapped to regions of previously identified *P. grisea* resistance genes but two mapped to regions with no reported blast resistance genes.

Materials and Methods

Two recombinant inbred lines (RIL's) (120 and 231 F7 advanced RIL's) of the cross between the resistant indica cultivar Oryzica Llanos 5 and the japonica susceptible cultivar Fanny were developed for this study. An initial set of 120 RIL was inoculated with different blast isolates representing the pathogen genetic lineages SRL-1 to SRL-6 from Colombia while a second set of 231 RIL was evaluated with the same blast isolates in separated experiments. Inoculations and evaluations were performed at the Rice Pathology greenhouse of CIAT according to the methodology described in other annual reports. Two evaluation methods, lesion type (LT) and percentage of disease leaf area (DLA) were used to score the blast resistance. One isolate named "killer", was recovered from O. Llanos 5 and observed to be highly aggressive and have a very broad virulence spectrum. This isolate has been used to detect minor resistance genes in the two populations. DNA of each RIL in the two populations was extracted at Kansas State University or CIAT for molecular analysis and restriction fragment length polymorphisms (RFLP) using five restriction enzymes and/or simple sequence repeats (SSR) were used as potential markers for the identification of the resistance genes present in Oryzica Llanos 5. Blast resistance genes to each genetic lineage of the pathogen were identified based on the phenotypic reaction and located on the different chromosomes of the rice genome. The genetic linkage map constructed from the RIL mapping population contains 350 markers including simple sequence repeats, and RFLPs. The chromosomal locations of the markers were determined using the Mapmaker program Version 2.0. Conversion of recombination fractions into centimorgans (cM) was performed using the Kosambi mapping function. Correspondence of linkage groups and the order of the markers on chromosomes were inferred based on the genetic linage map of rice reported in the literature and also from the rice physical map (www.gramene.org). An integrated genetic map with data from all the RFLP and SSR markers used was constructed to locate previously reported blast resistance

genes as well QTL identified in this study. Both composite interval mapping (CIM) and multiple interval mapping (MIM) techniques were used for QTL detection using QTL Cartographer package v2.5. Both QTL mapping methods were used to localize loci with major and minor effects on resistance. Several RIL's exhibiting QTLs associated with the partial resistance to the killer blast isolate were used to develop one or two backcrosses to the susceptible cultivar Fanny in order to generate near isogenic lines with the individual QTLs, which are being used to map these genes.

Results and discussion

Analysis (Chi-square) of the frequency distributions of disease leaf area affected and lesion type with the eight isolates indicated that no normal distributions were followed for any of the isolates for either trait. In general, all experiments showed a high percentage of individuals with disease leaf area affected below 20% among the eight blast isolates. The resistance segregation varied dramatically depending on the blast isolate used. All of the isolates were virulent on Fanny causing lesion types 3 or 4 and disease leaf area between 60 and 80% while O. Llanos 5 was immune except for one isolate, which was able to produce some lesion types 1, 2, and 3 but with disease leaf area affected that was typically less than 10%.
A total of 21 different loci were mapped, each associated with one or more of 58 statistically significant reductions in disease to one or more of the eight isolates used in the study. These loci were associated with LOD scores above the threshold value determined by the permutation test for one of the traits in at least one of the populations used. The QTLs mapped to nine of the 12 rice chromosomes, with none mapping to chromosomes 5, 7, or 10 (Figure 2). Of the 58 significant QTL identified, 36 (62%) were identified in one population and 22 (38%) in the other. Twenty (34%), corresponding to eight loci, were located in both populations. Thirty-eight were detected only in one of the populations; 23 in population 1 and 15 in population 2. None of the QTLs identified had statistically detectable effects on all eight-blast isolates, showing that all were isolate-specific. However, some traits required high LOD scores to be statistically significant because their abnormal distributions.

Twelve significant QTL were detected with six different blast isolates on Chromosome 8, being the higher number of QTL by chromosome detected. Most QTL on this chromosome were located in a cluster region around the centromere where several race-specific R genes have been reported. One of the detected QTL, with resistance effects for three isolates, was identified in the interval of markers RM 72 and RM 339, near the centromere. At least five race-specific resistance genes have been mapped in this region in previous studies including Pi-11, Pi-33, Pi-29, Pi-GD-1(t), and Pi-36. Pi-33 was previously mapped close to the SSR marker RM 72. This genes has been shown to confer resistance to isolates from lineages 1, 2, 4, and 6 but not 5 from Colombia. These results support the idea that O. Llanos 5 carries Pi-33 as suggested in our studies based on greenhouse inoculations with blast isolates carrying the corresponding avirulence gene (avr Pi-33). According to the results for at least three blast isolates, it seems likely that there are at least two resistance genes in this region of chromosome 8 in the cultivar O. Llanos 5.

Two loci on chromosome 6 were identified affecting resistance at least three blast isolates. One of the loci was located near the centromere. At least 10 resistance genes conferring high levels of blast resistance have been mapped in the centromere region of chromosome 6. Two of those, Pi-2

and Pi-z, were also suggested from our greenhouse inoculations of the cultivar O. Llanos 5 with blast isolates carrying the correspondent avirulence genes.
Three loci on chromosome 11 contributed to the resistance of at least 4 of the blast isolates. At least seven resistance genes have been mapped around this locus. Two of these resistance genes, Pi-k and Pi-sh have been predicted to possible be present in O. llanos 5 according to our greenhouse inoculation studies. Thus, these two genes alone could explain part of the resistance observed to some of the isolates used.

Two QTL on chromosome 12 affected resistance to at least three blast isolates. One of the QTL accounted for 62.7% of the variation observed in one of the populations. Several race-specific blast resistance genes have been mapped in the centromeric region of chormoseme 12. Pi-ta^2 is one of the genes that we have predicted to be potentially present in this region in the cultivar O. Llanos 5. The large effect this QTL has on the resistance to one of the isolates is consistent with the possibility that this gene is involved in the quantitative resistance of O. Llanos 5. However, the physical position of this gene has not been yet accurately determined so it would be premature to conclude that this gene controls the resistance observed in this region.

Two QTL affecting resistance to three isolates were identified on chromosome 9. No blast resistance genes have yet been reported at this locus. Additionally, two QTL were identified on chromosome 4.
Partial resistance to 4 blast isolates was controlled by two QTL on chromosome 3. One of these QTL affected resistance to three isolates and in all three cases the predicted resistant allele came from the susceptible parent Fanny. Another QTL was inherited form O. Llanos 5. No blast resistance genes have been previously reported on chromosome 3.
Although the results suggest that there are two loci conferring partial resistance to rice blast located on chromosome 3, further analysis will be needed for verification given that the effects were experiment (population) specific.

Two QTL affecting resistance to three isolates were identified on chromosome 1. There is no previous report of blast resistance genes in the regions detected.
Eight of the twenty-one loci had statistically significant effects in both populations, two on chromosomes 8 and 4 and one on chromosomes 6, 9, 11, and 12. Most of these loci correspond to map positions to which blast resistance genes have already been mapped.
As mentioned above, most of the QTL detected in the study map to locations previously identified as containing blast resistance genes with large, race-specific resistance effects or QTL with smaller effects on blast disease. This is consistent with the idea that the cultivar O. Llanos 5 carries multiple blast resistance genes with major and minor effects, and that those account for at least part of the high level of resistance that the cultivar has shown over time. Several others QTL mapped to positions where no blast resistance genes have yet been mapped, and all except for the locus on chromosome 9 were experiment specific; therefore, they should be regarded with cautious. These include QTL on chromosomes 1 and 8 and two QTL on chromosome 3. It is possible that some of the QTL found are involved in defense mechanisms different to Pi genes. Microsatellite markers spanning these new resistant loci are being integrated *in silico* to identify candidate genes on the last version of the physical map reported in the Gramene and Tigr databases. Predicted genes in these two databases such as NBS-LRR genes, receptor kinases and other protein kinases are considered good candidates for race-specific R genes. Other genes, such

as those coding for proteins commonly induced in defense reactions, were also considered as possible QTL. RILs carrying several QTLs associated with the partial resistance observed in Oryzica Llanos 5 and on the same RIL were backcrossed to Fanny and the BC1F1 and BC2F1 were evaluated and resistant plants selected in greenhouse inoculations with the killer isolate in 2004 and 2005. Selected lines are being advanced to the F3-F5 generations for DNA extraction, confirmation and possible cloning of the QTLs.

The molecular bases of Colombian rice cultivars conferring resistance to blast have not been documented. The cultivar O. Llanos 5 was developed by combining resistances from several cutivars with different sources of resistance and selecting for high levels of resistance to different genetic lineages of the pathogen. The presence of multiple genes with large effects made thorough characterization of all the QTL difficult, and the use of multiple blast isolates was a critical component of the analysis. It is difficult to determine whether some of the QTL represent Pi-type genes with race-specific effects or genes with potential nonspecific effects. Because O. Llanos 5 carries so many resistance genes, there are typically several genes conferring resistance to each of the isolates. The presence of genes conferring large resistance effects to a given isolate obscures the effects of genes with smaller effects and may make them impossible to detect. A nonspecific QTL with moderate effects may not show a statistically significant contribution to resistance in a population challenged with a blast isolate to which multiple genes with major effects were functioning.

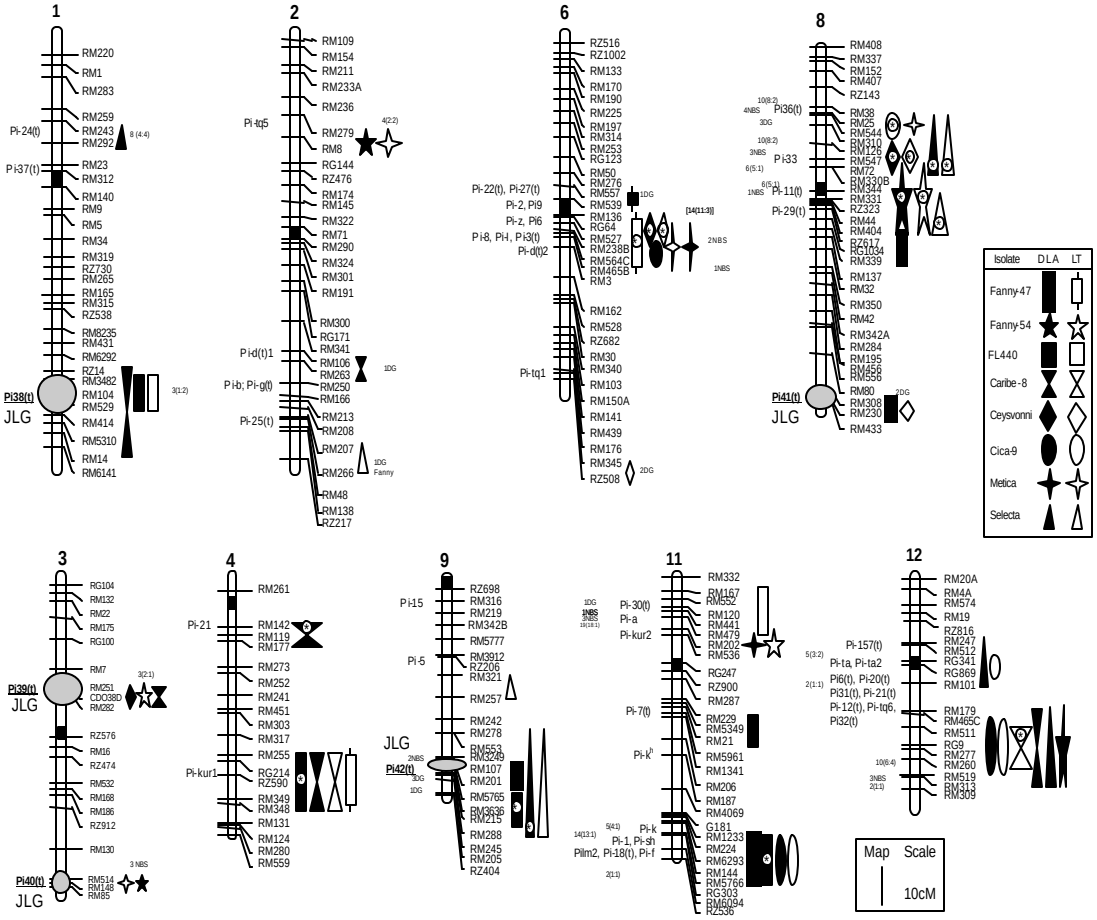
Conclusion

Blast resistance in the cultivar O. Llanos 5 was found to have very complex inheritance. The durable broad-spectrum resistance in O. Llanos 5 is associated with multiple major genes that induce resistance to different blast isolates. Eight resistance QTL were identified that were statistically significant in both of the two mapping populations analyzed. Many of the QTL identified in the present study mapped to regions where blast resistance had previously been mapped. Several of the blast resistance genes that mapped to these areas are though to be present in the cultivar O. llanos 5 based on controlled inoculations with blast isolates carrying the correspondent avirulence genes and are therefore good candidates for the genes controlling the resistance. One QTL on chromosme 9 was in a region where no blast resistance genes had been designated. All of the QTLs detected in this study for isolates other than killer were for lesion type blast resistance. LT is typically associated with QTLs with large effects (major, or “R” genes). In contrast all QTL identified by killer were for DLA. None of the major genes detected in Oryzica Llanos 5 are effective against the killer isolate, but O. Llanos 5 is still highly resistant. This resistance appears to be controlled by genes with small main effects. The killer isolate apparently allows these genes to be identified. As a whole, the observed durable resistance in O. Llanos 5 is the result from a combination of quantitative and qualitative resistance genes.

Future Activities

Evaluate the reaction of more RIL’s to the blast isolates already used in previous inoculations as well as new isolates exhibiting a compatible reaction with the cultivar O. Llanos 5. Continue analysis with microsatellite markers to identify and locate more blast resistance genes. Develop near isogenic lines with blast resistance genes present in Oryzica Llanos 5. Evaluations of

advanced generations of several backcrosses with molecular markers saturating regions associated with QTLs detected in the two RIL populations studied and inoculate them with different blast isolates to detect different blast resistance genes.



⊙ mean they were detected in both RIL populations

Jershon Lopez-gerena-Rice Blast QTL OL5

Figure 2. Mapping of QTL associated with resistance to *Pyricularia grisea* in the rice cultivar Oryzica Llanos 5. The map contains 256 markers. Approximate positions of Pi genes mapped in previous studies are indicated at the left of the chormosomes. QTL were identified for lesion type (LT) and disease leaf area affected (DLA) against eight blast isolates in two recombinant inbred line populations (RIL).

References

Correa-Victoria, F.J. & R.S. Zeigler, 1993. Pathogenic Variability in *Pyricularia grisea* at a Rice Blast " Hot Spot" Breeding Site in Eastern Colombia. Plant Disease 77: 1029-1035.

Correa-Victoria, F.J., D. Tharreau, C. Martínez, M. Vales, F. Escobar, G. Prado & G. Aricada, 2002. Combinación de genes en arroz para el desarrollo de resistencia durable a *Pyricularia grisea* en Colombia. Fitopatología Colombiana 26: 47-54.

Lopez-Gerena, J. 2006. Mapping QTL controlling durable resistance to rice blast in the cultivar Oryzica Llanos 5. Ph.D. Dissertation. Kansas State University. 128p.

- **Inheritance of the resistance to Pyricularia grisea in the rice lines Tres Marias and 75-1-127**

Highlights

- The number of major blast resistance genes present in the rice cultivars Tres marias and 75-1-127 was determined
- Resistance to the Colombia genetic lineage SRL-6 in the cultivar Tres Marias is complex and probably controlled by both qualitative and quantitative traits
- Resistance to blast in these two cultivars to the most prevalent genetic lineages of the blast pathogen in Colombia is mainly controlled by few major genes, which can be easily transferred to improved rice germplasm
- The rice cultivar Tres Marias frm Brazil can be a good source of new major and minor genes conferring durable resistance to blast

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Keywords: Blast (*Pyricularia grisea* Sacc) – inheritance studies - Resistance gene - Rice (*Oryza sativa* L).

Abstract

Rice blast is the main disease of the crop and development of resistant cultivars is one of the main strategies to combat the disease. The rice cultivars Tres Marias, an upland landrace from Brazil, and the line 75-1-127 with resistance derived from the wild rice species *Oryza minuta*, have been reported to exhibit a complete and stable resistance to blast in many parts of the world. These two lines have also exhibited complete resistance to Colombian blast populations, both in the field and under controlled inoculations in the greenhouse. In order to use these rice lines as potential sources of resistance, we have been conducting genetic studies to determine the number of genes controlling their blast resistance to different genetic lineages of the blast pathogen in Colombia. The potential use of these genes and future work to locate those genes in the rice genome is discussed.

Background

Rice blast caused by *Pyricularia grisea* is the main disease of the crop. Resistance incorporated into commercial cultivars is broken-down by the pathogen few years after their release. Several strategies have been proposed and developed to predict the durability of the resistance; however, the success of such strategies do not depend only on a good identification of resistance sources, but also on the knowledge and understanding of how this resistance is inherited from one generation to the other, as well as on the number and interaction of the resistance genes conferring stability.

Field and greenhouse evaluations of different rice nurseries at CIAT allowed the identification of potential sources of stable and durable resistance, which exhibited high levels of complete resistance under high disease pressure and pathogen diversity. Two of these rice cultivars, Tres Marias and 75-1-127 were used here to study the inheritance and genetic control of their stable blast resistance. Several reports indicate that the line 75-1-127 possesses a single gene, Pi-9, which confers broad and stable resistance to blast in many countries of the world. This gene was derived from the rice wild species *Oryza minuta.* Our experience suggests that there are no single genes able to confer resistance to all races of a blast population. Other authors suggest that the resistance found in wild species can be durable, especially if it has not coevolved with the target pathogen population. The Brazilian upland landrace, Tres Marias, has exhibited complete and durable blast resistance in many blast prone areas of the world, however, the genetic control to blast populations in Colombia is not known. We are pretending to elucidate the number and action of the blast resistance genes present in the cultivars Tres Marias and 75-1-127 and suggest possible strategies for their use as sources of blast resistance in rice breeding programs aiming at developing rice cultivars with durable resistance.

Material and Methods

Rice cultivars: Fanny (susceptible female parent); Tres Marias (unpland land race from Brazil, resistant source); 75-1-127 (resistant source carrying the resistance gene Pi-9). Blast isolates: Fanny 54 (genetic lineage SRL-6); Isolinea 6-7-1 (genetic lineage SRL-5); Oryzica Caribe 8-17-1 (genetic lineage SRL-4).

The rice cultivar Tres Marias is an unpland landrace from Brazil and is included as one of the best sources of blast resistance at IRRI's rice germplasm bank. The line 75-1-127 carries the blast resistance gene Pi-9 derived from the wild rice species *Oryza minuta,* which is considered to confer a broad and stable blast resistance. Both, Tres Marias and 75-1-127 were crossed to the susceptible parent Fanny to develop the F_2 and F_3 generations from each cross. Plants or lines from each cross were inoculated with the three blast isolates under controlled conditions and incubated under favorable considitons of relative humidity and temperature. Fifteen days after inoculation, each plant was evaluated for lesion type and percentage of leaf area affected. Each line had two replications with 10 plants each. Different tests were performed to determine and corroborate the number of resistance genes.

Results and Discussion

Blast resistance in the Brazilian landrace Tres Marias. The proportion of observed and expected resistant and susceptible plants in the generations F_2 and F_3 for the genetic cross between Fanny x Tres Marias are shown in Table 3. The results suggest that in the generation F_2 one dominant gene (3:1 ratio) seems to control the resistance to the blast isolates Fanny 54, Isolinea 6-7-1 and Oryzica Caribe 8 representing the Colombian genetic lineages SRL-6, SRL-5 and SRL-4, respectively. In the F_3, the segregation 3:1 for one gene is just confirmed for the resistance to the genetic lineage SRL-5. Although the 3:1 segregation for one gene was not statistically confirmed for the resistance to the lineage SRL-4, the low X^2 obtained suggests no to reject the hypothesis and consider the resistant control of one gene. The inheritance of blast resistance for the genetic lineage SRL-6 seems to be very complex and the segregation pattern in the F_3 did not follow any Mendelian segregation for 1-4 resistance genes.

The rice cultivar Tres Marias has been used as a donor of resistance in several rice breeding programs due to the stable blast resistance observed in this cultivar in the field over the years. Recent studies associate the high and stable resistance to rice blast in different rice cultivars with the presence of major and minor or quantitative trait loci (QTL) controlling partial resistance. In general, major genes are associated with qualitative resistance where a Mendelian segregation is easily observed. This type of segregation is in general not observed in the presence of QTLs and more specific studies based on molecular markers should be used to identify and locate them in the rice genome. Therefore, the difference between the F_2 and the F_3 segregation model could be due to the complex nature of the resistance in the cultivar Tres Marias to the blast isolate Fanny 54 (SRL-6). The presence of a single resistance gene controlling the isolates Isolinea 6-7-1 (SRL-5) and O. Caribe 8-17-1 (SRL-4) is an advantage for the use of Tres Marias as a source of resistance in a breeding program given that a single gene is easier to transfer to a commercial cultivar than more than one gene.

In general, heritability of blast resistance, being it qualitative or quantitative, has been reported to be high (32-82%) and easily transferred. Therefore, transferring the resistance to the genetic lineage SRL-6 from Tres Marias, despite its possible complex nature, is possible. We should investigate further, if all the resistance genes to SRL-6 present in Tres Marias should be together and complementing to confer durable resistance, with the major resistance genes present in the same cultivar and which controlled the lineages SRL-5 and SRL-4.Observed data on the blast reaction of the 156 F_3 rice lines to the blast lineages SRL-5 and SRL-4 yielded almost the same susceptible and resistant lines, suggesting that the same gene or alleles of the same locus, is controlling the resistance to these two lineages in Tres Marias. This was demonstrated using a Chi-square test (Table 4) for the hypothesis of independent segregation of resistance for each pair of isolates in the 156 F_3 lines and considering the reaction types resistant, segregating, and susceptible observed. No significant differences were found in the segregation pattern between isolates Isolinea 6-7-1 (SRL-5) and O. Caribe 8-17-1 (SRL-4). To verify if resistance in Tres Marias is determined by the same gene to all the blast isolates, genetic correlation coefficients were determined among isolates Fanny 54, Isolinea 6-7-1, and O. Caribe 8-17-1, computed from transformed data of the average of the percentage of leaf area affected of each individual line in the F_3 generation (Table 5 and Table 6). The results indicate different levels of correlation among the three isolates, where the magnitude of the genetic correlation between the isolates Isoline 6-

7-1 (SRL-5) and O. Caribe 8-17-1 –4) was very high (r = 0.89). These results suggest that both isolates are controlled by the same gene, however, resistance to Fanny 54 (SRL-6) is controlled by a different gene or genes.

Blast resistance in the rice line 75-1-127. This line derives its resistance form the wild rice species *Oryza minuta* and is supposed to carry just one resistance gene, Pi-9. Analysis of the number of resistant and susceptible plants in the F_2 generation (Table 7) indicates that a dominant and a recessive gene confer blast resistance to the blast isolates Fanny 54 (SRL-6) and Isoline 6-7-1 (SRL-5), while two dominant genes control the resistance to the isolate O. Caribe 8-17-1 (SRL-4). These results were corroborated in the F_3 generation where the epistatic and segregations models 13:3 and 15:1 fitted well the observed segregation of resistant and susceptible plants (Table 7). The literature indicates that the blast resistance gene Pi-9, is the only gene present in the line 75-1-127. This line has exhibited complete blast resistance in field trials conducted in at least 14 different Asian countries as well as in controlled inoculations at IRRI, Philippines. No compatible isolates with the line 75-1-127 were found, and therefore, the resistance of the line 75-1-127 and its resistance gene Pi-9, has been referred as broad and stable. This line is being used as a source of durable broad blast resistance in many countries of the world, especially in Asia. Our results demonstrate that the rice line 75-1-127 carries more than one resistance gene and not only Pi-9. Compatible isolates with this line were found after three years of field-testing at our experiment station Santa Rosa in Colombia (Annual Report 2005). The presence of additional resistance genes besides the gene Pi-9 could explain the broad spectrum of resistance and lack of susceptibility to the Asian blast populations.

A chi-square test (Table 8) of the hypothesis of independent segregation for each pair of isolates in the F_3 lines and considering the reaction types resistant, segregating, and susceptible, indicates a significant similarity (67.1%) for the blast reaction to the isolates Fanny 54 (SRL-6) and Isoline 6-7-1 (SRL-5) but not for the other two pairs (Table 8). These results suggest that the same genes present in the line 75-1-127 control the resistance to the genetic lineages SRL-6 and SRL-5, but at least one more and different gene controls the resistance to lineage SRL-4.

To determine the genetic relation for the blast reaction to the isolates used, the genetic correlation coefficients of the reaction of the F_3 lines were estimated for each pair of isolates. These coefficients were calculated with transformed data of the average percentage of leaf area affected, allowing determining the respective genetic covariance (Table 9 and Table 10). The genetic correlation observed for the blast reaction to Fanny 54 (SRL-6) and Isoline 6-7-1 (SRL-5) was significantly high (r = 0.8251), supporting our hypothesis that the same genes present in the cultivar control the resistance to the two genetic lineages of the pathogen. The genetic correlation for the other two pair of isolates were relatively low, although significant, suggesting that one common gene can control the resistance to the three isolates, but that the line 75-1-127 carries at least other gene that also confers resistance to the isolate O. Caribe 8-17-1 (SRL-4). Finally, our results indicate that the line 75-1-127 can be used as a donor of resistance to the genetic lineages SRL-6, SRL-5, and SRL-4, but to assure more stability, this resistance has to be based on all the genes present in the line and not only on the blast resistance gene Pi-9.

Conclusions

- The blast resistance present in the Brazilian land race Tres Marias to the Colombian blast lineage SRL-6 is complex and probably controlled by major and minor genes
- The blast resistance present in the cultivar Tres Marias to the blast genetic lineages SRL-5 and SRL-4 is controlled by one common and dominant resistance gene
- The complex nature of the resistance present in the cultivar Tres Marias suggests that this resistance can be a good source of durable blast resistance and therefore it is recommended to continue genetic and molecular work to elucidate the nature of this resistance and to use it in rice breeding programs
- The rice line 75-1-127 carries other resistance genes besides Pi-9
- Resistance of the line 75-1-127 to the genetic lineages SRL-6 and SRL-5 is controlled by one dominant and one recessive gene
- Two dominant resistance genes present in the line 75-1-127 control the resistance to the genetic lineage SRL-4
- It is possible that the blast resistance gene Pi-9 present in the line 75-1-127 confers resistance to the three genetic lineages SRL-6, SRL-5, and SRL-4, but this gene has to be accompanied with the other resistance genes present in the line to increase its resistance stability
- Given the different origin of the rice lines Tres Marias and 75-1-127, it is recommended to identify and compare the blast resistance genes present in each cultivar to determine their complimentarily and potential use of resistance gene combinations derived from both sources to develop rice cultivars with durable blast resistance

Future Work

The identification of the amount and location in the rice genome of the blast resistance genes present in the rice lines Tres Marias and 75-1-127 and reported here will continue. Recombinant inbred lines (RILs) will be developed and advance from individual plants selected within the F_3 lines used in these studies. Molecular microsatellite markers associated with the resistance genes will be identified for their use in a marker assisted selection breeding program for the introgression of these resistance genes into our improved rice germplasm.

Table 3. Observed and expected segregation ratios of resistant and susceptible plants in the F_2 and F_3 generations for the genetic cross between the rice cultivars Fanny x Tres Marias inoculated with three genetic lineages of *Pyricularia grisea*

		RICE BLAST ISOLATES (Genetic Lineage)								
		Fanny 54 (SRL-6)			**Isolinea 6-7-1 (SRL-5)**			**O. Caribe 8 (SRL-4)**		
Generation	**Reaction**	**Obs. No.**	**Exp. No.**	**? ^{2} (3:1)[1]**	**Obs.**	**Exp.**	**X^2 (3:1)**	**Obs.**	**Exp.**	**X^2 (3:1)**
	Resistant	147	149.3	0.03	139	140.3	0.01	135	130.5	0.16
F_2	**Susceptible**	52	49.8	0.10	48	46.8	0.03	39	43.5	0.47
	Total	199	199.0	0.14	187	187.0	0.04	174	174.0	0.62
				(5:3)			**(5:3)**			**(5:3)**
	Resistant	2266	1937	55.93	1982	1936	1.08	2037	1939.4	4.91
F_3	**Susceptible**	833	1162	93.21	1116	1162	1.80	1066	1163.6	8.19
	Total	3099	3099	149.14	3098	3098	2.88	3103	3103.0	13.10

[1] X^2 (0.05, 1) = 3.84; X^2 (0.01, 1) = 6.63

Table 4. Test of independence in the F_3 generation of the cross Fanny x Tres Marias inoculated with the rice blast isolates Fanny 54 (F54), Isolinea 6-7-1 (Isol 6-7-1), and Oryzica Caribe 8-17-1 (OC 8)

		Isolates F 54 and Isol 6-7-1		**Isolates F54 and OC 8**		**Isolates I. 6-7-1 and OC 8**	
Test	**Degrees of Freedom**	**X^2**	**Probability**	**X^2**	**Probability**	**X^2**	**Probability**
Chi-square	2	25.92	<0.0001	22.67	<0.0001	**0.2046**	**0.90**

Table 5. Analysis of variance of the percentage of leaf area affected in the F_3 generation of the cross Fanny x Tres Marias inoculated with the blast isolates Fanny 54 (F54), Isolinea 6-7-1 (Isol 6), and Oryzica Caribe 8-17-1 (OC 8)

Source of variation	**Degrees of Freedom**	**Mean Square(MS)**					
		F54 (x)	**F54.Isol 6 (x+y)**	**Isol 6 (y)**	**F54.OC8 (x+z)**	**OC8 (z)**	**Isol 6.OC8 (y+z)**
Block	1	0.0026	0.0099	0.0023	0.1490	0.1121	0.1467
Progeny	155	0.0373	0.6504	0.4495	0.5595	0.3771	1.5183
Error	311	0.0048	0.0273	0.0228	0.0205	0.0191	0.0341
Genetic variance		0.0162		0.2133		0.1790	

Table 6. Analysis of covariance of the percentage of leaf area affected in the F_3 generation of the cross Fanny x Tres Marias inoculated with the blast isolates Fanny 54 (x), Isolinea 6-7-1 (y), and Oryzica Caribe 8-17-1 (z)

Source of Variation	Degrees of Freedom	Mean Products (MP)		
		MP (x + y)	MP (x + z)	MP (y + z)
Block	1	…	…	…
Progeny	155	0.0818	0.0725	0.3459
Error	311	0.0410	-0.0017	-0.0039
Genetic covariance		0.0204	0.0371	0.1749
Correlation Coefficient	(R)	0.3466	0.6867	**0.8929**

Table 7. Observed and expected segregation ratios of resistant and susceptible plants in the F_2 and F_3 generations for the genetic cross between the rice cultivars Fanny x 75-1-127 inoculated with three genetic lineages of *Pyricularia grisea*

		RICE BLAST ISOLATES (Genetic Lineage)								
		Fanny 54 (SRL-6)			Isolinea 6-7-1 (SRL-5)			O. Caribe 8 (SRL-4)		
Generation	**Reaction**	**Obs. No.**	**Exp. No.**	**? ^{2} (13:3)[1]**	**Obs.**	**Exp.**	**X^2 (13:3)**	**Obs.**	**Exp.**	**X^2 (15:1)**
	Resistant	140	140	0.0004				149	162	0.286
F_2	**Susceptible**	32	32	0.0019				4	11	4.297
	Total	172	172	0.0024				173	173	4.583
				(13:3)			**(13:3)**			**(15:1)**
	Resistant	2095	2065	0.439	2173	2197	0.269	2459	2465	0.013
F_3	**Susceptible**	602	632	1.434	697	673	0.881	409	403	0.080
	Total	2697	2697	1.873	2870	2870	1.151	2868	2868	0.093

[1] X^2 (0.05, 1) = 3.84; X^2 (0.01, 1) = 6.63

Table 8. Test of independence in the F_3 generation of the cross Fanny x 75-1-127 inoculated with the rice blast isolates Fanny 54 (F54), Isolinea 6-7-1 (Isol 6-7-1), and Oryzica Caribe 8-17-1 (OC 8)

Test	Degrees of Freedom	Isolates F 54 and Isol 6-7-1		Isolates F54 and OC 8		Isolates I. 6-7-1 and OC 8	
		X^2	Probability	X^2	Probability	X^2	Probability
Chi-square	2	**0.80**	**0.671**	6.95	0.031	7.06	0.0293

Table 9. Analysis of variance of the percentage of leaf area affected in the F_3 generation of the cross Fanny x 75-1-127 inoculated with the blast isolates Fanny 54 (F54), Isolinea 6-7-1 (Isol 6), and Oryzica Caribe 8-17-1 (OC 8)

Source of variation	Degrees of Freedom	Mean Square(MS)					
		F54 (x)	F54.Isol 6 (x+y)	Isol 6 (y)	F54.OC8 (x+z)	OC8 (z)	Isol 6.OC8 (y+z)
Block	1	0.0089	0.0002	0.0063	0.0285	0.0056	0.0000
Progeny	166	0.1519	1.0182	0.4580	0.4117	1.1330	0.8516
Error	333	0.0149	0.0312	0.0205	0.0218	0.0099	0.0298
Genetic variance		0.0685		0.2188		0.0616	

Table 10. Analysis of covariance of the percentage of leaf area affected in the F_3 generation of the cross Fanny x 75-1-127 inoculated with the blast isolates Fanny 54 (x), Isolinea 6-7-1 (y), and Oryzica Caribe 8-17-1 (z)

Source of Variation	Degrees of Freedom	Mean Products (MP)		
		MP (x + y)	MP (x + z)	MP (y + z)
Block	1	…	…	…
Progeny	166	0.2041	0.0633	0.1303
Error	333	-0.0021	-0.0015	-0.0010
Genetic covariance		0.1010	0.0309	0.0646
Correlation Coefficient	(R)	**0.8251**	0.4759	0.5571

References

Correa-Victoria, F.J. & R.S. Zeigler, 1993. Pathogenic Variability in *Pyricularia grisea* at a Rice Blast " Hot Spot" Breeding Site in Eastern Colombia. Plant Disease 77: 1029-1035.

Correa-Victoria, F.J., D. Tharreau, C. Martínez, M. Vales, F. Escobar, G. Prado & G. Aricada, 2002. Combinación de genes en arroz para el desarrollo de resistencia durable a *Pyricularia grisea* en Colombia. Fitopatología Colombiana 26: 47-54.

Lopez-Gerena, J. 2006. Mapping QTL controlling durable resistance to rice blast in the cultivar Oryzica Llanos 5. Ph.D. Dissertation. Kansas State University. 128p.

Magia, J.M. 2007. Herencia de la resistencia a *Pyricularia grisea* Sacc. En dos líneas de arroz (*Oryza sativa*). Tesis de Maestria. Universidad Nacional de Colombia. 42p.

2B. Characterizing of the Complex of Rice Hoja Blanca Virus and *Tagosodes Orizicolus*

- **Understanding the Genetics of Resistance to Rice Hoja Blanca Virus.**

L.Calvert, I. Lozano, N. Villareal, L. Romero, L. Reyes & M. Lorieux.

Abstract

Key words: Rice Hoja Blanca Virus, *T. orizicolus*, resistance, micro-satellites, QTLs

Background

Rice hoja blanca virus causes cyclic epidemics which cause great losses. In order to develop varieties with resistance, colonies of viruliferous *T. orizicolus* were established in the mid 1980s.. The screening done at CIAT has been part of the rice breeding for Fedearroz, FLAR as well as other organizations. Fedearroz has successfully released several varieties with resistance to RHBV including Fedarroz 50, Fedarroz 2000, and Fedearroz Victoria 1 & 2. The technology of screening using a biological system is inconsistent and the colonies are costly to maintain. The use of viruliferous colonies for screening for resistance to RHBV has not been successfully transferred to other organizations and CIAT is the sole provider.

It is commonly thought that the major source of resistance to RHBV came from the Japonica variety Taku Iku 18. This was crossed with an Indica variety and the result is the line Colombia 1. This is a parent in all the crosses of commercial varieties with resistance to RHBV. Fedearroz 2000 has a higher level of resistance to RHBV than Colombia 1. Fedearroz 50 is slightly less resistance to RHBV than Colombia 1, but is highly resistant to the vector T. orizicolus.

Molecular studies were made to identify QTLs associated with resistance to RHBV and *T. orizicolus*. Crosses were made between the highly susceptible line WC 366 and Fedearroz 2000. A second cross was made between WC 366 and Fedearroz 50.
This studies identified a QTL for resistance to T. orizicolus on Cr 7 of Fedearroz 50. For resistance to RHBV a QTL on Cr 4 was identified for both Fedearroz 50 and Fedearroz 2000. An additional QTL for resistance to RHBV was identified on Cr 5 of Fedearroz 2000.

Material and Methods

Plant populations.

Cross 1. The F_1, F_2 y F_3 populations of crosses between the resistant variety Fedearroz (FD) 2000 (CT10323-29-4-1-1T-20) was crossed with the susceptible line WC 366 (IR65598-27-3-1). From 235 plants of the F_2 population, the F_3 seed was collected.

Cross 2. The F_1, F_2 y F_3 populations of crosses between the resistant variety Fedearroz (FD) 50 was crossed with the susceptible line WC 366 (IR65598-27-3-1). From 291 plants of the F_2 population, the F_3 seed was collected.

Evaluation for RHBV.

Ten rows each with 20 plants for a population of 200 were used for the evaluation of the F_2 generation as well as for 235 lines of the F_3 generation. Twenty-five days after planting, the rice lines were infested with a dosage of 1.5 insects per plant using viruliferous insects from the colony "Costa-CIAT". Five days after the infestation, the insects were collected and tested using ELISA in order to determine the virulence of the insects used in the evaluation. The remaining insects were eliminated and each week for four weeks the plants were evaluated for symptoms of RHBV.

Evaluation for resistance to *T. orizicolus*.

Each row consists of twenty plants of a line, and after 15 days, they are infested with approximately 10 *T. orizicolus* nymphs per plant. When the susceptible check Bluebonnet 50 dies, the lines are evaluated . Cica 8 was the intermediate control and Makalioka was the resistant control. The evaluation scale was a standard scale developed by IRRI (1996).

Micro-satellite analysis.

For the micro-satellite analysis, 20 F_3 plants from each of the 235 lines were grown. From each of the 231 populations that successfully germinated, 200 mg of tissue from each plant was collected at 20 days after planting. For each line the tissue sampled were placed together to make up the bulk samples. The samples were frozen in liquid nitrogen and stored at –80C until processed. The DNA was isolated and quantified. The PCR reactions for the micro-satellites were made with the commercial primers and the PCR products visualized with silver staining of the 6% polyacrylamide, 7M urea gels. The analysis calculated the distances with interval map using the program MapDistov17b.

Results and Discussion

Evaluation of the crosses.

The evaluation of 231 F3 families representing the F2 lines for RHBV and *T. orizicolus* from the cross FD 2000 X WC 366 were reported in the Rice AR 2005. The evaluation of 291 F3 families representing the F2 lines for RHBV and *T. orizicolus* from the cross Fd 50 X WC366 were reported in rice AR 2005.

The F2 plants of both crosses were evaluated using microsatellites. For the cross with Fedearroz 2000 four potential QTLs were identified for resistance to RHBV and three potential QTLs were identified for resistance to *T. orizicolus*. The QTL for RHBV

resistance on the short arm of chromosome 4 was highly significant. For the cross with Fedearroz 50, four potential QTLs were identified for resistance to RHBV and five potential QTLs were identified for resistance to *T. orizicolus*. The QTL on chromosome 4 for RHBV resistance was also identified in this cross.

The QTL that is located on the short arm of chromosome 4 is highly significant for resistance to RHBV. This QTL was identified in both the Fedearroz 2000 and the Fedearroz 50. A series of 16 micro-satellites were analyzed for Fedearroz 2000 cross and 14 were analyzed for the Fedearroz 50 cross. An interval map (figures 1A & 1B) profile the areas where the association with resistances is most significant. While the areas under the grafts are not the same, they do overlap. It is not clear if the QTL identified for Fedearroz 2000 and Fedearroz 50 are the same or distinct. The working hypothesis is that they represent the same QTL, and this will be determined by addition saturation of the region with additional markers which will allow the local of the resistance to be mapped more precisely.

On chromosome 7, there was a highly significant QTL for resistance to mechanical damage by the planthopper for Fedearroz 2000. An interval map (figure 3) profile the areas where the association is most significant. This QTL is very strong and additional markers are needed to better define the region most associated with the resistance. This QTL is absent in Fedearroz 2000. Fedearroz 50 is known to have antibiotic resistance and this QTL may be the region that contains the gene encoding this resistance.

On chromosome 5, there was a significant QTL for resistance to mechanical damage by the planthopper. An interval map (figure 2) profile the areas where the association is most significant. This QTL has two peaks but the level of saturation is still not enough determine if these are independent QTLs. While this QTL was not detected in Fedearroz 50, the effect of the very strong QTL on chromosome 7 could be masking the effect other resistance genes. An analysis to mitigate the effect of the QTL is being made to assure that this QTL is not present in Fedearroz 50.

Conclusions

1. A QTL associated with resistance to RHBV was located on the short arm of chromosome 4. This QTL appears to be similar on both Fedearroz 50 and Fedearroz 2000. The position of this QTL has been better defined by the addition of more markers.
2. In Fedearroz 2000, a QTL associated with resistance to *T. orizicolus* was located on chromosome 5. This QTL does not appear to be present in Fedearroz 50.
3. In Fedearroz 50, there is a very strong QTL that is associated with resistance to *T. orizicolus*.

Future Activitiens

1. For both crosses, additional markers are needed to fill in gaps and saturate the genomic maps. This may allow the association of additional QTLs with resistance to RHBV and/or *T. orizicolus*.
2. For the QTLs that have been identified, additional fine mapping needs to be done.
3. The markers that identified the QTLs need to be tested for their utility as molecular markers for resistance to RHBV or *T. orizicolus* need to be tested.

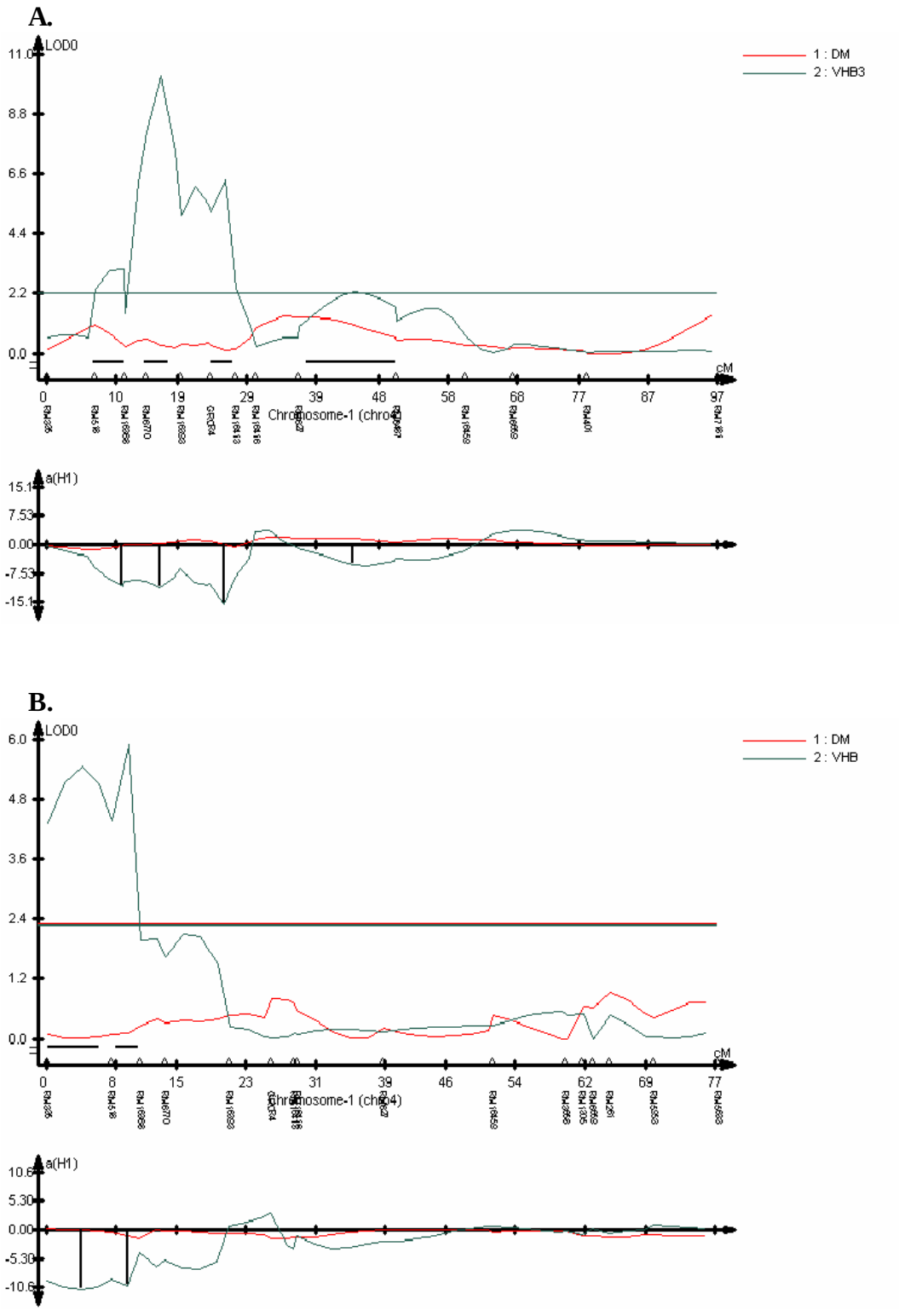

Figure 1. To more precisely map the association with resistance to RHBV A: an interval map calculated from 14 markers on chromosome 4 in the cross Fd50 X WC366, and B: an interval map calculated from 16 markers on chromosome 4 in the cross Fd2000 X WC366 were calculated.

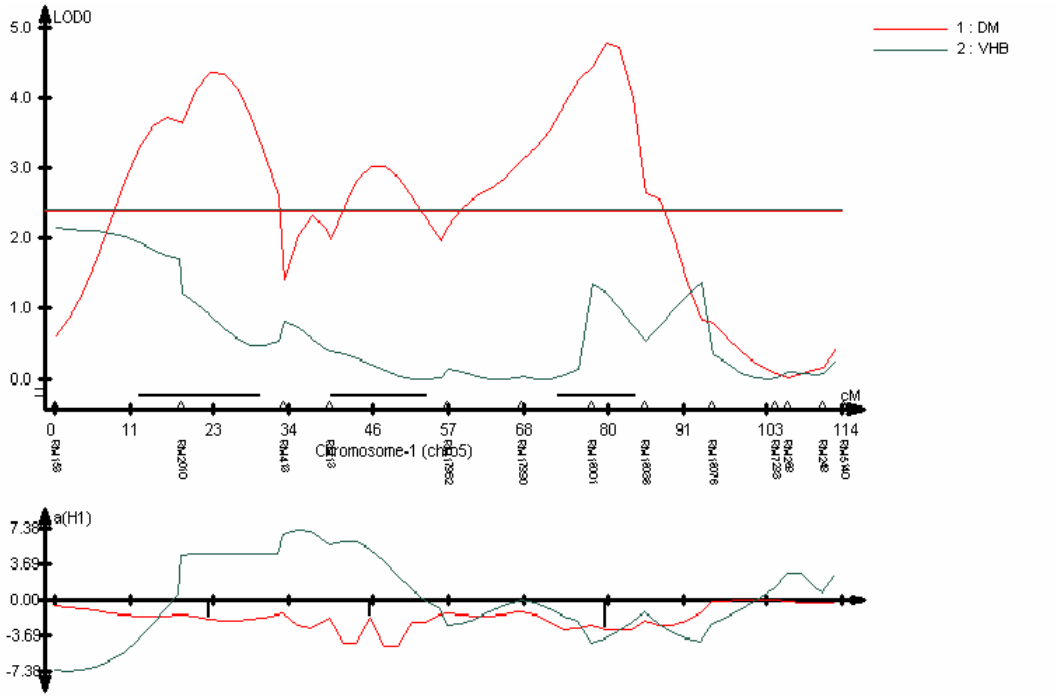

Figure 2. For the association with resistance to *T. orizicolus* an interval map calculated from 13 markers on chromosome 5 in the cross Fd2000 X WC366.

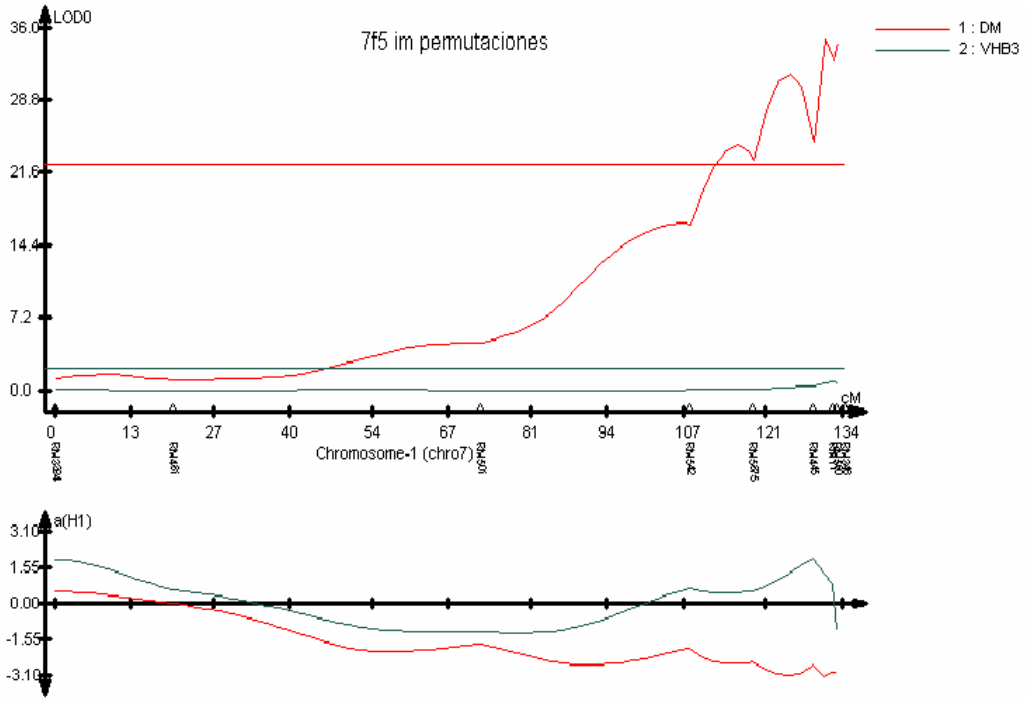

Figure 3. For the association with resistance to *T. orizicolus* an interval map calculated from 13 markers on chromosome 7 in the cross Fd50 X WC366.

References

Calvert, L.A., Lozano I., Villareal N., Romero L., Lorie uz M., Martinez C., Garavito A. 2006. Molecular analysis of the genetics of resistance to rice hoja blanca virus and its vector Tagosodes orizicolus. Phytopathology 96:S18

D.A. Cartwright, M. Troggio, R. Velasco, A. Gutin. 2007. Genetic Mapping in the Presence of Genotyping Errors. Genetics: Published Articles Ahead of Print, published on February 4, 2007 as 10.1534/genetics.106.063982.

Lorieux, M. 2007. MapDisto, A Free User-Friendly Program For Computing Genetic Maps. Computer demonstration given at the Plant and Animal Genome XV conference, Jan 13-17 2007, San Diego, CA. URL: http://mapdisto.free.fr.

Lincoln S, Daly M, Lander ES (1992) Constructing genetic maps with MAPMAKER/EXP 3.0, 3rd edn. Whitehead Institute Technical Report, Cambridge.

McCouch SR, Teytelman L, Xu YB, Lobos KB, Clare K, Walton M, Fu BY, Maghirang R, Li ZK, Xing YZ, Zhang QF, Kono I, Yano M, Fjellstom R, Declerck G, Scheider D,

Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.) DNA Res 9:199–207.

Wang, S., C. J. Basten, P. Gaffney and Z-B. Zeng, 2004 Windows QTL Cartographer 2.0 User Manual (http://statgen.ncsu.edu/ qtlcart/WQTLCart.html).

Zeigler R, Rubiano M, Pineda A (1988) A field screening method to evaluate rice breeding lines for resistance to the hoja blanca virus. Annals Applied Biology 112:151-158.

2C. Characterizing Rice Pest and Genetics Resistance

- **Final selection for RHBV resistance and yield potential of advanced breeding generation from crosses with transgenic rice resistant to RHBV in the field in 2006**

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Abstract

The Rice Biotechnology Project had generated transgenic rice lines with resistance to RHBV (Lentini *et al*., 2003) an endemic disease and of the main constrains of rice of Tropical America. Resistant transgenic lines carrying different source of resistance than the one already deployed in commercial varieties would allow increased production to a lower cost by reducing the need of insecticide applications commonly used to control *Tagosodes oryzicolus* (the virus vector). After stepwise selection from the original transgenic plants, including elimination of un-stable inheritance of RHBV resistance and plants with undesirable agronomic traits, the best lines were crossed with various varieties and derived progenies had been subjected to a breeding selection process in the field including evaluation for yield potential, other disease resistance (*Rhizoctonia* and *Pyricularia*), agronomic and grain quality traits. This report describes the final selection of the best lines, step after which these lines should be evaluated by third party and decision should be made on the fate of these materials for potential deployment to farmer fields.

Key Words: transgenic rice, RHBV, yield potential, field

Background

The transgenic RHBV resistant rice was generated by splicing in genes from the RHBV virus into Cica 8 variety (Lentini *et al*., 2003), selected by the breeders as potential parent donor of the transgene for future conversion of modern varieties because of its good grain quality, high productivity, and broad adaptation including low soil humidity, and acceptance to large and small resource farmers. In spite of this variety is still of preference by small farmers due to its good agronomic qualities and resilience to stress conditions, this variety is not currently grown commercially because of its high susceptibility to RHBV. The transgenic resistance incorporated is RNA-mediated and some plants show hypersensitive reaction when challenged with the RHBV virus (Lentini et al., 2003). Some of the transgenic lines outperform in RHBV resistance some of most currently grown commercial rice varieties. Attempts to transfer this resistance into other modern varieties through regular crossing indicated that the RHBV-N transgene is inherited and expressed independently of the genotype background. Thus this transgenic resistance could be used to complement the breeding resistance that has been deployed so far and does not protect plants when younger than 5 to 20 day-old. In addition to

selection for RHBV resistance and yield potential, the advanced lines were evaluated for tolerance to *Rhizoctonia*, resistance to *Pyricularia*, and grain quality traits. Progress in previous years led to a shift from the testing of concepts towards the final steps for its potential release to farmers' fields. Previous reports described the stepwise breeding selection in the field. Last year we reported the selection of advanced 8 advanced progeny lines derived from the original transgenic lines, and 8 lines derived from crosses between selected transgenic lines and with commercial varieties, for a total of 28 individual-plant selections. This year we report the evaluation and selection for RHBV resistance in the field, and final selection based on their RHBV resistance, tolerance to sheath blight and blast, grain quality and agronomic traits, and yield potential of T_7 and T_{10} advanced transgenic lines, and F_6 progeny plants derived from crosses between the transgenic lines and commercial varieties .

Materials and Methods

Evaluation of RHBV resistance in the field in 2006. Field evaluations were conducted using 12 T_7 and T_{10} advanced transgenic lines, and 16 F_6 families derived from crosses between the transgenic lines and the commercial varieties Fedearroz 50 or Oryzica 1. The selection of the original lines was based on its agronomic performance in the field in 2005. A row with 40 plants per line with 4 replicates was used for the RHBV evaluation. Controls consisted of RHBV resistant plants (Colombia 1, and Fedearroz 2000) and susceptile checks (Bluebonnet 50, Cica 8, Oryzica 1, Fedearroz 50, a cross Cica 8/ Fedearroz 50, and Cica 8/ Oryzica 1). Eighteen days after planting, plants were infested with dosages of 1.5 insects per plant using viruliferous insects from the colony "Tolima-CIAT". Five days after the infestation, the insects were killed, and the plants were evaluated for disease symptoms development at 30 and 45 after infestation.

Agronomic evaluation and selection of advanced generations of transgenic events and derived progeny plant from crosses. The same lines evaluated for RHBV resistance were also evaluated for agronomic performance in the field. Plants were transplanted in the field using 8 rows per lines of 21 plants per rows with 3 replicates. Agronomic traits were evaluated throughout the life cycle up to maturity. Tiller number, plant height, plant vigor, days to flowering, fertility, and yield was evaluated. Agronomic traits were evaluated according the scale IRRI (1996).

Results and Discussion

Cluster analysis of advanced crosses or self-cross transgenic lines using principal coordinates were conducted using data from the RHBV resistance evaluations with one dosage of 1.5 insects per plant and 3 replicates. Five F_6 generation lines derived from crosses with Oryzica 1, and five T_7 and T_{10} self progeny advanced transgenic lines were clustered in Groups 9 and 8 respectively, jointly with Fedearroz 2000 showing the highest level of RHBV resistance (score = 3) (Figure 1A and 1B, and Table 1). The RHBV resistance of these crosses and transgenic lines were significantly different from their corresponding non-transgenic controls (the controls clustered in other groups). The non-transgenic cross Cica 8/Oryzica 1 score = 5.7, and the non-transgenic control varieties

Cica 8, score = 7.7 and Oryzica 1, score = 7 were clustered in other groups with susceptible materials likewise the controls Cica 8/Fedearroz 50, score = 5.7; and Fedearroz 50, score = 7.7(Figure 1A and 1B, and Table 1). The susceptible transgenic control line A3-78-24 (which does not contain the RHBV transgene, internal control for the transgenic procedure) show the highest susceptibility as in earlier evaluations (score = 9).

The lines listed in Table 1 showed RHBV resistance score $\leq$ 5, have medium to long slender grains with low degree of white center and high amylose, tolerance to sheath blight (*Rhizoctonia sp)* and known resistance race-profile to *Pyricularia grisae* pathogen, as shown last year. Most lines with highest RHBV resistance level (score = 3) also showed promissory agronomic characteristics such as high plant vigor, intermediate days to flowering initiation and days to 50% anthesis, plant type, plant height, and tillering capacity.

The crosses with Fedearroz 50 were discarded by its genetic instability over generations, although some plants showed the highest vigor and agronomic performance (data not showed). No significant differences for agronomic traits and grain yield were found between the transgenic materials selected and the non-transgenic controls (crosses or commercials varieties Fedearroz 2000, Fedearroz 50, Cica 8, Oryzica 1 and Colombia 1)(Tables 1). Some lines showed low plant fertility (70%) likewise some of the commercial varieties (Fedearroz 50), but most lines showed fertility above 90%. Only lines with high fertility ($\geq$ 95%) were selected. Some lines with good agronomic traits and grain quality were discarded (5 of total 28 lines, 18%) because of its inconsistency response to RHBV. Most instability in RHBV response was discarded in earlier generations. It is important to highlight that at these advanced generations which had been subjected to at least 5 cycles of selection for RHBV resistance, 82% of the evaluated lines showed stable resistance. This stability is also seen in experimental designs sowing small plots of 0.8 m X 0.8 m of each line infested with virouliferous insects at a density of 1.5 insects per plant, where clear-cut resistance level differences are noted between the original Cica 8 susceptible variety and the lines selected with resistance to RHBV score $\leq$ 3 (Figure 2)

Based on this RHBV resistance profile, the agronomic performance including yield potential, and previous evaluations for sheath blight and blast resistance as well as for grain quality traits, three F6 generation lines from the cross with Oryzica 1, one T7 line and four T10 transgenic lines composed the final selection (Table 1, lines in bold letters). Of the 3 lines selected from crosses with Oryzica 1, two of them are sister lines; likewise, of the 5 transgenic lines selected four of them are sister lines (Table 1). Results suggest that the stability of traits over generation is genetically determined by the transgenic lines selected in early T_3 generation (generation selected to initiate the pedigree process). The final selections are derived from lines: A3-49-60-4-5; A3-49-60-12-3; A3-49-60-13-69; and A3-49-101-18-19. Furthermore, 6 of the 8 lines selected are derived from the original T_1 transgenic line A3-49-60 (Lentini et al., 2003).

Future Activity

The selected lines are currently being processed through anther culture to generate doubled haploid (complete homozygous) lines, convenient material for seed multiplication of replicated multi-location field trials and molecular genotyping.

References

Lentini Z., Lozano I, Tabares E., Fory L., Domínguez J., Cuervo M., Calvert L. 2003. Expression and inheritance of hypersensitive resistance to rice hoja blanca virus mediated by the viral nucleocapsid protein gene in transgenic rice. Theoretical and Applied Genetics 106: 1018-1026.

IRRI (1996). Standard evaluation system for rice. 4th edition. 52p

Table 1. RHBV resistance and agronomic performance in the field in 2006 of selected families from crosses (total 9) derived from crosses between Cica 8-RHBV transgenic resistant lines and commercial variety Oryzica 1, and Cica 8-RHBV transgenic resistant progeny (total of 14) derived from self cross.

Pedigree	Cluster		[1]Average RHBV	Yield (Kg/ha) Potential	[2]Vig	[3]DF	[4]DF 50%	Plant Height (cm)	[5]Tiller	F (%)
	C	L								
A3-49-101-18-19/Oryzica 1-14-M-10-1-2	8		4,3	10,912	3	98	105	105	3	95
A3-49-101-18-19/Oryzica 1-14-M-10-1-3	**9**		**2,3**	**10.975**	**3**	**98**	**103**	**110**	**3**	**95**
A3-49-101-18-19/Oryzica 1-14-M-10-2-4	8		4,3	9,508	3	94	98	110	3	95
A3-49-101-18-19/Oryzica 1-14-M-7-2-M-1	**9**		**3,0**	**8,643**	**3**	**90**	**96**	**100**	**3**	**98**
A3-49-60-4-13/ Oryzica 1-13III-6-5-M-1	9		3,0	6,864	3	99	104	120	3	70
A3-49-60-4-13/ Oryzica 1-13III-6-5-M-2	9		2,3	5,691	3	100	105	125	3	70
A3-49-60-4-13/ Oryzica 1-13III-6-5-M-3	1		5,0	7,431	3	98	105	120	3	70
A3-49-60-4-5/ Oryzica 1-15-15-11-M-3	**9**		**3,7**	**9,792**	**3**	**110**	**116**	**105**	**3**	**95**
A3-49-60-4-5/ Oryzica 1-15-15-11-M-6	10		5,0	17,508	3	107	112	115	3	95
A3-49-60-13-69-M-1-4-1		5	3,7	9,002	3	109	114	110	3	95
A3-49-60-13-69-M-1-4-2		**8**	**3,0**	**14,543**	**3**	**109**	**114**	**115**	**3**	**97**
A3-49-60-13-69-M-1-4-3		6	3,0	9,253	3	108	113	120	3	95
A3-49-60-12-3-20-M-13-2-1-M-1		7	4,3	8,534	3	98	105	115	3	97
A3-49-60-12-3-20-M-13-2-1-M-2		**8**	**3,7**	**12,814**	**3**	**98**	**105**	**118**	**3**	**97**
A3-49-60-12-3-20-M-13-2-1-M-3		5	3,7	10,596	3	107	112	118	3	95
A3-49-60-12-3-20-M-13-2-2-M-1		6	3,0	5,766	3	106	112	115	3	95
A3-49-60-12-3-20-M-13-2-2-M-2		**8**	**4,3**	**6,301**	**3**	**109**	**114**	**117**	**3**	**95**
A3-49-60-12-3-20-M-8-4-4-M-3		**8**	**3,0**	**15,141**	**3**	**105**	**110**	**125**	**3**	**95**
A3-49-60-12-3-20-M-8-4-4-M-4		5	3,7	8,532	3	102	106	115	3	90
A3-49-60-12-3-20-M-8-4-4-M-5		**8**	**3,7**	**7,326**	**3**	**105**	**109**	**115**	**3**	**97**
A3-49-60-12-3-20-M-8-6-4-M-1		5	4,3	5,507	3	102	106	125	3	95
A3-49-60-12-3-20-M-8-6-4-M-3		7	3,7	9,191	3	105	110	125	3	90
A3-49-60-12-3-20-M-8-6-4-M-4		6	5,0	8,987	3	105	109	125	3	90
Cica 8/ Fedearroz 50	5		5,7	9,793	3	105	110	120	3	90
Cica 8 / Oryzica 1	5		5,7	7,883	3	96	110	115	3	80
Cica 8	7	1	7,7	6,580	3	98	105	110	3	91
Fedearroz 2000	**9**	**8**	**2,3**	**15,429**	**3**	**99**	**105**	**115**	**3**	**95**
Fedearroz 50	3	1	7,7	7,434	3	105	112	120	3	70
Oryzica 1	5	1	7,0	9,555	3	94	101	100	3	95
Colombia 1	***10***	***6***	***4,3***	***10,085***	***3***	***101***	***105***	***155***	***3***	***85***
A3-78-24		4	9,0	7,490	3	107	113	110	3	95

[1] Average value score for resistance to the RHBV. [2] Plant vigor (1) higest vigor and (5) lowest vigor. [3]DF days to flowering initiation. [4] Days to 50% anthesis. F = mean fertility % per line. [5]Tillering ability (1) Extra vigorous and (9) Very weak plant.

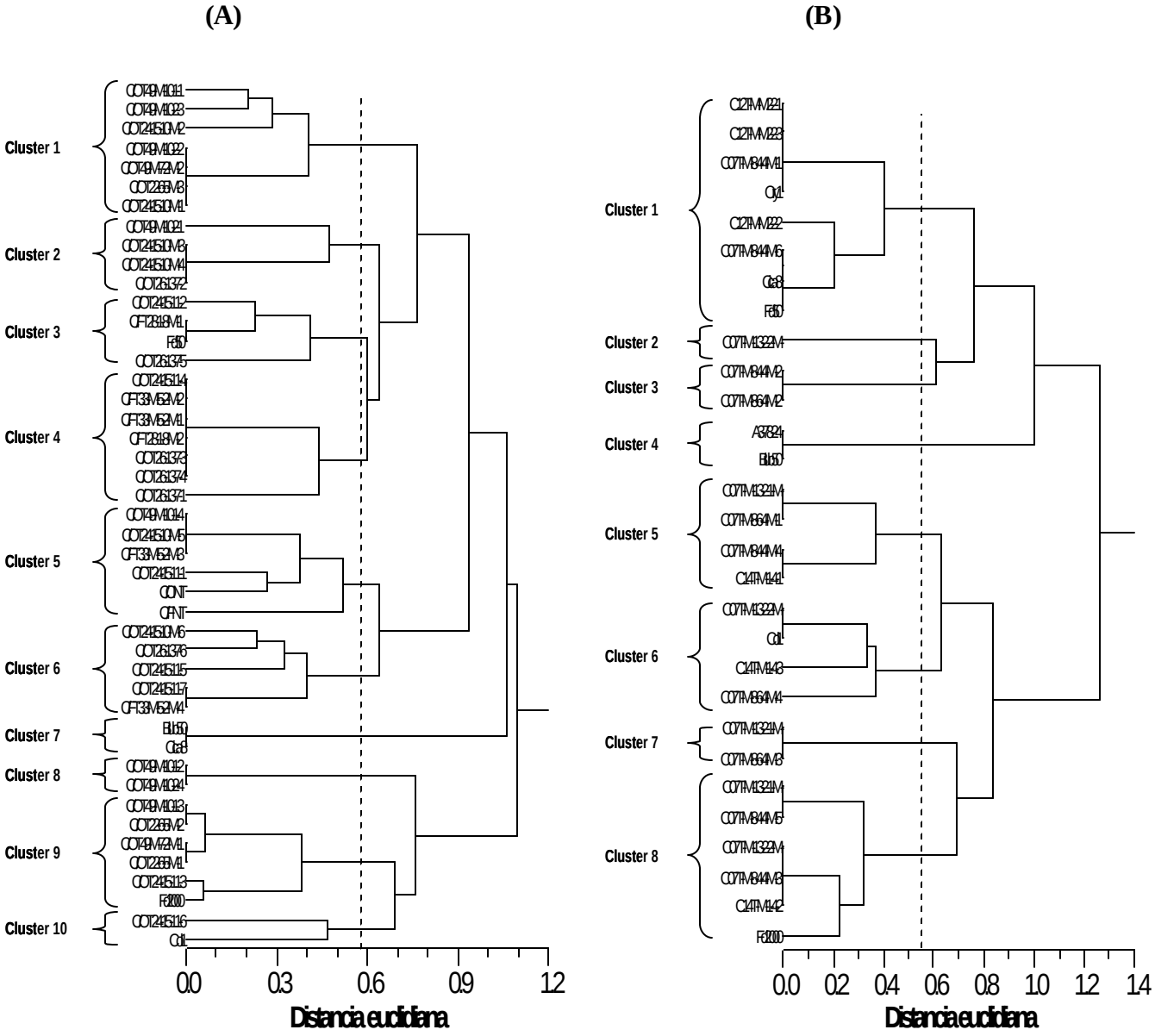

Figure 1. Similarity analysis for RHBV resistance response of 38 F_7 plants selected from crosses between selected Cica 8-RHBV transgenic resistant lines and Oryzica 1 (A), and 21 T_9 to T_{12} plants Cica 8-RHBV transgenic resistant lines (B) selected progeny plants derived from self cross. Controls consisted of varieties Fedearroz 2000 (highly resistant, score $\leq$3); susceptible checks of Oryzica 1 (score = 7); Cica 8 (score = 7.7); Fedearroz 50 (score = 7.7) and Bluebonnet 50 (highly susceptible, score = 9); and the non-transgenic cross Cica 8/Oryzica 1 and Cica 8/Fedearroz 50 (each score = 5.7).

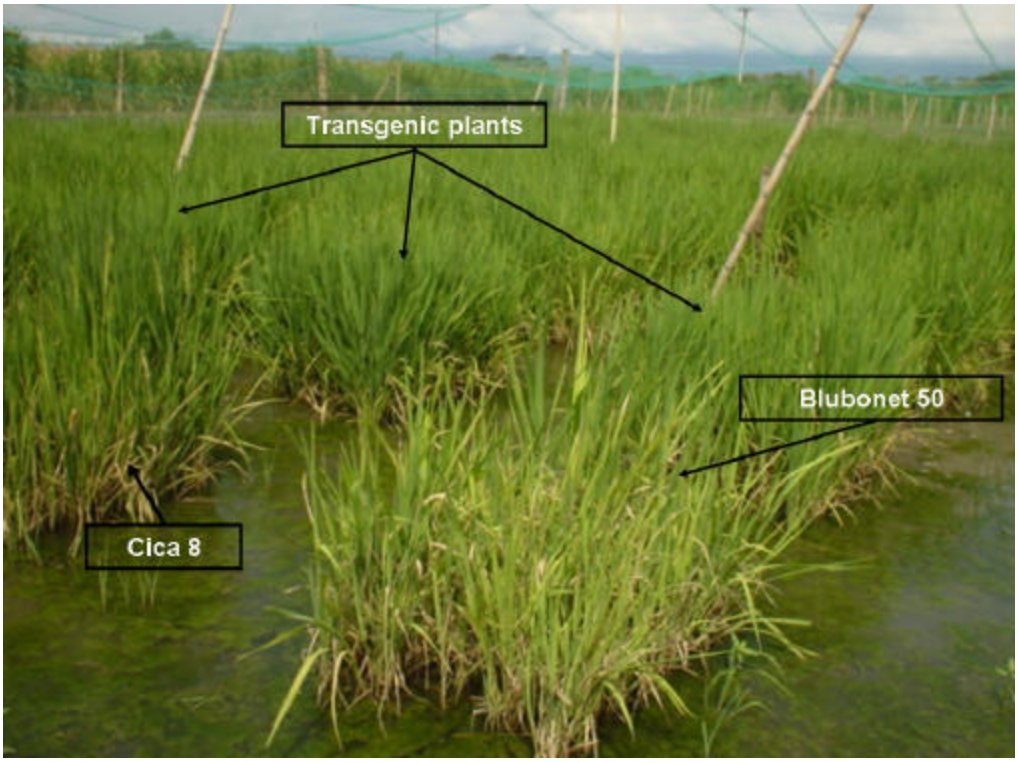

Figure 2. RHBV resistant transgenic lines (score $\leq$ 3) and susceptible varieties Cica 8 and Bluebonnet 50 planted in the field in small plots of 0.8 X 0.8 m and infested with virouliferous insects at a density 0f 1.5 insects per plant.

OUTPUT 3. ENHANCING REGIONAL RICE RESEARCH CAPACITIES AND PRIORITIZING WITH EMPHASIS ON THE SMALL FARMERS.

3A. Participatory breeding of upland rice in Nicaragua

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- NGOs, farmers' organizations and other institutions in Nicaragua: UNAG/PcaC, UCA Siuna, SERVITECA, CIPRES, PPB-MA program.

Source of funding: CIRAD, CIAT, MAE France

Abstract

With a strong involvement of local NGOs and farmers' organizations and using participatory crop improvement (PCI) approach and methods, the CIRAD-CIAT project in Nicaragua aims to take advantage of the diversified advanced lines and segregating synthetic populations recently developed by CIAT and CIRAD in Colombia as well as the new site-specific populations developed in-situ, in order to identify better-performing varieties matching the needs of the small and medium-scale upland rice growers. Research activities conducted during the last two years have permitted to identify well-performing and adapted lines for the existing upland cropping systems (manual as well as mechanized systems) and to give new variety options for the rice areas with increasing drought constraints. Among these lines, IRAT 364 and IRAT 366 will be released by the extension agency SERVITECA for small farmers in low inputs upland cropping systems; one variety for mechanized cropping systems and one early variety for areas with drought constraints will also be released by the national research institution during 2007. Moreover this research activity has generated other outputs concerning the development of methods, knowledge acquisition and capacity building, whose the most relevant results for 2006 are the creation of a national PCI network in Nicaragua and the publication of a special issue on PCI experiences in Latin America in the Agronomía Mesoamericana journal.

Key words: Participatory varietal selection, Participatory plant breeding, upland rice, small and medium-scale farmers, Nicaragua.

Background

This collaborative research project between CIAT and CIRAD started in Nicaragua in May 2002. The project proposes to develop and to apply participatory crop improvement (PCI) approaches and methods for two «model plants » -- i.e., rice and sorghum – for small and medium-scale farmers in low inputs cropping systems but undergoing progressive crop intensification and increased access to markets.

The specific objectives of the project are as follow:

1. To develop and apply new Participatory Variety Selection (PVS) and Participatory Plant Breeding (PPB) methods including population enhancement and recurrent selection.
2. To identify and develop new germplasm matching the needs of small and medium-scale farmers.
3. To enhance the partners' capacity on the participatory breeding approaches and methods.

Upland rice, otherwise known as aerobic rice, is an important food and cash crop in Central America. The total area of rice, upland and irrigated, in Central America totalizes 255,000 ha with a global paddy production of approx. 850,000 tons [1]. In Nicaragua, aerobic rice covers 55,000 ha and represents 66% of the total rice production area [2].

The most important constraints for upland rice production in Central America are:
- The lack of modern and performing varieties adapted to the diverse cropping systems, particularly for less favourable upland conditions and low inputs no mechanized cropping systems;
- Deficient weed control;
- Drought stress in some areas of the Pacific region;
- Acid soils and low solar radiation in the Atlantic regions;
- Pest and diseases, particularly blast, grain discoloration and mite complex;
- Insufficient grain quality for industry requirements and competitiveness with imported rice.

Materials and Methods

1. Germplasm enhancement and selection using participatory approaches

The introduction and evaluation in Nicaragua of exotic genetic resources from CIAT and CIRAD as well as the local development of new germplasm (lines and site-specific synthetic populations with male-sterile gene) form the core of the participatory breeding project.

1.1 Development of New Lines using Participatory Breeding (PPB)

At the Centro Experimental del Occidente (CEO), Posoltega, the participatory breeding schemes were started in 2003 from the PCT-4, PCT-11 and PCT-18 synthetic populations (CIRAD-CIAT program in Colombia) and from F_2s populations derived from specific crosses to match the needs of the mechanized cropping systems of the Pacific area. This work was followed-on during the 2004-2006 cropping seasons at the same site and with a stable group of trained expert-farmers. During the 2005 cropping season, another PPB scheme was started at this site using two new synthetic site-specific populations, PCTNic-1 and PCTNic-2, developed for this area.

At the Siuna and Bocay sites representing the manual cropping systems of the North-East and Atlantic regions, the participatory breeding schemes were started during the 2004 rainy season with two F_2s populations from crosses incorporating the local varieties Raizora Amarillo and Criolla Siuna and 63 S_1-S_3 progenies derived from the CNA-7 developed in Colombia. This work was continued in 2005 and 2006 only at the Siuna site because of better experimental conditions and institutional framework.

1.2 Participatory variety trials (PVS)

In 2006, 45 advanced trials including 106 lines or varieties were established on-station (8 trials) and on-farm (37 trials including validation trials) in five upland rice areas.

2. Experimental designs and cropping conditions

The breeding nurseries and PVS trials planted at the Posoltega and Siuna sites were managed following the common cultural practices and fertilizer doses applied by the farmers in each area. For the preliminary yield trials with a high number of lines, Federer incomplete block designs were used. For the more advanced yield trials, randomized complete block designs with three to four replications are commonly used.

3. Approaches and methods for PCI activities

The global approach and methods used for managing PVS and PPB activities has been described and discussed in different recent publications [3] [5] [6].

Results

1. Germplasm enhancement using participatory approach

1.1 Development of new lines using Participatory Breeding

At Posoltega, among the 88 S_3 and 33 F_5 progenies, derived from PCT-4, PCT-11 and PCT-18 populations and other crosses, evaluated during the 2006 season, the five expert-farmers and the breeders selected 57 S_4 and 33 F_6 plants respectively (Table 1). In a first evaluation of the 88 S_3 progenies for yield and other agronomic traits, we observed that the lines with higher yields and better plant types originate in the PCT-4 and PCT-18 populations. Among the S_1 progenies derived from the site-specific populations PCTNic-1 and PCTNic-2, the same expert-farmers and the breeders have selected 127 S_2 new progenies, almost exclusively from the first population. At this site, like in all the Pacific and Central regions, because of El Niño climatic phenomenon, the 2006 climatic conditions have been particularly unfavourable with a long and intense drought and high temperatures which affected the rice experiments during its reproductive phase.

At Siuna, among the 102 F_4 progenies derived from the crosses PCT-18/Raizora Amarillo and PCT-18/Criolla Siuna and other 143 S_3-S_6 progenies evaluated during the 2006 rainy season, the expert-farmers and the INTA breeder have selected 129 F_5 and 78 S_4-S_7 plants, here with a large preference for progenies derived from the PCT-11 population and from crosses with Raizora and criolla cultivars (Table 1).

1.2 Participatory Variety Selection

Mechanized cropping systems in favourable conditions

From all the advanced lines and varieties evaluated at Posoltega since 2003 with the regional committee of expert-rice growers, the two best lines were included in on-farm validation trials during this 2006 season. In these trials planted in eight localities of the North Pacific region, the POBL 1-38 line obtained an average yield of 4.9 t/ha and over-yielded the INTA Chinandega commercial variety in 19% (Table 2). This line also demonstrated high resistance to blast and to grain discoloration complex. During the 2003-2005 period, this line has been the best-praised by farmers during the participatory variety evaluations [7], this result indicates here a high degree of convergence between farmers' appreciations regarding their own selection criteria and the final agronomic performances of this line.

Low inputs cropping systems in climatic favourable conditions

In the North-East region (Wiwilí, El Cúa and Bocay localities), three CIRAD varieties were identified as the most promising materials for the local manual cropping systems, based on the agronomic results in on-farm trials and farmers' variety evaluation in the fields and at post-harvest stage [3] [6]. During 2005 and 2006, these varieties were evaluated in 500 m^2 plots in eleven validation trials managed under farmers' cropping practices. The two varieties IRAT 364 and IRAT 366 over-yielded the local check variety in 26 and 15 %, respectively (Table 3). IRAT 364 confirmed to be the best yielding but IRAT 366, which is slightly less productive, is the best praised by farmers for its adequate plant-type with strong stems, high lodging resistance, great panicle weights and better grain aspect. The main partner of this research in this region, the extension agency SERVITECA, has decided to register these two varieties IRAT 364 and IRAT 366 for their official release in 2007. The CIRAD-CIAT project has supported this initiative with the set-up of the variety descriptors and training the SERVITECA technicians on seed production.

In the same region, a set of very early lines has been evaluated on-farm during the two last seasons. Collaborative farmers asked for testing these early lines in the region in order to identify a variety which can allow two crops per year. Confirming preliminary results obtained in 2005, the lines PCT-4\SA\1\1>1479-M-1-M-1 and WAB758-1-1-HB-4 gave the most promising results concerning yields and farmers' acceptation for plant type and other agronomic traits (Table 4).

At the Siuna site, the experimental lines CT15944-10-4-3-3 and CT15944-10-4-3-1 and the varieties CIRAD 401 and CIRAD 400 confirmed in 2006 to be well-praised by local farmers; however the low precision of the 2006 agronomic trials didn't allow to determine yield differences among these four lines. Based on the grain quality tests (culinary and milling) to be achieved at the beginning of 2007, the best two materials will be included in validation trials during the 2007 season.

Low inputs cropping systems in areas with drought problems

In Nicaragua, very early materials (90 days or less to maturity) are of interest for the dry areas of the Pacific regions because they can be cultivated during the "postrera" second rainy season (mid-August to end of November) and so to avoid the common drought stress due to the "canícula" short dry season (mid-July to mid-August) and give new cropping options, compared with the current intermediate-cycle varieties, planted in June.
Following the participatory preliminary and advanced variety trials carried-out between 2003 and 2005, the best five lines have been included in validation trials in four departments during the 2006 "postrera" season. Because of the extreme drought in some areas, about 40% of the trials has been lost. Considering the results of all 2005 and 2006 available validation trials, the best lines for yields, agronomic traits (including lodging resistance and initial vigour), farmers' preferences and grain quality, are PCT-4\SA\1\1>1479-M-1-M-1, PCT-4\SA\1\1>516-M-6-M-3 and WAB758-1-1-HB-4. One of these lines will be launched in 2007 by INTA..

1. Strengthening the ongoing participatory crop improvement (PCI) activities in Nicaragua

With the concern of efficiency and sustainability of the current PCI activities on both rice and sorghum crops, we continued to strengthen the research framework with partners in Nicaragua. The different training and informative workshops coordinated with the NGO CIPRES and the regional PPB-MA program, have led to the creation of the Nicaragua Network on Participatory Crop Improvement of staple crops, which already includes INTA, five NGOs, three farmers' organizations and two universities. This network will be in the future a key partner for the diffusion of the know-how and germplasm generated by the project to new institutions and other regions.

As a permanent support to PCI activities, **9 workshops** for participatory variety evaluation or plant selection and for results sharing and activities planning have been achieved with collaborative farmers groups, INTA and NGO partners in 2006.

2. Strengthening partners' capacities on Participatory Crop Improvement

During the LII Programa Colaborativo Centroamericano de Mejoramiento de los Cultivos y Animales (PCCMCA) meeting held in Nicaragua, a half-day seminary on the concepts, objectives, methods and relevant outputs of PCI projects in Meso America (MA) was given for the participant Central American scientists; this seminary was prepared and implemented in coordination with the PPB-MA program. The project team also largely contributed to the edition of a special issue of the journal Agronomía Mesoamericana (Costa Rica) focused on participatory breeding research experiences in Latin America.
At regional level, the project team participated at the annual meeting of the PPB-MA program held in Cuba. In Guatemala, a one-day seminary on objectives, methods and relevant outputs of the PCI projects in Central America was given for the ICTA breeders and regional coordinators and a training workshop for evaluating rice varieties with farmers was carried out for the Arrozgua technicians (November 13-17, France funding).

3. Participation to conferences and meetings

- IV international conference and workshop of the "Grupo de Mejoramiento Genético Avanzado de Arroz (GRUMEGA) held at Chillán, Chile (February 27-March 4): 1 communication
- Annual PCCMCA meeting at Montelimar, Nicaragua (Abril 24-28): 5 communications
- Annual meeting of the Mesoamerican PPB Network at La Habana, Cuba (June 19-21): 1 communication
- External evaluation of the CIRAD research unit UPR-8 at Montpellier, France (November 22-23): 1 communication

4. Resource mobilization

A project for making-up a training course on PCI experiences and PVS methods in Guatemala and supporting PPB activities in Nicaragua has been funded (3,500 euros) by Regional Funds of the French Ministry of Foreign Affairs (MAE).
In 2006, we wrote or contributed to the writing of six concept-notes and project proposals, three of them are in the pipeline. A proposal for training courses and workshops on PCI approaches and methods addressed to the Meso-america and Andean countries has been discussed and elaborated with the PPB-MA program and the IPRA-CIAT project; it is in process to be presented to FAO for funding as a regional Technical Collaborative Program.

Conclusion

The diversified new upland rice germplasm from CIAT and CIRAD associated with PCI approaches is offering interesting results for improving and diversifying rice production in Nicaragua and Central America. Collaborative activities conducted in 2005 and 2006 have allowed to identify better-performing lines for matching the needs of existing upland cropping systems (manual as well as mechanized systems) and for giving new variety options to avoid drought constraints or to allow new cropping systems including rice crop. Among these lines, IRAT 364 and IRAT 366 for the low inputs cropping systems of the North-East and Atlantic regions, POBL 1-38 for the mechanized cropping systems of the Pacific region and the early lines PCT4/SA/1/1>1479-M-1-M-1 and/or WAB758-1-1-HB-4 should be officially released in Nicaragua during 2007.

Future Activities

In 2007, we plan to continue with our partners the participatory breeding schemes at the two main working sites (Posoltega and Siuna) and to support the validation and registration of the most advanced lines in Nicaragua. We also plan to share the new available germplasm (populations and elites lines) with new partners in Nicaragua and in the Central America region (Guatemala, Honduras, Haiti).

Other activities should be to mobilize funds and partners for implementing research activities in agronomy for taking better advantage of the new varieties into the current or new cropping

systems and for starting a participatory variety selection work on special high-value rice (aromatics and other) with the existing CIRAD and CIAT germplasm.

References

FAO 2005. Datos estadísticos de producción agrícola, FAOSTAT, http://faostat.fao.org/

Estadísticas de producción de granos básicos de Nicaragua. MAG-FOR 2002.

Trouche G. et al. 2004. Annual report for the research activity on upland rice participatory plant breeding in Central America.

Lhomme A. 2005. Creating varieties together. A technographic account of partnership in three participatory crop improvement projects in Nicaragua. Thesis of master on technology and agrarian development, MASK 18, Wageningen University. 80 p. 9 fig.

Trouche G. 2005. "Participatory Rice Breeding using Population Improvement: A New Methodology Adapted to the Needs of Small Farmers in Central America and the Caribbean. In E.P. Guimarães, ed. *Population improvement: a way of exploiting the rice genetic resources of Latin America*, pp. 99-110. Rome, Italie. FAO, CIRAD, CIAT, Embrapa Arroz e Feijao, DANAC.

Trouche, G.; Narváez-Rojas, L.; Chow-Wong, Z.; Corrales-Blandón, J. 2006. Fitomejoramiento participativo del arroz de secano en Nicaragua: metodologías, resultados y lecciones aprendidas. Agronomía Mesoamericana (CR) 17(3): 307-322.

Chow W., Z.; Trouche, G. 2006. Informe anual de actividades en arroz. Proyecto fitomejoramiento participativo en arroz y sorgo CIAT-CIRAD/INTA. 17 p. 35 tab.

Table 1: Progenies selected by the farmers and breeders at Posoltega, Pacific region and at Siuna, Atlantic region in Nicaragua during the 2006 rainy season.

Crosses or population sources	Posoltega		Siuna	
	Number of progenies evaluated	Number of plants selected	Number of progenies evaluated	Number of plants selected
Crosses				
PCT-18/INTA N-1	**33**	**32**		
PCT-18/Raizora Amarillo			66	46
PCT-18/Criolla Siuna			**35**	32
Populations				
PCT-4	51	39	44	29
PCT-11	12	10	79	88
PCT-18	25	8	13	7
CNA-7			7	5
PCTNic-1	136	118		
PCTNic-2	25	9		
PCTNic-3			1 pop S0	109
TOTAL		**216**		**316**

Table 2: Agronomic results in on-farm validation trials in mechanized cropping systems in favourable upland conditions, Chinandega region, Nicaragua 2006.

Line	Pedigree	Days to flowering [1]	Plant height [1] (cm)	Grain Yield (t/ha)	Check %	Frequency of farmers selection [2] (%)
POBL 1-11	BC2 EPAGRI 108/O. llanos 5	89	93	4.444	107	76
POBL 1-38	BC2 EPAGRI 108/O. llanos 5	85	100	4.919	119	82
INTA Chinandega	CT 12249-1P-1P	76	110	4.137	100	80

[1] Data of the 2005 advanced trials
[2] Average of farmers' evaluations between 2003 and 2005

Table 3: Agronomic results of the two varieties most praised by farmers in on-farm validation trials under manual cropping systems, Wiwilí-El Cúa, North-East of Nicaragua, 2005-2006.

Variety	Days of flowering	Plant height (cm)	Lodging !	Grain Yield (t/ha)	Check %	Average score of farmers' appreciation [1] (2004)
IRAT 364	85	140	1,0	3.01	126	3.2
IRAT 366	77	125	1,0	2.74	115	3.5
Local Check	98	148	1,7	2.19	100	1.9

! IRRI scale
[1] Farmers' appreciation of the variety concerning the five most important plant traits using a 1-4 evaluation scale where 1 =bad, 2 = acceptable, 3 = good and 4 = excellent

Table 4: Agronomics results and farmers' appreciation of eight early lines for manual cropping systems, North-East of Nicaragua, 2006.

Line	Days of flowering	Plant height (cm)	Grain Yield (t/ha)	Index of overall farmers' appreciation [1]	Frequency of farmers selection (%)
PCT-4\SA\1\1>516-M-6-M-3	62	91	4.796	2.3	0
PCT-4\SA\1\1>1036-M-6-M-2	68	98	4.721	2.5	22
PCT-4\SA\1\1>1479-M-1-M-1	69	93	4.526	3.1	89
CT 11891-3-3-3-M-1-2-2-M	59	86	4.590	1.2	0
CT 11891-3-3-3-M-5-1-1-M	60	94	4.070	1.2	0
PCT-4\0\0\0>721-M-2-M-4-M-2-M-5-M	69	98	4.503	2.3	56
WAB 758-1-1-HB-3	67	89	4.000	1.8	0
WAB 758-1-1-HB-4	69	93	5.241	2.9	89
Trial mean	65	93	4.556		
s.e.	0.72	3.3	561		
F-value for genotype	68 **	3.1 *	1 ns		
F-value for interaction locality x genotype	57.5 **	1.1 ns	0.5 ns		

*, ** significant at the 0.05 and 0.01 probability levels, respectively.

[1] Farmers' appreciation of the variety concerning the five most important plant traits using a 1-4 evaluation scale where 1 =bad, 2 = acceptable, 3 = good and 4 = excellent

3B. Upland rice germplasm improvement for Central America

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- CONARROZ and INTA Costa Rica: Marvin Vargas and Randolph Campos

***Source of funding*: CIAT IP-4, CIRAD and INTA**

Key words: Germplasm enhancement, upland rice, population improvement, capacity building, Central America.

Abstract

In Central America, the majority of the rice area is cultivated in upland conditions. Because of its central geographic situation and its representativeness for the agro-climatic conditions and the upland rice cropping systems of the region, Nicaragua is a good platform for in-situ germplasm improvement and screening of new CIAT and CIRAD lines developed in Colombia for matching the needs of the Central America region. After a two-years phase for testing a large diversity of new exotic germplasm and starting new breeding schemes using synthetic populations, three regional field selection workshops were organized in 2005 and 2006 in close collaboration with INTA, where the breeders of public and private institutions were invited to chose the progenies and advanced lines which can be useful for their country. Main outputs of this regional strategy are the strengthening of the national rice breeding programs and a better access of more specific improved germplasm and elites lines.

Background

Rice is the third staple crop after maize and bean in Central America Region. The total area of rice, upland and irrigated, grown in Central America totalizes 255,000 ha with a global paddy production of approx. 850,000 tons [1]. In Panama, Costa Rica and Nicaragua, the consumption of rice is fairly high (40-60 kg/year per capita). In Panama, Costa Rica, Nicaragua and Guatemala, upland rice accounts for up to 60 % of the rice production area (90 % in Guatemala). In Nicaragua, upland rice is cultivated in a great diversity of agro-climatic conditions and cropping systems, being representative of almost all the upland cropping systems existing in Central America. Consequently it is a good platform for germplasm improvement and preliminary screening of exotic materials for the region. Otherwise, the Rice National Research programs of Central America are requiring new sources of improved germplasm and training courses or workshop for strengthening their capacity in rice breeding. Since 2003, the introduction and evaluations of new CIAT and CIRAD germplasm in Nicaragua as well as the local development of new germplasm (site-specific populations and inbred lines) are managed

not only for matching the needs of Nicaraguan farmers but also as a strategy for germplasm improvement for the Central America region [2].

Materials and Methods

1. Germplasm development

1.1 Creation and improvement of synthetic populations

The development of three site-specific populations, as future source of new materials for matching the needs of the three main areas of upland rice production in Nicaragua, was achieved during the 2005 off-season. Conventional and participatory breeding schemes have been started in Nicaragua with these populations since the 2005 cropping season.

1.2 Development of New Lines

During the 2006 rainy season, 270 S_3-S_6 progenies derived from a breeding nursery received in 2004 from the program CIAT-CIRAD Colombia, 160 S_2 from the PCTNic-1, PCTNic-2 and PCTNic-3 populations, 42 S_6 progenies from PCT-18 and 72 S_6 progenies selected in the 2005 ION-CIAT upland nursery were planted on the Centro de Experimentación del Occidente (CEO) station, Posoltega, for observation and follow-up of the selection process using conventional breeding methods. The main objective of this breeding work is to generate lines with high level of diseases resistance, high yield potential and adequate grain quality for the mechanized and intensive upland cropping systems of Central America.

1.3 Observation Nurseries and yield trials

During the 2006 rainy season, five observation nurseries and four preliminary or advanced yield trials including 276 new introduced lines were managed at the CEO station:
Observation nurseries:

- 139 lines with high resistance to blast and/or grain discoloration complex
- 157 lines from conventional breeding and inter-specific crosses
- 94 lines BC3 O. glaberrima/Caíapo
- 25 lines derived from inter-specific crosses with O. glaberrima from Warda
- 45 S_7-S_8 lines from PCT-4 and PCT-11 populations

Yield trials**:**

- 20 S_7 lines from PCT-4 and PCT-11 populations
- 40 S_5 lines derived from PCT-18 (Participatory Breeding scheme managed at the San Dioniso site, regional trial planted at three contrasted sites)
- 12 elites lines for favourable upland conditions (indica + japonica)
- 12 elites early lines for zones with drought constraints (japonica)

2. Experimental designs and trials management

Breeding plots, observation nurseries and preliminary yield trials were all planted on-station at the CEO station. For these, the cultural practices and fertilizer doses recommended in Nicaragua for mechanized upland rice are applied. Since 2004, all the rice experimental area, is surrounded and interspersed with sprayer rows previously planted with a mixture of blast-susceptible varieties in order to increase the level of infestation of this disease, which is the main biotic constraint for rice production in this area. In the breeding and observation nurseries, the plots size is two rows of 5 m. long, with checks plots regularly interspersed. The preliminary yield trials are designed in the Federer incomplete blocks design.
The advanced yield trials are planted on-farm with a replication on-station; the randomized complete block design with three o four replications and plots of six rows of 5 m. long are commonly used.

Results and Discussion

In all the Pacific area of Nicaragua, the 2006 rainy season was exceptionally dry, due to the influence of the El Niño climatic phenomenon; on the rice experiments, drought and high temperatures stress were particularly intense during the end of the reproductive phase (16 days without rains), which caused high sterility problems and consequently reduced the yields compared to a normal year.

1. Germplasm improvement

1.1 New site-specific populations

The conventional and participatory breeding schemes developed from these populations since 2005 were followed up. Seeds of the PCTNic-1 population for upland favourable environments were sent to CENTA El Salvador.

1.2 Development of lines

At Posoltega, among the 270 S_3-S_6 progenies observed during the 2006 season, the CIAT-CIRAD and INTA breeders selected 136 new progenies for general adaptation, diseases resistance (mainly grain discoloration and sheath blight), productivity and grain format; the two-third of the selected lines originated in the PCT-11 population (Table 1). Among the other segregating materials, 32 S_3 progenies have been selected from the PCTNic-1, PCTNic-2 and PCTNic-3 populations (conventional breeding work), 14 S_6 progenies from PCT-18 population and 14 S_7 progenies from the 2005 ION-CIAT.

1.3 Observation nurseries and yield trials

Due to the extreme drought constraints, most of the new materials introduced in 2006 will be re-observed during the next cropping season. The 94 lines derived from O. glaberrima/Caíapo will be re-evaluated in 2007 in the low inputs manual cropping systems at the Siuna site (North-Atlantic region). In the preliminary and advanced trials, the materials which achieved the best results in the 2006 conditions are:

- Three S_7 lines: PCT-4\SA\2\1>44-3-1-1-1-M, PCT-11\0\0\3>1497-M-1-2-M-3-M and PCT-11\0\0\3>1497-M-1-2-M-2-M
- Three lines derived from CT15765 and CT15679 (also excellent in 2005 and very promising in 2006 in El Salvador and Costa Rica)
- Four early lines: CT11891-3-3-3-M-1-2-2-M, CT11891-3-3-3-M-5-1-1-M, WAB758-1-1-HB-4 and PCT-4\SA\1\1>1479-M-1-M-1

2. Strengthening the Rice Research Programs of Central America Region

Concerning capacity building, a 10-days training course on rice breeding methods is scheduled in Nicaragua for May 2007 in the framework of the TCP-FAO project TCP/RLA/3102 (A)**.** In 2006, two field selection workshops were achieved in Nicaragua in close collaboration with INTA and the Technical Mission of China Taiwan, the first one in April during the annual PCCMCA meeting at Sebaco (irrigated rice) and the second one in October in two localities, the CEO station (upland) and the Finca Los Cocos at Malacatoya (irrigated). At these selection workshops have participated breeders and agronomists of Nicaragua, El Salvador, Costa Rica and Panama.

Following the selection workshop held in October 2005 in Nicaragua [2], the materials selected by the participants have been multiplied during the 2006 off-season and distributed during the second trimester of 2006:

- 46 advanced lines and 80 segregating progenies to CENTA
- 25 advanced lines and 52 segregating progenies to IAASA
- 74 advanced lines and 101 segregating progenies to CONARROZ and INTA Costa Rica

Conclusions

Since 2003, the CIAT-CIRAD project has supported the INTA Rice program to strengthen its breeding capacity for the upland mechanized cropping systems of the Pacific regions and to build-up a stable breeding program for the upland low inputs cropping systems of the Center and Atlantic areas. This effort has been achieved through the introduction of new germplasm (inbred lines, segregating progenies and broad genetic base populations), the development of new site-specific populations, the implementation and training on new breeding methods, including population improvement. This would set up the core activity for sustaining conventional and participatory breeding activities for Nicaragua and more widely Central America through regional field selection and training workshops and seed distribution from Nicaragua. Results of this activity for the NARS is the strengthening of their capacity in rice breeding and a major access to new diversified improved germplasm; at the present, promising lines, firstly introduced

and evaluated in Nicaragua, are in pre-launching phase in Nicaragua but also in El Salvador and Costa Rica.

Future Activities

Depending of the available funds and the future of the CIAT rice project, the activities planed for 2007 could be**:**

- Strengthening the current breeding and germplasm screening platform put in place in Nicaragua
- One regional course on rice breeding (TCP-FAO project, May 2007)
- Two regional workshops of field selection addressed to rice breeders of the Central America region (May and October 2007).

References

FAO 2005. Datos estadísticos de producción agrícola, FAOSTAT, http://faostat.fao.org/

Trouche G. et al. 2006. Annual report for the research activity on Upland rice germplasm improvement for Central America.

3C. Annual Report Summary

1. Research Highlights 2006

- Evidence is shown for the usefulness of combining near-isogenic progeny analysis with rice genome information available in public databases to identify molecular markers highly linked to blast resistance genes in rice. Although a limited number of polymorphic markers can be expected when near-isogenic lines are used as progenitors, here we found six polymorphic markers in a region of only 13 cM surrounding the blast resistance gene *Pi-1(t).* Additionally, two of these markers (RM1233*I and RM224) were closely linked to the gene. Our results support the utility of these DNA markers in MAS and gene pyramiding rice breeding programs addressing the improvement of blast resistance in rice cultivars; and eventually to map based cloning of the gene. The speed, simplicity and reliability of PCR based approaches make microsatellite analysis on agarose gels an attractive tool for marker-assisted selection in rice breeding programs aiming at developing durable rice blast resistant cultivars.
- Combining near-isogenic progeny analysis with rice genome information available in public databases is a very valuable approach to identify molecular markers highly linked to blast resistance genes in rice.
- Six polymorphic markers in a region of only 13 cM surrounding the blast resistance gene *Pi-1(t)* were found. Additionally, two of these markers (RM1233*I and RM224) were closely linked to the gene. Our results support the utility of these DNA markers in MAS and gene pyramiding rice breeding programs addressing the improvement of blast resistance in rice cultivars.
- The speed, simplicity and reliability of PCR based approaches make microsatellite analysis on agarose gels an attractive tool for marker-assisted selection in rice breeding programs aiming at developing durable rice blast resistant cultivars.
- We have demonstrated that polymorphic markers linked to resistance genes in near-isogenic populations, can also be expected to detect polymorphism and presence of linked genes in commercial rice cultivars with certain level of inbreeding.
- Our studies indicate that blast pathotypes evolve losing many avirulence genes to defeat almost any resistance gene combination
- The Brazilian rice land race Tres Marias exhibiting stable blast resistance for many years in Brazil and Colombia carries at least one gene that confers resistance to the Colombian blast lineages SRL-4 and SRL-5.
- The resistance of the cultivar Tres Marias to the lineage SRL-6 was found very complex and did not follow a Mendelian segregation in the F_3 generation. QTL studies using molecular markers need to be conducted to elucidate the number of genes responsible for the stable resistance of the cultivar.
- The rice line 75-1-127 reported with wide spectrum of blast resistance to many blast populations of the world carries at least three different resistance genes and not only the Pi-9 gene derived from *O. minuta.*
- Resistance to blast in the rice line 75-1-127 is controlled by a dominant and a recessive gene to the blast genetic lineages SRL-6 and SRL-5 and by two dominant genes to the genetic lineage SRL-4.

- The blast resistance in the cultivar Oryzica Llanos 5 (durable blast resistance for more than 15 years) was found to have very complex inheritance.
- The durable broad-spectrum resistance in the rice cultivar Oryzica Llanos 5 is associated with multiple genes of major and minor effects that induce resistance to different blast isolates.
- Twenty-one QTL present in nine chromosomes were detected and associated with resistant traits in Oryzica Llanos 5. Most but not all of the QTL occurred in the same genomic regions of other genes that had been reported in the literature. None of the QTLs was effective against all blast isolates and all were isolate specific
- One QTL mapped to a region on chromosome 9 where no blast resistance genes had yet been mapped. Another QTL near the bottom of rice chromosome 11 was found to be significantly associated with partial resistance.
- Advanced breeding lines (generation F_7-F_{11}) with transgenic-resistance to RHBV (comparable to Fedearroz 2000 and Colombia 1) combining high yield potential, good grain quality, tolerance to *Rhizoctonia* and characterized profile for strain resistance to pyricularia. These plants are ready to be evaluated by peers and to decide potential process for deployment to farmers. First product selection at completion, phase out, ready for decision on the fate for potential delivery to third parties.
- Transgenic lines carrying *DREB* genes. Hundreds of independent transgenic events carrying various transgenes for tolerance to abiotic stress (temperature, drought) generated and characterized molecularly**.** The demonstrated competitive technical capacity for rice genetic transformation was reviewed by Japanese peer pioneer in development of gene technology in rice, recognition allowed CIAT to enter in new proposal in collaboration with JIRCAS, IRRI and CIMMYT and funded by MAFF Japan..
- Chloroplast and nuclear sequences selected and tested for genome and species characterization of *Oryza* allowing characterization of species composition and direction of gene flow in samples collected in farmers' fields in Colombia and Venezuela.
- High through-output methodology PCR-real time based for analysis of gene flow in rice at landscape level optimized. Set up international collaboration on experimental design and data collection for gene flow at landscape level that may allow adaptation of expert model systems for tropical conditions, applicable tool for biosafety decision process by competent authorities.
- Near-completion of a clean lab for handling and preparing rice samples for iron and zinc analysis, establishment of a methodology for running iron and zinc analysis in rice at CIAT, and establishment of base lines for iron and zinc.
- Validation of SNP markers to be used for the screening of rice genotypes having contrasting levels of iron content in the polished grains.
- Understanding of meiotic process of F1 hybrids between *O. sativa* x *O. latifolia* including abnormalities in spindle formation, chromosome segregation and cytokinesis leading to polyads formation, which give rise to unviable pollen and sterility, and chromosome elimination.
- Wide segregation for desirable traits including grain quality and high fertility was observed in F5 generation in crosses involving *O. latifolia*, and a large number of plant selections was made for further testing.

- Identification of rice cultivars having 2-3 times more iron than commercial milled rice bought by consumers
- Evaluation of about 13,000 breeding lines and identification of promising lines for CIAT-ION nurseries
- Promising interespecific breeding lines with high yield potential, tolerance to main diseases and good grain quality were identified and included in the CIAT-ION nursery made available to NARs in 2006. Out of the 194 lines, 65 were from interspecific crosses.
- Two varieties for low inputs upland cropping systems in process to be registered by a private partner of the project (launching at mid-2007)
- Following convincing validation trials carried out in 2005 and 2006, future launching by the Nicaraguan agriculture research institute (INTA) of a very early line for upland areas with drought constraints and a line for favorable upland mechanized cropping systems
- Follow-up of the participatory plant breeding schemes with associated farmers and NGOs in two areas of Nicaragua
- Creation of a national Participatory Crop Improvement (PCI) network in Nicaragua
- Publication of a special issue about PCI experiences in Latin America in the Agronomía Mesoamericana journal
- Seed distribution to public and private research institutions in Nicaragua, El Salvador, Guatemala and Costa Rica
- Two field selection workshops held in Nicaragua for the Central America breeders
- Two very promising lines for favourable upland and irrigated cropping systems in pre-launching trials in Nicaragua, El Salvador and Costa Rica

2. Problems encountered and their solutions

1. In July 2006, CIBIOGEM (Mexico National Biosafety Secretary) indicated the impossibility of Mexico to participate in implementation of the project entitled: The Latin America: Multi-country capacity-building for compliance with the Cartagena Protocol on biosafety. USD 5 million. Donor: GEF-World Bank. The decision was communicated to the World Bank, and modifications of activities were jointly adjusted without affecting the outcome of the multi-country project.
2. The main problem encountered for 2006 was the elimination of funding from the Colombian Government to the Rice Project and the reduction of core budget assigned to the Project by CIAT. Special Projects were funded during 2006 that will help to cover part of the gap in funding but will not be enough for future budget reduction expected to be implemented for 2007-2008. Special projects do not generally fund costs of personnel, which do not solve all the problems of budget reductions at the Center
3. Transaction costs continue to be too high. It is very hard to keep up with breeding, coordination and supervision of diverse activities, and field work due to too many meetings, travel and paper work. Even my support team feels overloaded. We have tried to more carefuly divide and assign responsibilities among support staff and field workers to do our job without sacrificing efficiency, quality and quality of life.
4. As last year this year we managed to keep going our core breeding activities by using other sources of funding, especially from AgroSalud, and Cenicafe.

5. The stability and sustainability of the Rice Project continues to be a major concern. We have to be more creative and original in approaching the Latin American rice sectors to obtain additional funding for our core activities. There are sectors that actually are not contributing to funding research activities but that are willing to contribute if an adequate mechanism is proposed to them.

3. Indicators

1. List technologies, Methods, & tools

- Advanced breeding lines (generation F_7-F_{11}) with transgenic-resistance to RHBV (comparable to Fedearroz 200 and Colombia 1) combining high yield potential, good grain quality, tolerance to *Rhizoctonia* and characterized profile for strain resistance to pyricularia. These plants are ready to be evaluated by peers and to decide potential process for deployment to farmers.
- Transgenic lines carrying *DREB* genes with putative better recovery from water stress than non-transgenic lines
- Methodology for regeneration of somatic embryoids from mango nucellar fruit tissue of various commercial cultivars applicable for genetic transformation
- Rice germplasm with different blast resistance genes was distributed to different countries in Latin America
- A screening method for evaluating sheath blight tolerance in rice populations was developed in collaboration with rice researchers from the USA and implemented for identification of new sources of resistance and identification of QTL controlling tolerance to the pathogen

Varietal Releases

In Bolivia, the first upland/aerobic commercial variety selected from the enhanced composite population PCT-4 was officially released in 2006 as **ESPERANZA**. The variety is adapted both to manual upland and mechanized aerobic rice ecosystems. This variety has Pedigrí CT8240-1-5-2P-M-1P/CT8008-3-12-3P-1X//CT9509-17-3-1-1-M-1-3P-M

In Chile, the first commercial variety, **RQuila 28**, adapted to the temperate irrigated rice ecosystem, coming from the enhancement and selection of the population PQUI-1 was proposed for official released in early 2007

In Salvador, the commercial variety released was **CENTA A-8**, released by ANAR. This variety came from the cross CT 11519/CT 11492 and Pedigrí CT 122249-3-4-3-3P-1P

In Nicaragua, the commercial variety released was **ANAR 2006**, released by ANAR. Pedigrí CT8240-1-5-2P-M-1P/CT8008-3-12-3P-1X//CT9509-17-3-1-1-M-1-3P-M

In Panamá, the commercial variety released was **IDIAP 54-05**, released by IDIAP. This variety came from Pedrigrí CT9682-2-M-14-1-M-1-3P-M-1/CT10825-1-2-1-3-M//CT8222-7-6-2P-1X

and also the variety **IDIAP 145-05** was released under Pedigrí CT8008-16-31-3P-M//CT9682-2-M-14-1-M-1-3P-M-1/CT11008-12-3-1M-4P-4P

In Venezuela, the commercial variety released was **CENTAURO**, released by FUNDARROZ, INIA AND FLAR. This variety came from the cross ECIA38-2-4-2-5-6/CT822-7-6-2P-1X/FB0007-3-1-6-1-M and Pedigrí FL00984-8P11-2P-2P-M-M

Germplasm Distributed

Seed increase of 983 upland lines for shipment to 25 Institutions from 12 Countries.

Electronic Modules

Development of the web site of the Working Group on Advanced Rice Breeding (GRUMEGA by its Spanish acronym) **http://www.grumega.org**

2. Publication list

a. In Referred Journals

1. Flórez-Ramos C.P., **Z. Lentini*,** M.E. Buitrago, and J. Cock. 2006. Somatic Embryogenesis and Plantlet Regeneration of Mango (*Mangifera indica* L.). *Acta Horticulturae* (In Press)
2. Ruiz J.J., **Z. Lentini*,** V. Segovia, M. Buitrago, C. Flórez, and J. Cock. 2006. *In vitro* Propagation and Regeneration of *Solanum quitoense* (Lulo) Plants and their Use as Elite Clones by Resource Farmers. Somatic Embryogenesis and Plantlet. *Acta Horticulturae* (In Press).
3. Fuentes, J.L., **Correa-Victoria, F.**J., Escobar, F., Prado, G., Aricapa, G., Duque, M.C., and Tohme, J. 2006. Microsatellite markers linked to the blast resistance gene *Pi-1* in rice for use in marker assisted selection. Euphytica (accepted)
4. Jia, Y., **Correa-Victoria, F**.J., McClung, A., Zhu, L., Wamishe, Y., Xie, J., Marchetti, M., Pinson, S., Rutger, N., and Correll. J. 2006. Rapid determination of rice cultivar responses to the sheath blight pathogen *Rhizoctonia solani* using a micro-chamber screening method. Plant Disease (accepted)
5. Lopez-Gerena, J., **Correa-Victoria, F**.J., Prado, G., Tohme, J., Zeigler, R., and Hulbert, S. 2006. Mapping QTL affecting partial resistance and identification of new blast resistance genes in rice (*Oryza sativa*). Theor. Appli. Genet. (submitted)
6. **Trouche, G.; Narváez-Rojas, L.; Chow-Wong, Z.; Corrales-Blandón, J. 2006**. Fitomejoramiento participativo del arroz de secano en Nicaragua: metodologías, resultados y lecciones aprendidas. Agronomía Mesoamericana (CR) 17(3): 307-322.
7. **Trouche, G.; Hocdé, H.; Aguirre-Acuña, S.; Martínez-Sanchez, F.; Gutiérrez-Palacios, N. 2006**. Dinámicas campesinas y fitomejoramiento participativo: el caso de los sorgos blancos (Sorghum bicolor, L. Moench) en la region Norte de Nicaragua. Agronomía Mesoamericana (CR) 17(3): 407-425.

b. In Books

- Calvert L.A. and **Z. Lentini.** 2007. Rice Hoja Blanca Virus. *In:* Characterization, Diagnosis and Management of Plant Viruses. Vol. 4: Grain Crops and Ornamentals. Govind P. Rao, Claude Bragard and Benedicte S.M. Lebas (Editors). Stadium Press ILLC, Texas, USA. ISBN 1-933699-34-5. p: 85-99.
- Marc Châtel, Yolima Ospina and Gilles Trouche. 2006. Impact of the rice synthetic population breeding project for Latin America and the Caribbean. In: France and the CGIAR. Delivering Scientific Results for Agricultural Development. Chapter 1: Scientific Partnerships. Producing more and better food. Publication coordinated by Daniel Rocchi, Liaison Officer at the CGIAR Secretariat in Washington.Washington, U.S.A. CGIAR, p.44-47.

c. Conferences and Workshops

- Lentini, Z*. 2006. Biotecnología y Riesgos Fitosanitarios *Invited Key-note lecture*. 2do Curso Internacional sobre Riesgos Fitosanitarios para la Agricultura Colombiana. Cali, Colombia December 2006. Funded by MADR Colombia.
- Coordination and Execution of Course: Capacitación para el Fortalecimiento de la capacidad institucional del Ministerio de Ambiente, Vivienda y Desarrollo Territorial y Autoridades Ambientales Regionales en materia de Biotecnología y Bioseguridad Ambiental de OGM con énfasis en Plantas Transgénicas. Abril 26, 27, y 28 de 2006. Funded by Colombia GEF/WB Biosafety Project.
- Trouche, G.; Hocdé, H.; Aguirre S. 2006. Sélection participative des sorghos au Nicaragua : approche et dispositifs. *In:* Lançon J., Weltzien E., Floquet A. Eds. Gestion du partenariat dans les projets de sélection participative. Actes de l'atelier Recherche 14-18 Mars 2005, Cotonou, Benin: 159-173.
- Lancon, J.; Bertrand, B.; Clément-Demange, A.; Hocdé, H.; Nouy, B.; Trouche, G. 2006. What determines the stakeholders'participation in plant breeding programs? Cases studies in the South. *In:* Lançon J., Weltzien E., Floquet A. Eds. Gestion du partenariat dans les projets de sélection participative. Actes de l'atelier Recherche 14-18 Mars 2005, Cotonou, Benin: 179-193.
- Taller de selección de material genético de arroz de secano y de riego. Villavicencio-Colombia. August 15-18, 2006. 61 participants from 12 countries (Bolivia; Colombia; Costa Rica; Cuba; Dominican Republic; France; Guatemala; Madagascar; Nicaragua; Panama; Peru and Venezuela)
- Correa-Victoria, F.J. 2006. Improving Blast Resistance for Upland Rice in Colombia: a Challenging Task. 31st Rice Technical Working Group Meeting. The Woodlands, Texas, February 26-March 1, 2006.
- Correa-Victoria, F.J. 2006. Identification of molecular markers for pyramiding rice blast resistance genes. Second Research Coordination Meeting. Nanjing, China, April 10-14, 2006.
- Correa-Victoria, F.J. 2006. Avances en la investigación en enfermedades del arroz: *Pyricularia grisea.* II Congreso Brasilero de la Cadena Productiva del Arroz. VIII Reunión Nacional de Pesquisa de Arroz. EMBRAPA, Brasilia 26-28 de Abril, 2006. (Invited speaker).

- Correa-Victoria, F.J. 2006. Situación del complejo acaro-hongo-bacteria en el arroz. Segundo Congreso Arrocero. San José, Costa Rica, Junio 29-30, 2006. (Invited speaker).
- Correa-Victoria, F.J. 2006. Using rice differentials with known blast resistance genes for pathogen characterization and improving rice cultivars in Latin America. Rice Blast Workshop IRRI-JIRCAS. IRRI, Los Baños, Philippines, August 29-30, 2006.

In Proceedings Scientific Meetings
Proceedings in refereed book

- Lentini, Z. 2006. Estimating Likelihood and Exposure (***Invited Moderator Session V, Key-note lecture***). Proceedings from the 9th International Symposium on Biosafety of Genetically Modified Organisms of the International Society for Biosafety Research (ISBR). Editor: USDA, USA. Jeju, South Korea; September 24th -29th, 2006.
- **Lentini Z*,** D. Debouck, A.M. Espinoza, and R. Araya. 2006. Gene flow analysis into wild/weedy relatives from crops with center origin/ diversity in tropical America. Proceedings from the 9th International Symposium on Biosafety of Genetically Modified Organisms of the International Society for Biosafety Research (ISBR). Editor: USDA, USA. Jeju, South Korea; September 24th -29th, 2006 (*In Press*)

d. Posters

- Marc Châtel, Yolima Ospina & Cooperadotes de ALC Alianzas Estratégicas en Mejoramiento Genético de Arroz Para América Latina y el Caribe: Resultados del Proyecto Regional de Mejoramiento Poblacional. II Congresso Brasileiro da Cadeia Produtiva de Arroz - II CBC Arroz. VIII Reunião Nacional de Pesquisa de Arroz - VIII RENATA. Brasilia-Brasil, 26 - 28 de abril de 2006.
- Marc Châtel, Yolima Ospina and LAC Cooperators. Strategic alliances in rice breeding for Latin America and the Caribbean: Results of a regional rice synthetic population improvement project. Section 1.3 Breeding for aerobic and unfavourable conditions. International Rice Research Conference (IRRC). New Delhi, India. October 9-13, 2006

e. Oral Presentations

1. **Lentini, Z*.** 2006. Biotecnología y Riesgos Fitosanitarios ***Invited Key-note lecture***. 2do Curso Internacional sobre Riesgos Fitosanitarios para la Agricultura Colombiana. Cali, Colombia December 2006.
2. **Lentini, Z*.**2006. Estimating Likelihood and Exposure (Moderator Session V, ***Invited Key-note lecture***). the 9th International Symposium on Biosafety of Genetically Modified Organisms of the International Society for Biosafety Research (ISBR). Jeju, South Korea; September 24th -29th, 2006.
3. **Lentini Z*,** D. Debouck, A.M. Espinoza, and R. Araya. 2006. Gene flow analysis into wild/weedy relatives from crops with center origin/ diversity in tropical America. (***Invited Key-note lecture***). The 9th International Symposium on Biosafety of Genetically Modified Organisms of the International Society for Biosafety Research (ISBR). Jeju, South Korea; September 24th -29th, 2006.

4. **Marc Châtel.** Semana Nacional del Arroz. Santa Cruz de la Sierra, Bolivia, 18-20 de enero del 2006.
5. **Marc Châtel,** Lee Calvert & Cooperadores "El proyecto Arroz del Cirad en CIAT: 10 años de colaboración regional". IV Conferencia-Taller Internacional de Mejoramiento Poblacional en Arroz. Chillán, Chile. 27 febrero - marzo 3, 2006
6. Nourollah Ahmadi, **Marc Châtel**. A glance at Rice research at CIRAD Twenty-first Session of the International Rice Commission. Chiclayo, Peru. 3-5 May 2006

f. Internal Seminars

- Latin America: Multi-Country Capacity Building in Biosafety. Where do we stand?. Thematic Areas, Organizational Set up, and Implementation Arrangements. December 11, 2006
- Adaptation and regional standardization of methodology for large scale monitoring of gene flow. May 24. 2006
- Biosafety in Centers of Biodiversity: Brazil, Colombia, Costa Rica, Mexico, and Peru. February 3, 2006.

g. Policy briefs

Preparation of 2 documents related to the establishment by the TCP-FAO TCP/RLA/3102 (A)Project of a network called "Red de Mejoramiento Genetico de Arroz para las Américas" (RedMeGAA)

(1) Red-MeGAA Status and Rules
(2) Red-MeGAA Material Transfer Agreement

3. Trips made during 2006

- Washington D.C., Final revision and legal negotiation of Project Appraisal Document with the World Bank for project entitled: *The Latin America: Multi-country capacity-building for compliance with the Cartagena Protocol on biosafety. USD 5 million. Donor: GEF-World Bank.* The document was approved by the World Bank and final negotiation reached. Currently project is in final phase for receiving GEF CEO Endorsement for final approval (total USD 5 million). The project development included coordination with internal team at CIAT (SB2, SB1, Impact assessment, GIS, IPM), with support from CIAT Project Office, Finance, Human Resources, Legal Department and Procurement, with external task team at the World Bank (Matt McMahon, Indira Ekanayake, Teresa Roncal, Alexandra Horst, Danish Aryal, Anna Roumani, José Martínez) and regional partners (Costa Rica, Colombia, Peru and Brazil).
- Bolivia, January 2006.Semana Nacional del Arroz.
- PANAMA (January 31-February 2; May 4-6); Dec 28-29). FONTAGRO Project Meeting and field visits.
- Chile, February 2006. IV GRUMEGA Conference
- February 2-11.2006. Santa Cruz, Bolivia. Visit to Ciat-Bolivia and Aspar to observe rice activities in several places and assess progres made.

- Feb.26-28. Participation in 31st Rice Technical Working Group Meeting in Houston, Texas . Poster presentation on "High iron and zinc rice lines".
- USA (Feb 26-March 1). Rice Technical Working Group and RiceCap Meeting.
- Brazil, April 2006. II Congresso Brasileiro da Cadeia Produtiva de Arroz - II CBC Arroz. VIII Reunião Nacional de Pesquisa de Arroz - VIII RENATA.
- April 3-4.2006. James Stangulis visited CIAT for discussions/suggestions on Fe/Zn analysis.
- April 21-28,2006. Managua, Nicaragua. Attend and present a paper at LII Reunión Anual PCCMCA on activities related to High iron and zinc rice lines. A workshop was held to discuss the role and activities related to nutrition.
- CHINA (April 10-14). IAEA Research Coordination Meeting.
- BRASIL (April 26-28). Annual Rice Research Meeting.
- Peru, May 2006. Twenty-first Session of the International Rice Commission. Chiclayo, Peru. 3-5 May 2006
- June 28-30,2006. San Jose, Costa Rica. Present a paper on the CIDA –Agrosalud Project at the II International Rice Conference organized by CONARROZ.
- USA (June 4-10). Marker Assisted Selection Workshop.
- COSTA RICA (June 29-30). National Rice Congress.
- August 15-24,2006. Two workshops were held in Santa Rosa, Villavicencio, Colombia to present breeding activities in rice biofortification to participants coming from different institutions in Latin America. About 100 people attended and selected breeding lines for testing under local conditions.
- PHILIPPINES (August 29-30). Rice Blast Workshop.
- September 17-22,2006. Brazil. Visit to CNPAF-Goiania ,and EMBRAPA-Meio-Norte in Teresina to observe field activities and discuss activities related to CIDA-Agrosalud Rice Biofortification project.
- Jeju, South Korea; September 24th -29th, 2006. To participate a Invited Session Moderator and Speaker in the 9th International Symposium on Biosafety of Genetically Modified Organisms of the International Society for Biosafety Research (ISBR). Gave two lectures and presented overview of chaired Sessions. Proceeding in press Editor: USDA, USA.
- October 7-14, 2006. Attend International Rice Congress in New Delhi, India to keep up on new developments in rice research and HP+biofortification.
- MEXICO (Oct 25-28). FLAR Meeting.
- November 2-3, 2006. Workshop held in CIAT to discuss impact assessment on Agrosalud activities and future work plans.
- November 14-21,2006. La Habana . Cuba. Presented rice biofortification activities to 30 people attending the International Rice Breeding Course. Visit our AgroSalud collaborators from IIA, goverment officials and representatives of the nutrition and health sectors. Discussions on PhD thesis proposal to be carried out by Violeta Puldon.
- Cuba, November 2006. Proyecto TCP/RLA/3102
- December 4-5, 2006. Workshop held in CIAT with representatives from Venezuela interested in joining AgroSalud activities starting in 2007. Good possibilities for additional funding.

4. Training list conducted during 2006

- Marc Châtel, Yolima Ospina. Mejoramiento Genético de Arroz. Selección Recurrente utilizando Androesterilidad Genética: un nuevo Método de Selección. Primer curso de capacitación en fitomejoramiento genético de arroz: Proyecto TCP/RLA/3102(A). Sancti Spiritus-Cuba. 30 de octubre al 10 de noviembre del 2006.
- Major activities in several areas of Colombia, Panamá, Costa Rica and Nicaragua under a FONTAGRO Project dealing with the management of the new invasive pest in rice associated with the bacteria (Burkholderia glumae)-acaro (Steneotarsonemus spinki)-hongo (Sarocladium oryzae) complex.
- Training individual scientists from Argentina, Peru, and Mozambique for a period of one-two months on rice pathology at CIAT. Training of other national and international scientists or students for periods of one week on rice pathology. One day training of students from different Universities on rice pathology.

Interim training of students of Partners Institutions at CIAT

1. Pedro José González. Universidad Católica de Quito, training on molecular analysis of rice and gene flow.

5. Resource mobilization list

a. List of proposals funded, dollar value of contract and donor

- Gene Flow Analysis for Environmental safety in the Tropics. CIAT – University of Costa Rica – Hannover University and BBA, Germany. Donor: EURO 450,000 (2005-2007).
- Development and evaluation of drought-tolerant rice transgenic plants. GCP SB3 USD 70,000 (2005-2006)
- The Latin America: Multi-country capacity-building for compliance with the Cartagena Protocol on biosafety. PDF-B: Development of PAD (Project Appraissal Document). Donor: GEF-World Bank. USD 260,000 (Nov 2005-April 2007)
- Latin America: Multi-country capacity-building for compliance with the Cartagena Protocol on biosafety (Brazil, Colombia, Costa Rica, Peru). USD 5 million. Donor: GEF-World Bank
- Impacto ambiental de la adopción del arroz resistente a las imidazolinoas en sistemas productivos contrastantes de América Latina (AL). INIA-UCV-CIAT. USD 420,000. Donor: Fontagro.
- Capacitación en fitomejoramiento genético e intercambio de germoplasma para utilizar los recursos genéticos del arroz en América Latina y el Caribe TCP/RLA/3102 (A) USD 340,000.00. FAO
- High iron and zinc rice lines. AgroSalud. CIDA-Cananda US$230,000.
- Interspecific bridges to get full access to genetic diversity found in O. glaberrima: GCP , US 300,000 total; about US$ 80,000 for CIAT. To get started in 2007.
- Cenicafe. Technical assistance to the Coffee Genome funded by MADR: US15,000.
- Identification and expression analysis of genes important for iron translocation to the rice grain , hp+ us 15,000.

- Reducción del uso y desarrollo de resistencia a plaguicidas en el cultivo del arroz y fríjol en Colombia, Venezuela y Ecuador. FONTAGRO. US$ 224,000 (2006-2008)
- Manejo del complejo acaro-hongo-bacteria, nuevo reto para arroceros centroamericanos. FONTAGRO. US$ 360,000 (2006-2008)
- Identify and use candidate genes and other molecular markers linked to quantitative trait loci which control milling quality and resistance to sheath blight disease. USDA National Research Initiative Competitive Grants Program. CIAT US$ 47,000 (2005-2007).
- Phenotype evaluation of mutant collection for sheath blight resistance within the commissioned research project PI: Dr. Mathias Lorieux/Dr. I. Manabu (2006-2007). US$ 8,000
- Rice breeding for disease resistance and grain quality in Cuba. IAEA. US$ 30,000 (CIAT US$ 3,750). Within a Project on Pyramiding of mutated genes contributing to crop quality and resistance to stress affecting quality. Project for 15 countries and several crops (US$ 750,000 for five years).

6. List of Partners

- Agrosalud network(Fedearroz-Colombia, IIA-Cuba, CIAT-Bolivia and Aspar, MAN-B(Mision Alianza Noruega)
- CONABIO, UNAM, Mexico
- CONAM, Peru
- CONAGRO
- Corpoica
- CALESA-Panamá
- CNPAF-Embrapa,
- CTA-Embrapa
- CTA-Embrapa Brazil
- Cornell University
- Dominican Republic
- Dale Bumpers Rice Research Center
- DANAC-Venezuela
- EMBRAPA, Brazil
- ETH, Switzerland
- Fedearroz
- FIBA-Argentina
- HP+ IRRI
- IDIAF
- IDIAP
- INTA-Nicaragua
- IDIAP-Panama
- ICA
- INIA Uruguay
- INIA, DANAC-Venezuela
- INIA-Perú

- Institute von Humboldt, Colombia
- INIA-Uruguay
- IRGA
- IRD CIRAD-Francia IAEA-Austria
- IRRI-Philippines
- INTA
- IRD, CIRAD-Francia
- IAEA-Austria
- JIRCAS, Japan
- KSU
- LSU
- PRI, University of Wageningen, The Netherlands
- Phil-Rice Philiipines
- RiceTec
- SENUMISA and CONARROZ-Costa Rica
- Semillas el Aceituno
- Texas &AM Univ.
- Universidad Rio Grande do Soul-Brazil
- University of Arizona
- USDA-USA.
- U. Nacional
- U. de Antioquia-Colombia
- U. of Arkansas
- University of Costa Rica, Costa Rica
- UCV, IVIC, INIAP, Venezuela
- University of Hanover, BBA, University of Braunschweig, Germany
- Warda,

8. List of Visitors

8.1 Distinguished visitors-2006

Name	Institution	Activity	Attended By	Date
Dr. Alain Audebert Dra. Danielle Clavel	CIRAD –CA Rice Breeding and Management Agrobiodiversity in Savannah Enviroments	Rice Program	Lee Calvert, Marc Chatel, Zaida Lentini, Gilles Trouche	January 21-28
Dr. Federico Cuevas Dr. Jose Ré	Rice Tec, USA	Rice Program Flar	Lee Calvert Luis E. Berrio	February 20-24

Dr. Gordon McNeal Financial Consultant	XCG International Consulting Group, Inc Canadá	Rice Program	Lee Calvert	March 21
Dr. Bryan Harvey Dr. Hartmann and Dr. Lena Lange	IITA Incoming Board Chair	Biotechnology, Bean, Rice Projects	Lee Calvert	April 28th , 2006
Martin Price	Executive Director ECHO	Rice Project	Lee Calvert	February 27th up to march 4th
Dr. Rutabanzibwa Gratian Bamwenda, Dr. Roshan Said Abdallah	General Director Technical Director TPRI-TCU	Rice Project	Lee Calvert Fernando Correa	February 7-11
Dr. Modesto Reyes , Dr. Genaro A. Reynoso	Director Recursos Fitogenéticos CENTA-IDIAF	Rice Pathology	Gustavo Prado	February 13-14
Dr. Alexandre Nunes Cardoso, Ph.D	Multilateral Cooperation International Cooperation Coordination EMBRAPA	Rice Project	Lee Calvert	June 8, 2006

Mallam Adamu Bello Honorable Minister of Agriculture and Rural Development of the Federal Republic of Nigeria Mr. Samson Ayodele Adeniran Nigerian Ambassador to Venezuela, Colombia and Ecuador, Based in Caracas Dr. Kenneth Nwosu Executive Director, Nacional Root Crops Research Institute, Umudike, Nigeria Ms. Abdulmumuni Binta Lami Sada Deputy Director, National Poverty Eradication Programme, Nigeria Mr. Ismaila Aliyu Numan Special Assistant to the Minister of Agriculture and Rural Development, Nigeria	Nigeria	Rice program	Marc Chatel César P. Martínez	August 2nd, 2006
Dr. Ron Brlansky	Professor at the U. of Florida Citrus Research and Education Center		Lee Calvert	September 4 -8th
Dra. Jan Leach	Dra. Jan Leach, Incoming President of the American Phytopathological Society, USA		Lee Calvert Marc Chatel Fernando Correa Gilles Trouche	September 11, 2006
Dra. Maria del Carmen Muñoz	Interventoría del Ministerio de Agricultura		Lee Calvert Ivan Lozano Natalia Villarreal	February 14, 15 y 16

James Correll	University of Arkansas. Plant Pathologist. Director of Applied Plant Genomics. Coordinated Agricultural Project	Rice Project	Fernando Correa	September 8 up to 20th

8.2 List of visiting scientists-2006

Name	Institution	Activity	Supervised By	Date
Fabiana Consolo	Argentina	Análisis de información de marcadores moleculares sobre variedades Argentinas de Arroz. Relacionarlo con info sobre virulencia a pyricularia.	Fernando Correa Myrian C. Duque	January 11-20
Adam Famoso	Cornell Univ. Visiting Scientist	Rice Project Thesis "Evaluation of the Panicle Structure in *Oryza glaberrima*".	Mathias Lorieux, César P. Martínez Jaime Borrero	February 13-March 3rd
Vanina Lilian Castroagudin	Instituto Nacional de Tecnología Agropecuaria. Centro	Rice project Capacitación en Caracterización del hongo en *Rhizoctonia solani* e identificación de Fuentes de resistencia en arroz	Fernando Correa	June 18th up to 28th 2006

Lydia Mae Child	Cornell University	Rice Project Research on "Inulin in Rice" (under-graduate) did some work on rice phytates.	Cesar Martinez	July 4, 2006 up to January 3rd, 2007
Aurelie Rakotofiringa	ENSAIA	Investigación Mejoramiento de Arroz	Marc Chatel	Julio 26th up to August 25th 2006
Fernando Cattaneo	INTA-Argentina	Capacitacion especializada en Mejoramiento delCultivo de Arroz	Fernando Correa	Junio 19 al 28
Orlando Peixoto de Morais José de Almeida Pereira	Embrapa Arroz – Brasil	Rice Project	Lee Calvert Zaida Lentini Joe Tohme Jaime Lozano Marc Chatel Rafael Posada César P. Martínez Jaime Borrero	
Grupo SYNGENTA Dr. Julián Valero	Syngenta	Rice Project	Lee Calvert Fernando Correa Luisa Fory Gonzalo Zorrilla Edgar Corredor Luis E. Berrio César P. Martínez Jaime Borrero	September 22, 2006

8.3. List of visitor students-2006

Name	Institution	Activity	Attended By	Date
Prof. Suarez, Rocio	Universidad del Quindío	Rice Pathology	Gustavo Prado	June 1st
Isaac Cieza Ruiz	INIEA – Perú	Rice Pathology, Rice Imrprovement	Lee Calvert, Gustavo Prado, Luis Reyes,	August 15-25, 2006

Aleida Janet Vigil	INIA, Perú	Rice Pathology, Rice Improvement, FLAR, Entomology, Virology, Biosafety´s Projects	Lee Calvert Fernando Correa Gustavo Prado César Martinez Jaime Borrero Luis A. Reyes Luisa Fory Manuel Quintero Iván Lozano Luz E. Romero Pilar Hernandez	September 24 th up to October 13rd
Mónica Ruidiaz	Colinagro, Venezuela Universidad del Tolima	Rice Pathology, Rice Improvement, Entomología	Gustavo Prado, Jaime Borrero, Luis A. Reyes	October 27-2006
Prof. Oscar Checa (31 students)	Universidad de Nariño	Rice Improvement	César P. Martínez Jaime Borrero	November 14- 15, 2006
Prof. Consuelo Montes Prof. Víctor Felipe Terán	Universidad del Cauca	Rice Improvement	César Martínez Jaime Borrero	May 25, 2006
Prof. Hector F. Ramos	Universidad Nacional De Colombia Sede Palmira	Rice Improvement	César P. Martínez Jaime Borrero	May 18, 2006

8.4. List of students

BSc Thesis

Name	Supervisor	University	Title
David Pulgarin	Fernando Correa (CIAT) Gustavo Adolfo Garcia Henao (Univ. de Antioquia, Medellín, Colombia)	Univ. de Antioquia, Medellín, Colombia	Characterization of rice blast resistance genes in Latin American and Caribbean rice varieties.

Erick Giovanni Hernández.	Zaida Lentini	Universidad Francisco de Paula Santander Facultad de Ciencias Agrarias y del Ambiente. Plan de Estudios de Ingeniería Producción Biotecnológica Cúcuta.	Caracterización genotípica y fenotípica de accesiones de arroz rojo *(Oryza sativa f. spontanea*) procedentes de los Departamentos de Tolima y Valle del Cauca. Noviembre 2005. Tesis Meritoria
Mabel Morales.	Zaida Lentini	Universidad Javeriana, Bogotá, Colombia.	Identificación y Caracterización de especies de los complejos *Oryza sativa* y *Oryza officinalis.* Tesis en Curso.
Alicia Milena Velásquez	Zaida Lentini	. Universidad Javeriana, Bogotá, Colombia.	Rastreo de flujo de genes en campos comerciales de arroz utilizando marcadores moleculares y resistencia a herbicidas. Tesis en Curso.
Mónica Fernández	Lee Calvert	Universidad del Valle	Caracterizacion molecular de variedades de arroz (Oryza sativa) resistentes y susceptibles al virus HB mediante el uso de marcadores moleculares microsatélites
Yamid Sanabria Góngora	César P. Martínez	Universidad del Tolima, Facultad de Ciencias Básicas	caracterización morfológica, citogenética y molecular de una accesión del género oryza y evaluación de introgresiones en progenies f_1, bc_2 y bc_3 originadas de cruces con *Oryza sativa* L**.**

MSc Thesis

Name	Supervisor	University	Title
Rosana Pineda	Zaida Lentini	Universidad Nacional de Colombia	Transgenic Genes Flow for Rice Blast. April 2005
Natalia Labrin	Lee Calvert	Centro Agronómico Tropical de Investigación y Enseñanza – CATIE, Costa Rica	Maestria en Agricultura Ecológica en el área de Genética de la Resistencia en Variedades de Arroz (Oryza Sativa) Venezolanas al Virus de la Hoja Blanca. Enero 18-Octubre 2006
Kiliany Andrea Arcia Moreno.	Zaida Lentini	Universidad Nacional, Sede Palmira.	Caracterización de especies silvestres del género *Oryza* colectadas en condiciones naturales y campos de arroz en el Estado Portuguesa, Venezuela. Tesis en Curso.
Andrés Eloy Blanco.	Zaida Lentini	Programa sándwich Universidad Nacional, Sede Palmira/ Universidad Central de Venezuela, Maracay, Venezuela.	Caracterización de especies silvestres del género *Oryza* colectadas en condiciones naturales y campos de arroz en el Estado Guárico, Venezuela. Tesis en Curso.
Olga Ximena Giraldo	César P. Martínez	Universidad Nacional de Colombia-Palmira	Maestría en Ciencias Agrarias.Fitomejoramiento Tesis" Identificación de Marcadores SNPs para Biofortificación en Arroz

Ph.D. Thesis

Name	Supervisor	University	Title
Luis Armando Castilla. Fedearroz	César Martínez	Universidad Nacional de Colombia.	Interspecifical lines rice evaluation (Oryza spp) and inoculación con bacterias fijadoras de nitrógeno A. Chrooccum and A. Amazonense en un suelo de la meseta de Ibagué, Tolima
Francisco Amela	Cesar Martínez	Instituto de Investigación Agraria de Mozambique (Mozambique Nacional Rice Program)	Maestria en Ciencias Agrarias en el area de Mejoramiento de Arroz

9. Awards 2006

Award	Granted To	Organization	Date
Premio Semilleros ADN Agro-Bio 2006	Luz E. Romero Lee A. Calvert	AGROBIO	Bogotá, November 9, 2006
Reconocimiento proyecto de arroz a la labor consejo municipal general Saavedra	Marc Chatel y Lee Calvert	Consejo municipal General	Bolivia
National Award in Phytopathology "Rafael Obregon", 2006. Professional category. Research: "Identification of microsatellite markers linked to resistance genes to *Pyricularia grisea* in rice"	Fernando J. Correa Victoria, Jorge L. Fuentes, Fabio Escobar, Gustavo Prado, Girlena Aricapa, Myriam C. Duque	Given by the National Association of Colombian Phytopathologists "ASCOLFI".	