CIAT

Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

Annual Report 2008

SBA-4: RICE

For Internal Circulation and Discussion Only

March 2008

Output 1: Enhanced gene pools

Output 2: Integrated crop, pest and disease management

Output 3: Intensification and diversification of rice cropping systems for small farmers.

Output 1: Enhanced gene pools

1A IRRIGATED RICE

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

César P. Martínez, Jaime Borrero, Silvio James Carabali,

Roger Taboada, Jose Luis Viana, Lázaro Narvaez, Violeta

Puldon, Angel Adames, Alejandro Vargas, and J.Tohme

Source of funding: CIDA-Canada and CIAT_Core

Abstract

A fast-track approach is under way to screen breeding lines and traditional/ improved rice varieties to find rice germplasm with higher iron and zinc content in milled rice. A total of 5743 milled rice samples including breeding lines from CIAT and NARs were sent to the Ciat's service lab for iron and zinc analysis using the atomic adsorption method. Some rice lines were identified presenting between 5 and 7 ppm of iron, which are being evaluated by our AgroSalud partners for local adaptation. In Brazil, EMBRAPA identified traditional rice cultivars with up to 12.6 and 42.2 ppm of iron and zinc respectively, which are being used as parents in their breeding program. Breeding activities under way in Colombia, Dominican Republic, Brazil, Cuba, Nicaragua, Bolivia, Panama, and at CIAT are on schedule and will meet the goals of delivering **nutrivars** as planned by 2009-2010. Bolivia and Cuba will be releasing the first **nutrivars** by February and June/2009, respectively followed by Brazil, Nicaragua and Dominican Republic in 2010. Bolivia, Cuba and Brazil are already producing enough seed of **nutrivars** for delivery to end users. Demostration plots and field days are being conducted by our partners to present nutrivars to farmers , as well as contacts with the news media to promote AgroSalud. GxE studies are under way to determine the effects of climatic and soil conditions on the expression of iron and zinc in the rice grain, and to establish agronomic practices for the nutrivars.

Introduction

Our aim is to increase the content of iron and zinc in milled rice, using conventional methods of breeding, including methods of bulk and pedigree selection, backcrossing, population breeding and mutagenesis. On a fast track(short term phase), 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. A crossing program (medium- long term phase) is also under way 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, and Nicaragua. More recently, Panamá joined AgroSalud.

We also work in the identification of molecular markers(SNPs) associated with high iron and zinc content in the rice grain to be used in a marker assisted breeding program. Breeding materials developed by the AgroSalud Project are shared with our collaborators for evaluation and selection by collaborators under local conditions and best lines will be released as varieties. This report presents the results of several activities carried out in 2008.

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. Different activities including evaluation of rice germplasm and breeding lines, assessment of NIR technology to speed up analysis of iron and zinc in the rice grain, development of segregating populations via recurrent selection, correlation of the iron and zinc content in brown and milled rice, use of the statistical design " increased blocks by Federer" in the analysis of iron and zinc content in the rice grain, and development of a database for iron and zinc data were carried out.

Results and Discussions

Development of segregating populations to increase iron and zinc in the rice grain in addition to other desirable traits, **and germplasm exchange**. A total of 238 top crosses were made for evaluation in 2009. This crossing program, including some breeding lines we got from IRRI HP rice project(Table 1) was started to recombine desirable traits found in elite lines derived from diverse interspecific crosses. A total of 2738 segregating breeding lines were sown and evaluated at Ciat-Palmira and Santa

Rosa for agronomic traits, tolerance to main insect pests and diseases, grain quality, and yield potential; 972 promissing lines were identified. These are lines derived from crosses between *O.sativa* and other wild rice species. Two yield trials with selected lines were planted and harvested in April/2008 at CIAT-Palmira. Based on agronomic traits, yield potential and iron/zinc data best promissing lines were selected for distribution to our AgroSalud partners(Table 2).Recurrent selection is being used by Embrapa, Fedearroz and CIAT to increase iron and zinc in the rice grain; preliminary data are encouraging.

Table 1. Breeding lines from IRRI Harvet Plus Project used as progenitors in Agrosalud.

Pedigree	Fe (mg/kg)	*std*	Zn (mg/kg)	*std*
IR68144-2B-2-2-3-1-120	4.84	*1.76*	17.45	*1.38*
IR68144-2B-2-2-3-1-166	5.23	*2.04*	17.49	*3.27*
IR69428-6-1-1-3-3	4.36	*1.15*	22.21	*0.78*
IR75862-206-2-8-3-B-B-B	5.43	*3.81*	21.43	*6.55*
IR75862-221-2-1-2-B-B-B	3.85	*1.33*	19.11	*4.90*

Average of five evaluations Laboratorio de servicios analiticos del Ciat.2008

Table 2. List of breeding lines with best performance in two yield trials carried out in Palmira. CIAT.2008.

Pedigree	Vg	Bl1	Bl2	LSc	BS	NBl	GlD	Hb	Tag	clk	Len	Fl	Amy	Exc	Fe	Zn	Fe (mg/kg)	Zn	Mean(kg/ha)
CT18245-18-2-4-2-7-M	5	1	1	3	3	3	3	7		0.2	L	110	29.3	60.5	5.7	13.7	3.5	14.5	6958
CT14543-10-M-3-3-2V-2-M	5								3	0.2		110	24.0	61.1	5.1	13.4	4.5	13.2	7609
CT18664-9-10-2-1-M	5	2.3	4	5	3	1	3	9		0.2	L	107	30.9	61.2	4.4	14.0	3.6	15.5	7128
MAT CANDU												130	30.0	59.5	4.1	13.0	4.5	15.2	5692
CT17379-32-5-1-4-1-4-2-M	5	1	1	1	1	1	3	5		0.4	L	93			3.6	14.3	4.0	17.5	4619
CT17334-13-3-1-3-1-2-1-M	3	4	3	3	3	3	1	5	1	0.4	L	110	31.2	67.0	3.5	13.3	3.6	15.8	5758
CT18375-9-5-1-1-7-M	5	2.3	5	3	5	3	5	9		0.2	L	108	30.2	60.8	3.4	16.0	4.2	19.3	4303
CT18232-5-9-1-1-4-4-M	1	1	1	3	3	3	3	7		0.2	L	110	30.4	56.5	3.1	15.2	4.4	20.5	5489
CT17334-13-3-1-3-1-4-1-M	3	4	3	3	3	3	1	3	1	0.2	L	108	33.5	45.2	3.4	14.6	3.4	19.0	4993
Arroz 9666								3				105	33.0	31.0	3.4	9.9	4.1	11.3	6134
CT18375-9-6-1-2-4-M	5	1.3	1	5	5	5	3	9		0.2	L	110	32.1	47.8	3.4	13.1	3.5	17.6	5195
CT18614-10-6-2-4-3-M	5	1	3	3	1	3	1	7		0.2	L	110	32.9	49.3	3.4	14.7	3.8	18.7	5105
CT18375-9-6-1-3-1-M	5	1.2	3	5	5	5	3	9		0.4	L	111	33.0	49.5	3.3	12.6	4.5	14.4	5599
CT17334-13-7-1-5-M-6-M-9-M	3	1	2	1	3	3	3	7	1	0.8	L	110	32.5	38.3	3.1	13.3	3.9	12.0	6136
Lineas IRRI- JDB-4-M								7		0.2	L	105	28.5	55.0	3.1	11.6	3.1	12.2	6881
CT17330-M-2-5-M-2-1-1-M	5	2	2	1	5	3	3	7		0.2	L	98	35.0	64.3	2.9	10.8	3.9	13.8	5960
CT17251-1-5-3-2-1-1-2-2-M	3	2	2	5	3	1	3					107	33.0	36.8	2.6	10.2	4.5	18.9	5397
Fedearroz 50	1	2	3	3	3	3	3					112	30.3	64.2	3.6	15.9	4.3	10.5	5494
IR64																	4.8	10.9	

A total of 5743 samples of milled rice including breeding lines from CIAT and NARs were sent to the Ciat's service lab for iron and zinc analysis using the atomic adsorption method. Some rice lines were identified at CIAT presenting between 5 and 7 ppm of iron(Table 3) whilst EMBRAPA-CNPAF identified traditional rice cultivars with up to 12.6 and 42.2 ppm of iron and zinc, respectively. This data need to be confirmed; however, this material is being used by CNPAF as parental sources in their breeding program.

It has been documented that in wheat the expression of iron and zinc in the grain depends on several factors including climatic and soil conditions. This type of studies are conducted locally by our partners in Cuba, Brazil, Colombia, Dominican Republic, and Nicaragua. This information is needed to make recommendations to farmers as agronomic practices for biofortified rice lines. Prelimary data from Nicaragua and Colombia are presented in Tables 4, and 5, which seems to indicate small differences in the expression of iron and zinc in milled rice depending on genotype, locations, and growing conditions.

Table 3.Some agronomic traits and nutrient content of breeding lines showing good performance in Colombia and Nicaragua.

T2008-188

	Santa Rosa 2003								Santa Rosa 2008								Ciat 2008					
PEDIGREE	VG	Bl1	Bl2	Fl	LSc	BS	NBl	GD	VG	BL1	BL2	FL	LSC	BS	NBL	GD	VG	FL	HB	Kg/ha.	Fe (mg/kg)	Zn (mg/kg)
CT16658-5-2-2SR-2-3-6	3	3	3	84	1	1	1	1	1	3	4	86	1	1	3	3	3	109	3	4906.4	5.27	13.02
CT16658-5-2-3SR-2-1-M	3	2	3	85	1	1	1	1	1	3	3	85	1	1	3	3	3	109	3	6092.6	5.22	12.95
CT16658-5-2-3SR-3-1-3	3	3	4	83	3	1	3	1	1	2	3	85	1	1	3	3	3	113	3	5891.5	5.14	12.79
CT15691-4-3-4-2-1-2-M	5	1	2	110	3	3	3	5	3	2	3	88	1	1	3	1	1	113	1	4632.5	5.95	12.64
CT15691-4-3-4-3-2-2-M	5	3	4	94	1	1	3	1	3	2	3	89	1	1	3	3	3	118	1	5307.7	5.90	8.79
CT15659-4-3-1-2-3SR-1-1	3	2	4	92	1	1	3	1	1	2	4	91	1	1	3	3	3	118	1	4258.4	4.90	11.78
CT15659-4-3-1-3-4SR-2-1	3	3	4	92	1	1	3	1	1	1	3	91	1	1	3	1	3	118	1	4475.5	5.12	11.18
CT15696-3-4-1-1-3SR-1-2	3	5	4	96	1	1	3	1	3	2	3	91	1	1	5	3	3	113	1	7296.6	6.45	9.28
CT15696-3-4-1-1-3SR-2-2	3	5	4	96	1	1	3	1	3	2	4	93	1	1	5	3	3	113	1	6459.5	6.59	8.66
CT15716-6-1-2-3-2SR-M-4	3	4	5	88	3	5	3	3	3	5	6	87	3	5	5	3	3	113	3	6088.2	4.05	9.87
CT15717-7-1-1-1-2SR-M-2	3	4	3	90	1	1	3	3	3	5	6	87	3	5	5	3	3	113	3	9153.0	4.66	9.51
CT14544-1-M-2-3-3-M-M	3	4	3	97	3	3	5	3	3	3	4	85	1	1	3	1	3	116	3	5518.7	5.99	19.42
CT14544-1-M-2-4-1-M-M	3	4	3	97	3	3	3	3	3	3	3	97	1	1	3	3	3	109	3	4754.5	5.41	20.94
CT18141-6-4-2-2-4-M	3	3	5	81	1	1	1	1	3	3	3	92	1	1	3	1	3	113	5	5268.5	4.98	9.15
CT18145-7-1-1-3-1-M	3	2	2	81	3	3	3	1	3	1	2	85	1	1	3	3	3	109	5	6728.7	5.05	10.86
CT18148-6-9-3-3-2-M	3	3	5	83	5	3	3	3	1	3	3	90	1	1	3	3	5	113	5	7935.2	6.33	9.25
CT18148-6-9-5-1-2-M	3	3	3	82	3	5	3	3	1	3	3	93	1	1	1	1	5	109	5	9149.4	7.12	9.78
CT18148-6-9-5-1-3-4-M	3	3	3	81	3	5	5	3	1	2	3	93	1	1	3	3	5	109	5	7752.2	6.38	10.19
CT18148-10-3-2-2-3-M	1	3	4	78	1	3	3	1	1	3	4	89	1	1	3	5	3	109	5	6738.2	5.01	10.18
CT18148-10-3-2-4-1-M	3	4	5	81	3	3	3	3	3	3	4	88	3	1	3	5	3	109	5	7643.4	5.28	10.24
CT18148-10-3-6-1-6-M	3	3	4	81	3	3	3	3	1	3	3	91	1	1	3	3	3	109	3	8441.3	4.24	10.18
CT18148-10-3-6-4-6-M	1	5	5	79	1	3	3	3	3	3	5	90	1	1	3	3	3	109	3	7828.0	4.56	9.91

Table 4. Genotype x Environment experiments conducted by INTA. Nicaragua in two locations: Sebaco(irrigated) and Posoltega(upland). Nicaragua 2008

Genotypes	Irrigated *Sebaco*				Upland *Posoltega*			
	Fe (mg/kg)	*sdt*	Zn (mg/kg)	*sdt*	Fe (mg/kg)	*sdt*	Zn (mg/kg)	*sdt*
FLO 3001-MP2-1P-3P-M	5.47	*0.48*	14.70	*0.56*	5.97	*0.26*	10.78	*1.03*
FLO 3724-3P-5-1P-M	6.00	*0.20*	17.68	*1.63*	6.43	*1.73*	14.60	*2.95*
FLO 3779-4P-9-3P-1P-M	6.63	*1.42*	16.29	*1.22*	5.61	*0.28*	14.65	*3.86*
FLO 3801-1P-1-1P-2P-M	5.23	*0.69*	17.17	*0.94*	5.29	*0.11*	15.07	*0.98*
FLO 4052-2P-3-2P-2P-M	5.47	*0.32*	16.01	*0.33*	7.25	*0.91*	14.62	*0.92*
CIWINI	4.74	*0.74*	13.81	*0.63*	5.28	*0.45*	11.67	*0.80*
CT 15679-17-2-7-5-5-M	5.38	*0.08*	22.53	*0.94*	5.41	*0.68*	13.44	*1.20*
CT 15679-17-1-1-1-4-M	4.09	*0.41*	20.99	*0.57*	5.54	*0.77*	12.27	*1.08*
CT 15679-3-4-2-3-3-M	4.69	*0.19*	20.82	*0.41*	5.57	*1.06*	15.09	*0.76*
CT 15691-4-5-2-2-1-M	5.78	*1.36*	16.24	*0.74*	7.53	*0.67*	13.04	*1.41*
ISA-40	5.30	*0.41*	15.44	*0.23*	7.51	*2.81*	12.24	*0.34*
CT 15679-17-1-4-5-2-M	4.47	*0.53*	20.67	*1.17*	5.81	*1.87*	14.21	*1.03*
INTA DORADO	6.45	*0.88*	12.37	*0.52*	7.63	*1.18*	11.40	*6.08*
FEDEARROZ-50	5.36	*0.62*	20.20	*0.25*	6.92	*1.06*	12.76	*4.48*
Mean	4.98		16.05		5.77		12.36	

Table 5. Performance of breeding lines in two contrasting environments in Colombia. Fedearroz. 2008

Genotypes	VG	FL	HT	Exc	Lpan	Pan/ m2	GrII	Est %	P1000	Yield Kg/ha	CIAT		AIPE	
											Fe (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
CT18238-23-1-2-3-3-1-M	3	92	100	1	22	700	41	24	30	4566	3.99	13.39	8.05	16.75
CT18238-23-6-1-4-1-2-M	3	89	97	2	22	833	78	18	25	6410	4.26	13.21	6.69	17.18
CT18232-5-8-2-2-2-1-M	3	89	102	1	24	500	81	30	26	6307	3.51	12.26	7.17	15.21
CT18238-23-1-3-3-1-4-M	3	92	93	1	27	800	84	26	25	7482	4.32	15.52	7.08	16.06
CT18614-10-6-2-4-3-M	3	91	104	2	23	467	91	17	26	9017	3.37	14.74	7.13	17.97
CT18614-4-1-2-3-1-M	1	82	119	3	26	900	71	11	27	9250	4.04	13.37	7.25	18.60
CT18238-23-1-1-3-2-1-M	3	92	97	2	23	667	81	26	21	3274	3.54	16.08	7.36	16.41
CT18238-23-1-1-2-3-3-M	1	92	96	3	23	767	59	19	23	4116	3.67	14.26	7.63	16.28
CT18238-23-1-3-2-4-1-M	3	92	89	1	26	1067	69	16	23	8619	3.97	14.02	6.99	16.04
CT18235-2-2-1-4-3-3-M	5	90	107	4	22	833	57	25	24	4756	4.38	13.31	8.53	16.62
CT18617-6-2-2-2-2-M	5	89	94	2	23	600	77	26	23	8119	3.59	12.69	6.53	17.19
CT18247-11-5-2-3-2-1-M	3	91	89	1	22	800	54	29	25	6294	3.70	16.46	9.52	19.47
CT18614-9-4-1-1-3-M	3	76	104	2	24	933	78	20	24	6689	4.92	12.90	7.03	18.15
CT17334-13-3-1-3-1-2-1-M	3	89	105	1	25	667	84	14	26	8644	3.53	13.34	7.87	17.39
CT17334-13-3-1-3-1-2-2-M	3	91	98	2	25	967	74	18	25	8279	3.83	14.77	7.95	18.84
CT17334-13-3-1-3-1-4-3-M	3	89	92	1	22	1067	72	15	25	6531	3.47	15.16	7.87	16.76
CT17334-13-3-1-4-1-1-2-M	5	89	93	1	22	1067	67	22	26	9048	3.18	12.88	7.46	18.59
CT17334-13-3-1-4-1-1-3-M	5	89	94	5	24	1033	68	18	27	8141	2.81	11.96	7.88	17.88
CT17334-13-3-1-4-1-1-4-M	5	90	90	1	23	1167	65	19	26	8858	3.21	12.00	8.11	17.26
CT17334-13-3-1-2-3-3-2-1-M	3	92	111	1	23	933	82	20	26	8293	2.84	11.02	8.85	18.14
CT17334-13-3-1-2-3-5-1-2-M	7	94	100	3	29	467	122	31	26	3302	2.92	12.12	9.62	18.45
CT17334-13-3-1-3-5-3-4-4-M	5	92	93	1	23	833	54	25	25	7352	2.77	10.44	9.11	17.09
CT17334-2-1-6-2-2-1-3-2-M	5	89	94	2	23	900	64	19	25	6372	3.80	15.27	9.06	18.20
Fedearroz 50	3	91	91	2	21	567	64	16	27	8446	2.45	15.98	7.77	17.04
Fedearroz 60	3	90	79	2	24	1100	78	17	28	9082			7.51	12.62
F-edearroz 473	3	92	80	1	19	800	65	34	27	8754			7.47	13.33

Our rice partners in Bolivia and Cuba are very much committed to releasing the first **nutrivars** (**rice lines having more iron and zinc compared to local base line, but not yet the target level)** by 2009, to be followed by releases in Brazil, Nicaragua, Dominican Republic, and Panama in 2010. On Februay 6/2009 our patners in Bolivia will be releasing Azucena and Saavedra 27(CT16659-8-2C-1-3-5-1); Azucena will be recommended for planting by small farmers who produce rice mostly for their own consumption while Saavedra 27 will be recommended for medium to large farmers, who produce rice under irrigated and rainfed conditions. Saavedra 27 will be the first variety coming out of an interspecific cross(Perla/ *O.rufipogon*) to be released in Latin America and according to estimates run by our nutricionist Helena Pachon contains 18% and 28% more iron than Paitití and Tari(popular commercial varieties in Bolivia), respectively, and 17% and 5% more zinc compared to Paitití and Tari, respectively. By growing and consuming Saavedra 27 Bolivians could have more iron and zinc in their diet. Besides, data

from regional trials indicate that Saavedra 27 has a 27% yield increase over Epagri 7, the more popular rice variety grown by farmers in Bolivia;from the food security perspective Saavedra 27 could also make a good impact on improving the productivity of rice in Bolivia.

Assessment of technologies and equipment to speed up analysis of iron and zinc .

CNPAF-EMBRAPA and CIAT conducted some work to optimize the utilization of a modified Kett Rice Mill, donated by James Stangoulis - Flinders University, through HarvestPlus;this mill can be used for small sample sizes (around 10 g) of dehulled rice, results were promissing, and no indication of contamination was found. However, samples run through the Kett Mill did not reach the degree of witness preferred by consumers and the iron and zinc content was higher when compared to samples milled with the Modified Suziki Mill used in our Agrosalud laboratoty. Some work was done in our laboratory at CIAT to determine and control factors influencing the inconsistent results we are getting in iron and zinc values via the atomic adsorption method and to get familiar with NIRS before it is used as a high throughput technique. Results suggest that the size of the particles found in the rice flour is a key factor determining the mineral content in a given sample. Rice flour is not homogenous and different varieties produce rice flour having particles of different sizes. These results suggest that some adjustment in milling time, and selection of a representative size of the rice flour particles could produce more consistent data. More work will be conducted in 2009 in collaboration with Thomas Zum Felde at CIP with funding from HP to stablish an adequate calibration curve for NIRs.

Development of a Database

A database has been developed to facilitate access of iron and zinc data as well as to main agronomic data of rice materials in a fast and structured form. This database is in Excel and constitutes a very easy, simple and friendly tool that can be handled by users; it is a dynamic database, where the stored information can be modified and updated as new data becomes available. Main characteristis are:

-Fast and agile consultations of agronomics characteristics, pedigree information and the content of micronutrients iron and zinc.

-It allows operations like updating, addition of data and consultations.

- It provides minimum and maximum values, averages values for iron and zinc of evaluated materials.

- It allows sorting the materials by anyone of the registered characteristics.

-It displays in graphic form the values of iron and zinc of the evaluated materials.

At present, the database has a record of 8500 materials, and is being fine tuned with new applications aimed at offering more information to the users. In the future this database will be in the webpage of the project available to the rice community.

Future activities

Activities will depend on funding since funding by CIDA-Canada will terminate in March 2010. Lobbying is under way to try to convince CIDA to fund a second phase. In rice we are meeting the goals of identifying and releasing rice varieties with higher iron and zinc content comapred to base line. Better lines are in the pipeline. Our partners are highly motivated and committed to meet Agrosalud goals. Priority will be given in 2009 to releasing nutrivars, seed multiplication and distribution to farmers and in collaboartion with our partners and Agrosalud's economist to monitor economic and social impact of the adoption and comsumption of biofortified rice.

1A.2. Evaluation and selection of breeding populations

S. J. Carabalí, J.Borrero, E.A.Torres, César P.Martinez

Source of funding: MADR-Colombia, AgroSalud and CIAT-core

Abstract

Pre-breeding or the transfer of valuable traits from exotic or wild parents to elite lines is a key activity in the CIAT rice breeding program. New breeding activities were started to generate new populations using a combination of diverse parental sources and breeding populations were advanced using a shuttle breeding strategy. Additionally, these populations were screened for disease reactions and quality traits. The final product is the CIAT International Observational Nursery (CIAT-ION), which is distributed to several partners. Also, experimental populations for research purposes were developed.

Background

Wild species from genus *Oryza* are a valuable source of new genes for breeding cultivated rice. However, the transfer of useful traits from wild species to those cultivars has been very limited. Some examples like the Grassy Stunt virus resistance gene from *O. nivara* (Khush et al. 1977) and the *Cytoplasmatic Sterility* genes from *O. perennis* to generated hybrid rice (Lin and Yuan 1978) are reported as successful transfer of interesting traits.

Several wild species are highly resistant to all well-known biotypes of *Brown Plant-hopper* (BPH), *White Backed Plant-hopper* (WBPH), and *Green Leaf-hopper* (Heinrichs et al.1985). Recently Brar et al (2002) reported that some genes like bacterial resistance, blast resistance, tungro virus resistance and tolerance to acid soils conditions can be transferred from wild species to cultivated rice. Recent data (Xiao et to the, 1996) suggest that some wild species of rice contains genes that will allow to increase yield potential and improve grain quality of modern rice varieties. For example, the use of wild parents in breeding wheat, by CIMMYT, has given high levels of resistance or tolerance to *Septoria*, *Fusarium*, drought, heat, salinity and flooding. Also, they have found higher concentration of Iron and Zinc in the wild wheat types than in cultivated wheat.

Recently, an increase of so-called secondary diseases affecting rice has been observed in Latin America. Some diseases like *Rhizoctonia solani*, *Sarocladium oryzae*, *Bipolaris oryzae*, *Polymyxa graminis*, *Gaeummanomyces graminis* and *Burkholderia glumae* are now are causing high yield looses in several areas in Latin America and the Caribbean. To face these new challenges not only better agronomic practices are needed but new breeding populations have to be developed. Therefore, CIAT has proposed a strategy to transfer resistance genes from wild species to elite lines using conventional breeding methods and appropiate molecular tools.

Materials and Methods

Diverse experimental lines coming from different programs including FLAR, CIAT, IRRI, and Warda and commercial varieties like Fedearroz 50, Fedearroz 2000, Fanny, Cica 8, Caiapó, Curinga, were crossed with wild species such as *O. rufipogon*, *O.glaberrima*, *O. barthii*, *O. latifolia*, and *O.meridionalis*.

The CIAT Rice Breeding Program strategy includes a backcross approach (2-3 backcrosses), pedigree rows and shuttle breeding between contrasting locations. Additionally, breeding populations are screened for quality traits and pest resistance under controlled conditions in CIAT Palmira. Some Fedearroz experimental fields are used for planting and selection under specific conditions.

In the first semester of 2008, a total of 5112 pedigree rows from different generations (F_3,F_4,F_5,F_6,F_7) were evaluated in Santa Rosa (Table 1), which is a hot spot for blast and secondary diseases. A total of 1536 individual plants were selected. These materials were sown at CIAT (Palmira) by the end of 2008. Additionally, they were evaluated to Hoja Blanca virus, white belly and amilosa in Palmira.

Table 1. Populations evaluated in Santa Rosa during semester A 2008.

Population	Generation	Evaluated lines	Selected lines
INTERESPECIFIC CROSSES	**F_3,F_4,F_5,F_6,F_7**	**2154**	**767**
INTRODUCTIONS	**F_7**	**258**	**50**
BIOFORTIFICATION	**F_5,F_6**	**819**	**150**
BLAST RESISTANCE	**F_4**	**446**	**342**
TAGOSODES RESISTANCE	**F_5**	**208**	**57**
INTRODUCED POPULATIONS	**F_4,F_5,F_7**	**522**	**100**
RECURRENT SELECTION	**S_2,S_5**	**172**	**20**
MONTERIA SELECTION	**F_4,F_5**	**469**	**50**
CHARACTERIZATION MADR		**64**	
Total		**5112**	**1536**

At the same time advanced populations were planted in Palmira with the purpose of seed increase and selection under non-disease pressure. Lines showing disease resistance, fertility and lodging tolerance in Santa Rosa and good plant type and agronomic characters were selected and harvested in Palmira. Santa Rosa seeds were used only for grain quality evaluation.

In Palmira, F_1 and BC_1F_1 populations from the Fedearroz- Colombian Ministry of Agriculture and biofortification projects are now under evaluation.

Table 2. Evaluation and selection of segregating populations at CIAT Palmira 2008.

Population	Generation	Evaluated lines	Selected lines
INTERESPECIFIC CROSSES	**F_5,F_6,F_7**	**1360**	**267**
INTRODUCTIONS	**F_7**	**258**	**192**
BIOFORTIFICATION	**F_1,F_5,F_6**	**844**	**419**
TAGOSODES RESISTANCE	**F_5**	**208**	**161**
INTRODUCED POPULATIONS	**F_5,F_7**	**408**	**100**
RECURRENT SELECTION	**S_2,S_5**	**172**	**149**
SELECTION-MONTERIA	**F_4,F_5**	**469**	**221**
CHARACTERIZATION MADR		**84**	**84**
CROSSES MADR		**34**	**599**
Total		**3837**	**2192**

Results and discusion

Blast reaction**.** Figure 1 shows responses from different populations to the *Pyricularia* pathogen at Santa Rosa experimental station. The material could be characterized as: resistant 39.78%, intermediate 35.11 and susceptible 25.10%.

Most blast resistant populations came from the interspecific crosses with 57.03% of resistant lines; recurrent selection and populations developed for blast resistance also showed good performace for blast. Most susceptible ones were introductions and biofortification populations with 70.24% and 53.39% of susceptible materials.

Hoja Blanca virus reaction. In Figure 2 the susceptibility of different populations is shown for VHB; 15.82% of the lines were resistant, 18.01% had an intermediate susceptibility and 66.17% were susceptible.

Populations with the higher number of resistant lines came from inter specific crosses with the 49.46% of resistant lines; while resistance blast populations and plant introductions showed the less numbers of resistant lines with the 8.52%.

Figure 1. Summary of Leaf Blast reaction at Santa Rosa 2008**.**

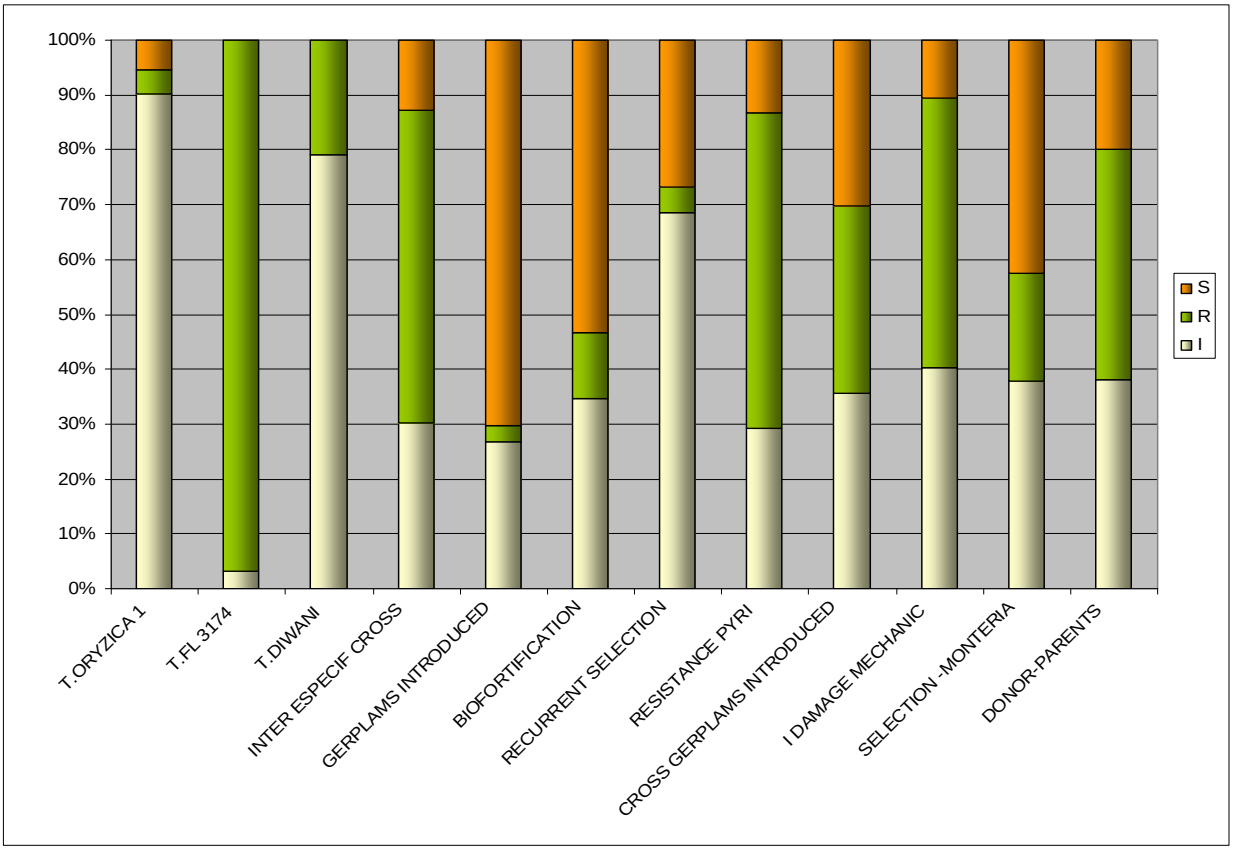
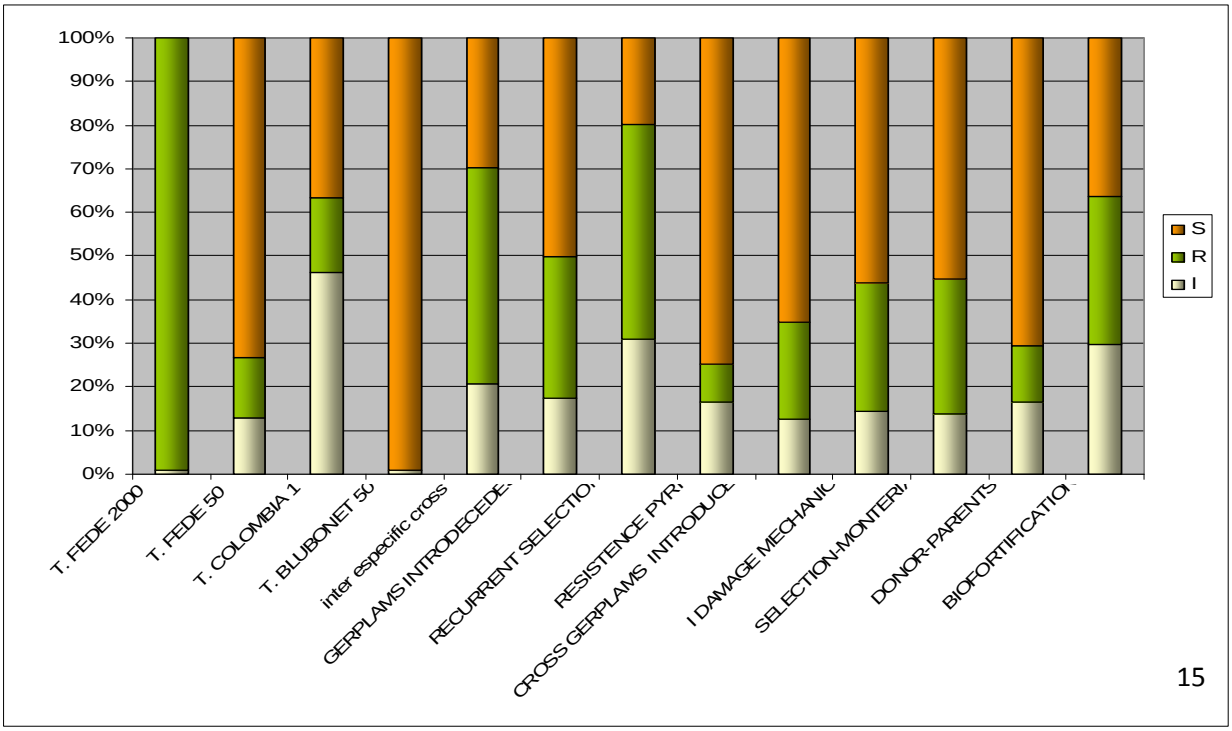


Figure 2. VHB Reactions of segregant populations at CIAT Palmira 2008



Milling quality

Advanced lines, which are candidates to CIAT ION nursery, were evaluated for milling quality using a Rice Miller McGill # 2 and a Satake Test Rice Grader. A summary of results is presented in figures 3 and 4. Even though these lines are derived for interspecific or elite x exotic crosses, they have very interesting values; more than 80% had a percentage of whole grain (head) over 60% and 11.21% had an excellent grain appearance similar to Fedearroz 60.

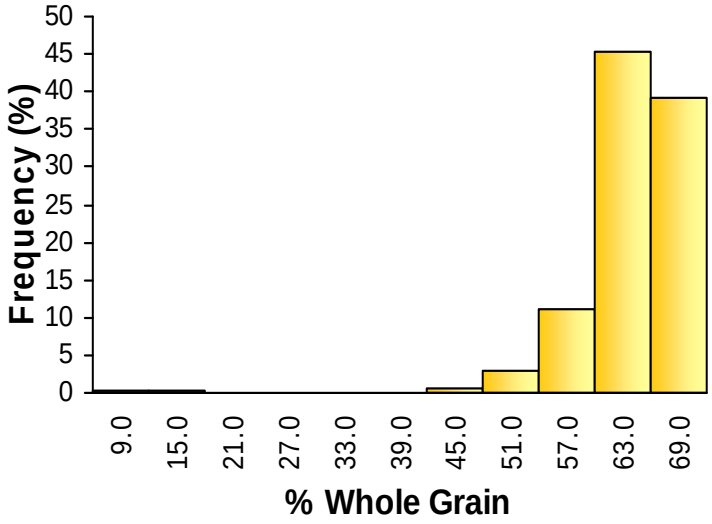


Figure 3. Distribution of percentage of whole grain (head) in advanced lines CIAT-ION candidates, n=320.

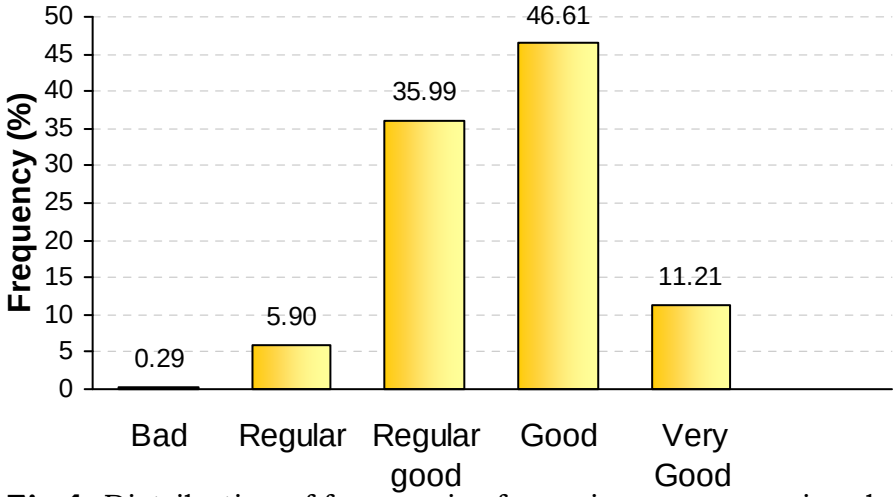


Fig 4. Distribution of frequencies for grain appearance in advanced lines CIAT – ION candidates, n=320.

Production of RIL's.

A total of 786 RIL's are being multiplied this 2008B semester. They belong to ten single crosses and have been advanced using SSD. These crosses were made between contrasting parents for several traits including long panicles, intermediate stature, stay green, grain quality and one thousand grain weight.

Formation of new populations

Breeding populations are the raw material in a breeding program. In 2008 a total of 1130 new crosses were done as three way or top crosses, backcrosses or to form new recurrent selection populations. They included: 900 single cross and backcrosses for the Fedearroz- Colombian Ministry of Agricultures project, 77 single crosses to develop lines with higher iron and zinc, and 150 backcrosses to develop drought tolerant materials.

Future plans

All populations under advance will be screened in Santa Rosa for blast and secondary diseases. At the same time these populations will be evaluated to VHB in Palmira.

RIL's from one population will be evaluated for agronomic, yield and yield components, and grain quality in several environments in Colombia.

CIAT ION nursery will be distributed to national programs in Latin America.

References

Brar D.S., Bui Chi Bun, B. Nguyen, Z. Li, M. Jones and G.S. Khush. 2002. Gene

transfer from wild species and molecular characterization of alien introgression in rice. Abstracts International Rice Congress. 16-20 September, 2002. Beijing, China. p.69.

Heinrichs, E. A., F. G. Medrano and H. R. Rapusas, 1985. Genetic evaluation for insect resistance in rice. International Rice Research Institute, P. 0. Box 933, Manila, Philippines. p. 356.

Khush GS, Ling KC, Aquino RC, Aguiero VM (1977). Breeding

for resistance to grassy stunt in rice. In: 3rd Int. Cong.

SABRAO, Canberra, Australia. *Plant Breeding Papers*. 1-4:

3-9.

Xiao, J. et al. 1998. Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150:899-909.

1A.3. Mean Generation Analysis for Panicle length in Rice

Collaborators

Cesar Martinez, Jaime Borrero and Edgar Torres – CIAT Rice Program

Francisco Amela – Rice Program Mozambique MS Student

Abstract

The genetic control of panicle length and other traits was investigated using generation means analysis in two locations during semester B 2007. The contrasting genotypes FL01028 and Norin 22 were crossed and the F1, F2, BC1 and BC2 generations produced. These experimental materials were planted in Jamundi and CIAT Palmira under transplanting conditions in a RBD with four reps. Plot size was different depending on the generation. Results indicated the existence of significant genotype by environment interaction for all traits including panicle length. A model including additive by additive and additive by dominance gene action explained the observed variation in panicle lenght. Dominance direction was always positive; however, in Palmira it was not different from zero but in Jamundi it was very important. For other traits the better models always included epistatic terms.

Keywords Panicle length, mean generation analysis,

Background

Rice genotypes with bigger panicles have been proposed as a way to increase the yield potential in rice. For example, in the 90's the International Rice Research Institute (IRRI) proposed the new plant type concept, which means a plant type with fewer tillers and bigger panicles (Kush, 2005). Hybrids in rice also have bigger panicles as mentioned by Yang (2007) who reported that the yield advantage in hybrids was associated with large sink size due to large panicles and the capacity to maintain a balance between panicles number per unit of area and spikelet number per panicles. CIAT- FLAR strategy to increase the yield potential also includes increasing the number of grains per panicles.

There is an intuitive relationship between long panicles and yielding ability and some authors like Laza et al (2004) mentioned that cultivars with intermediate number of grains per panicles (100-115 spikelets per panicle) has more yielding ability than cultivars with small panicles (60-80 spikelets per panicle). However,

Visperas (2000) mention that in some cases long panicles result in low number of panicles per square meter and more sterility. Additionally, genotypes with long panicles have the tendency to be taller than normal panicles genotypes and tend to be more susceptible to lodging .

Several studies have shown that panicle length is a polygenic trait. De-Lin and Yin (2004) reported that traits like panicle length and number of spikelets per panicle show quantitative inheritance with the allele dispersion between parents being very important to explain heterosis. For these authors the dominance variance is more important than additive variance in genetic variation for panicle length; on the other hand, for the number of spikelets per panicle epistatic effects were important. Ando et al (2008) indentified 38 quantitative trait loci (QTL´s) with small additive effect distributed on 11 chromosomes related with five morphological components of panicle architecture. These authors concluded that the cumulative effects of QTL´s distributed throughout the genome represent the major genetic basis of panicle architecture in rice.

The objective of this work was to estimate genetic parameters related to panicle length, number of primary and secondary ramifications per panicle, weight of 1000 grains, panicle number per plant, branches number per plant and percentage of sterility in an indica – japonica cross.

Material and Methods.

Parents were selected based on their panicle length. Long panicle parent was FL1028 that is the long panicle donor used in the FLAR breeding program. Short panicle donor was Norin 22 a japonica variety with small panicles. The F1 cross was small by long panicle. Generation mean analysis was performed using the six generations P1, P2, F1, F2, BC1 and BC2.

Field experiments were planted at CIAT 3°30´N, 76°30´W and 965 masl and Jamundi 3°14´N, 76°31´W and 1004 masl using transplanting during semester 2007B. The six generations were planted in randomized blocks design with four reps. For P1, P2 and F1 the experimental plot had four rows 5.24 length 30 wide; F2 and BC´s were planted in 10 and 8 rows plots respectively. Data were taken in individual plants in central rows. For no segregating populations the plant numbers were 25 in CIAT and 15 in Jamundi. In F2, a total of 100 plants per rep in Palmira and 80 plants per rep were measured. In the case of BC1 and BC2 the number of plants were 50 in CIAT and 40 in Jamundi.

Data were taken on traits: Panicle length (PL), number of tillers per plant (Ti), number of panicles per plant (Pan), number of primary branches per panicle(Primbr), number of secondary branches per panicle (Secbr), one thousand grains weight (GW) and sterility percentage (Spfert).

For each character, by location variance analysis was done using SAS Proc GLM. Weighted regression analyses were used to test the importance of gene effects. When additive dominance effects models were adequate to explain observed variability, additive and dominance variances, broad sense heritability, narrow sense heritability and medium degree of dominance were estimated. For all traits FL01028 was considered the parent 1 (P1); however, for tillers number per plant (Ti) and panicles number per plant (Npan), Norin 22 was the P1 because his higher mean for these traits.

Results and Discussion

Location and joint variance analysis showed significant differences for generations in all traits indicating the release of genetic variability when FL01028 and Norin 22 were crossed. In the same way, generation x location interaction was significant or highly significant depending on the trait, indicating a differential response from generations to environmental changes. High sterility was observed in F1 and segregating populations, which could be attributed to indica – japonica incompatibility.

Table 1. Mean squares and significance from joint variance analysis for: Panicle Length (PL), number of tillers per plant (Ti), number of panicles per panicle (Npan), number of primary branches in the panicle (Primbr), number of secondary branches (Secbr), one thousand grains weight (GW), and sterility percentage (Spfert).

Traits	**Site**		**Blocks**	**Generation**		**Site X Generation**		**Experimental Erro**	**Simple Error (Plants in plots)**	
	CM	**P**	**CM**	**CM**	**P**	**CM**	**P**	**CM**	**CM**	**CV(%)**
Pl	498.39	**	9.57	2622.37	**	159.72	**	11.58	5.08	9.76
Ti	1227.25	**	41.56	2898.92	**	1224.69	**	176.73	51.99	27.66
NPan	13606.05	**	172.17	21735.44	**	2135.16	**	247.92	84.63	31.44
Primbr	1048.46	**	4.84	2829.49	**	76.09	**	13.30	4.16	18.57
Secbr	31960.46	**	248.38	15520.81	**	3886.58	**	230.21	42.01	25.15
Gw	853.69	**	19.67	56.65	**	43.62	*	13.18	11.82	13.32
Spfert	3.07	**	0.17	11.36	**	1.45	**	0.06	0.04	22.95

Because of significant interaction between location and generation, genetic analyses were done per location.

For panicle length, a joint scale test using only m, a and d parameters showed that a model considering only additive and dominance effects was not adequate to explain the observed variation. The same situation was observed with other traits (data non show) (**Table 2**).

Table 2. Scaling test for additive dominance model adequacy.

CIAT-Palmira				
Generation	**Observed**	**Expected**	**Weight**	**$X^{2‡}$**
P1	26.86	26.80	33.040	0.127
P2	16.12	16.71	65.076	22.653
F1	25.08	25.81	50.104	26.365
F2	24.71	23.78	58.209	50.377
RC1	25.69	26.31	50.903	19.738
RC2	22.52	21.26	39.205	62.469
	X^2cal (3)			181.73**
Jamundí				
Generation	**Observed**	**Expected**	**Weight**	**X^2**
P1	27.17	26.18	24.754	24.385
P2	15.68	15.45	42.496	2.399
F1	24.92	24.06	40.449	29.673
F2	21.44	22.44	48.771	47.945
RC1	24.39	25.12	34.403	18.028
RC2	19.94	19.75	21.587	0.735
	X^2cal (3)			123.17**

‡ X^2 $(Observed-Expected)^2$*Weight

Weighted regression analyses were done in order to test the importance of genetic effects. The used weight was $1/(standar\ error)^2$ as recommended by Kearsey and Pooni (1998). In some traits as tillers number, panicles number and one thousand grain weight; the model does not explain the variation (non significant F). However, for panicle length, grains per panicle, number of primary branches and number of secondary branches, the analyses indicate that models considering not only additive and dominance effects, but also additivexadditive additivexdominance and dominancexdominance effects were adequate for explaining the data (Table 3).

Table 3. Gene effects and significance for models considering additive, dominance and interaction effects for panicle length.

Parameter	Panicle Length		Grains per Panicle		Primary Branches		Secondary Branches	
	CIAT	Jamundi	CIAT	Jamundi	CIAT	Jamundi	CIAT	Jamundi
m	24.43**	17.89**	132.80**	66.65**	13.22**	5.09*	40.06*	10.51*
a	5.37**	5.74*	76.54**	71.44**	5.30**	5.32**	12.55*	8.09*
d	0.68ns	6.99*	61.37**	79.13**	-1.83 ns	10.9 ns	-9.01 ns	10.59 ns
aa	-2.92 ns	3.51 ns	xxxx	60.27**	-1.8 ns	5.03 ns	-17.33 ns	6.89 ns
ad	-4.39 ns	-2.65 ns	xxxx	-32.18**	1.49 ns	xxx	14.72 ns	xxx
dd	xxx	xxx	-34.99**	xxx	xxx	-6.15	xxx	5.15 ns
R^2	0.9999	0.9997	0.999	0.9999	0.9999	0.9997	0.9996	0.9997
Adjusted R^2	0.9993	0.9986	0.9997	0.9998	0.9997	0.9984	0.9980	0.9985
CV	3.32	1.67	0.57	0.44	1.55	4.38	3.59	2.81

**,* and ns: Highly significant, significant and non-significant according to *t* test

These results indicate that in this cross FL01028xNorin 22, the epistasis is acting on the genetic architecture of panicle related traits.

For panicle length, dominance direction was positive; however, the importance of each parameter was dependent on location, for example, dominance effects were very important in Jamundi but not in CIAT. In the same way, the sign of additive by additive effects was positive in Jamundi and negative in Palmira. Dominance by dominance interaction was always negative. These results are consequence of genotype by environment interaction. However, in Palmira where there was much better control over experimental conditions only additive effects were different from zero indicating that this trait is highly heritable.

In conclusion, panicle size is a trait with high heritability under good environment and agronomic conditions.

References:

Ando, T.; Yamamoto, T.; Shimizu T.; Ma XF.; Shomura, A.; Takeouchi, Y.; Lin, SY. and Yano, M. 2008. Genetic dissection and pyramiding of quantitative traits for panicle architecture by using segmental substitution lines in rice. Theoretical and Applied Genetics 116(6) 881-890.

De-Lin, H.; Yan, L. 2004. Genetic Analysis of Heterosis for Number of Spikelets per Panicle and Panicle Length of F1 Hybrids in japonica Rice Hybrids. Rice Science, 2004 Vol.11 No.3 P.255-260.

Kearsey, M.J. and Pooni, H.S. 1998. The Genetical Analysis of Quantitative Traits. Staley Thornes Publishers. Cheltanham, United Kingdom. p. 379

Kush, G.S. 2005. What it will take to feed 5,0 billion rice consumers in 2030. Plant Molecular Biology 59: 1 – 6.

Singh. R.K. and Chaudry B.D. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers. New Delhi, India. p. 315

Visperas, R. M.; Peng, S.; Khush, G. S.; Pamplona, A. 2000. Relative performance of new plant type lines during the dry and wet seasons. Phil. J. Crop sci. 25 (Suppl. 1):51.

Yang, W.; Peng, S.; Laza, R.; Visperas, R. and Dionisio-Sese M. 2007. Grain yield and yield attributes of new plant type and hybrid rice. Crops Science 47: 1393 – 1400.

1A.4. Evaluation of Long Panicles Lines in Several Environments

Edgar Torres, Silvio James Carabali-CIAT Rice Program

Collaborators

Luis Eduardo Berrio FLAR, Cristo Pérez FEDEARROZ Colombia, Juan Figueroa ASOPORTUGUESA Venezuela, Funding: CIAT-Core, FLAR, Fedearroz and Fundarroz, Eduardo Graterol, Carlos Lozada DANAC Foundation Venezuela

Abstract

Increasing number of grains per panicles is a strategy followed by the FLAR breeding program to increase yield potential in rice. The objective of this study was to evaluate contrasting experimental rice lines and checks in several locations in Colombia and Venezuela for yield, yield components, and agronomic traits. Twelve genotypes were evaluated in yield trials in CIAT Palmira, La Victoria Experimental Station, La Carolina Farm in Portuguesa Venezuela, and Farm 178 in Calabozo Venezuela during the dry season 2007-2008. Results showed several long panicles lines with higher yields than checks in all locations; however, no line was superior in all environments because significant gxe interaction. In general, number of grains per panicles was positively associated with yield, but negatively with number of panicles per square meter indicating compensation between yield components. Also, long panicles lines had higher index harvest in Palmira and higher yields across locations. There was no relationship between number of grains per panicles and stability, some long panicles had high yield but were unstable and some had high yields and were stable. These results indicate that breeding for more grains per panicle or "paniculas largas" is a correct strategy to increase yield potential in rice; however, it is necessary to take in account the interrelationships and compensations between yield components, while maintaining good plant type and short plant stature, tolerance to lodging and low panicle sterility. These results, suggest that a strategy based in specific adaptation could be more successful.

Key words: Yield potential, long panicles, yield components.

Background

Increases in rice yield has been growing in at a low rate after the green revolution. For example, Peng et al (1999) concluded that empirical breeding has resulted in the maintenance of yield potential in the tropics of about 10 $Mgha^{-1}$ since IR8 release. In the same way, Peng et al (2000) found an annual gain of 75 to 81 kg ha^{-1} in Philippine cultivars and interestedly, the last cultivar released had similar yield with the first release (IR8). A similar situation was observed in several experiments done in Venezuela during the rainy and dry crop season in 2007, they showed an annual yield increase between 30.4 to 33.7 kg ha^{-1} (Pieters and Graterol 2008 personal communication). Recently, Tabien *et al* (2008) reported

linear growing for rice yield in Texas from 1944 to 1992; there, the rice yield increases were depending on Nitrogen level, at 190 Kg/Ha the annual rate was 42 kg ha^{-1} and at 95 kg ha^{-1} N it was 26.3 kg ha^{-1}. These results indicate that rice breeding probably has been concentrated in stress tolerance and grain quality rather than yield *per se* and that it is necessary to put more efforts in breeding for yield potential in order to face growing demand and climate change.

Recently, several approaches have been proposed to increase yield potential. Because modern semi-dwarf varieties produce excessive unproductive tillers and leaf area which causes mutual shading and reduced canopy photosynthesis and sink size, the International Rice Research Institute (IRRI) proposed the concept of a new plant type. This new ideotype was characterized by low tillering capacity (9-10 tillers), no unproductive tillers, 200 – 250 grains per panicle, dark green leaves, and a vigorous and deep root system (Kush, 2005). However, after intensive evaluation of these materials, the results were disappointing because of low biomass production and poor grain filling (Peng, et al 1999). Japanese researchers also attempted to increase yield potential by modifying plant type, modeled after the Korean Tongil type varieties; it was characterized by short culms, relatively fewer panicles with a very large number of grains per panicle. (Yonezawa, 1997). Hybrid rice was also developed to break the yield potential barrier. Hybrid cultivars have been showing consistently a yield advantage of 10 – 20% over the best inbred varieties (Cheng, et al 2007). Yan et al (2007) reported that the yield advantage in hybrids was associated with large sink size due to large panicles and the capacity to maintain a balance between panicles number per unit of area and spikelet number per panicles.

Because of the importance of yield potential for FLAR and CIAT partners in Latin America, they developed a strategy to increase yield, which has two sequential approaches. The first is to close the gap existing between potential and actual yield in existing varieties by better agronomic practices. These practices reduce six major constraints: inappropriate seeding dates, heavy seeding densities, deficient insect and weed control, poor fertilization practices, and late establishment of permanent irrigation. The second approach is to develop more productive varieties by increasing sink size through large panicles while maintaining high tillering capacity, associated with dark- green, slow senescing leaves (Jennings PR, 2007).

The objective of this work was to evaluate contrasting rice experimental lines, for number of grains per panicle and number of tillers by square meter, in four environments in Venezuela and Colombia during the dry season 2007-2008.

Materials and Methods

Twelve rice genotypes were used in this study; eight were experimental F_7 lines selected because of their differences in terms of yield components observed during the long panicle yield evaluation in 2007 (CIAT Rice Program Annual Report 2007). There were two common checks Fedearroz 50, a widely cultivated variety, and FL01028 the long panicle donor from FLAR tropical breeding program; additionally, there was a local check in each environment.

The experiment was established in two locations in Colombia, CIAT Palmira 3°30´09”N 76°22´21”W 965 masl and La Victoria Experimental Station 8°48´58”N 75°50´45”W 14 masl; and two locations in Venezuela, Calabozo Irrigation System Farm #178 at 8°45´11”N 67°32´43”W 80 masl in Guárico State and La Carolina Farm at 9°27´17”N 69°07´03”W 145 masl in Portuguesa State; during the 2007 – 2008 dry season. A complete randomized blocks design was used with three blocks for each site and plot size of 20 m^2 (5x4). Seeding rates of 100 kg ha^{-1} and pre-germinated seeds in puddle soil were used in Palmira, La Carolina and Farm 178 and 180 kg ha^{-1} with dry seeding in Monteria. A general fertilization scheme was used and the doses were 250 Kg ha^{-1} N, 60 Kg ha^{-1} P_2O_5, 90 kg ha^{-1} K_2O. Nitrogen was split three times with 70% before permanent flooding, which was applied immediately after fertilizer application at 30 days after seeding and maintained until near harvest. Weeds, insects and rodents were chemically controlled when necessary.

Days to 50% of flowering were taken in each plot. At maturity one sub-plot 0.25 m^2 within each plot were sampled by cutting plants at the soil level. Plants were threshed by hand and separated into grain and straw. In each straw sample the total and effective (bearing a panicle) culms were counted. The grain sample was oven dried at 70 °C for three days and filled and empty spikelets were separated and counted. Finally, filled grains per square meter, total grain per square meter, spikelets per panicle, 1000-grains weight, sterility percentage, and the harvest index were calculated. In Palmira the harvest index was determined. The grain yield was determined for 12 m^2 area in each replication and adjusted to a moisture content of 14 percent.

The percent of sterility was transformed using the arsine of the square root. In order to establish the significance of locations, genotypes and the genotype by environment interaction a combined analysis, in which genotypic effects were considered fixed and sites and blocks in sites were considered as random effects, was done using the model (1).

$$Y_{ijk} = \mu + l_j + b_{k(j)} + g_i + l*g_{ij} + e_{ijk} \quad (1)$$

The General Linear Model procedure of SAS (SAS Institute, 2002) was used for the location and combined analysis. Yield stability was studied using the AMMI analysis (Gauch, 1992), by using a SAS program written by Burgueño *et al* (2003).

Results and discussion

Calabozo location was eliminated from the combined analysis because of lack of homogeneity of variance. Locations effects were highly significant ($p<0.01$) for yield, yield components and flowering, indicating than they are very different in terms of performance of the rice cropping. In the same way, genotypic effects and genotype by environment interaction were highly significant ($p<0.01$) for all

characters illustrating not only differences in all characters between tested lines but also differential response of cultivars at different locations (table 1).

Table 1. Mean squares for grain yield (kg ha $^{-1}$), days to 50% flowering (Fl), number of panicles per square meter (Pan m^{-2}), number of grains per panicle (G/pan), sterility percentage (Spfert.) and 1000 grain weight (GW) at three locations in dry season 2007-2008.

Source	df	Kg ha^{-1}	Fl	Pan m^{-2}	G/Pan	Spfert	GW
Locations	2	100327187.1**	1176.5**	541093.078**	5461.90**	0.238**	29.58**
block(loc)	6	243840.37ns	3.29ns	3015.79ns	142.77ns	0.004ns	2.33ns
Genotypes	91	1810150.69**	70.13**	54871.01**	2716.36**	0.039**	31.97**
GenxLoc	182	2068448.83**	9.94**	11543.55**	430.68**	0.017**	10.38**
Pooled error	52	389394.4	1.86	3437.31	127.86	0.004	1.84
Total	86						
CV (%)		9.30	1.66	12.49	11.3	12.48	5.78
Mean		6706.30	81.86	469.46	89.9	23.89	23.22

** significant at 0.01 level, ns non-significant by *F*-test

Several experimental lines outyielded the checks in each location but none was superior in all enviroments. In Monteria, line 3817 and Fedearroz 50 showed significant superior yielding ability; in Palmira, line 3371 was the best in terms of yield and in Portuguesa line 3399 had the highest yield. Palmira had the highest yield mean across genotypes and Monteria location had low mean. (Table 2).

Table 2. Grain yield (kg ha^{-1}) at three locations during the dry season 2007-2009

Line	Montería	Palmira	Portuguesa
3371	3259.75 b	9163.57 a	7955.52 ab
3399	3858.41 ab	8960.88 ab	8767.13 a
3481	4136.71 ab	6887.24 bc	7349.57 abc
3602	5157.53 ab	7632.34 abc	7674.71 abc
3614	4794.93 ab	8563.14 ab	7232.14 bc
3817	5217.16 a	8310.15 ab	7552.08 abc
3834	4057.45 ab	8911.58 ab	7763.6 ab
3860	4893.64 ab	5764.22 c	6237.48 c
Fedearroz 50	5253.53 a	7303.94 abc	8411.56 ab
FL01028	5194.68 ab	8311.95 ab	7026.2b c
Location Mean	4582.38	7980.9	7597.00

Means followed by different letters are significant different according with REGWQ multiple range test.

Across locations, lines 3817, 3371, FL01028 and 3399 had more grains per panicle and lower number of panicles per square meter. In contrast, lines 3602 and 3614 showed few grains per panicle and higher number of panicles per square meter. Other genotypes were in the middle of this pattern. In relation to sterility, lines 3371, 3602, Fedearroz 50 and 3860 had higher sterility percentage; on the other hand, line 3399 was more fertile. Line 3614 had heavier grains; in contrast, 3371 and 3860 had light grains. There were several lines with high harvest index, such as 3834, 3399, 3371, 3817 and FL1028. Finally, lines 3399 and 3817 had yields over 7000 kg ha^{-1} across environments. (table 3).

Table 3. Mean values for grain yield (kg ha $^{-1}$), number of panicles per square meter (Pan m^{-2}), number of grains per panicle (G/pan), sterility percentage (Pest), 1000 grain weight (GW), harvest index (IH) and days to 50% flowering (Fl), across three locations in dry season 2007-2008.

Gen	Kg ha^{-1}	Pan m^{-2}	Gpan	Pest	P1000	IH‡	FL
3371	6792.95	382.56	103.44	33.09	21.82	0.50	82.78
3399	7195.47	405.22	97.21	15.48	22.70	0.52	78.33
3481	6124.51	452.17	91.70	23.08	24.41	0.45	81.11
3602	6821.53	636.67	61.25	29.45	24.30	0.46	84.56
3614	6863.40	525.11	66.01	18.84	27.62	0.46	77.00
3817	7026.46	422.06	118.91	23.69	21.54	0.50	83.89
3834	6910.88	460.55	93.50	18.81	23.37	0.53	81.67
3860	5631.78	538.78	78.43	25.86	21.08	0.43	80.78
Fedearroz 50	6989.68	469.33	88.72	27.66	22.63	0.47	86.33
FL01028	6844.28	401.45	105.03	24.32	22.86	0.50	82.11

‡ Harvest index measured only in Palmira

Relationships between traits were depending on the location; however, some very clear tendencies appear. Yield was positive related with grain per panicle and negatively with panicles per square meter in Palmira and Portuguesa, but both relationships were significant only in Palmira; in contrast, it had a positive and significant correlation with panicles per square meter in Monteria. Yield was positively related with one thousand grains weight in the three locations but it was no significant. Panicles per square meter and grains per panicles were negatively related with grains per panicle in the three locations, but it was significant only in Palmira and Portuguesa. A similar negative correlation was observed in grain per panicle and one hundred grain weight; however, it was not significant. Negative no significant relationship also was observed between yield and percentage of sterility (Table 4).

Table 4. Phenotypic correlations between grain yield (kg ha $^{-1}$), number of panicles per square meter (Pan/m^2), number of grains per panicle (G/pan), sterility percentage (Spfert.), 1000 grain weight (GW)

		Kg ha^{-1}	Pan/m2	Gpan	Pest	P1000
Kg ha^{-1}	Montería	1.0	0.73*	-0.09^{ns}	-0.28^{ns}	0.35^{ns}

	Palmira	1.0	-0.79**	0.69*	-0.43ns	0.48ns
	Portuguesa	1.0	-0.09ns	0.17ns	-0.07ns	0.34ns
Pan/m2	Montería		1.0	-0.56ns	-0.17ns	0.40ns
	Palmira		1.0	-0.89**	-0.54ns	-0.07ns
	Portuguesa		1.0	-0.85**	0.05ns	0.14ns
Gpan	Montería			1.0	0.09ns	-0.62ns
	Palmira			1.0	-0.67*	-0.14ns
	Portuguesa			1.0	0.37ns	-0.46ns
Pest	Montería				1.0	-0.52ns
	Palmira				1.0	0.26ns
	Portuguesa				1.0	-0.72*
P1000	Montería					1.0
	Palmira					1.0
	Portuguesa					1.0

** Correlation coeficiente (r) highly significant (p<0.01), * significant (p<0.05) ,ns non significant

The AMMI analysis of the genotype by environment interaction indicated two IPCA as significant, IPCA 1 and IPCA 2; which explained 80,6 % and 19 % of the variability respectively.

The AMMI2 graph (Figure 1) show that the genotypes studied differ not only for mean yields, but also for their interaction effects. Genotypes plotted closer to the origin like 3481, 3602, 3614 and 3817; had similar yields in all environments, which mean more stability. In the other hand, lines far apart were sensitive to environmental variation and had a large interaction. In the same way, environments were very different for yield and interaction effects. Because of the length of their spokes the three environments exerted a strong interaction and due to their opposite position, they had very different pattern of interaction on genotypes. Lines and environments con similar signs of IPCA 1 score had positive interaction and thus higher yield of the genotype at the particular location, for example lines 3371 and 3399 perform very well in Palmira and Portuguesa but they were poor performers in Monteria; in the other hand, line 3860 had good yields in Monteria and low yield in Palmira and Portuguesa.

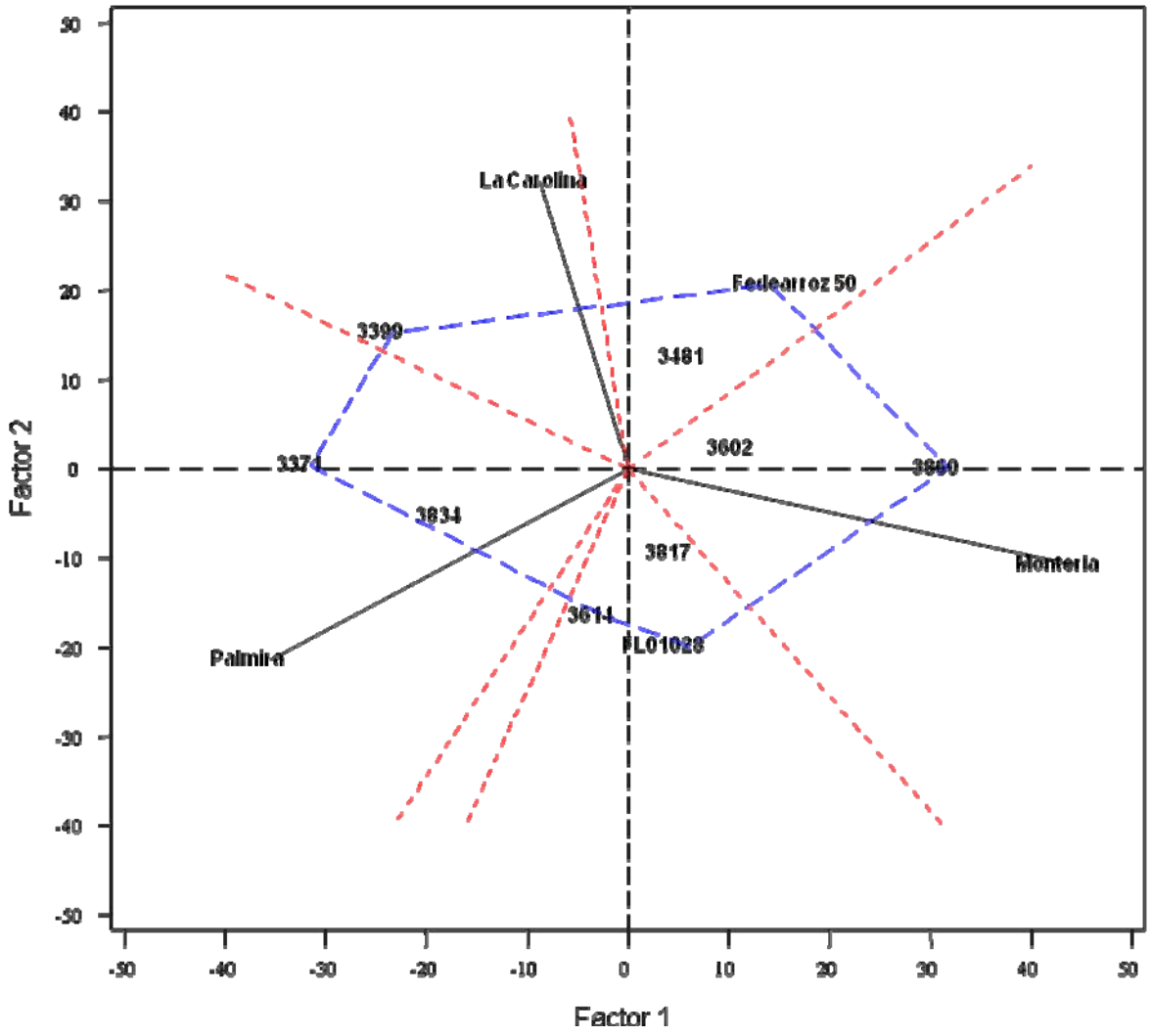


Fig 1. AMMI biplot for yield kg ha^{-1}

The genotypes evaluated were different in relation with their number of grains per panicle and number of panicles per square meter. Lines 3817, 3371, FL1028 and 3399 can be considered as long panicles types. In contrast, lines 3602 and 3614 have high number of panicles per square meter. Other materials can be considered as intermediate type. Interestingly, lines 3817 and 3371 had consistently bigger panicles than the original source FL1028 indicating the positive genetic gain for this trait achieved in the FLAR tropical program.

No one material had higher yields in all locations; however, there was a trend; lines with higher number of grains per panicle also had higher yields in each location, for example, 3817 in Monteria, 3371 in Palmira, and 3399 in Portuguesa. Also, across locations 3399 and 3817 had the highest yields. Additionally, lines with large panicles had a better harvest index at Palmira(lines 3399, 3817, 3371 and FL1028). These results indicate that breeding for more

grains per panicle or “paniculas largas” is a correct strategy to increase yield potential in rice.

However, there were negative relationships between yield components; panicles per square meters and one thousand grains weight were negatively related with number of grains per panicle. It probably means that yield is a complex trait; the rice plant should have a balance between the components in order to achieve a high yield. Also, some long panicle lines like 3371 were kind of taller and lodging susceptible. Additionally, there wasn´t a clear relationship between grains per panicle and percentage of sterility; which is important, because not necessarily more grains per panicle means more sterility, but line 3371 had high sterility in some locations. In consequence, in breeding for more grains per panicle it is necessary to be carefully with: the interrelationships and compensations between yield components, plant type, plant height, lodging susceptibility and panicle sterility.

In relation to stability, lines 3371, 3399 and FL01028 had interaction with the environment; in contrast line 3817 was more stable. In consequence, more grains per panicle doesn´t mean more interaction with the environment. However, to take advantage of this trait a strategy based in specific adaptation can be more successful.

References

Burgueño, J.; Crossa, J. and Vargas, M. 2003. Graphing GE and GGE biplots. Kang. M (ed.). Hanbook of formulas and software for plant geneticists and breeders. Food Products Press. New York, 2003. pag 193-204.

Cheng, S.H.; Zhuan, JY.; Fang, Y.; Du, JH.; and Cao, LY. 2007. Progress in research and development on hybrid rice: A super domesticate in China. Annals of Botany. 100: 959 – 966.

Jennings, PR. 2007. Rice revolutions in Latin-America. Rice Today, April – June 2007, 38.

Kush, G.S. 2005. What it will take to feed 5,0 billion rice consumers in 2030. Plant Molecular Biology 59: 1 – 6.

Peng S.; Cassman, K.; Virmani S.; Seehy, J. and Khush G. S. 1999. Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. Crop Science 39: 1552- 1559.

Peng, S.; Laza RC.; Visperas, RM.; Cassman, K.G and Khush, G.S. 2000. Grain yield of cultivars and lines developed in Phillipines since 1966. Crop Science 40: 307-314

Tabien, R.E; Omar S.; Samonte, PB. and McClung A. 2008. Improvement in Texas since the release of cultivar Bluebonnet in 1944. Crop Science 48: 2097-2106.

Yang, W.; Peng, S.; Laza, R.; Visperas, R. and Dionisio-Sese M. 2007. Grain yield and yield attributes of new plant type and hybrid rice. Crops Science 47: 1393 – 1400.

Yonezawa, K. 1997. Yield components. In: Matsuo, T.; Futsuhara, Y.; Kikuchi, F. and Yamaguchi, H. (Eds). 1997. The science of rice plant. Volume Three: Genetics. Food and Agriculture Policy Research Center, Tokyo, Japan. p. 1003.

Genetic analysis of agronomic traits, yield components and grain quality in *Oryza barthii* derivatives

Edgar Torres, Silvio James Carabali – CIAT Rice Program

Marisel Agredo Berrío – Student Universidad Del Cauca.

Abstract

Wild species are very important sources of valuable traits in breeding rice; however, little is known about their performance in crosses with elite lines. The objective of this work was to study the genetic control of agronomic, yield components and grain quality traits using *O. barthii* derivatives and FLAR elite lines. According with their 1000 grains weight, the parents were divided in light-weighted parents (elite lines) and heavy-weighted parents (barthii derivatives), and crossed using a partial diallel analysis approach. Parents and hybrids were evaluated under transplanted conditions in Palmira in 2008 for agronomic, yield components and grain quality traits. Results indicated that additive effects were more important for all traits and SCA effects were significant only for sterility percentage, percentage of chalkiness and one hundred milled grains weight. *O. barthii* derivatives did not differ in their GCA effects for one thousand grains weight and one thousand milled grains weight, which means probably common genes for these traits coming from the wild parent. However, they were different in their *gca* effects for earliness, plant height, number of tillers, panicle length, number of grains per panicle, percentage of sterility, percentage of milled grains, percentage of whole grain and percentage of chalkiness. This results show that *O. barthii* derivatives can be a useful source of positive alleles especially for one thousand grains weight. Also, even though reproductive barriers or F1 sterility was present in these crosses, some elite lines were more compatible and produced near fertile progenies, indicating the existence of compatibility alleles in the elite germplasm.

Key words: wild relatives, partial diallel analysis, yield components, grain quality

Background

The Oryza wild species represent a potential source of new alleles for improving yield, quality and stress resistance. Some authors have proposed that exploitation and utilization of the favorable alleles of wild rice that were lost or weakened during domestication of cultivated rice might be able to overcome the yield plateau (Tian, *et al* 2006). For example, Martinez *et al* (2004) found that O. *rufipogon* and *O. glaberrima* are sources of positive alleles for yield, grain quality and disease resistance. Similar findings were reported by Xiao et al (1998) who found transgressive segregants that outperformed the original elite hybrid for several traits in a BC_2 interspecific testcross population, eventhough the *O rufipogon* accession was inferior for all traits studied. Consequently, the CIAT rice project is working in the transference of specific positive alleles from rice wild relatives to improved germplasm.

According to Yoshida (1981) rice yields are determined by the number of effective panicles per unit of area, number of spikelet per panicle, one thousand grain weight and percentage of filled spikelet. However, in breeding for yield potential, the effect of grain weight and percentage of filled spikelets have received little attention.

The African wild specie *Oryza barthii* is an interesting source of several traits including one thousand grains weight. Commercial varieties with heavier grains, weight around 28 grams per 1000 grains; however, *O. barthii* derivatives achieve higher weights in order of 33 to 34 grams, which means an increase of 5 grams over commercial varieties. With 36000 filled grains per square meter, it could adds 1,8 tons per hectare, maintaining the number of panicles per square meters around 450, the number of grains per panicle around 100 and the panicle fertility about 80%. Hence, increasing grain weight might help to increase the yield potential in rice.

The objective of this experiment was to study the performance of *O. barthii* derived lines as parents for yield, yield components and grain quality traits, crossed with elites FLAR lines as testers using a partial diallel analysis approach.

Materials and Methods

Six *O. barthii* derivatives were crossed with three elite FLAR lines (Table 1) using a partial diallel design. The FLAR elite lines were designed as light-weighted grain group and the wild derivatives as heavier grain group. Manual crosses were made between groups and in only one direction (light x heavier).

Table 1. Genetic material used in the experiment.

Code (i or j)	Origin	Pedigri	Cross
1	BCF 2107	Centauro	Ecia 38-2-4-2-5-6/CT8222-7-6-2P-1X//Fedearroz 50
2	BCF 2246	FL06733-12P-4-3P-IP	FL03186-1P-11-1P-3P-M/FL03197-22P-4-1P-2P-M//FL01028
3	BCF 2251	FL06899-1P-6-1P-1P	FL03199-26P-3-1P-2P-M-1P/FL03186-1P-7-2P-1P-M//FL03197-22P-4-1P-2P-M-1P
1’	HI85B2975	CT17238-1-1-1-2-1-7-1	Lemont*5/O. barthii//CT10310-15-9-2P-3-1T-2P-1
2’	HI85B2993	CT17238-1-1-1-2-4-3-3	Lemont*5/O. barthii//CT10310-15-9-2P-3-1T-2P-1
3.	HI85B2995	CT17238-1-1-1-2-4-4-2	Lemont*5/O. barthii//CT10310-15-9-2P-3-1T-2P-1
4’	1836	CT15068-5-10-1-1	Oryzica Llanos 5*2/O. barthii
5’	1845	CT15081-6-10-2-2	Oryzica Llanos 5*2/O. barthii
6’	1846	CT15081-6-10-2-3	Oryzica Llanos 5*2/O. barthii

The experimental design was a completed randomized blocks with three reps and experimental plots of one row with seventeen plants each. The nine parents and the eighteen crosses were transplanted in Palmira field using distances of 30x30. Weeds were controlled with 3,36 kg ia ha^{-1} propanil, 3kg ia ha^{-1} butaclor and 0.19 kg ia ha^{-1} bentazon 10 days after seeding (dds). Chemical fertilizers applied were: 4 gr. m^{-2} N, 6 gr. m^{-2} P_2O_5, 4.5 gr. m^{-2} K_2O and 1.5 gr. m^{-2} Zn, as basal after transplanting; 10 gr. m^{-2} N. and 4.5 gr. m^{-2} K_2O in dry soil (15 ddt); and 6 gr. m^{-2} N at panicle initiation. Permanent flooding was applied immediately after fertilizer application and maintained until near harvest.

The experimental materials were evaluated for agronomic, yield and yield components and grain quality parameters: Days to 50% flowering (FL), Plant height (PH), production per plant (g/plant), number of panicles per plant (Ti), number of grains per panicle (Gpan), one thousand grain weight (GW), panicle length (PL), percentage of sterility (Spfert), percentage of total milled rice (MR), percentage of head rice (HR), percentage of chalkiness (YPB), one thousand milled grains weight (Pdes) and amylose content (AC). A McGill No. 2 miller was used for milling purposes and a Satake Test Rice Grader for classification. The percentage of chalkiness was measured weighting the grains with spot > 1 (scale) in the whole grain sample. A NIRS method was used to measure apparent amylose content.

Data analysis was made by the ¨partial ST¨ model (Geraldi and Miranda Filho, 1988) as explained by Torres and Geraldi (2007). According with that model, the total genotypes sum of squares was partitioned into parents, crosses and parents vs crosses. Additionally, parents were partitioned into light grains parents(LG), heavier grains parents(HG) and light grains vs heavier grains. Variation due to crosses was partitioned into general LGCA (light group,gi), general HGCA (heavier group gj) and specific (sij) combining ability. Significance of general and specific combining ability was tested with an error term considering only hybrid evaluation. Mid-parent heterosis was tested for significance using *F*-test for the contrast parents *vs* crosses in the analysis of variance.

Results and discussion

There was a high percentage of sterility in many hybrids; in consequence, production per plant was not considered for analysis.

Variance analysis revealed the existence of high divergence between the parents used in this study. *O. barthii* derivatives were very different from FLAR elite lines and among them, for agronomic, yield related and grain quality traits; however, both groups were similar in the number of grains per panicle. In a similar way, FLAR elite lines had differences among them for all characters, with exception of plant height and number of panicles per plant. The highly significant differences between parents groups allowed the use of the partial diallel approach (Tables 1 and 2).

Hybrids were different for all characters (Table 1 and 2) with the exception of amylose content indicating the release of genetic variability when *O. barthii*

derivatives and FLAR elite lines were crossed, and the feasibility of the genetic analysis. General combining ability effects for heavier grains parents were highly significant for all traits with the exception of GW and Pdes. On the other hand, GCA effects for light-weight parents were significant for FL, PL, GPAN, GW, Spfert, MR, HR, YPB and Pdes. Specific combining ability effects were significant only for sterility percentage, percentage of chalkiness and one hundred milled grains weight; indicating that specific interactions between alleles had little importance for other traits. These results indicate that both groups of parents are diverse and have differences in their breeding values with more importance for additive effects in the genetic control of all characters; however, for Spfert, YPB and Pdes, there are non-additive effects acting in the genetic control of these traits. Interestingly, *O. barthii* derivatives did not differ in their GCA effects for one thousand grains weight and one thousand milled grains weight, which means common genes for these traits coming from the wild parent, and probably few genes governing these traits.

The contrast Hybrid vs parents was highly significant for all traits with the exception of amylose content indicating the existence of significant mid-parent heterosis. Also, it means the presence of non-additive effects acting in the genetic control for these traits or allele dispersion between parents.

General combining effects (Table 4) for a parent is determinant for his average progeny performance and it is a measure of his breeding value. Depending on the trait, positive and significant value means good parents. O. barthii derivatives show a clear division according with the cross, Lemont/barthii or O. Llanos 5/ barthii, the first group has positive values for earliness, number of grains per panicle and the second group was good for short plant height, high tillering, whole grain percentage and less chalkiness. Interestingly, Lemont/barthii lines had more compatibility with FLAR elite lines as showed by their negative effects for sterility. In the FLAR elite lines side, BCF 2251 had positive effects for earliness, one thousand grain weight, one thousand milled grains weight and percentage of whole grain; BCF 2107 was the best parent only for shorter stature and BCF2246 showed positive values for panicle length, number of grains per panicles, fertility, percentage of milled grains and less chalkiness.

Mean values are shown in Table 5. As mentioned before, mid-parent heterosis effects were significant, indicating the presence of dominance acting in the genetic control of all traits evaluated or allele dispersion between parents. Dominance directions were positive for all characters with the exception for days to 50% flowering and percentage of chalkiness.

Results suggest that *O. barthii* derivatives can be a useful source of positive alleles especially for one thousand grains weight. Also, even though reproductive barriers or F1 sterility limits the use of wild relatives, some elite lines were more compatible and produced near fertile progenies, indicating the existence of compatibility alleles; which is an important point to choose parents for crosses with wild derivates.

Differences between Lemont and O. Llanos 5 derivatives illustrate the key point that genetic background of inferior recurrent parent for desirable character may have a negative effect on the performance of their progenies; which means that it

is necessary to select recurrent parents without severe defects in agronomic fitness.

Grain quality has been a bottleneck in hybrid breeding; however, this results show that exist positive dominance for percentage of whole grain and better appearance, which favor hybrids use. Additionally, excellent hybrids in terms of grain quality (more whole grains and better translucence) were obtained by crossing good x good, indicating also the importance of dispersion of positive alleles between parents.

Table 2. Mean squares from partial diallel analysis for: Days to 50 % flowering (FL), Plant height (PH), number of panicles per plant (Ti), panicle length (PL), number of grains per panicle (Gpan), one thousand grain weight (GW), and percentage of sterility (Spfert).

Sources	df	FL	PH	Ti	PL	GPAN†	GW	Spfert‡
Blocks	2	90.48**	17.623^{ns}	106.42**	2.82^{ns}	2990.33**	0.50^{ns}	0.01^{ns}
Genotypes	26	43.44**	180.73**	99.41**	5.82**	11091.94**	19.53**	0.12**
.Parents	8	64.20**	188.73**	217.96**	9.98**	15187.52**	42.47**	0.045**
..Light Parents	2	48.78**	15.70^{ns}	25.67^{ns}	6.33*	29980.35**	19.71**	0.042**
..Heavier Parents	5	60.10**	269.93**	299.79**	7.01**	12307.60**	4.31**	0.033**
..Light vs Heavier	1	115.57**	128.81**	193.42**	28.43**	3.02^{ns}	278.76**	0.09**
.Hybrids	17	13.70**	159.85**	46.48**	3.87**	8688.98**	8.70**	0.12**
..GCA Light	2	58.02**	22.00^{ns}	15.63^{ns}	12.93**	41114.70**	62.77**	0.18**
..GCA Heavier	5	20.77**	521.17**	129.51**	4.78*	11413.99**	2.23^{ns}	0.26**
..SCA	10	1.31^{ns}	6.76^{ns}	11.14^{ns}	1.60^{ns}	841.33^{ns}	1.12^{ns}	0.04**
.Hybrids vs Parents	1	382.72**	471.77**	50.81*	6.23*	18490.79**	20.30**	0.77**
[1] Error A	52	3.96	8.97	10.60	1.28	479.92	0.95	0.0029
[2] Error B	34	5.11	7.38	9.89	1.35	566.59	1.16	0.0027
Total	80							
CV (%)		2.018	2.67	13.36	4.09	8.03	3.23	6.81
Mean		98.56	112.146	24.38	27.686	272.90	30.14	50.11

† Freedom degrees for error A in GPAN and Spfert are 51; ‡ Percent of sterility transformed by arcsin(square root (x)); [1] General term error; [2] Error term for partial diallel anlysis considering only hybrids; **, *, ^{ns}: Significant at 1%, 5% and non-significant, respectively by *F*-test.

Table 3. Mean squares from partial diallel analysis for percentage of milled rice (MR), percentage of head rice (HR), percentage of chalkiness (YPB), one thousand total milled grains weight (Pdes) and amylose content (AC).

Sources	**df**	**MR‡**	**HR‡**	**YPB‡**	**Pdes‡**	**AC‡**
Blocks	2	0.00015*	0.0123**	0.0016ns	0.000ns	0.0002ns
Genotypes	25	0.00022**	0.0098**	0.021**	11.61**	0.0007**
.Parents	8	0.00048**	0.0190**	0.027**	23.00**	0.0016**
..Light Parents	2	0.00015*	0.0019*	0.0019ns	7.11*	0.0001ns
..Heavier Parents	5	0.00049**	0.0261**	0.0319**	3.96ns	0.0010**
..Light vs Heavier	1	0.0011**	0.0179**	0.0497**	150.0**	0.0072**
.Hybrids	16	0.0001**	0.0039**	0.0161**	6.55**	0.0025ns
..GCA Light	2	0.00019**	0.0039**	0.0564**	30.86**	xxxxxxx
..GCA Heavier	5	0.00014**	0.0092**	0.0125**	2.34ns	xxxxxxx
..SCA	9	0.00004ns	0.0007ns	0.0053**	3.35*	xxxxxxx
.Hybrids vs Parents	1	0.00003ns	0.0314**	0.0622**	0.29*	0.0002ns
[1]Error A	47	$4x10^{-5}$	0.0005	0.0017	1.65	0.0001
[2]Error B	29	$3x10^{-5}$	0.0003	0.0013	1.26	xxxxxxx
Total	74					
CV (%)		0.58	2.37	14.65	5.72	2.13
Mean		71.38	63.78	8.58	22.4	29.1

‡ Percentages transformed by arcsin(square root (x)),[1] General term error; [2] Error term for partial diallel anlysis considering only hybrids; **, *, ns: Significant at 1%, 5% and non-significant, respectively by *F*-test.

Table 4. Estimates of general combining ability effects for: Days to 50 % flowering (FL), Plant height (PH), number of panicles per plant (Ti), panicle length (PL), number of grains per panicle (Gpan), percentage of sterility (Spfert), one thousand grain weight (GW), one thousand milled grains weight (Pdes) , percentage of total milled rice (MR), percentage of head rice (HR), percentage of chalkiness (YPB), and amylose content (AC).

Code	FL	PH	Ti	PL	Gpan	Spfert	GW	PDes	MR	HR	YPB
1	2.04**	-1.21*	-0.71^{ns}	-0.60*	22.49**	-3.66**	-1.72**	-1,67**	-0.39**	-0.87*	-1.82**
2	0.69^{ns}	0.96^{ns}	-0.35^{ns}	0.97**	32.4**	-7.31**	0.27^{ns}	0.31^{ns}	0.21*	-0.97*	-1.99**
3	-1.35**	0.25^{ns}	1.06^{ns}	0.37^{ns}	54.89**	10.97**	1.99**	1.36**	0.19^{ns}	1.84**	3.82**
1’	0.91^{ns}	7.41**	-3.03**	-1.20**	20.98**	15.93**	0.27^{ns}	0.31^{ns}	0.46^{ns}	-1.65**	0.86^{ns}
2’	0.57^{ns}	5.66**	-3.96**	0.20^{ns}	38.07**	12.63**	0.40^{ns}	0.76*	0.16^{ns}	-2.66**	1.96**
3’	-2.13*	7.29**	-3.28**	0.09^{ns}	33.49**	15.95**	0.47^{ns}	0.31^{ns}	0.16^{ns}	-4.18**	2.03**
4’	0.76^{ns}	-9.16**	3.805**	0.64^{ns}	48.20**	16.26**	-0.86*	0.77^{ns}	-0.65**	1.72*	-2.50**
5’	2.20**	-4.41**	3.905**	0.87*	25.67**	13.96**	0.10^{ns}	0.09^{ns}	0.38*	3.43**	0.85^{ns}
6’	0.65**	-6.79**	2.56*	0.02^{ns}	-18.67*	14.27**	0.17^{ns}	0.70^{ns}	0.13^{ns}	3.34**	1.49^{ns}

**, *, ^{ns}: Significant at 1%, 5% and non-significant, respectively, by *t*-test

Table 5. Means values for: Days to 50 % flowering (FL), Plant height (PH), number of panicles per plant (Ti), panicle length (PL), number of grains per panicle (Gpan), percentage of sterility (Spfert), one thousand grain weight (GW), one thousand milled grains weight (Pdes) , percentage of milled rice (MR), percentage of head rice (HR), percentage of chalkiness (YPB), and amylose content (AC).

Genotype	FL	PH	Ti	PL	Gpan	Pest	GW	PDES	MR	HR	YPB	AC
1	106.00	111.46	28.73	26.60	346.13	32.24	22.88	18.00	70.34	62.51	5.75	31.33
2	107.67	109.73	23.66	26.80	267.20	36.81	24.00	18.00	70.18	64.64	6.33	31.27
3	100.00	114.26	28.73	24.00	146.73	16.47	27.77	20.66	71.36	67.25	8.24	30.46
$\mu_{i.}$	104.56	111.82	27.04	25.80	253.35	28.51	24.88	18.89	70.63	64.80	6.77	31.02
1’	96.00	117.93	11.73	25.66	290.26	36.03	32.44	23.33	71.78	55.47	9.00	26.36
2’	96.33	112.26	13.00	26.73	308.06	25.34	31.78	24.00	70.08	48.48	16.68	26.73
3’	96.00	116.66	12.80	28.13	323.73	34.78	32.22	24.66	71.04	50.08	15.53	26.30
4’	103.33	97.26	34.53	29.26	177.46	47.74	29.77	22.00	72.10	66.51	2.60	28.30
5’	105.00	100.53	28.46	29.46	212.20	51.07	30.88	24.00	72.80	67.52	16.55	29.90
6’	104.33	98.46	27.66	28.93	191.73	48.17	33.11	25.33	73.22	68.27	15.81	29.73
$\mu_{.j}$	100.17	107.18	21.36	28.03	250.57	40.52	31.70	23.89	71.84	59.39	12.70	27.89
1x1’	98.00	119.53	22.66	25.73	348.26	28.85	28.22	20.00	71.09	62.88	5.88	30.43
1x2’	97.67	120.66	21.40	27.26	352.26	26.61	28.66	21.33	70.61	61.92	6.02	29.20
1x3’	97.33	118.8	17.80	25.86	319.93	30.71	29.77	22.66	71.31	60.96	7.40	29.10
1x4’	100.67	103.53	29.40	28.40	260.06	79.64	27.33	20.00	70.29	66.36	4.16	29.70
1x5’	100.67	109.06	29.53	28.26	266.46	76.49	28.89	20.66	71.20	68.16	5.84	29.17
1x6’	100.00	104.26	24.57	28.06	288.86	75.67	29.77	20.00	70.72	68.94	3.57	30.06
2x1’	95.00	122.8	20.20	27.60	329.20	34.51	30.22	23.33	72.21	62.67	7.82	29.33
2x2’	96.66	119.66	21.00	28.06	360.00	37.43	30.44	22.66	71.41	61.12	8.35	29.17
2x3’	94.33	123.35	22.96	29.86	372.06	32.05	30.44	23.33	70.72	58.77	8.86	29.20
2x4’	96.66	106.13	27.40	29.13	252.06	63.39	29.33	22.00	70.77	66.67	1.59	30.67
2x5’	98.66	109.66	28.33	29.93	294.46	63.11	30.89	22.66	71.94	69.60	2.30	29.63
2x6’	96.66	107.26	27.66	28.42	287.53	65.61	30.00	22.00	71.89	69.49	2.33	30.10
3x1’	95.33	121.46	22.86	26.66	235.93	58.90	32.22	24.66	71.94	66.56	10.48	27.30
3x2’	95.00	118.2	20.53	27.66	252.40	68.04	33.55	25.33	71.36	66.03	13.09	28.10
3x3’	93.00	121.26	24.20	27.60	258.93	59.34	32.66	22.00	71.36	64.80	11.44	28.50
3x4’	96.00	104.4	29.43	28.00	193.73	75.71	32.22	.	.	.	.	.
3x5’	98.33	109.6	28.66	28.00	212.53	72.26	32.00	24.00	71.84	69.60	10.90	29.03
3x6'	96.33	109.66	30.26	27.06	218.06	71.50	32.22	23.33	71.52	68.91	11.67	28.50
μ_{ij}	97.02	113.85	24.94	27.86	283.48	56.66	30.49	22.35	71.30	65.50	7.16	29.25
H	-5.34	4.35	0.73	0.95	31.52	22.14	2.20	0.96	0.07	3.40	-2.58	-0.21
LSD (0.05)	3.26	4.95	5.35	1.85	36.00	8.00	1.59	2.70	0.9	0.06	5.00	1.85

References

Geraldi, IO and Miranda Filho JB. 1988. Adapted models for the analysis of combining ability of varieties in partial diallel crosses. Brazilian Journal of Genetics. 11(2): 419 – 430.

Martinez, C.; Borrero, J.; Almeida, A.; Duque. M.C.; Correa-Victoria,F.; Silva, J. and Tohme, J. 2004. Utilization of new alleles from wild rice species to improve rice cultivars in Latin America. Poster available in: www.ciat.cgiar.org.

Tiang, F.; Li, D.J.; Fu, Q.; Zhu, Z.; Fu, Y.; Wang, X. and Sun, C. 2006. Construction of introgression lines carrying wild rice (*O. rufipogon*) segments in cultivated rice (*O. sativa*) background and characterization of introgressed segments associated with yield related traits. Theoretical and Applied Genetics 112: 570-580

Torres, EA. And Geraldi IO. 2007. Partiall diallel analysis of agronomic characters in rice (*Oryza sativa* L.). Genetics and Molecular Biology 30 (3). 605 -613.

Xiao, J.; Li, J,; Grandillo, S.; Ahn, SN.; Yuan, L.; Tanskley, S. and McCough S. 1998. Identification of trait-improving quantitative trait loci from a wild rice relative, *Oryza rufipogon*. Genetics 150: 899-909.

Characterization of exotic and elite germplasm

Edgar Torres, James Carabali, Duque Myriam, Cuasquer Juan – CIAT Rice Program

Nelson Amezquita – FEDEARROZ

Marisel Agredo Berrio – Student Universidad Del Cauca

Abstract

Transferring traits of economic importance from exotic to elite germplasm is a key component in rice breeding. The objective of this work was to characterize a group of exotic an elite materials for yield, yield components and grain quality traits in two locations in Colombia; blast and secondary diseases in Santa Rosa and reaction to Hoja Blanca virus and Tagosodes under controlled conditions in Palmira. It was possible to identify several sources for yield, earliness, short stature, high tillering, long panicles, heavier grains, high whole grain percentage, low chalkiness and resistance to Blast, Sheath Blight, Hoja Blanca virus. The analysis based in PCA or pedigree showed the existence of considerable diversity between selected materials.

Key words: pre-breeding, germplasm characterization

Background

Increasing the genetic variability is the goal of pre-breeding work. Selection within crop species for adaptation to different growing regions and uses has resulted in distinct pools of genetic variation in cultivated rice. These gene pools confer adaptive characteristic in some situations, but are considered unadaptive in others (Osborn et al, 2007). Even the definition of exotic implies non adaptation; exotic germplasm is a useful source of alleles when in existing cultivated gene pools the necessary allelic variation for breeding is lacking (Osborn, 2007). The transference of valuable traits form exotic to elite germplasm is named pre-breeding, which is a strategic phase of genetic improvement because provide the raw material for further advances. Because the donor germplasm generally is inferior in agronomic performance, pre-breeding work is sometimes a difficult, long term and often unsuccessful process.

Characterization of potential donors is an important step in the pre-breeding process. Description of germplasm based in useful attributes are immediately advantageous to practical plant breeding programs because they indicate where useful variation may be found (Skovmand, *et al* 2001).

The objective of this work was to characterize several elite and exotic genotypes for agronomic, yield and yield related, disease reaction and grain quality traits.

Materials and Methods

A total of sixty one genotypes with diverse origin were used for the characterization process. There were thirty seven exotic and twenty four FLAR elite lines. Traits considerered for initial selection in exotic germplasm were: one thousand grain weight, milling yield, blast resistance, sheath blight resistance, lodging tolerance, fertility and field resistance to panicle blight (*Burkholderia* sp). Selection for elite genotypes was based on adaptation to Colombian rice growing conditions, which means high yield, good grain quality and resistance to Hoja Blanca virus and Sogata (*Tagosodes oryzicolus*).

Elite and exotic materials were planted in Palmira and Saldaña for agronomic, yield and yield components and grain quality evaluation. The experimental design was augmented blocks with common checks, each block had a size of fourteen materials with ten lines and four common checks. Each material was transplanted using 30x30 distances and the experimental plot had four rows, 5 meters long. The measured variables were: Days to 50% flowering; plant height average from five plants, number of tiller per plant average from five plants, number of grains per panicle average from five panicles, number of grains per panicles average from five panicles, percent of sterility, one thousand grain weight, grain yield adjusted to 140 g kg^{-1} percentage of total milled grains and percentage of head rice. McGill # 2 and Satake Test Rice Grader were used for milling purposes.

These materials were also planted in Santa Rosa to evaluate leaf and neck blast, secondary disease reactions and amylose content. Additionally, reaction to rice hoja blanca virus was evaluated in a field mass screening using three reps and reaction to Tagosodes was evaluated in greenhouse controlled conditions in CIAT Palmira.

Statistical analysis for agronomic, yield and yield components and milling quality, were done as recommended by Duarte (2000). Blocks and error effects were considerered random and genotypes as fixed. To facilitate analysis, a dummy variable named type was created, with two categories T for check and P for line. The statistical model was

$$y_{ijk} = u + b_j + t_k + g_{i(k)} + e_{ij}$$

In this model

t_k= type effect, with k= 1, 2; depending on the treatment type, check or line.

$g_{i(k)}$= genotype inside treatment effect,

SAS Proc´s GLM and MIXED were used to produce ANOVA and adjusted means with inter block recovery information.

The genetic diversity present in genotypes was assessed using clustering analysis. The first evaluation was done by using a Principal Component Analysis based in adjusted means across locations for agronomic, yield and yield components, disease reaction and grain quality and the Mean Euclidian distance for grouping.

The second grouping was based in the coefficient of parentage; it was estimated using the BROWSE module in the IRIS (International Rice Information System) program; to group genotypes, UPGMA distance was used, and the dendogram was constructed using the TRE function of NTSYS program.

Results and Discussion

Genotypes showed variability for all traits studied in both locations. In Palmira, analysis of variance indicated significant differences in all traits for genotypes. For tested materials (named progenies) there were significant differences in all traits with the exception for whole grain percentage. However, in Saldaña there were no differences for genotypes and progenies not only for whole grain percentage but also for sterility and milled grain percentages (Tables 1 and 2).

Several materials with good characteristics were identified. In general elite lines had higher yields across locations than exotic ones; some lines like Pankaj, Palmar, Araure 3, Eloni, CT19558-2-17-1-4-3-M, and CT19298(83)-1-13-2-1-M had good yields. Elite lines FL04168-3P-12-2P-2P-M, FL03787-3P-5-1P-2P-M, FL03187-16P-11-2P-3P-M-1P-M, FL05564-8P-1-2P-2P-M, and FL06613-20P-2AI-2P-2P-M had higher yields in both locations. For days to 50% flowering, exotic materials flowered more early; in Cocodrie, Cypress and Tres Marias flowering was less than 80 days. Colombian landraces, Palawan and Suakoko were tall materials while Cocodrie, Cypress and FL05372 lines were the shortest ones. Elites lines like FL06885-4P-3-2P-2P-M, FL03191-5P-13-3P-3P-M, FL03174-8P-7-2P-2P-M and the exotic Pankaj, were high tillering. CT lines CT18528-12-1-1-3 and CT18528-12-1-1-4; Colombian landraces Miramono, Monolaya, Inamono, and Cola de Vaca; and FLAR line FL06613-20P-2AI-2P-2P-M had the largest number of grains per panicles. Barthii derivatives lines CT17238-1-1-1-2-1-7-1, CT17238-1-1-1-2-4-3-3, CT17238-1-1-1-2-4-4-2, CT15068-5-10-1-1, and CT15081-6-10-2-2 had heavier grains. In terms of grain quality USA varieties Cocodrie, Cypress and Drew had the best performance in the percentage of whole grain. For blast resistance, several exotics like: Cypress, Tres Marias, CT18726-1-3-3-3-3, CT18247-12-8-1-2-2-3, CT18247-23-2-2-1-3-2, CT18526-15-2-1-1, CT18528-12-1-1-3, CT19558-2-17-1-4-3-M; and elites as: FL00147-8P-6-15P-M, FL03174-8P-7-2P-2P-M, FL04168-3P-12-2P-2P-M, FL05372-7P-1-2P-1P-M, FL05372-7P-4-4P-M-2PY, FL05516-8P-3-5P-1P-M; were highly resistant to leaf and neck blast under field conditions in Santa Rosa. The most tolerant Sheath Blight genotype under greenhouse conditions was Araure 3; also, other materials like CT15068-5-10-1-1, Palmar, Ligerito playero and FL00147-8P-6-15P-M showed some tolerance to sheath blight. For Hoja Blanca Virus the best materials were: CT17238-1-1-1-2-1-7-1, CT18247-23-2-2-1-3-2, FL05372-7P-4-4P-M-2PY, FL05516-8P-3-5P-1P-M, FL05564-8P-1-2P-2P-M, FL06883-9P-6-5P-5P-M and FL06885-4P-3-2P-2P-M. There were CT15081-6-10-2-2, Jasmine, Tres Marias, CT18247-12-8-1-2-2-3, CT19298(83)-1-13-2-1-M, FL03188-7P-5-3P-1P-M, FL03191-5P-13-3P-3P-M, FL05516-8P-3-5P-1P-M, FL06883-9P-6-5P-5P-M, FL06885-4P-3-2P-2P-M. In general, all materials had intermediate or high

amylose content with the exception of Jasmine and CT19558-2-17-1-4-3-M (Table 3). Results showed that several important commercial traits are present in the evaluated genotypes.

The pedigree analysis taking account of shared ancestry revealed very interesting patterns. The studied genotypes can be divided in several groups that share few genes with parentage coefficient less than 0,06 . One of them is composed by FLAR and CIAT lines, which could be named as predominantly indica. Other included CT17238 derivates, US-Varieties, Eloni, and IRGA 417, which are predominantly japonica. Oryzica Turipana, Suakoko, Palawan and Colombian landraces were considered as individuals groups. Data indicate the considerable diversity existing between these materials and the opportunity to use exotic materials which are less related with CIAT -FLAR germplasm to widening the genetic basis of cultivated rice. However, the more distant materials are tropical japonicas that in general have incompatibility when crossed to indicas.

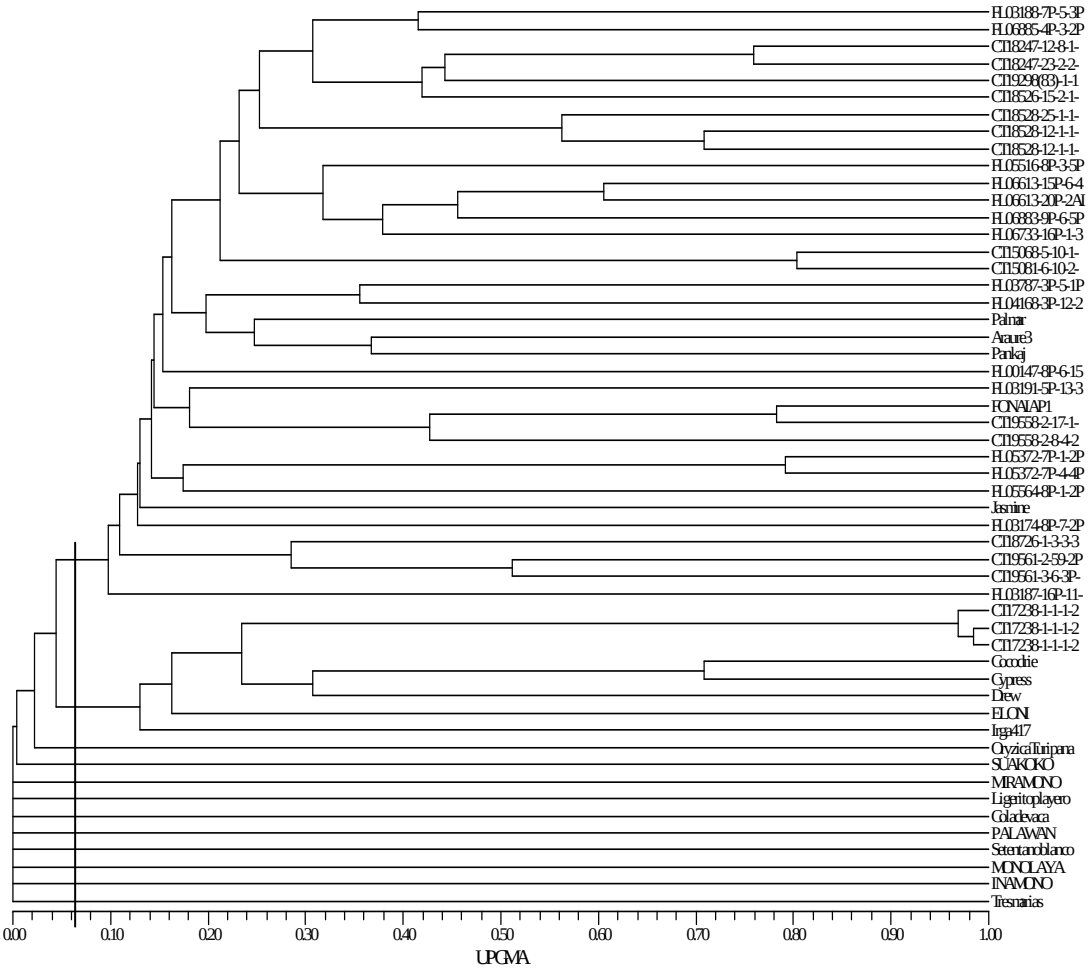


Fig 1. Grouping of elite and exotic materials using UPGMA based in Coefficient Parentage as genetic distance.

The principal component analysis using agronomic, yield and yield components, diseases reaction and grain quality traits indicated that 83.38% of variability could be explained by six main components. Those components allow grouping of studied genotypes in six clusters (fig 2). Group 1 was composed of 35 genotypes and included FLAR elites, CIAT lines, Eloni, Fonaiap 1 and Palmar. This group had the higher yields. Group 2 was formed by low yielding tall materials including Colombian landraces and Palawan. CT17238 lines which are Lemont/Barthii derivatives, were included in the third group; they were characterized by higher grain weight and high susceptibility to sheath blight. Group 4 was formed by CT15068-5-10-1-1, CT15081-6-10-2-2, FL00147-8P-6-15P-M, Irga 417, Jasmine, Ligerito playero, Tres marias; it was the second in terms of percentage of whole grain. Tropical japonicas like Cocodrie, Cypress, Drew and Oryzica Turipana formed the group 5; they had the highest percentage of whole grain and earliness. Group 6 was formed by CT18528-12-1-1-3, CT18528-12-1-1-4 and SUAKOKO; it was lowest yielding group.

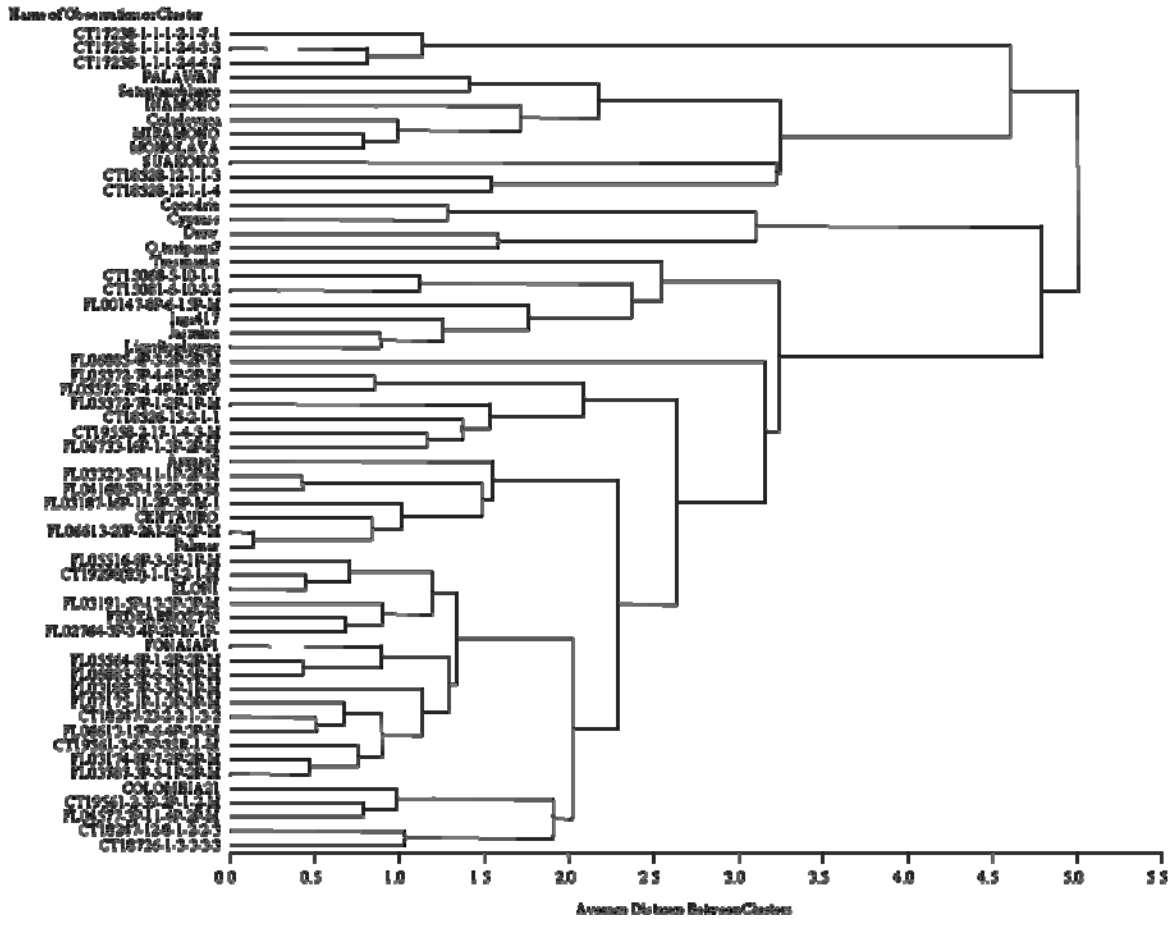


Fig 2. Grouping of elite and exotic materials based in PCA analysis, the distance was the square of the euclidian distance

References

Duarte J.B. 2000. Sobre o emprego e a analise estatística do delineamento em blocos aumentados no melhoramento genético vegetal . Chapter Three: Métodos de analise estatística para blocos aumentados com aplicação no melhoramento de plantas. Tese (Doutorado) – Escola Superior de Agricultura Luiz de Queiroz, Piracicaba-SP, Brasil 293 p.

Osborn, T.C.; Graham, E. and Braun, C.J. 2007. Insights and innovations from wide crosses: Examples from Canola and Tomato. Crop Science 47(S3): S228-S237.

Skovmand, B.; Reynolds, M.P. and Delacy, I.H. 2001. Searching genetic resources for physiological traits with potential for increasing yield. In: Reynolds, M.P.; Ortiz-Monasterio, J.I. and McNab, A. Application of physiology in Wheat breeding. Mexico, DF.: CYMMIT.

Table 1. Mean squares from Palmira ANOVA for: Yield (Kg ha^{-1}), Days to 50 % flowering (FL), Plant height (PH), number of panicles per plant (Ti), number of grains per panicle (Gpan), panicle length (PL), one thousand grain weight (GW), percentage of sterility (Spfert), percentage of milled rice and percentage of whole grain (HR).

Sources	**df**	**Kg ha^{-1}**	**FL**	**PH**	**Ti**	**Gpan**	**PL**	**GW**	**Spfert**	**MR**	**HR**
Block	5	558614.00*	5.84^{ns}	15.17*	4.13^{ns}	364.41^{ns}	0.77^{ns}	0.01^{ns}	0.003^{ns}	0.00004^{ns}	0.002^{ns}
Genotypes	62	2053260.6**	81.314**	385.57**	22.89*	4470.93**	6.19**	0.089**	0.019**	0.0007**	0.003*
Checks vs Progen	1	4682440.0**	140.25**	1524.94**	13.24^{ns}	35.73ns	7.49**	0.0006^{ns}	0.140**	0.0ns	0.003^{ns}
Cod(type)	61	1997719.20	79.59	363.88	22.94	4537.78	6.19	0.091	0.016	0.0007	0.003
Checks	3	593924.0*	73.35**	27.02*	29.40^{ns}	14362.0**	4.76**	0.059**	0.003^{ns}	0.001**	0.015**
Progenies	58	2073765.00**	80.15**	381.08**	22.55*	3964.08**	6.26**	0.092**	0.017**	0.0007**	0.0027^{ns}
Error	14	143647.50	3.69	4.98	8.84	677.12		0.0086	0.0042	4x10^{-5}	0.0015
Total	81										
CV		6.15	1.76	2.08	13.22	10.99	2.8	3.53	11.75	0.7	4.44
Mean		6161.37	111.21	107.14	22.49	236.74	24.24	2.62	27.92	70.71	58.32

**, *, ^{ns}: Significant at 1%, 5% and non-significant, respectively, by *F*-test.

Table 2. Mean squares from Saldaña ANOVA for: Yield (Kg ha^{-1}), Days to 50 % flowering (FL), Plant height (PH), number of panicles per plant (Ti), number of grains per panicle (Gpan), panicle length (PL), one thousand grain weight (GW), percentage of sterility (Spfert), percentage of milled rice and percentage of whole grain (HR).

Source	**df**	**Kg ha^{-1}**	**FL**	**PH**	**TI**	**GPAN**	**PL**	**GW**	**Spfert**	**MR**	**HR**
Block	5	3045060.7*	0.46^{ns}	23.36^{ns}	5.11^{ns}	235.22^{ns}	0.45^{ns}	0.012^{ns}	0.0078^{ns}	0.0004^{ns}	0.0023^{ns}
Gen	63	3272898.2**	27.99**	292.38**	20.17**	5488.11**	5.06*	0.12**	0.018^{ns}	0.0004^{ns}	0.007^{ns}
Checks vs Prog	1	13132981**	259.08**	850.88**	15.14^{ns}	331.66^{ns}	0.18^{ns}	0.009^{ns}	0.032^{ns}	0.0007^{ns}	0.002^{ns}
Cod(type)	62	3077641.00	23.94	282.36	20.21	5593.94	5.16	0.12	0.02	0.00040	0.01
Checks	3	191795^{ns}	60.87**	100.46*	30.38*	16423.00**	6.82*	0.09**	0.016^{ns}	0.0022^{ns}	0.04^{ns}
Progenies	59	3223999.0**	21.88**	292.73**	19.50**	4775.46**	5.05*	0.12**	0.016^{ns}	0.0004^{ns}	0.005^{ns}
Error	14	653907.70	1.55	20.96	3.24	742.15	1.84	0.01	0.01	0.00026	0.00
Total	83										
CV		11.29	1.49	3.84	10.94	11.52	5.0	3.79	21.13	1.63	6.92
Promedio		7156.85	82.38	119.0	16.45	236.52	27.08	2.70	20.57	70.28	56.16

**, *, ^{ns}: Significant at 1%, 5% and non-significant, respectively, by *F*-test.

Table 3. Means values across locations for yield (Kg ha^{-1}), days to 50% flowering (FL), plant height (PH), number of tiller per plant (Ti), number of grains per panicle (Gpan), average reaction Hoja Blanca virus (HB), average reaction to *Tagosodes oryzicoulus* (Tag) and average reaction to Sheath Blight (SHB)

Code	Pedigree	Kg ha^{-1}	FL	PH	TI	Gpan	P100	GE	HB	Tag	SHB
MADR/1	CT17238-1-1-1-2-1-7-1	4675.320	94.21	114.84	10.23	314.60	3.44	51.98	4.00	5.00	91.50
MADR/2	CT17238-1-1-1-2-4-3-3	4758.930	95.71	111.64	10.03	330.80	3.44	47.09	6.00	7.67	97.00
MADR/3	CT17238-1-1-1-2-4-4-2	5512.855	96.21	115.64	11.03	333.20	3.34	49.94	5.50	7.67	90.50
MADR/4	CT15068-5-10-1-1	5687.840	98.71	107.92	25.83	170.10	3.39	62.22	7.50	4.33	47.60
MADR/5	CT15081-6-10-2-2	6253.570	99.31	112.91	22.27	208.10	3.46	62.89	7.00	1.00	59.50
MADR/6	Cocodrie	3897.290	73.81	90.14	16.23	192.00	2.15	64.79	7.00	9.00	66.25
MADR/7	Cypress	5109.360	78.01	91.63	21.55	146.20	2.36	64.79	7.50	9.00	72.10
MADR/8	Drew	4953.075	89.16	109.65	14.41	328.60	2.25	67.29	7.50	9.00	82.50
MADR/9	Irga 417	6876.610	86.31	93.81	17.65	208.40	2.56	62.44	6.50	1.67	56.00
MADR/10	Jasmine	6782.005	90.16	99.33	16.37	210.30	2.80	60.85	7.50	1.00	52.10
MADR/11	Palmar	7575.490	99.51	113.86	19.57	293.10	2.15	56.64	5.00	2.33	48.60
MADR/12	Araure 3	7663.755	101.16	107.83	21.27	204.80	2.45	62.88	6.50	9.00	39.45
MADR/13	Pankaj	9127.180	104.16	120.53	24.05	209.20	2.45	48.09	5.50	1.67	49.25
MADR/14	Tres marias	4283.065	70.71	132.44	18.89	157.20	2.79	59.92	7.50	1.00	48.00
MADR/15	O. turipana 7	5877.260	96.16	107.73	16.43	242.30	2.50	60.26	6.50	9.00	71.75
MADR/16	CT18726-1-3-3-3-3	6530.055	97.01	115.16	17.85	235.30	3.30	63.13	5.00	8.33	65.75
MADR/17	CT18247-12-8-1-2-2-3	5494.680	99.01	114.86	19.85	230.90	2.65	54.30	6.50	1.00	67.60
MADR/18	CT18247-23-2-2-1-3-2	6121.950	96.31	118.77	21.81	176.80	2.75	62.66	4.50	1.67	55.75
MADR/19	CT18526-15-2-1-1	5072.765	96.51	93.32	21.27	246.70	2.35	58.60	7.00	9.00	66.56
MADR/20	CT18528-12-1-1-3	3049.060	102.51	108.36	20.37	366.20	2.10	54.19	7.00	9.00	67.25
MADR/21	CT18528-12-1-1-4	5029.945	100.31	101.81	12.89	425.60	2.36	47.77	6.50	9.00	60.70
MADR/22	FONAIAP 1	7272.740	91.31	101.31	17.85	207.80	2.91	56.43	5.50	7.67	49.60
MADR/23	ELONI	7456.185	95.51	101.16	20.85	212.20	2.80	44.61	6.00	2.33	50.10
MADR/24	SUAKOKO	5007.770	100.51	152.12	21.85	205.20	2.36	45.12	6.50	7.67	51.75

Table 3. Continuation... Means values across locations for yield (Kg ha^{-1}), days to 50% flowering (FL), plant height (PH), number of tiller per plant (Ti), number of grains per panicle (Gpan), average reaction Hoja Blanca virus (HB), average reaction to *Tagosodes oryzicoulus* (Tag) and average reaction to Sheath Blight (SHB)

Code	Pedigree	Kg ha	FL	PH	TI	Gpan	P100	GE	HB	Tag	SHB
MADR/25	MIRAMONO	4735.490	99.31	166.17	12.99	304.90	2.70	54.21	6.50	9.00	65.94
MADR/26	MONOLAYA	4556.195	101.01	160.82	11.11	337.80	2.75	56.00	7.00	9.00	72.60
MADR/27	INAMONO	5310.430	101.66	176.43	13.75	321.00	2.60	61.28	7.00	9.00	53.60
MADR/28	PALAWAN	5592.670	93.81	147.87	12.25	272.40	2.95	58.87	7.00	9.00	69.35
MADR/29	CT19561-2-59-2P-1-2-M	6606.415	98.31	99.37	19.63	204.80	2.80	52.23	4.00	1.67	71.67
MADR/31	CT19558-2-17-1-4-3-M	7388.240	97.71	111.02	21.35	248.50	2.70	56.37	5.50	9.00	67.50
MADR/32	CT19561-3-6-3P-3SR-1-M	5912.770	97.21	102.04	23.31	204.00	2.64	68.03	5.00	2.33	52.20
MADR/33	CT19298(83)-1-13-2-1-M	7290.590	99.51	110.42	20.95	219.00	2.65	51.58	6.00	1.00	55.70
MADR/35	Ligerito playero	5378.625	81.51	118.83	20.45	131.20	2.50	54.16	5.50	1.67	46.60
MADR/36	Cola de vaca	5382.790	94.16	163.23	10.45	368.70	2.50	51.97	7.50	9.00	69.00
MADR/37	Setentano blanco	3821.015	91.01	164.83	14.91	244.00	2.70	61.61	7.00	9.00	85.50
MADR/38	FL00147-8P-6-15P-M	6386.440	96.21	97.04	20.13	139.20	2.54	62.73	7.50	1.67	48.75
MADR/39	FL03174-8P-7-2P-2P-M	7242.025	98.01	106.36	24.83	188.40	2.54	57.26	5.50	3.00	55.75
MADR/40	FL03188-7P-5-3P-1P-M	7379.425	96.51	106.76	21.09	192.80	2.79	61.86	7.00	1.00	53.25
MADR/41	FL03191-5P-13-3P-3P-M	7943.495	98.81	116.57	24.39	161.10	2.70	55.47	6.00	1.00	54.35
MADR/42	FL03787-3P-5-1P-2P-M	8972.145	88.00	109.12	18.75	192.60	2.50	54.84	5.50	2.33	56.75
MADR/43	FL04168-3P-12-2P-2P-M	9142.510	103.01	118.06	23.15	237.00	2.15	59.52	5.00	2.33	47.70
MADR/44	FL03187-16P-11-2P-3P-M-1P-M	8567.465	98.81	108.71	23.81	181.30	2.46	54.77	5.50	9.00	51.35
MADR/45	FL05372-7P-1-2P-1P-M	6947.520	92.51	91.36	17.79	201.80	2.44	58.07	5.00	8.33	72.75
MADR/46	FL05372-7P-4-4P-M-2PY	6863.205	100.71	98.12	17.97	207.20	2.49	54.92	4.50	9.00	72.00
MADR/47	FL05516-8P-3-5P-1P-M	6969.610	98.81	109.67	18.49	227.20	2.70	58.91	4.50	1.00	58.75
MADR/48	FL05564-8P-1-2P-2P-M	8092.215	95.51	111.32	22.37	218.20	2.65	60.75	4.50	9.00	62.15
MADR/49	FL06613-15P-6-4P-3P-M	6674.485	99.81	112.41	21.17	159.90	2.66	60.43	5.00	3.00	61.25
MADR/50	FL06733-16P-1-3P-2P-M	5307.705	93.16	100.45	17.31	256.80	2.60	64.26	6.00	5.00	78.50

Table 3. Continuation... Means values across locations for yield (Kg ha^{-1}), days to 50% flowering (FL), plant height (PH), number of tiller per plant (Ti), number of grains per panicle (Gpan), average reaction Hoja Blanca virus (HB), average reaction to *Tagosodes oryzicoulus* (Tag) and average reaction to Sheath Blight (SHB)

Code	Pedigree	Kg ha	FL	PH	TI	Gpan	P100	GE	HB	Tag	SHB
MADR/51	FL06883-9P-6-5P-5P-M	7297.220	96.81	116.77	21.75	238.90	2.40	60.89	4.50	1.00	69.33
MADR/52	FL06885-4P-3-2P-2P-M	7423.070	99.81	109.27	30.09	149.90	3.20	57.23	4.50	1.00	56.60
MADR/53	FL06613-20P-2AI-2P-2P-M	8585.185	96.81	117.91	18.59	305.00	2.41	61.66	5.50	6.33	56.65
MADR/55	CENTAURO	6461.760	101.66	106.73	21.11	314.50	2.40	58.27	7.00	1.00	56.75
MADR/56	COLOMBIA 21	6436.320	95.51	105.96	16.25	233.60	2.75	51.57	4.00	3.67	62.75
MADR/57	FEDEARROZ 733	8541.365	98.81	114.31	21.01	205.70	2.81	61.53	4.50	1.67	51.75
MADR/60	FL02764-3P-3-4P-2P-M-1P-F12	7199.390	98.81	114.41	24.21	211.10	2.56	58.02	4.50	3.67	49.00
MADR/61	FL03323-5P-11-1P-2P-M	9315.345	98.31	105.81	23.65	230.70	2.51	56.66	6.00	7.67	40.85
MADR/62	FL04577-3P-11-4P-2P-M	6506.755	100.01	107.42	18.29	214.20	3.00	57.82	3.50	1.00	58.50
MADR/63	FL05372-7P-4-4P-2P-M	6600.190	94.81	101.97	18.73	264.50	2.40	57.85	3.50	5.00	60.00
MADR/65	FL07175-1P-1-3P-3P-M	7217.240	91.51	109.06	25.75	187.50	2.70	56.65	4.33	2.33	51.56
Testigo1	FL06733-2P-1	6992.87	95.20	107.33	16.87	315.0	2.66	51.98			
Testigo2	Fedearroz 50	7146.34	103.33	111.17	19.85	222.7	2.67	62.96			
Testigo3	Fedearroz 473	7270.03	98.34	104.12	22.13	234.5	2.55	45.75			
Testigo4	Fedearroz 60	7298.64	100.17	105.41	21.48	186.2	2.79	61.41			

Table 4. Reactions to Leaf Blast (BL1 and BL2), Neck Blast (NBL), Leaf Scald (Lsc), Brown Spot (Bs), grain decoloration (GD) and amylose content, evaluated in Santa Rosa during semester A 2008.

Code	BL1	BL2	NBL	Lsc	Bs	GD	Amy
MADR/1	5	5	7	1	3	3	25.7
MADR/2	5	5	7	1	3	3	26.3
MADR/3	5	5	7	1	3	3	26.5
MADR/4	4	5	7	1	1	5	27.7
MADR/5	4	5	5	1	1	5	28.3
MADR/6	2	3	5	3	1	1	26.0
MADR/7	3	2	3	3	3	3	27.0
MADR/8	4	4	5	3	3	1	26.9
MADR/9	5	5	9	3	3	3	30.4
MADR/10	5	7	9	3	1	3	23.5
MADR/11	4	5	7	1	3	5	26.9
MADR/12	7	8	5	1	3	1	29.9
MADR/13	7	8	***	***	***	***	***
MADR/14	1	1	1	3	1	3	31.7
MADR/15	4	3	3	1	3	1	20.5
MADR/16	1	1	3	1	9	5	31.2
MADR/17	1	1	3	1	5	3	32.0
MADR/18	2	1	3	1	1	3	31.4
MADR/19	3	2	3	3	3	7	27.2
MADR/20	1	3	3	3	5	5	32.0
MADR/21	3	3	3	5	3	5	30.9
MADR/22	5	5	5	3	3	3	33.0
MADR/23	5	5	3	1	3	3	29.1
MADR/24	5	5	5	3	5	5	31.8
MADR/25	7	8	5	1	3	3	26.9

Table 4. Continuation... Reactions to Leaf Blast (BL1 and BL2), Neck Blast (NBL), Leaf Scald (Lsc), Brown Spot (Bs), grain decoloration (GD) and amylose content, evaluated in Santa Rosa during semester A 2008.

Code	BL1	BL2	NBL	LSC	BS	GD	Amy
MADR/26	7	8	5	1	1	3	25.9
MADR/27	7	8	5	1	3	3	26.9
MADR/28	6	7	5	1	3	1	29.0
MADR/29	4	5	5	3	1	3	33.0
MADR/31	2	1	3	1	1	3	24.70
MADR/32	4	3	3	3	5	7	32.10
MADR/33	5	5	5	3	3	3	30.60
MADR/35	6	6	5	3	3	5	26.70
MADR/36	7	7	7	1	3	5	29.80
MADR/37	7	7	5	1	3	5	26.90
MADR/38	3	3	3	1	1	5	32.00
MADR/39	2	3	3	1	1	5	26.90
MADR/40	4	5	7	1	1	5	32.50
MADR/41	4	4	3	3	3	3	31.30
MADR/42	2	2	5	3	3	3	31.10
MADR/43	1	2	3	3	1	3	30.70
MADR/44	5	5	3	3	5	3	29.90
MADR/45	2	1	3	3	3	5	28.80
MADR/46	2	3	3	3	3	7	30.70
MADR/47	2	2	3	3	1	5	32.00
MADR/48	3	5	5	3	3	3	32.00
MADR/49	4	5	3	1	3	3	32.50
MADR/50	5	6	5	1	3	3	30.70
MADR/51	3	5	5	1	3	3	33.00

Table 4. Continuation... Reactions to Leaf Blast (BL1 and BL2), Neck Blast (NBL), Leaf Scald (Lsc), Brown Spot (Bs), grain decoloration (GD) and amylose content, evaluated in Santa Rosa during semester A 2008.

Code	BL1	BL2	NBL	LSC	BS	GD	Amy
MADR/52	3	3	5	1	1	3	33.00
MADR/53	4	5	7	1	3	3	32.80
MADR/55	2	4	5	1	1	3	33.00
MADR/56	3	6	7	1	1	3	32.60
MADR/57	2	5	5	1	1	3	30.80
MADR/60	1	4	5	1	1	3	30.90
MADR/61	1	5	5	1	1	5	32.50
MADR/62	2	3	3	1	3	3	31.50
MADR/63	2	3	3	1	3	5	32.80
MADR/65	3	4	5	1	5	3	26.90

1B UPLAND RICE

1B.1. Rice Collaborative Project between CIAT and Cirad

Marc Châtel, Yolima Ospina, Francisco Rodriguez, Daniel Guzmán
Alain Audebert and Cécile Grenier
Lac Cooperators

Funding: Cirad, CIAT and LAC Cooperators

Introduction

Partnership between Cirad and CIAT is crucial to develop and implement new breeding methods as is the case of rice synthetic population breeding.

Through this partnership the collaborative project is able to attend Latin American and Caribbean (LAC) partners of the public and private rice research sectors in delivering genetic resources (basic, site-specific synthetic populations and promising lines), training and networking.

Decentralized breeding through the development of site-specific populations with LAC partners leads to the integration and implementation of the new breeding method in their national breeding programs and to the release of commercial varieties both for upland and irrigated rice ecosystems.

Implementation of new activities

Breeding genetic resources development through the improvement by recurrent selection of rice synthetic populations, is associated with eco-physiology and molecular markers.

The association of the 3 disciplines mentioned above aims at more efficient rice breeding methods for water use efficiency.

Eco-physiology and Breeding

In the framework of the Cirad rice project at CIAT, the eco-physiology experiments are coordinated by the project and implemented by Alain AUDEBERT through

field-work missions at the Santa-Rosa Experimental Station, Villavicencio-Colombia.

Cirad and CIAT are developing new activities in rice breeding for water use efficiency. The implementation of this activity started in 2006 thanks to three (4) field-work missions of Dr. Alain AUDEBERT, eco-physiologist at Cirad Montpellier.

Selection is based on field-yield and, in the curse of the selection process, the screening methods are refined by selection of relevant traits (morpho-pheno-physiological traits).

The 2006 preliminary experiment conducted during the growing season, at La Libertad Research Station-Villavicencio, aimed at characterizing a group of potential recurrent population genitors. Thirteen cultivars showed morpho-pheno-physiological diversity for rice crop behavior in rain-fed conditions. Three groups with homogeneous morphology traits were define according the roots-shoot allometric rate. The experiment was also used for obtaining specific cultivar parameter for SaraH rice crop growth model and for eco-physiological methodology training course for CIAT field technicians.

During the dry off-season 2007-2009 (October 07 - February 08) a set of fifty (50) targeted potential parents for the development of a new synthetic population were evaluated for morpho-pheno-physiological traits. A new and very expensive equipment (NEC Infra-red Camera) purchased by Cirad was used to characterize the canopy temperature of the same rice varieties when grown under no water limitation and drought stress condition applied during 15 days after panicle initiation. The results showed that genetic variability exists which is a very important result for using this trait for breeding purpose.

During the dry off-season 2008-2009 (October 08 - February 09) large scale phenotyping was set-up through the evaluation of 400 (four hundred) S_1 lines coming from 4 different synthetic populations selected in S_0 during the cropping season 2008 (April September). Adjustments of the statistical design were proposed by Myriam Christina DUQUE (CIAT) and field screening methods with the IR Camera by Alain AUDEBERT were implemented because of the huge number of lines to be evaluated (total of 800 lines) when grown under no water limitation and drought stress condition applied during 15 days after panicle initiation.

Another equipment (Diviner 2000) purchased by Cirad was used at field condition for monitoring soil moisture using permanent access PVC tubes, a sensor and a data display.

The two equipments, NEC IR Camera and the Diviner 2000 permit, at field condition, to monitor the water status of the rice lines (low canopy temperature = plant transpiration = good physiological status) and moisture monitoring of the soil. In fact the water use efficiency depends of 3 main factors which are the soil moisture content, the plant water content and the air moisture content for which it is needed to buy 2 automatic weather station placed on each field plots (no water limitation and drought stress condition).

Marker Assisted Recurrent Selection (MARS)

Marker Assisted Recurrent Selection (MARS) activities coordinated by the project starts starting off-season 2008. Cécile GRENIER from Cirad was out-posted at CIAT in mid-2008 and is already implementing this activity in close collaboration with the Cirad breeder and the eco-physiologist.

Results

Breeding 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. Upland synthetic 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.

During the cropping season 2008, four (4) populations were evaluated and individual S_0 genotypes selected for line development and off-season evaluation for water use efficiency (Alain AUDEBERT) and molecular characterization (Cécile GRENIER).

A set of 1500 inbred lines at different generation stages of development (from S_1 up to S_8) were characterized and selected for next selection steps. They are the starting point for promising line development and distribution to partners.

Breeding, Eco-physiology and Molecular Markers

During the dry off-season 2007 (October 07 - February 08) a set of fifty (50) targeted potential parents for the development of a new synthetic population were evaluated for morpho-pheno-physiological traits and also using an infrared camera and carbon isotopes determination. Data analysis are reported by Alain AUDEBERT in an other chapter of this CIAT-Rice project annual report.

During the dry off-season 2008 (October 08-February 09) four hundred (400) S_1 lines selected during the cropping season 2008 (April-September) from four (4) existing synthetic populations will be screened for drought tolerance during the reproductive stage using infrared imaging, photosynthesis evaluation and carbon isotopes. This activity is to be reported next year.

Also, a preliminary experiment is to be conducted for direct screening, under water stress, of individual plants within a synthetic population. The aim is to evaluate by infrared imaging the possibility to speed-up recurrent selection breeding based on evaluation-selection of S_0 individual plants and the subsequent recombination of the best genotypes.

Marker Assisted Recurrent Selection (MARS) activities coordinated by the project are to be implemented, starting off-season 2008 using the 400 S_1 lines phenotypes for general agronomic traits and water use efficiency.

Commercial line release

If, as it is mentioned above, the project is implementing new research activities, we do not stop developing genetic resources for different ecosystems and cooperators. In fact this is the finality of the project.

Upland rice:

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. Seed production started during the year 2007 and commercial seed was made available in 2008, both for small farmer's and mechanized upland rice ecosystems.

New lines are in the pipe line for final agronomic evaluation. The best one(s) will be tested next year in demonstration plots before to become new cultivar(s).

Promising lines
Lowland Rice:
In **France**, different inbred lines selected from the aromatic synthetic population PCAQ-1 are under evaluation. Some S_6 lines show good agronomic characteristics and are strongly aromatic. For more information about the behavior of theses lines, please report to the complete results of the evaluations are presented in the "Rapport d'activité 2008" of Guy CLEMENT.

In **Chile**, a line coming from the enhanced synthetic population PQUI-1 (temperate climate rice ecosystem) was registered in 2008 and is called RQUILIA 23.

Networking GRUMEGA: Please look at www.grumega.org

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. We were planning the V GRUMEGA Conference to be held in 2008. But, because of lack of funds, the Conference was postponed.

In a near future, the new implemented activities and methods used (eco-physiology and Marker assisted Recurrent Selection) will be networked with LAC partners.

Future activities

The way forward in rice synthetic population improvement
The improvement of variety's 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 tools (Eco-physiology and Molecular Markers) to increase the efficiency of recurrent selection breeding mainly targeting water use efficiency and climate change.

The results of theses research activities will be shared with LAC and others potential partners through the GRUMEGA network.

In 2009 we will fully be engaged in the Cirad ATPd Bios project *"Diversité des caractères d'adaptation aux contraintes hydriques et thermiques chez le riz (Oryza sativa L.) : Phénotypage à grande échelle dans le cadre d'études d'association"*

"Diversity of adaptative traits to drought and high temperature in rice (Oryza sativa L.): Large scale phenotyping in the framework of genetic of association"

During the 2009 season we will seed increase the 181 accessions received from Cirad Montpellier in late 2008.
During the off-season 2009 at the Santa Rosa Experimental Station-Villavicencio, theses accessions will be characterized at field condition for water use efficiency by Alain AUDEBERT and by molecular markers at CIAT-Palmira biotechnology laboratory by Cécile GRENIER.

Conclusion

In a same physical sites (Santa Rosa and Palmira Experimental Stations) and in close collaboration with CIAT, the rice project of Cirad at CIAT is implementing novel breeding methods assisted by eco-physiology and molecular markers tools for the development of rice genetic resources for better water use efficiency. This is well in line with the thematic of the Cirad's Research Unit *"Agro-Ecological Adaptation and Varietal Innovation* (ĀIVA) and the Rice Outcome Line of CIAT.

Publications

Châtel M., Ospina Y., Rodriguez F., Lozano V.H., Delgado H. 2008. Upland rice composite population breeding and selection of promising lines for Colombian savannah ecosystem = Melhoramento populacional de arroz de terras altas e seleçao de linhagens promissoras para as condiçoes de savana da Colombia. Pesquisa agropecuaria tropical, 38 (1) : 1-5.

Marc Châtel; Elcio.P. Guimarães; Yolima Ospina; César.P. Martinez; Jaime Borrero: Mejoramiento del arroz de secano para América Latina.

Book chapter accepted. Libro de Arroz . CIAT Publication.

Marc Châtel; Elcio P. Guimarães; Yolima Ospina; Francisco Rodríguez; Víctor Hugo Lozano : Mejoramiento de poblaciones de arroz de secano empleando selección recurrente y desarrollo de variedades

Book chapter accepted. Libro de Arroz . CIAT Publication.

1B.2. Rice breeding for drought tolerance. Recurrent selection for population improvement based on molecular markers and an ecophysiological model

Audebert Alain, Chatel Marc, Ospina Yolima, Rodríguez Francisco

Source of funding : CIRAD, CIAT

Abstract

Cirad and CIAT develop a new breeding project based on the recurrent selection for drought resistance. Selection is primarily based on yield and, in the course of the selection process; the screening methods are refined by additional secondary relevant traits (morpho-pheno-physiological traits). The preliminary experiment conducted during the dry season in 2007, in the Santa Rosa research station in Villavicencio (Colombia), is used to adjust the phenotyping method based on Infra-red thermography. Sixty cultivars from the recurrent genitor population were tested. These cultivars were chosen at the construction of the recurrent population according to various morpho-pheno-physiological criteria. Results showed a genetic diversity for canopy temperature. This diversity was observed on both irrigated and stress treatments. Canopy temperature was directly related to soil humidity.

Key words :

Drought resistance, Recurrent selection, Oryza sativa, Upland rice, Latin America

Background

Enhanced crop production under limited water supply depends on a subtle dosage of various physiological mechanisms and plant traits according to timing, intensity and duration of the water 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 method based on direct selection for grain yield still produces the

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 in the selection process. Integration of CIRAD and CIAT's expertise in rice recurrent selection and drought physiology are 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 aims at creating new rice breeding population (BP), using already available information on loci, alleles, phenotypic traits of interest regarding drought tolerance in rice. This population will also be used to develop breeding methods making use of molecular markers and ecophysiological criteria for enhancing recurrent selection (RS) for drought tolerance and water use efficiency. Crop development models will also be used to analyze and predict the behaviour of advanced breeding lines in 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. This 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 (populations with a broad genetic base and advanced lines) with improved drought tolerance or improved water use efficiency, (ii) validated methods of molecular marker and crop model based selection for drought tolerance and (iii) a better understanding of the physiological and genetic bases of mechanisms of drought tolerance.

The experiment was done during the dry season (2007-2008). The objectives were :

- The physiological field characterisation of potential parents for the development of the new synthetic population

- Seed multiplication of the potential genitors

- Field phenotyping development based on infra-red temperature

Materials and Methods

The experiment was done at the CIAT experimental station Santa Rosa, Villavicencio, Colombia. Sixty(60) varieties were tested in a complete randomized block design; four control cultivars were used and replicated in the four blocks. Experimental plots had a 2 square meter area. Seeds were sowed at 20 x 20 cm intervals on October 8th and 9th. 2 treatments were applied *i.e.* with irrigation and without irrigation. For the stress treatment, irrigation was interrupted from 65 to 80 day after sowing (between panicle initiation(PI) and flowering stage).

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

Sampling and measurements were done at the end of the drought treatment (78-80 days after sowing). Yield components were determinated for all cultivars. Canopy temperature was obtained by using the infra-red thermographer TH 7800 (NEC, Japan).

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

The canopy temperature values obtained for each of the tested variety with the Infra-red equipment showed that there is a genetic variability for this criterion in both treatments (with and without drought) (Fig 1). With 8 degrees between the extreme values (30-38.7 °C), this diversity was wider for the stress treatment compared to the irrigated treatment with a 4 degrees range (28.5-32.7 °C).

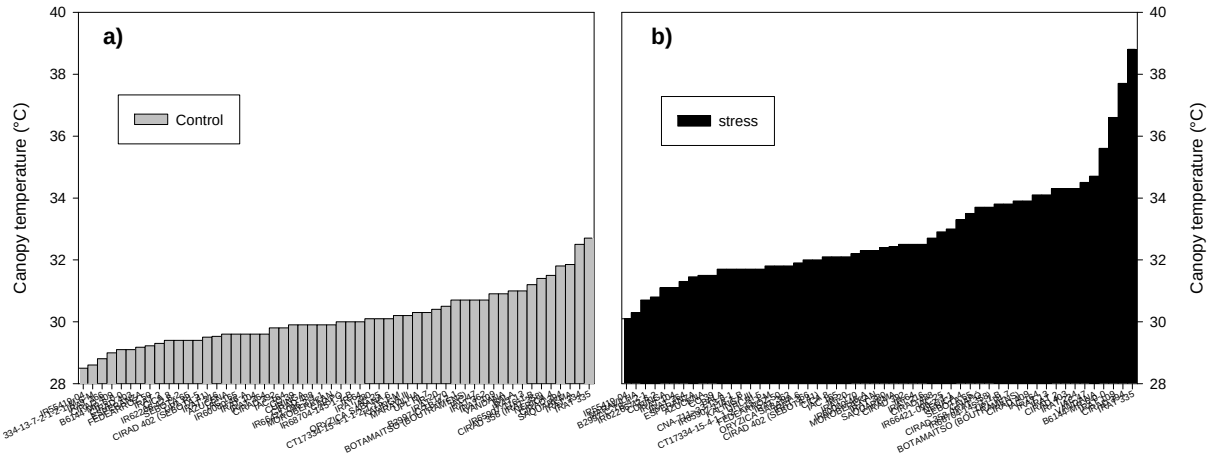


Figure 1 : Histograms of the 60 cultivar for the canopy temperature a) irrigated treatment and b) stress treatment.

The varieties canopy temperature did not rank similarly in both treatments. However, particular cultivars with low canopy temperature in the irrigated treatment also showed similar canopy temperature in the stress treatment (IR55419-04, Esperanza, IRAT 104, IRAT 364, IRAT 366, Fedearroz50). These

cultivars thus seem to display a better transpiration ability under both stressed and non-stressed water availability conditions.

Canopy temperature differences between the two treatments were highly significant (fig 2). At the end of the drought period, canopy temperature for the stressed varieties was in average 2.68 °C higher than the average canopy temperature for the control cultivars, respectively (32.8 and 30.2 °C). .

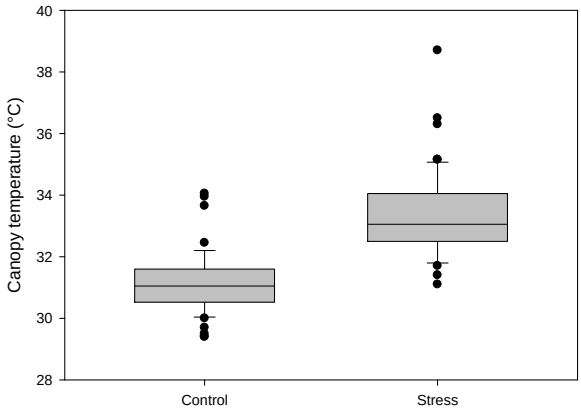


Figure 2 : Canopy temperature box plot built with 60 varieties for each treatment (irrigated and stress).

To be able to compare varieties, the temperature difference between treatments (irrigated and stress) was analyzed for each variety. The frequency histograms obtained also shows the existence of a genetic variability for this criterion (Fig 3).

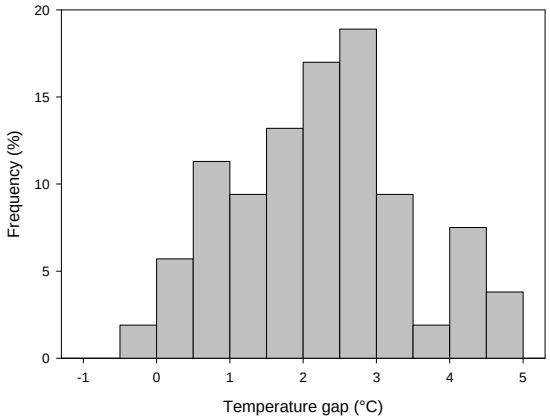


Figure 3 : Frequency histograms for the temperature gap between the two treatments (irrigated and stress), based on sixty cultivars evaluated.

The representation of all the values obtained during the experiment according to soil humidity (Fig 4) enables us to confirm the relevance of this criterion (canopy temperature) to characterize drought effect on plants. Indeed canopy temperature was negatively correlated to soil humidity. We observed that the dryer the soil, the higher the canopy temperature was. Soil humidity in the two treatments was well differentiated: in average 18 % Hr and 12.5 HR respectively for irrigated and stressed treatments.

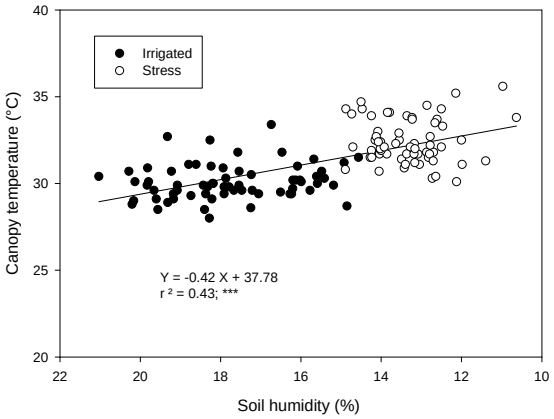


Figure 4 : Relationship between canopy temperature and soil humidity based on sixty cultivars.

However cultivars could present different behaviour. This diversity of reaction towards soil humidity explains the relatively weak R ²value. Figure 5 shows the relation between canopy temperature and soil humidity for the four control varieties. It seemed that for these control cultivars, two behaviours could be observed. At the same soil humidity, some cultivars always showed higher canopy temperatures (Saquarema). A more detailed study of this cultivar indicates that Saquarema differs from the other varieties by a higher dry matter content (shoots and roots).

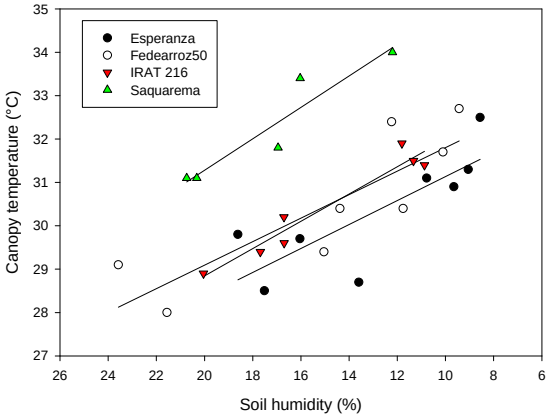


Figure 5 : Relationship between canopy temperature and soil humidity for the four referenced cultivars.

The figure 6 presents the relationship between canopy temperature 80 days after sowing (near flowering stage) and grain yield. Results showed the negative effect of high temperature on grain production. The four reference cultivars used present the same effect. Nevertheless IRAT 216 and Esperanza showed the highest yield with the irrigated treatment but also the higher temperature and yield variation with the stress treatment.

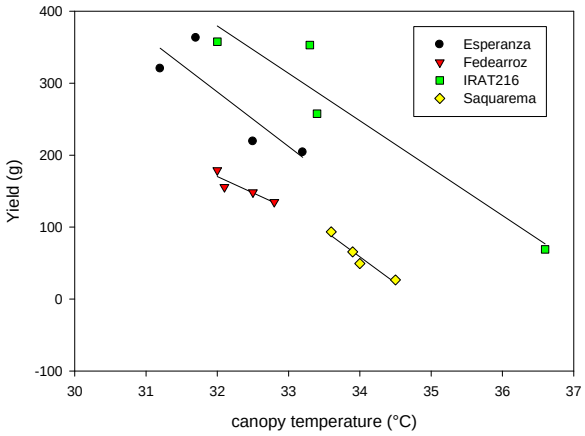


Figure 6 : Relationship between grain yield and canopy temperature for the four reference cultivars.

Conclusion

The experiment confirmed the effect of water deficit on canopy temperature. This effect was significantly different from the irrigated treatment. Canopy temperature was conversely proportional to soil humidity whatever the variety. Grain yield was also conversely proportional to canopy temperature.

From these results, canopy temperature could be used as a breeding criterion to screen drought tolerant lines. Furthermore, the frequency histograms confirms the existence of genetic diversity on this variable.

The best grain production under stressed condition was obtained with cultivars Oryzica Sabana 6 and 10, Curinga, UPL RI-7, Bala and B6144-MR-6-0-0. Under irrigated conditions Bala, UPL RI-7, Sebota 65, B2997-C-TR-4-2-1, Esperanza, and B6144-MR-6-0-0 presented higher grain yields.

Future Activities

The collaborative Cirad-Ciat project will continue during the 2008-2009 dry season. The planned activities are:

- Building-up allogamous breeding populations through crossings with a source of genic male sterility.

- Phenotyping tests for canopy temperature on 400 lines derived from existing recurrent population

- Test of the canopy temperature methodology to screen individual plants for drought tolerance in a breeding population.

1C. BIOTECHNOLOGY ACTIVITIES RELATED TO ENHANTED GENE POOLS

1C.1. Genotyping SNP and SSR Markers for Biofortification in Rice

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

Introduction

Gross. *et al.*, 2003, identified genes involved in the production of proteins participating in the transport and storage of iron. However, allelic variants affecting the iron content in the grain have not yet been elucidated. This project aims to identify those allelic variants by means of molecular markers (SNPs and SSRs) located within the genes involved in iron homeostasis.

Materials and methods

Content of iron and zinc in the plant material

We are currently working with samples from IRRI and CIAT germoplasm banks, a wild rice species (*O.rufipogon*), and the Latin American bank of germoplasm. These samples are grown at CIATs greenhouse so that the evaluation of iron and zinc is possible. The methodology used for obtaining the rice flour was implemented by the Agrosalud Rice Laboratory at CIAT. In order to evaluate the content of Iron and Zinc the rice flour thus obtained is sent to the CIAT-Analitycal Service Laboratory using the atomic absorption method. The process we followed is illustrated in figure 1.

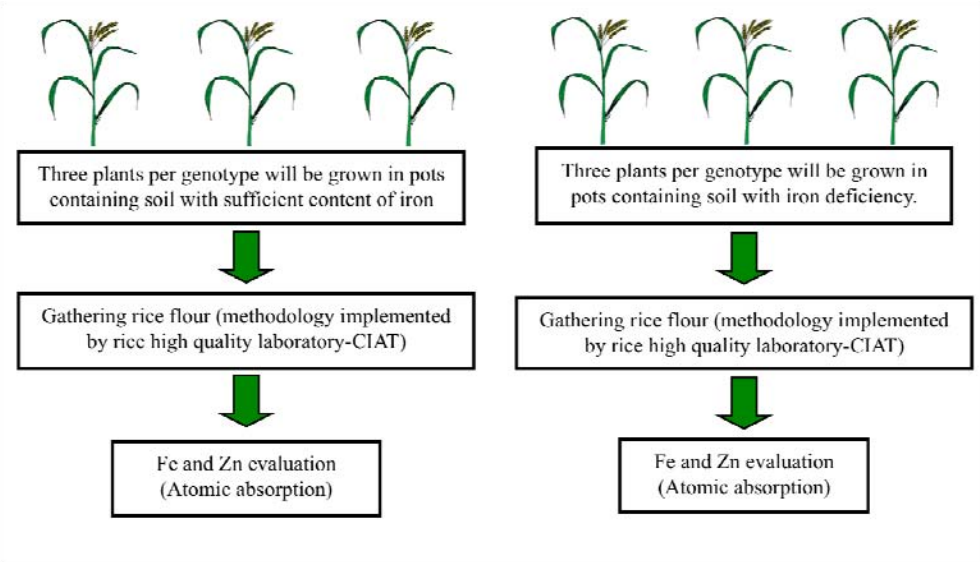


Figure 1. Process to evaluate the content of Iron and Zinc the rice flour.

Results

SNP genotyping in the plant material

Forty-four markers in 47 samples of rice were genotyped. Table 1 presents the 44 markers evaluated according to their polymorphism.

SNPs genotyping in the plant material.

Polymorphism	IUB Code[1]	SNP Type	Quantity
C/T o T/C	Y	Transition	30
A/C o C/A	M	Transversion	2
A/G o G/A	R	Transition	8
G/T o T/G	K	Transversion	1
C/G o G/C	S	transversion	3

Table 1. SNP markers genotyped in 47 sample of rice.
[1]international Union of pure and Applied chemistry

Standardization and evaluation of SSRs markers in the plant material

Those selected SSR markers are located within genes involved in iron homeostasis in rice (Gross. *et al.*, 2003); these markers are available at the gramene database (www.gramene.org). For the standardization process the experimental PRC conditions were adjusted; six rice genotypes were used as controls, three of them belonged to the *Oryza sativa* subsp. *japonica* (Koshihikari, Nipponbare and Caiapo), two to *indica* subsp. (93-11 and BG90) and the remaining samples belonged to the African rice species *Oryza glaberrima*. We

have evaluated 19 SSR markers in 117 samples of rice. Table 2 presents the 19 markers identified in 15 out of 43 genes involved in iron homeostasis.

SSRs evaluated in the plant material.

Primer SSR	Gen	Chromosome
RM15638	IRT1, IRT2	3
RM20592, RM20603	NRAMP3	6
RM7033, RM12390	NRAMP4	2
RM10481, RM10483, RM10485	YS1	1
RM12345	YS3, YS4	2
RM13647	YS5	2
RM16858	YS8, YS9	4
RM7409, RM18189	YS17	5
RM17474	ZIP4	4
RM6321, RM12254, RM12255, RM12261	ZIP6	1
RM18864	ZIP8, ZIP9	5

Table2. A total of 19 markers identified in 15 out of 43 genes involved in iron homeostasis.

Future work

We are planning to

- Keep on genotyping the SNPs for which PCR and SBE primers have been designed,
- continue the evaluation of the SSRs located within the other 28 genes, and also to
- identify allelic variants that may be affecting the iron content in rice grain by using SNPs and SSRs markers in genes involved in iron homeostasis.

Bibliography

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.

1C.2. Exploring wild diversity in rice through development of introgression line libraries

J. D. Arbelaez – L. Moreno* – P. Rangel* – M. Cissoko* – J. Kimball – C. P. Martinez – J. Carabali – S.R. Mc Couch – J. Tohme – M. Lorieux*

**These authors contributed equally to the work*

Partners:
IRD-UMR5096
Cornell University
Embrapa-CNPAF
Fedearroz
WARDA

Project funded by:
The Generation Challenge Program
IRD
CIAT

Background

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

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

CSSLs are particularly valuable when complex, quantitatively inherited phenotypes are the breeding target. Because they represent permanent (inbred) genetic resources that can be easily replicated by seed and distributed to collaborators working in different environments. Each set of CSSLs consists of a relatively small number of lines that can be evaluated in replicated trials. They are constructed to provide maximum power of statistical analysis because each

line can be compared to all others or may simply be compared to the recurrent parent, making it possible to extract a great deal of valuable information from a relatively small number of lines crops. For phenotypes that are difficult to measure, or require repeated evaluation over years and environments, the ability to focus quickly on a small number of lines is a critical component of success (Ghesquière et al, 1997).

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

Wide spread utilization of *O. sativa* relatives remains limited due to the fact that: (1) no extensive study has been carried out to explore the range of allelic diversity in any of the *Oryza* AA genome relatives, (2) the genetic basis of heterosis or transgressive variation in interspecific crosses remains largely unknown, (3) interspecific crossing barriers have hampered full utilization of rice relatives for breeding and genetic studies, (4) very few genomic resources have yet been developed to facilitate breeding efforts using *O. sativa* relatives. In particular, the lack of a cost effective, high throughput marker system that targets gene-based polymorphisms impedes efforts to efficiently and systematically select the best introgression lines and to evaluate the gene content of those lines in the context of comparative cereal genomics.

Results

We are currently developing introgression lines from two cultivated x wild crosses, where the wild species are *O. meridionalis and O. rufipogon.* Two other populations with *O. barthii* and *O. glumaepatula* are being developed at WARDA, Benin and Embrapa-CNPAF, Brazil, respectively.

O. sativa *x* O. meridionalis

Laura T Moreno - CIAT

Interspecific cross: *O. sativa* ssp. tropical *japonica* cv. Curinga x *O. meridionalis* acc. OR44

The development of a Chromosome Segment Substitution Line (CSSL) library for the interspecific cross between the cultivated rice *O. sativa* BRSMG Curinga and the wild species *O. meridionalis* OR44, is now at its final stage and results from this research will be available during the second semester 2009.

During 2008, the foreground and background selection of a BC2F1 population of the interspecific cross mentioned above, allowed the selection of 70 lines that were backcrossed with the recurrent parent (*O. sativa* BRSMG Curinga) to obtain BC3F1 seeds. These lines were used for a preliminary foreground selection implementing the optimized set of molecular markers previously optimized. This molecular genotyping allowed the selection of 1-3 plants out 6 with the desire introgression fragment for each chromosomal location. Anther culture (androgenesis) was used to develop double haploids for 140 BC3F1 lines selected during the preliminary foreground selection process. Results demonstrated a wide range of green plant recovery percentage (3-52%) in 92% of the BC3F1 lines processed. Nevertheless, observation of transplanted lines to the field will be necessary to estimate the percentage of double haploids recovery. The remaining BC3F1 lines that could not be processed through anther culture will be advanced by self crossing to obtain homozygous lines for the final set of CSSLs. Selection of plants at the greenhouse with specific diploid features was done to transplant potential double haploid lines to obtain at least one DH per BC3F1 family.

In 2009, BC3DH seeds will be harvested, and foreground and background molecular selection will be performed using an Illumina platform-based Single Nucleotide Polymorphism DNA chip developed by Cornell University, and SSR validation if required. This is the first time at CIAT that an interspecific population with a wild donor parent is used to produce Double Haploids.

O. sativa *x* O. rufipogon

Juan David Arbelaez – Fedearroz/CIAT

Interspecific cross: *O. sativa* ssp. tropical *japonica* cv. Curinga x *O. rufipogon* acc. IRGC105491

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

To accomplish this task, two main activities were carried out during the first semester of 2008. The first was the genetic background checking of the BC_2F_1 population between Curinga and *O. rufipogon*, and the second was the backcross of this population with recurrent parent Curinga to generate a BC_3F_1 population.

160 BC_2F_1 plants were chosen to be background-selected from a 600 BC_2F_1 population. These plants contain a total of 50 desirable introgressed fragments

from the wild parental *O. rufipogon* that represents its whole genome. The background selection helps to narrow down the number of plants that contain the desirable introgressed fragments according to the fracition of recovery of recurrent background. With a set of 130 SSRs markers 60 BC_2F_1 plants were selected to be backcrossed. These plants have recovered as much as 88% of the recurrent genome, values similar to those expected theoretically. Using these 60 plants, a population of 600 BC_3F_1 plants was generated (10 plants per family).

In the second semester of 2008, this BC_3F_1 population was genotyped for a foreground selection, this type of selection allowed us to check those plants that keep the desirable wild introgressed fragment. From each family, three plants were chosen to develop a *doubled haploid* population (total of 180 plants) according to the foreground selection. These 180 plants were taken to the field and the first panicles from each plant were harvested to generated the doubled haploid plants at the Anther Culture Lab. At the same time these plants were backcrossed to obtain a BC_4F_1 population. This BC_3F_1 DH population will be taken to the field by the first semester of 2009.

Conclusion

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

We hope that the development of full-genome coverage CSSL populations will contribute significantly to the set of genetic and genomic tools available for breeding and gene discovery in rice.

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

- Four interspecific genetic maps were developed,

- Four cultivated x wild BC1F1 populations were genotyped,

- Four cultivated x wild BC2F1 populations were derived,

- A software (CSSL Finder) was designed for the specific purpose of helping at developing CSSL lines,

- Several students and research assistants were trained,

- Four students do shuttle research between their respective centers and Cornell University,

- The international collaboration between several ARIs, CG centers and NARS was strengthened,

- Several publications are in preparation.

References

Ghesquière, A., J. Séquier, et al. (1997). "First steps towards a rational use of African rice, Oryza glaberrima, in rice breeding through a 'contig line' concept." Euphytica 96: 31-39.

Gur A and D Zamir (2004) Unused natural variation can lift yield barriers in plant breeding. Public Library of Science 2(10):1610-1615

Lorieux M, Ndjiondjop MN, Ghesquière A (2000) A first interspecific *O. sativa* x *O. glaberrima* microsatellite-based genetic linkage map. *Theoretical and Applied Genetics* 100: 593-601.

Lorieux M (2005) CSSL Finder, a free program for managing introgression lines. URL: http://mapdisto.free.fr/

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/

Moncada M.P., C.P. Martínez, J. Tohme, E. Guimaraes, M. Chatel, J. Borrero, H. Gauch Jr. and S.R. McCouch (2001) Quantitative trait loci for yield and yield components in an *O. sativa* x *O. rufipogon* BC_2F_2 population evaluated in an upland environment. Theor Appl Genet 102:41–52

Nelson, JC (1997) QGENE: software for marker-based genomic analysis and breeding. Mol. Breed. 3:239–245

Sun, C.Q., X. K. Wang, Z. C. Li, A. Yoshimura and N. Iwata (2001) Comparison of the genetic diversity of common wild rice (*O. rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers Theo. Appl. Genet. 102:157-162

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

Andrés Gutiérrez, Maria Fernanda Alvarez, César Martínez, James Carabalí, Joe Tohme and Mathias Lorieux

Partners:
IRD-UMR5096

Project funded by:
The Generation Challenge Program
IRD
CIAT
The French Ministry of Foreign Affairs

Background

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

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

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

Methodology and Results

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

A BC_3F_1 population was obtained at CIAT HQs from the cross between Caiapo (an elite tropical *japonica* from Brazil) and *O. glaberrima* acc. IRGC103544 (alias MG12) (IRRI genebank). From these lines, anthers were collected and a population of 695 lines BC_3F_1 doubled-haploid (DH) was obtained through *in vitro* culture (Zaida Lentini).

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

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

On the other hand, in order to optimize and to purify the genetic background of the 64 selected lines and to reduce the number of introgressed genomic fragments from *Oryza glaberrima*, 59 of these lines were chosen and backcrossed to Caiapo and selfed to obtain BC_4F_2 lines. For each of these 59 BC_4F_2 lines, at least 60 individuals were planted in the field (in total 4200 BC_4F_2 individuals). With the aim to identify plants carrying a single target fragment, these 4200 materials were grouped in bulks and evaluated with 2-3 microsatellite markers that flank the target *O. glaberrima* segments. Thus, 47 bulks were chosen and we will select the single plants that contain the target fragment and that contain the highest fraction of recurrent genetic background.

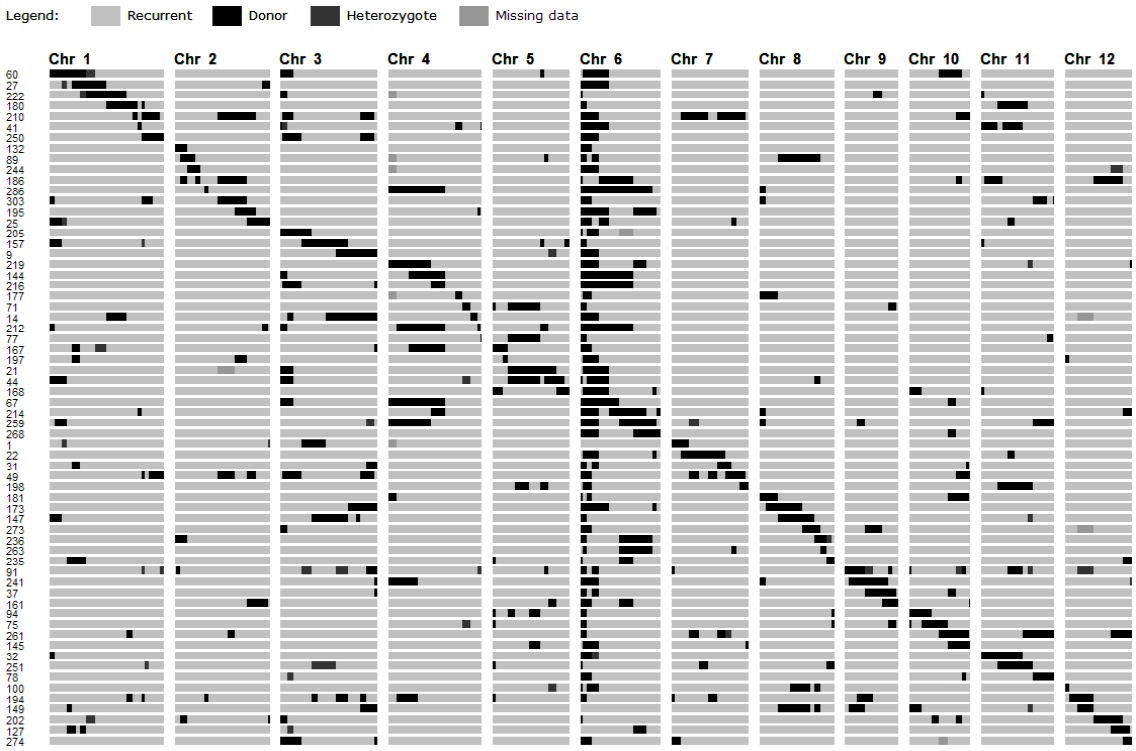


Figure 1. Graphical representation of the genotypes of 64 BC_3DH lines selected from a library of 312 lines. The 12 rice chromosomes are displayed vertically. There are covered by 125 evenly dispersed SSR markers. The genotypes are displayed horizontally. Gray-scale legend indicates the allelic status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

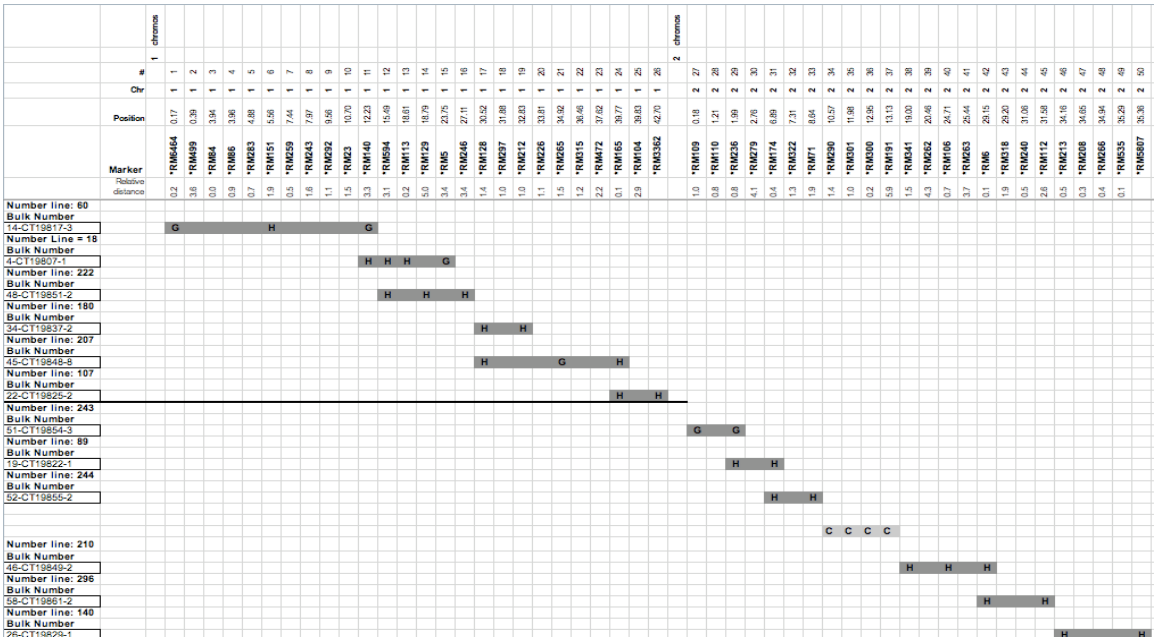


Figure 2. Bulks chosen to be opened. On the left, number of bulk, each one with a target fragment (represented by gray rectangles) flanked by 2-3 SSRs marker (above). Only chromosome 1 and 2 are showed.

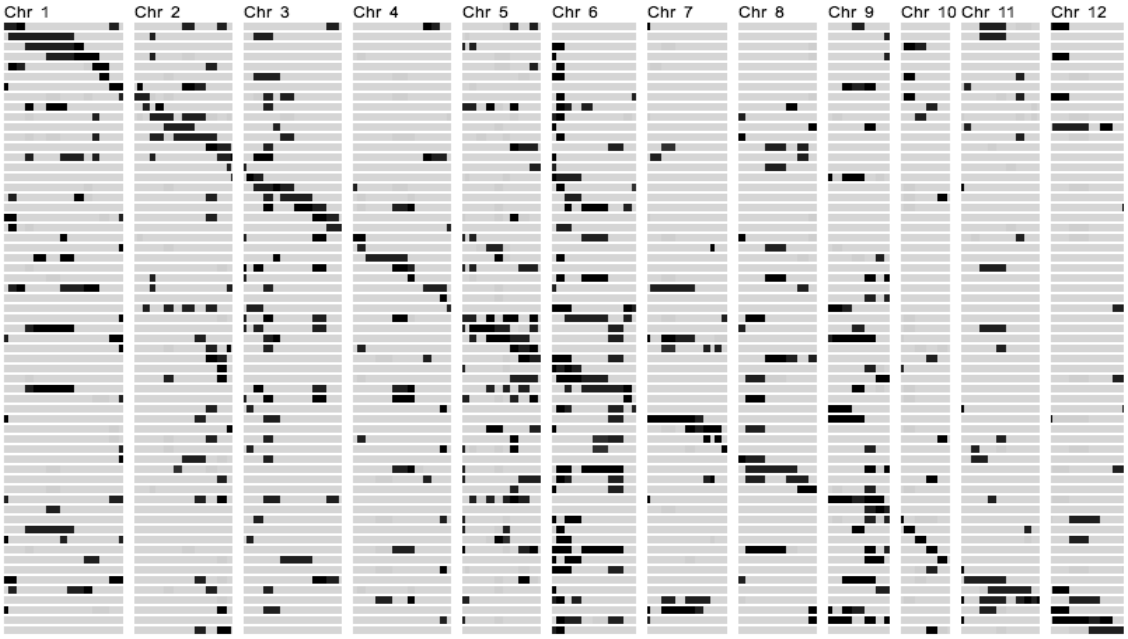


Figure 3. Graphical genotypes of 61 CSSLs lines selected by CSSL Finder. The 12 chromosomes of rice are displayed vertically. They are covered by 115 evenly dispersed SSRs marker. CSSLs lines are displayed horizontally. Black rectangles indicate homozygous introgressions from *O. glaberrima*, light gray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

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

A population made of BC_2F_4 and BC_3F_3 lines (Pre-CSSLs) was developed at IRD, Montpellier, France through marker-assisted backcrossing from the cross IR64 (*O. sativa* ssp. *indica*) x TOG5681 (*O. glaberrima*). These sub-populations were analyzed with 153 well-distributed SSRs marker for their genomic content at CIAT. The SSRs were selected using the Paddy Map database (http://mapdisto.free.fr/PaddyMap/)).

The search for a set of lines as candidate for CSSLs was done using the CSSL Finder program (Lorieux 2005). The following parameters were taken into account: size of introgressed segments, minimum number of segments that cover the genome and treatment of heterozygotes as homozygotes for the donor allele. As a result, 61 lines were selected using 115 of 153 SSRs marker that showed an even distribution across the twelve rice chromosomes (Figure 3). These lines covered the complete *O. glaberrima* genome with introgressions, except for some

small regions on chromosomes 4 and 10. The lost segments are currently being recovered from a new BC_2/BC_3 population derived at CIAT from the same cross.

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

We used the same methodology based on DNA bulks — the one used for the Caiapo x MG12 population — to choose single plants carrying the target fragment. Sixty-one bulks were selected using CSSL Finderand will be evaluated with 2-3 SSR markers that flank the target segments of *O. glaberrima.*

Conclusions

This project allowed us to advance significantly in the construction and evaluation of CSSLs libraries between the two cultivated species of rice, in both *indica* and *japonica* genetic backgrounds. Both population are almot ready for distribution to partners.

The CSSL Finder program was useful to optimize the choice of candidate lines that optimize both foreground and background genetic content. It also will allow us to detect and compare the gene or QTL locations discovered with those two populations for various traits of importance.

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

References

Ebitani T., Takeuchi Y., Nonoue Y., Yamamoto T., Takeuchi K. and Yano M. (2005) Construction and Evaluation of Chromosome Segment Substitution Lines Carrying Overlapping Chromosome Segments of *indica* Rice Cultivar "Kasalath" in a Genetic Background of *japonica* Elite Cultivar "Koshihikari". *Breeding Science* 55: 65-73

Eshed Y. and Zamir D. (1995) An Introgression Line Population of *Lycopersicon pennellii* in the Cultivated Tomato Enables the Identification and Fine Mapping of Yield-Associated QTL. *Genetics* 141: 1147-1162

Howell P.M., Marshall D.F. and Lydiate D.J. (1996) Towards developing inter-varietal substitution lines in *Brassica napus* using marker-assisted selection. *Genome* 39:348-358

Li J., Xu P., Hu F., Zhou J., Deng X., Wan J. and Tao D. (2005) Mapping of QTLs for pollen stertility in *Oryza sativa-O. glaberrima* interspecific hybrid. 5th International Rice Genetics Symposium and 3rd International Rice Functional Genomics Symposium. IRRI P155: 131

Lorieux M. (2005) CSSL Finder, a free program for managing introgression lines. URL: http://mapdisto.free.fr/

Zhang Xia, Zhou Shaoxia, Fu Yongcai, Su Zhen, Wang Xiangkun, Sun Chuanqing (2006) Identification of a drought tolerant introgression line derived from Dongxiang common wild rice (*O. rufipogon* Griff.) *Plant Mol Biol* 62: 247–259

1C.4. Paddy Genes Book, a database of rice T-DNA insertion lines phenotypes

J. Lozano – M. Bouniol* – M. Brito – E. Guiderdoni – A. Ghesquière – M. Lorieux*

**These authors contributed equally to the work*

Partners:
IRD
Cirad
Génoplante

Project funded by:
The Génoplante consortium
IRD

Background

In the framework of its work plan for functional analysis of cereal genomes, the Génoplante consortium decided to construct a rice T-DNA insertional mutagenesis collection (Sallaud et al 2003). Rice was chosen as a model species because of its small genome and because of all the genomic resources available for this species (ESTs, genetic maps, complete sequence, etc.). The lines were produced in Cirad laboratories, and grown in Cirad and IRD greenhouses, in Montpellier, France. The present work carried out at CIAT as a collaboration with Génoplante consists in: (i) a systematical phenotypic evaluation of the mutant collection, with production of an associated phenotypic database, and (ii) the multiplication of seeds for the entire collection, for later distribution to all laboratories interested in rice functional genomics. We focus here on the first topic.

Methodology

Screenhouse

The T_0 plants were produced at Cirad and grown in Cirad and IRD glasshouses in Montpellier, France. Twenty-five T_1 seeds per T_0 plant were received at CIAT and were sown in a screenhouse. Sowing was carried out in eight batches of 1,250 lines, with about three weeks delay between the batches. The seeds were pre-treated by heat for three days at 50 °C to break dormancy, and planted in plastic trays with a mixture of CIAT (67 %) and Santander de Quilichao (33 %) soils. Germination was determined at ten days after sowing (DAS). The first phenotypic observations were carried out at 18-20 DAS, with counting of the number of

individuals presenting the mutant phenotype. A list of possible phenotypic traits was established from data mining of several rice phenotypic databases (www.gramene.org, www.grs.nig.ac.jp/rice/oryzabase, www.irri.org/genomics), and was used as a guide for observations. An English-Spanish-French lexical of botanical and agronomic terms was established to facilitate phenotype identification.

Field

Two fields of two hectares each were prepared following the requirements of the ICA (Instituto Colombiano Agropecuario). The entire surface was covered by nets to avoid any damage or seed dissemination that could be caused by birds. The plantlets were transplanted at 25 DAS. A basic fertilization composed of Mono-Ammonium Phosphate, Iron Sulfate, Potassium Chloride and micro-elements was applied. The field was irrigated two times a week. Control lines of Nipponbare cv. were planted for each 10 T-DNA lines in order to facilitate the comparison with wild phenotype. Phenotypic analyses were carried out at different ages, using the list of possible traits as a guide. A first round of observation was done when the plants were approximately 45 days old. A second evaluation was done at flowering, while the ultimate observation was done at maturity. This maximized the chances to detect phenotypic variations, as various traits could be observed at only one of these stages. Moreover, this permitted to follow the evolution of a suspected phenotype at early stage and possibly confirm or invalidate it.

Results

Mutant Phenotypes

A total of 27,832 lines were grown since the beginning of the project.

The overall mutant phenotypes percentage was 19.4%. Numerous lines showed chlorotic or albino plantlets, with associated deficiency in leaf development. General abnormal development was also frequently observed. The most common phenotypes included several types of albinism, sterility, dwarfism more or less pronounced, chlorotic leaves, rolled leaves, awning, modified leaf shape, white streaks, lesion mimics, general abnormal development, late flowering, round hull, modified tillering.

Redundancy of phenotypes was frequently observed between two or more lines. This is probably due to the fact that these lines proceed from the same transformation event. We thus applied a correction to the calculation of the percentage of observed mutant phenotypes. If several lines proceed from the same

callus and share at least one trait, only the first entry is retained. This led to a corrected estimation of 6.2% of mutant phenotypes (screen house + field).

Database Set Up

A local database of all data relative to growth conditions, germination, flowering, and phenotypic observations was set up. This database, called *Paddy Genes Book*, is mainly used as a working tool to facilitate data entry and compilation. However, it also can be used for data browsing, as it permits the display of information by mutant bar code number or CIAT number. Several options for searching for lines or traits according different criteria are available. Moreover, the database offers tools for computing basic statistics over traits and lines.

New features were recently implemented in the database, which allow us to link the phenotypic information to the FST positions. It is possible to make searches using the Trait Ontology instead of trait codes. These features permit fast and powerful identification of candidate genes or gene families that can be confirmed later on by line tagging.

This database also displays photographs of the mutant phenotypes (see screenshots for details). More than 36,000 pictures are available.

A flat data file is regularly extracted form the database in order to fill the Génoplante Oryza Tag Line database (Larmande et al 2008), which is available online at http://urgi.versailles.inra.fr/OryzaTagLine/.

Conclusion

The overall mutation rate was higher than it is currently observed in other mutant collections, where visual phenotypic screening typically identifies about 3 to 5% of mutants. A part of that excessive mutation rate could be eliminated by clone redundancy analysis. T-DNA insertion is probably not responsible for all the variation observed. Indeed, it is well known that other sources of mutation like the Tos 17 retrotransposon are positively activated by in vitro culture of rice. Moreover, discrepancies in germination dates and seed quality, mainly due to the growth conditions of the T0 plants, may be responsible for apparent mutations, notably Retarded Growth (RG), tillering, height and delayed flowering. Also, in some cases we chose to include some doubtful data, as it is preferable to eliminate false-positive data after more detailed analyses for a specific trait than to miss real data.

The overall process of seed multiplication and phenotypic analysis worked very well. The timetable was respected, and valuable phenotypic data were produced. The phenotypic database will constitute a precious tool for selecting lines for functional genomics studies. The project is now completed and we hope that the resource produced will constitute a useful tool for the rice research community.

References

Larmande P, Gay C, Lorieux M, Perin C, Bouniol M, Droc G, Sallaud C, Perez P, Barnola I, Biderre-Petit C, Martin J, Morel J, Johnson A, Bourgis F, Ghesquière A, Ruiz M, Courtois B, Guiderdoni E (2008) Oryza Tag Line, a phenotypic mutant database for the Génoplante rice insertion line library. Nucleic Acids Res 36 :D1022-D1027

Sallaud C, D Meynard, J van Boxtel, C Gay, M Bès, J P Brizard, P Larmande, D Ortega, M Raynal, M Portefaix, P B F Ouwerkerk, S Rueb, M Delseny, E Guiderdoni. 2003. Highly efficient production and characterization of T-DNA plants for rice (*Oryza sativa* L.) functional genomics. Theoretical and Applied Genetics 106:1396-1408.

Figure 1. Screenshots of the Paddy Genes Book phenotypic database

Figure 1a. Main menu

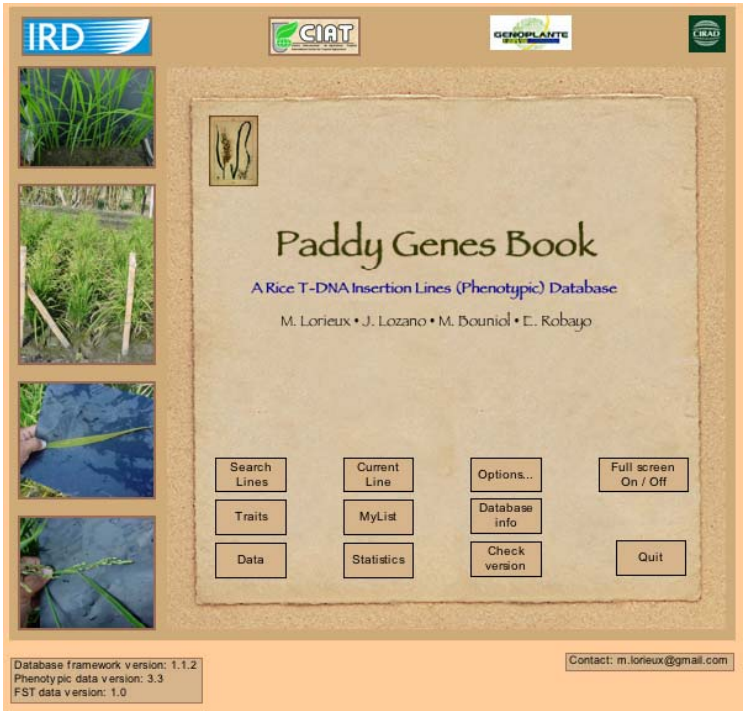


Figure 1b. Trait browsing

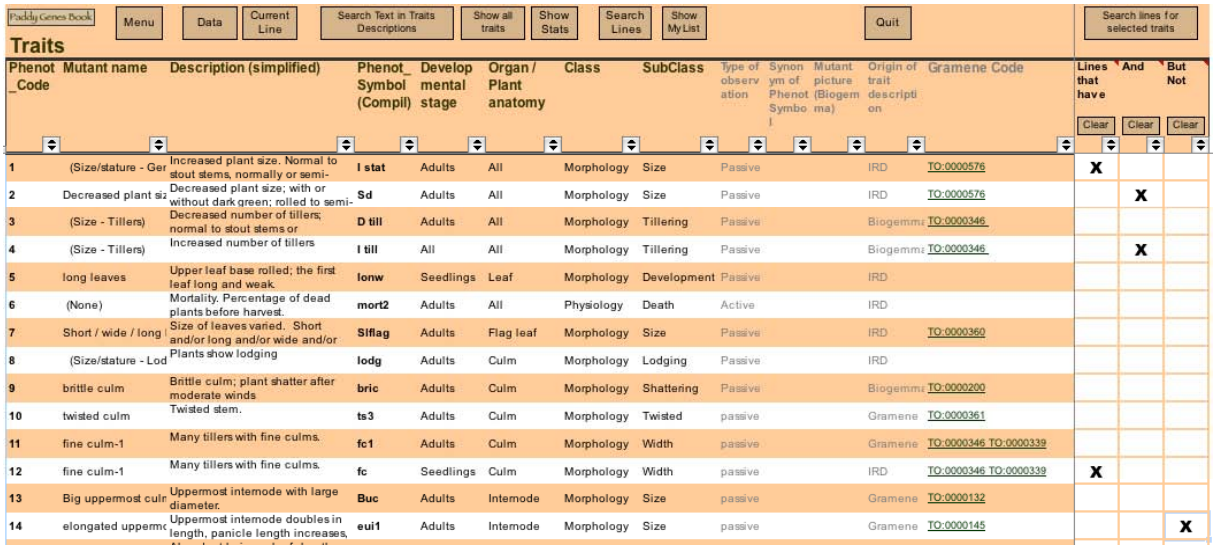


Figure 1c. Search for lines by trait code or full text

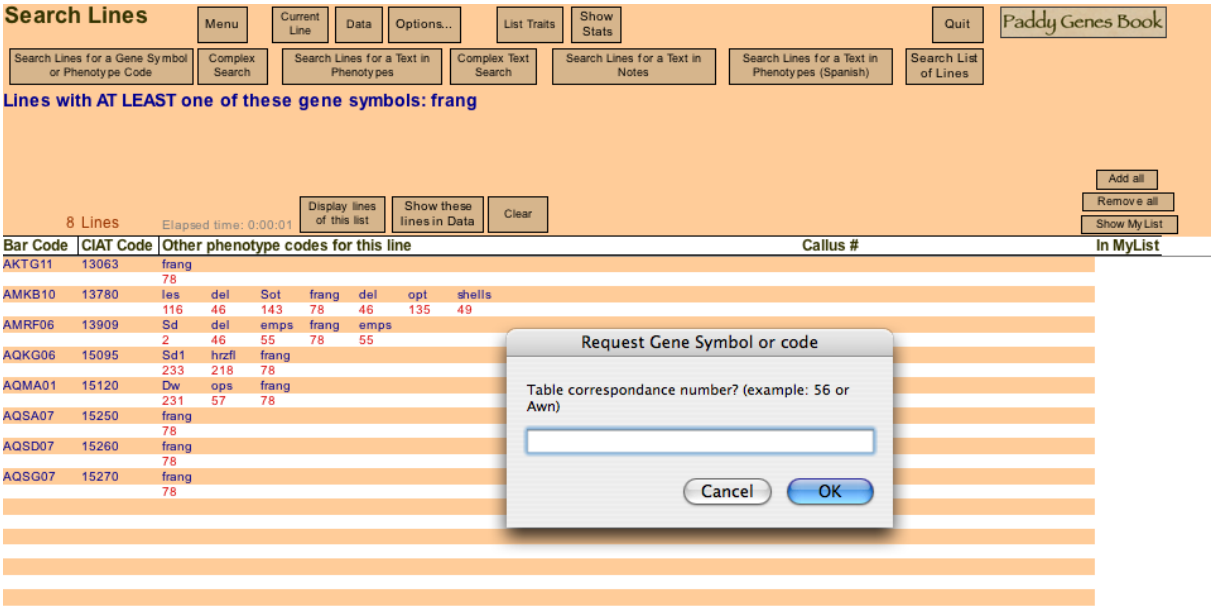


Figure 1d. Statistics on traits and lines

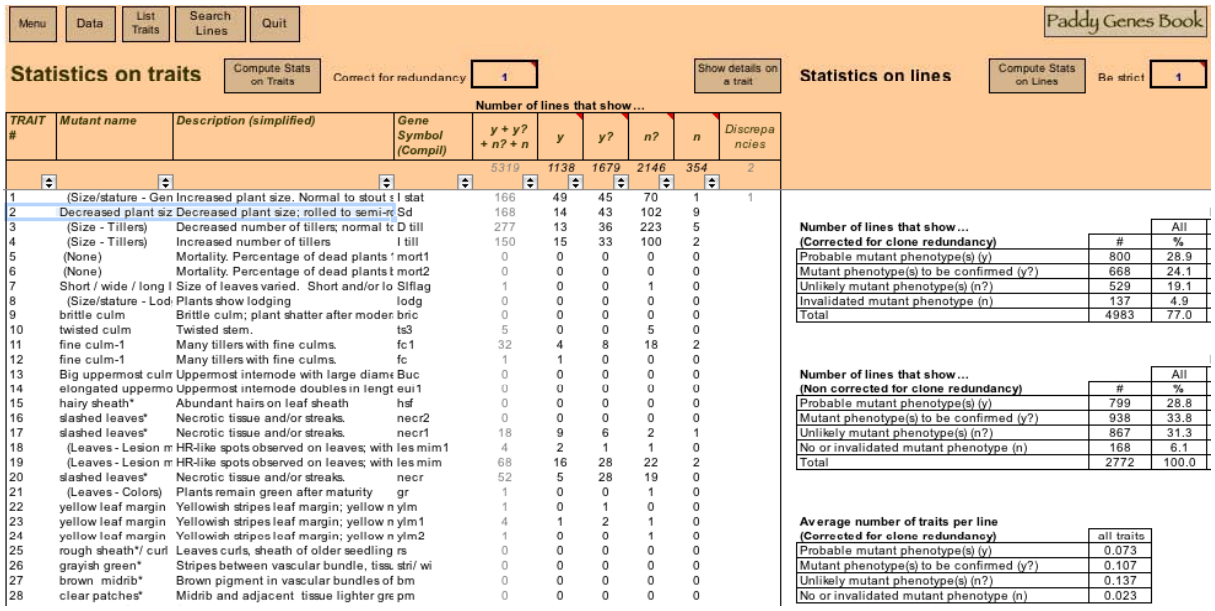


Figure 1e. Display of phenotype, codes, statistics, heading date graphics and photographs for each line

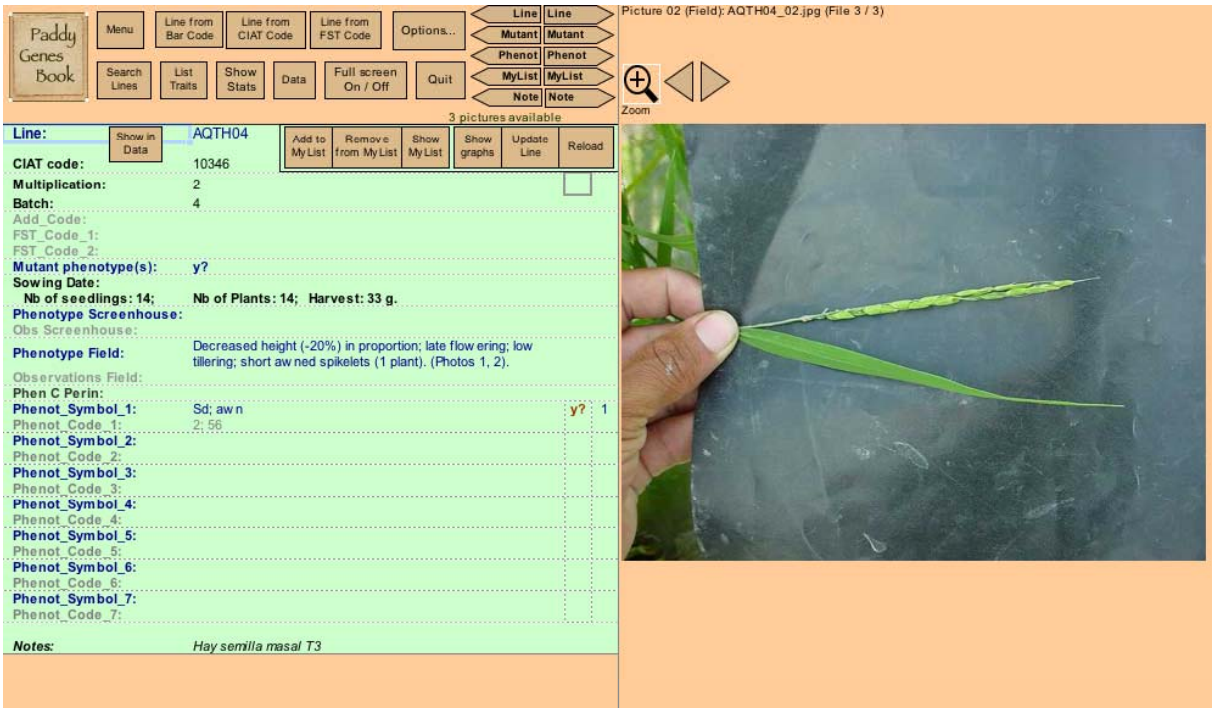
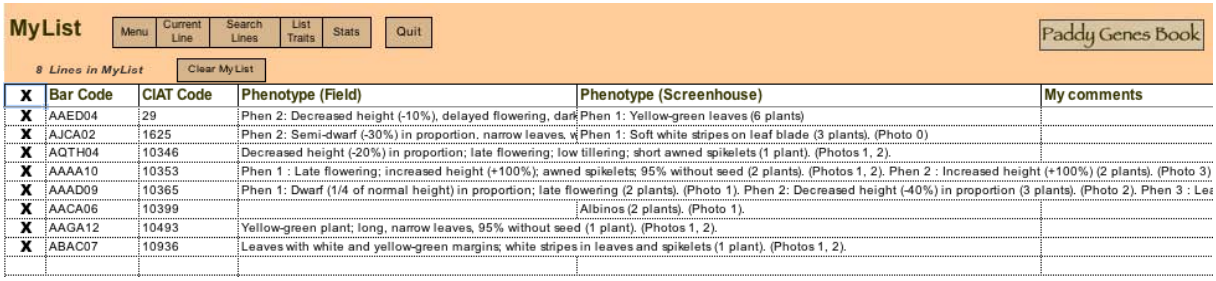


Figure 1f. Customized search list



MyList

Menu | Current Line | Search Lines | List Traits | Stats | Quit

Paddy Genes Book

8 Lines in MyList

Clear My List

X	Bar Code	CIAT Code	Phenotype (Field)	Phenotype (Screenhouse)	My comments
X	AAED04	29	Phen 2: Decreased height (-10%), delayed flowering, dark	Phen 1: Yellow-green leaves (6 plants)	
X	AJCA02	1625	Phen 2: Semi-dwarf (-30%) in proportion, narrow leaves, w	Phen 1: Soft white stripes on leaf blade (3 plants). (Photo 0)	
X	AQTH04	10346	Decreased height (-20%) in proportion; late flowering; low tillering; short awned spikelets (1 plant). (Photos 1, 2).		
X	AAAA10	10353	Phen 1 : Late flowering; increased height (+100%); awned spikelets; 95% without seed (2 plants). (Photos 1, 2). Phen 2 : Increased height (+100%) (2 plants). (Photo 3)		
X	AAAD09	10365	Phen 1: Dwarf (1/4 of normal height) in proportion; late flowering (2 plants). (Photo 1). Phen 2: Decreased height (-40%) in proportion (3 plants). (Photo 2). Phen 3 : Lea		
X	AACA06	10399		Albinos (2 plants). (Photo 1).	
X	AAGA12	10493	Yellow-green plant; long, narrow leaves, 95% without seed (1 plant). (Photos 1, 2).		
X	ABAC07	10936	Leaves with white and yellow-green margins; white stripes in leaves and spikelets (1 plant). (Photos 1, 2).		

1C.5. Development of protocol for drought phenotyping in rice

Contributors: Jagadish Rane, C.E. Manrique, Alva Lucia Chavez, Manabu Ishitani

Source of Funding: MAFF-JAPAN

Rationale

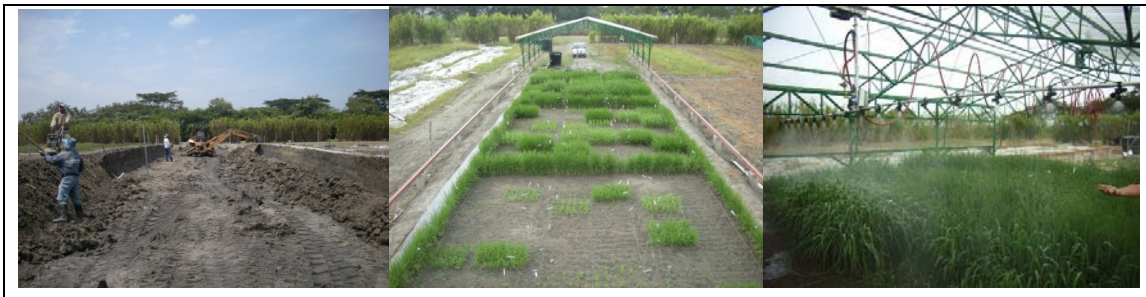
Drought is a major constraint for sustaining the crop productivity across the world. Ever increasing demand for food and water and predicted adverse effect of change in future climate emphasize need for improved technologies to avoid global food crisis. While the conventional plant breeding approaches had limited gains, gene technologies are expected to complement conventional research efforts to improve productivity under water limited environment. Successful demonstration of such technologies is largely restricted to controlled environments and pot studies and needs to be tested under field conditions under stringent conditions to avoid biosafety risks and to comply with biosafety regulations. Curinga, the most promising upland rice cultivar of Latin America, is known for its adaptation to drought environments and hence this was chosen for transformation with proven genes that impart drought tolerance. The extent of drought tolerance at various stages of growth of Curinga under a simulated drought environment has not been documented. Further, it is also necessary to monitor and maintain desired level of soil moisture regime during experimentation. Hence, efforts were made to develop screening protocol for drought tolerance in rice at CIAT-Palmira under biosafety screenhouse and confined fields.

Key Words: drought, upland rice, transgenic rice, phenotyping

Establishing phenotyping for drought tolerance in confined field at CIAT Palmira

Permission from National Biosafety Committee has uniquely placed CIAT to be a global platform to evaluate gene technology under confined field. To make best use of this opportunity, our initial efforts were focused on improving the facilities

such as rainout shelter under confined field conditions. Preliminary experiments with four non transgenic check varieties viz Curinga, Azucena, Inta Chinnandega and IR 64 revealed that it is difficult to simulate drought experienced by upland rice on CIAT soil. Hence, it was felt necessary to use restructured soil that resembles the soils of upland rice ecosystem. Based on our experience with big tray experiment and available data on soil that exist in upland conditions, it was decided to use acid soil of Quilichao, alkaline soil of CIAT and river sand in 2:1: 2 proportions by weight to restructure the soil. To start with, an existing rainout shelter facility was used to create experimental area of 7 m X 15 m with 0.35 cm deep restructured soil. Suitability of this soil for drought screening was tested with four non transgenic rice cultivars. Based on this experience, efforts were made to create larger experimental area that can permit screening of large number of lines. Initially, 1 m high bunds with 1 m width at the top and 1.5 m at the bottom were created to make 30m X 8m X 1m pool that can be filled with restructured soil. Different components of soil were measured in big trucks and thoroughly mixed by spreading it over a stretch of 50 m X 5 m road and then mixing with the levelers, rotavators and excavators. Then the soil mix was used to fill the pool. Before filling the pool, 10 cm layer of dead rock was laid at the bottom of the pool to separate the native soil from the restructured soil. Chemical analysis of soil revealed homogeneity across the experimental area.



Rainout shelter with boom system and 1 m deep new soil mix for drought screening at CIAT Palmira.

This structure now has the rainout shelter, which also supports boom irrigation system for regulated delivery of irrigation. Soil moisture regime is monitored by using Aquapro system through a network of one meter long several access tubes inserted into the soil across the experimental area.

Experiments are in progress to evaluate the response of 10 non transgenic check varieties to drought imposed at seedling stage and also around flowering stage under rainout shelters.

Biosafety post harvest processing facility

To avoid biosafety risks while processing the harvested transgenic material biosafety post harvest facility was created. The facility at present has seed processing room, seed preparation room with instruments exclusively for transgenic material. Standard operating procedures are followed to transport material harvested from biosafety screenhouse and the confined fields to the biosafety post harvest facility and also while disposing the wastes.

Establishing protocol for evaluation of transgenic events at early stages of growth in biosafety screenhouse

Since it was clearly shown that plants transformed with genes such as DREB can tolerate drought under controlled environment elsewhere, our major focus was on evaluation of the technology under natural field conditions. However, it was felt necessary to have a phenotyping protocol for biosafety screenhouse for validation of the effect of genes on response of promising transgenic events and of the expression of promising gene constructs identified through field screening.

Materials and Methods

Growth environments:

Big trays with two meter diameter and 30 cm depth designed at CIAT were successfully used to simulate upland drought environment at early stages of rice plants in the biosafety screen house. A series of experiments were conducted to determine extent of drought tolerance in Curinga and other 3 check varieties viz. IR-64, Azucena and INTA-Chinnandega. In each of the experiment, one big tray was used to impose drought and the other was used as irrigated control where soil moisture was maintained at field capacity. Completely randomized design was followed to layout the experiment in each of the tray with 5 radial blocks and 4-8 reps in each block. Drought was imposed by withholding irrigation as shown in Table 2 to evaluate the plant response at seedling and at vegetative stage. Plants were reirrigated when most of the Curinga plants were severely affected to evaluate the recovery from drought. Leaf rolling and soil moisture was recorded regularly and biomass in plants were recorded after two week.

Table 2 Summary of drought intensity and plant response in different experiments

Experiment	Drought Imposed	Soil Moisture below 20%(v/v)	Initiation of leaf rolling	Severe drought symptom	Plants sampled after reirrigation	Curinga plants with poor Recovery (<1 New Leaf)	% Curinga plant with poor Recovery
Seedling –I	9 DAS	23 DAS	25 DAS	43 DAS	57 DAS	28/45	62
Seedling –II	11 DAS	27 DAS	27 DAS	38 DAS	60 DAS	24/45	59
Vegetative – I	17 DAS	27 DAS	35 DAS	50 DAS	62 DAS	23/28	82
Vegetative – II	22 DAS	35 DAS	32 DAS	47 DAS	62 DAS	25/28	89

Based on the results the screening protocol was developed for identifying rice transgenic events superior to non transgenic Curinga in the biosafety screenhouse. The protocol involves use of soil mix that contains soil from St Quilichao, CIAT and river sand in 2:1:2 by weight, exposure to drought by withholding irrigation two week after sowing till severe symptom appear in Curinga and then reirrigation to allow for recovery. This protocol is being presently used to screen existing transgenic events of Palmar and CT 6241.

MAFF of Japan funded project entitled "Development of Abiotic Stress Tolerant Crops by DREB Genes" entered into its second year. Our goal in this project is to evaluate gene technology in upland rice by transgenic approach under water-limited conditions and to select superior lines under normal as well as drought conditions. Our focus was to establish a medium-throughput rice transformation pipeline and drought phenotyping platform. To establish the pipeline and platform, laboratory information management system (LIMS) was fully implemented and an automated rainout shelter was designed and built.

These enhanced our capacity for data management as well as phenotyping for drought. As to the biosafety concern, Colombian biosafety committees issued a generic permit for rice and cassava for field testing at the CIAT-Palmira station in March of 2008. Standard Operating Procedures (SOP) was prepared and is being implemented to fulfill our responsibility for transgenic research. To enhance

NARS capacity of transgenic research, MOU was signed between CIAT and Fedearroz to conduct transgenic research.

Future Activities

- Establishing screening protocol for drought tolerance at seedling stage and at flowering stage in confined field.

- Drought screening of single copy homozygous events of Curinga transformed separately with different gene constructs.

Output 2: Integrated crop, pest and disease management

2A RICE PATHOLOGY

2A.1. Molecular Detection of the *Pi-1*(t) Rice Blast (*Pyricularia grisea*) Resistance Gene in Advanced Lines of Rice (*Oryza sativa* L) Phenotipically Selected Under Field Conditions

L. Gil, J. G. Gallego, J. Tohme, M. Lorieux, F. Correa, G. Prado, C. Martínez, G. Aricapa, M.C. Duque.

Source of funding: CIAT Core and AgroSalud, CIDA

Abstract

Rice blast caused by *Pyricularia grisea* is one of the most devastating diseases affecting crop production and is worldwide distributed. One of the most efficient strategies to control the disease is pyramiding more than one resistant gene in the same variety. CIAT has developed an advanced population BC2F6 which was evaluated phenotipically under field conditions from crosses between isogenic line CT13432-107 containing the resistance genes *Pi-1*(t), *Pi-2*(t), and *Pi-33*(t) and Fedearroz 50 containing *Pi-2*(t), *Pi-33*(t), *Pi-z*, *Pi-zt*, *Pi-ta2*, *Pi-sh*, *Pi-k* and *Pi-b* resistance genes. The purpose of these crosses was the introduction of Pi-1 gene into Fedearroz 50 background. In this study a set of SSRs molecular markers were evaluated for its association with *Pi-1*(t) resistance gene. A BC2F6 population was evaluated first for its phenotypical reaction against 14 isolates of *P. grisea* under greenhouse conditions.

Data showed that SSRs are able to identify lines containing markers associated to Pi-1 gene. However, many lines in which markers associated to Pi-1 were found to show different degrees of resistance, suggesting the need for the development of functional markers that could detect more accurately the presence of the gene. To see if there is a phenotypic-genotypic relationship, a statistical test was applied to QTLs ANOVA1 analysis which evaluated the interaction between major and minor (QTLs) resistance genes in the population challenged with 14 pathogen isolates. In addition, variation on the hypersensitive response imparted by major gene Pi-33 was found. This suggests possible gene interaction between the major gene and other minor genes to respond to a given pathogen isolate. This type of response has not been described before and could be interesting to do more research in this matter.

Introduction

Rice blast caused by *Pyricularia grisea* (Cook) Sacc, anamorph stage *Magnaporthe grisea*, is the major disease affecting rice. The most efficient way to control the disease is the use of resistant varieties. This is the continuous effort of plant breeding programs. One successful strategy to face the high variability of the pathogen is the deployment of plant materials containing the resistance genes that control the infection of the most frequent combination of avirulence genes on the pathogen population. This strategy is known as lineage exclusion. This can be accomplished by means of gene pyramiding and results in a broad range and durable resistance (Correa-Victoria et al., 2002).

The resistance gene *Pi-1*(t), identified on LAC23 variety, confers complete resistance to a big number of isolates from Latin America when is combined with other resistance genes as *Pi-2*(t) and *Pi-33*(t) (Correa-Victoria., 2002). Independently, *Pi-1* (t) confers resistance to pathogen isolates belonging to lineage SRL-4, whereas Pi-2(t) and *Pi-33*(t) confer resistance to lineages SRL-5 and SRL-6. From mapping analysis, it has been shown that Pi-1(t) is located on chromosome 11 on the rice genome and close to Npb181 (3.5 cM) and RZ536 (14.0 cM) markers.

CIAT rice program is implementing a breeding plan using marker-assisted selection. The goal is to pyramid *Pi-1(t)*, *Pi-2(t)* and *Pi-33(t)*, to develop highly resistant materials that could be used to control the disease in Latin America. Here, we describe the results obtained in the evaluation of molecular markers associated to the Pi-1(t) resistance gene in a population consisting of 276 lines. These individuals were obtained from crosses between a CT-13432-107 isogenic line containing (*Pi-1(t)*, *Pi-2(t)* and *Pi-33(t)*) genes, and Fedearroz 50 which has a broad set of resistance genes but *Pi-1(t)*.

Materials and Methods

Plant Material

The methodology used in this study is divided into two stages: First, phenotypic evaluation of plant materials; and second their molecular characterization. The second one was carried out by using microsatellites molecular markers.

Lines subject of study were 1,300 BC2F6 lines coming from crosses CT13432 isogenic line X Fedearroz50 (figure 1). This population was selected as resistant under field conditions in Santa Rosa (Meta) experimental station where pathogen inoculum pressure and variability are very high.

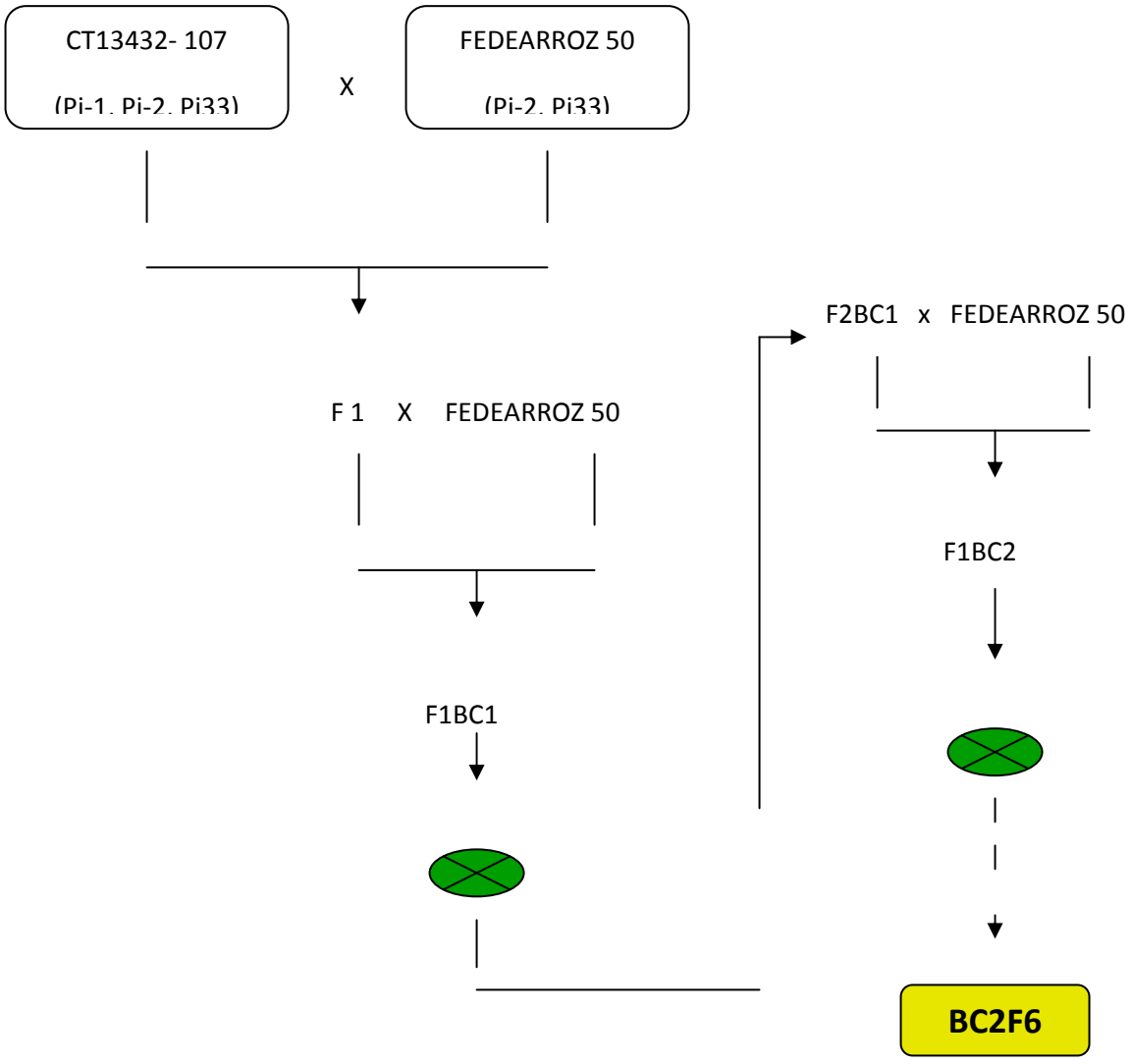


Figure 1. Description of crossing processes developed to obtain BC2F6 population.

Fungal strains and Inoculation assay

Fourteen *P. grisea* isolates were selected as the most virulent of each lineage. Some of them were completely unknown in their reaction against the tested population. Parental lines and lines related to *Pi-1*(t) gene were also included (Table 1).

Table 1. *Pyricularia grisea* isolates used in this study.

No.	Isolate	Avirulence Gene	Lineage
1	Fanny 54	Pi 33	SRL – 6
2	Selecta 320 (1)	Unknown	SRL – 6
3	O. Yacú 9 (19-1)	Pi 1	SRL – 6
4	Isolinea 6 (7-1)	Pi 2	SRL – 5
5	Isolinea 22 (3-1)	Pi 2	SRL – 5
6	Oryzica Caribe 8 (17)	Unknown	SRL – 4
7	Metica 1 (33-18)	Unknown	SRL – 3
8	Oryzica Llanos 5 (237-2)	Unknown	SRL – 2
9	Cica 9 (151-1)	Unknown	SRL – 2
10	Cica 9 (52-1)	Unknown	SRL – 1
11	Cica 9 (15)	Unknown	SRL – 1
12	CT13432 – 107 (25-1)	Unknown	Unknown
13	75-1-127 (7)	Unknown	Unknown
14	FEDEARROZ 50 (76-1)	Unknown	Unknown

Rice seeds were sown on 5-inches diameter pots (10seeds /pot) using sterile soil. Each experiment was repeated twice, and each replication was treated as an experiment. Twenty plants per line were evaluated with each isolate. Susceptible cultivar Fanny and isolines carrying *Pi-1* (t), *Pi-2*(t), and *Pi-33*(t) were used as controls.

Twenty days old plants were inoculated and arrange into plastic chambers, 18 pots per chamber, and infected with a 5 x 10^5 spores/ml suspension. Chambers

were sealed after inoculation to maintain relative humidity >80% which favors infection process. Evaluation was done 8 days after inoculation.

Each line was evaluated for lesion type (LT) and percentage of foliar area affected (FAA). For LT a visual scale from 0 to 4 was used, including intermediate values like 2, 3 and 3, 4. For FAA the scale range was from 0 to 100% (Table 2)

Table 2. Evaluation scale used on materials inoculated with *P. grisea*. HR, highly resistant; R, resistant; I, intermediate; S, susceptible; HS, highly susceptible.

LT	% FAA	Reaction
1	> 0	HR
2	≤ 10	HR
2	> 10	R
2,3	≤ 10	R
2,3	> 10	I
3	≤ 8	I
3	> 8	S
3,4	≤ 8	S
3,4	> 8	HS
4	≤ 5	S
4	> 5	HS

Plant Molecular Characterization

Rice materials were sawn (10 seeds/well) on 96-wells trays containing sterile soil. After 30 days of germination, leaves were collected for DNA extraction.

DNA was extracted using the method described by Lorieux *et al.* (2002). Quality and quantity of DNA was assessed by agarose gels and later on diluted to 4 ng/ul and stored at 4°C.

Selection of polymorphic markers for *Pi-1*(t) gene was carried out over parental and isogenic lines. A set of 12 SSRs markers was selected from GRAMENE data base (www.gramene.org). This selection took into account results reported by Fuentes *et al.* (2007) and also location inside of 1Mb range (Figure 2).

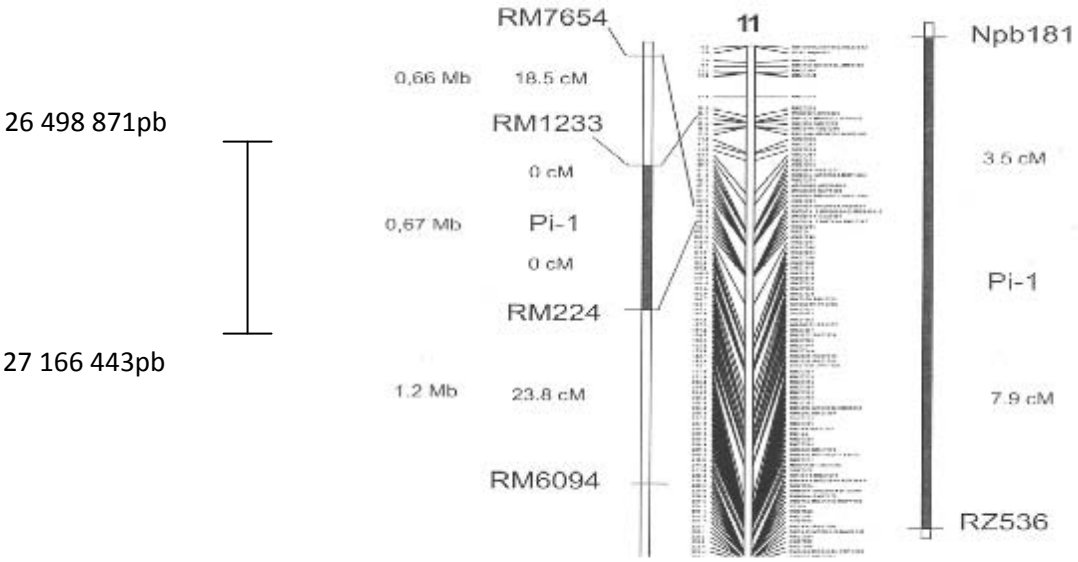


Figure 2. Physical map of a section of chromosome 11 showing SSRs RM1233 and RM224 markers associated with Pi-1(t) gene in a range of 1Mb.

Conditions for each marker were standardized using gradient profiles on 96-wells plates using a PTC – 225 DNA Engine Tetrad cycler, with six temperature ranges. Amplification was performed using the following profile: Initial step of 3 min. at 94°C; followed of 30 cycles of 3 secs. At 94°C; 45 secs, at 50°C to 61°C depending of each pair of primers; 1 min. at 72°C; and a final extension step of 5 min. at 72°C.

PCR products were analyzed on 4% polyacrilamide denaturing gels using Sequi Gen (BIO-RAD) electrophoresis equipment and silver stained according to Basam *et al.* (1991) protocol. Plant population was evaluated using RM 1233, RM 224 y RM 27273 SSRs polymorphic markers.

For this study evaluation scale was simplified by using only 3 categories (Table 3). This new scale did not include intermediate values but the higher number for 2, 3 and 3, 4 values is only taken into account. In this way the scale of qualitative evaluation will show only R, I, and S ranges. For quantitative evaluation was performed using LT and FAA percentage.

Table 3. Evaluation scale used to screen plant population.

LT	% FAA	Reaction
1	> 0	R
2	≤ > 10	R
3	≤ 8	I
3	>8	S
4	≤ > 5	S

Multiple correspondence analysis (MCA) was run over these data, which allowed the spatial positioning of individuals according to their response to the pathogen.

For molecular analysis, haplotype combinations found in the population were clustered and the frequency of them was registered. Tridimensional chart was used to represent all the individuals according to their molecular profile after the MCA to evaluate the biological correlation of the results.

Correlation between genotypes and phenotypic variation was established using variance analysis from MapDisto v.1.7 (Lorieux 2007) (http://mapdisto.free.fr) package used for QTLs analysis.

Results and discussion

A population obtained from crosses between CT13432-107 isogenic line (*Pi-1*(t), *Pi-2*(t), and *Pi-33*(t)) and Fedearroz 50 (*Pi-2(t)*, *Pi-33*(t) *Pi-z*, *Pi-zt*, *Pi-ta2*, *Pi-sh*, *Pi-k* and Pi-b) was evaluated using phenotypic data. The objective of this analysis was the introgression of *Pi-1*(t) gene into Fedearroz 50 cultivar. Response frequency of 276 lines inoculated with different isolates is shown in Table 4.

As expected, this population showed high levels of resistance, only isolates 12 and 14 showed I and S reaction (Table 4). All 276 lines showed full resistance to 5 isolates belonging to SRL-5, SRL-3, SRL-2, and SRL-1 *P. grisea* lineages. Interestingly, the population also showed lines with high frequency of resistance to other 6 isolates, except for few intermediate and susceptible cases (Table 4). These six isolates represent SRL-6 and SRL-4, which together with SRL-5 and SRL-2, are reported as the most frequent lineages in Colombia Correa *et al.* (2002).

These results confirm the predictions of broad expectrum resistance when a pyramiding strategy is used (Zeigler *et al.* 1994). One the other hand, the fact that some lines showed to be susceptible to isolates 12 and 14 confirm not only their hypervirulence but also their low frequency in the field. This can be explain by the hosts that they were isolated, CT13432-107 (*Pi-1(t)*, *Pi-2(t)*, *Pi-33(t)*) and Fedearroz 50 which have 8 known major resistance genes. It seems that the use of materials with diverse set of resistance genes promotes the mutation of the correspondent avirulence genes on the pathogen. The accumulation of these mutations also affects the fitness of these strains in the field.

The observation of susceptible lines in this screening also can be explained by the method that was used to select these lines in the first place. In our case field data was used. This fact contributes to the selection of lines as resistant to isolates that are present in the field in very low frequency. That seems to be the case of isolates 12 and 14. Thus, when the same screening is carried out under control conditions and using pure cultures of these isolates these lines can turn susceptible.

Table 4. Reaction frequencies observed in 276 lines against different isolates.

No.	Isolates	Avr Gene	Observed Frequency R	Observed Frequency I	Observed Frequency S
1	Fanny 54	Pi 33	259	**17**	
2	Selecta 320 (1)	?	273	2	**1**
3	O. Yacú 9 (19-1)	Pi-1	274	1	**1**
4	Isolinea 6 (7-1)	Pi-2	276		
5	Isolinea 22 (3-1)	Pi-2	275	1	
6	Oryzica Caribe 8 (17)	?	275	1	
7	Metica 1 (33-18)	?	276		
8	**Oryzica Llanos 5 (237-2)**	**?**	**228**	**47**	**1**
9	Cica 9 (151-1)	?	273	3	
10	Cica 9 (52-1)	?	276		
11	Cica 9 (15)	?	276		
12	**CT13432 – 107 (25-1)**	**?**	**41**	**221**	**14**
13	75-1-127 (7)	?	276		
14	**FEDEARROZ 50 (76-1)**	**?**	**83**	**136**	**57**

Overall, control plants behaved as expected. In contrast, isogenic line CT13432-54 which is predicted to contain *Pi-2*(t) resistance gene not only showed resistance to *Avr-Pi-2* isolates (4 and 5) but also to an Avr-Pi-1 isolate (3). The same line also showed resistance to the rest of isolates but *Avr-Pi-33* isolate (1) and isolate 12 with unknown Avr gene (Table 5). Isoline C101A51, predicted to have *Pi-2*(t), showed to be resistant to *Avr-Pi-2* isolates (4 and 5) as was expected, but surprisingly also showed resistance reaction to isolates 7 and 13 with unknown Avr genes. With the remaining isolates I and S reactions were observed (Table 5). Based on data obtained with isolate 3, having *Avr-Pi-1* gene, we could speculate that isoline CT13432-54 also has *Pi-1*(t) resistance gene.

Table 5. Evaluation of reaction of isogenic and parental lines after infection with 14 isolates

Line	Code	Genotype	Isolate													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
C103TTP (Pi-1)	292	. . . . . .	S	R	R	S	S	R	R	R	R	R	R	S	S	R
CT13432-68 (Pi-1)	285	. . . . . .	S	R	R	S	S	R	R	R	R	I	R	S	S	R
C101LAC (Pi-1, Pi-33)	283	135 135 134 134 147 147	R	R	R	S	S	R	R	R	I	R	R	I	R	R
C104LAC (Pi-1)	291	135 135 134 134 147 147	S	R	R	S	S	R	R	R	R	R	R	S	S	R
CT13432-107 (Pi-1, Pi-2, Pi-	278	135 135 134 134	R	R	R	R	R	R	R	R	R	R	R	R	R	I

33)		147 147														
CT13432-54 (Pi-2 / Pi-1)	286	135 135 134 134 147 147	S	R	R	R	R	R	R	R	R	R	R	S	I	R
C101A51 (Pi-2)	282	135 135 134 134 147 147	I	I	S	R	R	I	R	S	S	S	S	I	R	S
CT13432-267 (Pi-2)	294	147 147 140 140 165 165	S	S	S	R	R	S	R	S	S	S	S	S	R	S
Fedearroz 50	277	155 155 140 140 153 153	R	R	R	R	R	R	R	R	R	R	R	I	R	I
O.Llanos 5	287	155 155 142 142 153 153	R	R	S	R	R	I	R	R	R	R	R	R	R	S

Molecular analysis could detect only one polymorphic marker RM27273 in parental lines and related controls. That means that analyzed genomic region is highly conserved and more molecular markers should be evaluated to be able to discriminate the differences in this region. The RM27273 marker is located between two SSRs markers at a distance of 0 cM reported by Fuentes *et al.* (2007) (Table 6).

This study contributed to the refinement of the set of molecular markers linked to Pi-1 gene that was previously described by Fuentes *et al.* (2007). These results will help in the marker-assisted selection for *Pi-1*(t) gene. Population evaluation for the three described markers RM224, RM27273, and RM1233 showed a homogeneous heterocigocity frequency pattern among them (Table 6).

Table 6. Percentage of heterocigocity obtained for each of the four analyzed markers.

Locus	Chromosome Position (bp)	Recurrent Parental		Donor Parental		Observed Heterocigocity	
		%	k	%	k	%	K
224*	27 166 443	63.03	179	21.83	62	4.58	13
27273	26 808 963	63.73	181	21.48	61	5.99	17
1233*	26 498 871	72.18	205	7.04	20	15.49	44

*SSRs markers suggested by Fuentes *et al.* (2007) to be implemented in a marker-assisted selection program of a population generated from two isoline crosses.

K = frequencies

Every selected marker showed a different locus associated to *Pi-1*(t) gene ranging from 135 to 147bp (Table 7).

Table 7. *Pi-1* associated alleles found by evaluation of each marker.

Locus	Molecular Marker SSR	Associated allele
L1	RM 224	135 bp
L2	RM 27273	134 bp
L3	RM 1233	147 bp

Obtained allelic profiles allowed us to build haplotype combinations which were used to assign genotypes associated to *Pi-1*(t) gene. In this way five different genotypes were found, one of them represented all the alleles for this gene and 4 of them showed to be heterozygous (Table 8).

Table 8. Genotypes found on 276 lines representing at least one allele associated to *Pi-1*(t).

GENOTYPES	#	L1 a1	L1 a2	L2 a1	L2 a2	L3 a1	L3 a2	K
	1	135	135	134	134	147	147	11
	2	135	135	134	134	**147**	**153**	21
	3	135	135	**134**	**140**	147	147	3
	4	**135**	**155**	134	134	**147**	**153**	1
	5	**135**	**155**	**134**	**140**	**147**	**153**	2
K = frequency						**TOTAL**		**38**

L = locus per marker

Multiple correspondence analysis (MCA) showed 4 groups derived from the R^2 calculation where 0.96 values were considered as significant. Evaluated data explained 96% of the clustering.

MCA of all alleles present in 276 lines, including control lines, allowed to establish the most over-represented alleles on the population. As an example, 135bp allele is present on 30.35% of the population, whereas 134bp and 147bp were found in 74.32 and 75.1% of the population, respectively. Alleles coming from Fedearroz 50 showed to be highly represented in the population. Thus, 155bp appeared on 74.32% of the lines, 140bp on 31.13%, and 153bp on 75.1% of the lines. Results obtained from MCA show that SSRs molecular markers designed for *Pi-1*(t) detection make possible the identification of lines that carry alleles associated to this gene. However, in spite of their allelic combination associated to *Pi-1*(t), they were also present on lines showing I and S reactions.

Uniform population used for this study requires a sensitive and accurate molecular method using functional markers as SNPs. These markers facilitate the prediction of resistant genotypes during phenotypic selection being useful when resistance is imparted by several genes at the same time Ingvardsen *et al.* 2008).

In order to determine associations between each evaluated locus and obtained phenotypes in each experiment, variance analysis (single criteria F test) was run taking analyzed marker as criteria.

Variance analysis showed that studied population had statistically meaningful differences to the response to the isolates in different experiments (F >15 for 4 out of 14). Where experiments represent two inoculation repetitions as described on materials and methods.

Mean values for genotypic groups (Fedearroz homozygous, CT13432-107 homozygous, and heterozygous) were scored for meaningful isolates. The means were calculated from the phenotypic scale of lesion type (1 and 2 = R; 3 = I; 4 = S). Values from 0 to 20 denote resistance, 20 to 30 intermediate, and ≥30 values denote susceptibility.

Obtained data from 3 evaluated loci showed that lines homozygous for Fedearroz 50 were over-represented on the population compared with CT13432-107 homozygous. Heterozygous genotype was the less frequent (table 9) what is expected for a BCF6 population that has been phenotipically selected for its retention of Fedearoz 50 genetic background.

Table 9. Frequency of lines with Fedearroz 50 homozygous, CT13432-107 homozygous and heterozygous genotypes for 4 isolates, considering 3 evaluated markers.

Isolate	SSR	No. Homozygous Fedearroz	No. Homozygous CT13432-107	No. Heterozygous
Fanny 54	RM224	177	60	14
	RM27273	178	59	17
	RM1233	203	18	44
O. Llanos 5	RM224	177	60	14
	RM27273	178	59	17
	RM1233	203	18	44
CT13432-107 (25-1)	RM224	177	60	14
	RM27273	178	59	17
	RM1233	203	18	44
Fedearroz 50 (76-1)	RM224	177	60	14
	RM27273	178	59	17
	RM1233	203	18	44

Averages of phenotypic reactions showed variations among Fedearroz 50 homozygous, CT13432-107 homozygous and heterozygous genotypes against used isolates. Frequency profiles were similar for all three loci, for that reason we focused only on the result obtained for the first locus with each isolate (Table 10).

In the second experiment with Fanny 54 isolate, obtained averages corresponded to a resistance reaction with variations in lesion type. Dominance of CT13432-107 compared to Fedearroz 50 alleles was also observed because heterozygous showed similar values to CT13432-107 homozygous.

The average for lesion type for the first locus with Fedearroz 50 associated alleles was 12.93, which suggests a resistance reaction with lesion type 1; lines with CT13432-107 associated alleles had an average of 19.583 which suggests a resistance reaction with lesion type 2; heterozygous showed average similar to CT13432-107 (20.07) which suggests a dominant relationship previously mentioned.

Isolate Fanny 54 (Avr-Pi33) showed variations inside a resistance reaction. These results can be produce by plant responses modulated by a QTL that could be nearby of Pi-1(t) gene. However, effect of QTLs over a major resistance gene function is not known yet. This interaction could be subject for further studies to elucidate the effect of QTL-related genes on the gene-for-gene interaction.

Table 10. F test to determine genotype/phenotype correlation from averages obtained for lesion type (LT) and alleles for each tested locus.

* F meaningful.

SSR	Promedio TL homocigotos Fedearroz50	Promedio TL homocigotos CT13432-107	Promedio TL Heterocigotos	F	R2	A	D
			Fanny 54 - Experimento 2				
RM224	12.93	19.58	20.07	20.43	0.14	3.326	3.81366
RM27273	12.90	19.54	20.24	21.17	0.14	3.322	4.01467
RM1233	13.58	21.11	20.05	19.21	0.13	3.767	2.70172
			O. Llanos 5 - Experimento 1				
RM224	18.19	3.53	13.07	54.03	0.30	-7.329	2.20872
RM27273	17.92	3.80	12.18	48.07	0.28	-7.060	1.32030
RM1233	16.97	5.33	7.23	23.89	0.15	-5.816	-3.92215
			CT13432-107 (25-1) - Experimento 2				
RM224	26.33	29.567	28.57	14.67	0.11	1.617	0.62143
RM27273	26.39	29.576	28.82	14.74	0.11	1.594	0.84157
RM1233	26.69	29.056	29.75	11.74	0.08	1.180	1.87493
			Fedearroz 50 (76-1) - Experimento 2				
RM224	28.80	6.500	16.86	242.83 *	0.66	-11.151	-0.79399
RM27273	28.48	6.712	16.71	199.95	0.61	-10.883	-0.88881
RM1233	26.33	10.667	13.77	43.31	0.25	-7.834	-4.72809

Observe partial resistance of -107 (25-1) and Fedearroz 50 (76-1) against isolate O. Llanos 5 (237-2) can be explained by a hypothetical specific partial resistance. This would involve the action of major genes for specificity, and QTLs for partial resistance.

Evaluation of specificity of a partial resistance was first described by Wang *et al.* (1994), who evaluated isolates establishing compatible interactions. Later on, Zahirul *et al.* (2004) studied the specific interaction in partial resistance by using 3 isolates establishing compatible interactions with the host. From the first study was concluded that several loci that conferred partial resistance were not specific, and in the second study was concluded that partial resistance was due to specific interaction effects.

Population response involving major and minor genes can be explained under two possible scenarios; inespecific partial resistance can be due to interactions of one or more QTLs with NBS-LRR genes (Zahirul *et al.* 2004). Clusters of putative resistance genes have been reported along chromosome 11 (Wang *et al.* 2001). López-Gerena (2006) also reported 27 NBS-LRR candidates mapping between 831 137pb and 28 284 056pb position of chromosome 11, when O. Llanos 5 was evaluated against FL440 isolate. Evaluated region for Pi-1(t) is located between 26 498 871pb and 27 166 443 position on the same chromosome, suggesting that

this population can contain considerable number of NBS-LRR genes that interact to confer inespecific resistance. On the other hand, specific partial resistance can be due to a combination of all major genes contained by Fedearroz 50.

Experiment 1 with O. Llanos 5 (237-2) isolate and lines with Fedearroz 50 associated alleles showed resistance with lesion type 2. Heterozygous showed resistance with lesion type 1, suggesting additive effect of Fedearroz 50 alleles with the ones coming from CT13432-107. Fedearroz 50 and O. Llanos 5 share the same resistance genes Pi-k and Pi-sh closely linked to Pi-1(t) (López-Gerena, 2006) and O. Llanos 5 isolate should have Avr genes that are not recognized by this cultivar. We can speculate that obtained resistant lines could be originated by the interaction of NBS-LRR domains of genes linked to Pi-1(t), coming from Fedearroz 50, and their correspondent Avr genes present on O. llanos 5 isolate.

Intermediate or partial resistance could be generated by interaction of QTLs present on Fedearroz 50 that might work in a polygenic or inespecific fashion. Moreover, specificity of Avr genes present on O. Llanos 5 is unknown; it can be also hypothesized that these genes can be interacting with Fedearroz 50 major genes (Zahirul *et al.* 2004).

Experiment 2 with CT13432-107 (25-1) isolate showed a dominant response of CT13432-107 genotype over Fedearroz 50. Phenotypic average reaction for CT13432-107 was 29.567 and for Fedearroz 50 was 26.33. Heterozygous showed an average of 28.57 pointing out CT13432-107 dominance. Intermediate reaction frequencies were high (221 plants) as is shown in Table 4, indicating a prevalence of partial resistance in the interaction between the pathogen and its host. Previous studies put on evidence that *Pi-1(t)*, *Pi-2(t)* and *Pi-33(t)* resistance genes were defeated by CT13432-107 (25-1) isolate. This is explained by mutation on AVR genes making them undetectable by the plant resistance machinery.

This fact suggests a residual effect of major resistance genes present on the population turned into a specific partial resistance as suggested by (Hu *et al.* 1997; Liu *et al.* 2002; Wang *et al.* 2004).

Experiment 2 with Fedearroz 50 (76-1) isolate showed a strongly resistance reaction with CT13432-107 homozygous, whereas Fedearroz 50 homozygous showed an intermediate reaction. Heterozygous exhibited additive effect of CT13432-107 alleles with those present on Fedearroz 50. F value for locus 1 was significantly high (242.84) which suggest a strong effect of a QTL that could be *Pi-1*(t) by itself. Speculations like Fedearroz 50 (76-1) isolate could contain Avr-Pi-1 can arise from this result.

Population lines where total resistance with no variation on hypersensitive response was observed are not candidates for interaction between *Pi-1*(t) linked

genes or QTLs using the same isolates (Table 4). Their resistance reaction should be due to the presence of *Avr-Pi-2* and *Avr-Pi-33* in used isolates, and that can be recognized by *Pi-2* (t) and *Pi-33*(t) inherited from Fedearroz 50 parental line. This adds up by the fact that isolate 4 and 5, which have *Avr-Pi-2*, were avirulent on the population.

Resistance to most of the isolates was given by major resistance genes coming from Fedearroz 50. However, genes linked to *Pi-1*(t) can be interacting to interfere with the pathogen infection process.

Considering the results obtained from Multiple Correspondence Analysis for molecular data and genotype/phenotype correlation is important to notice that introgression of gene of interest can be only guaranteed if a marker-assisted selection strategy is used. This tool based on markers linked to the target gene is not influenced by environmental conditions or pressure selection that allows its use on different developmental stages or type of tissue. Screening would be faster decreasing the time necessary for new cultivars development (Ingvardsen *et al.* 2008).

Recombination can occur between the marker and the gene of interest which could bias the selection when molecular markers are used. This can be solved by the use of phenotypic marker (Andersen and Lübberstedt 2003; Ingvardsen *et al.* 2008). However, gene sequence should be available to allow the identification of polymorphisms associated with the trait. In our case, phenotypic markers were not used because *Pi-1*(t) is not cloned yet.

Conclusions

SSRs molecular markers designed for the *Pi-1*(t) resistance gene detection allowed the identification of rice lines that contain alleles in common and are associated to the gene of interest, but did not detect specific haplotypes associated to resistance trait.

Lines proportion with genotypes associated to Fedearroz 50 (recurrent parental) was higher than the ones observed for CT13432-107 (donor parental) as expected for a BC2F6 population.

Intermediate reaction observed on some lines challenged with *P. grisea* isolates suggests a potential inespecific interaction between QTL's and major resistance genes that conserve some specificity against mutant *avr* genes.

Pathogenicity studies done by Rice Pathology Laboratory have demonstrated the effectiveness of gene pyramiding strategy, specifically *Pi-1(t)*, *Pi-2(t)* y *Pi-33(t)*, to

control infection by *P. grisea*. But is necessary a detailed characterization of isolates that are recognized by *Pi-1*(t) gene and could support the results obtained from molecular detection analyses.

Recommendations

Detection of *Pi-1*(t) in the studied population requires the use of functional markers (SNPs) that permit the detection of haplotypes specific for *Pi-1*(t) inside the population.

Any breeding program aimed to gene pyramiding for resistance should be supported by marked-assisted selection tools in order to guide the selection process for the introgression of a gene of interest on a desired cultivar.

References

Bassam, B.J. *et al*. 1991. Fast and sentitive silver staning of DNA in polyacrilamide gels. Anal. Biochem. 196**:** 80-83.

Correa, F., Tharreau, C.P., Martínez, C.P., Valdes, G., Prado, G. 2002. Identification of molecular markers associated with the blast resistance genes Pi 1, Pi 2 y Pi 33 and their incorporation into commercial rice varieties through h backcrossing and marker assisted selection (MAS). Annual Report. Project IP-4: Improve rice germplasm for Latin America and the Caribbean. CIAT. 78-79.

Fuentes, J.L, Correa-Victoria, J.F., Escobar, F., Prado, G., Aricada, G., Duque, M.C., Tohme, J. 2007. Identification of microsatellite linked to the blast resistance gene Pi-1 (t) in rice. Euphytica 160: 295-304.

Hu, G., Webb, C.A., Hulbert, S.H. 1997. Adult-plant phenotype of the *Rp1-DJ* compound rust resistance gene in maize. Phytopathology 87:236-241.

Ingvardsen, C. R., Schejbel, B. and Lübberstedt. 2008. Functional markers in resistance breeding. Progress in Botany 69 : 61 – 87.

Liu, G., Lu, G., Wang, G.L. 2002. Two broad spectrum blast resistance genes Pi-9(t) and Pi-2(t) are physically linked on rice chromosome 6. Mol Genet Genomics 267:472-480.

López-Gerena, J. 2006. Mapping QTL controlling durable resistance to rice blast in the cultivar Oryzica Llanos 5. Kansas State University. Department of Pathology College of Agriculture. Manhattan, Kansas. PhD thesis. 142p.

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/

Wang, G.L. MacKill, D.J., Bonman, J.M., McCouch, S.R., Champoux, M. C., Nelson, R.J. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistance rice cultivar. Genetics 136:1421-1434.

Zahirul, I., Talukder, Therreau, D. and Price, A.H. 2004. Quantitative trait loci analyses suggest that partial resistance to rice blast is mostly determined by race-specific interactions. New Phytologist 162:197-209.

Zeigler R. S., Tohme, J., Nelson, R., Levy, M and Correa-Victoria, F. J. 1994. Lineage Exclusion: A Proposal for Linking Blast Population Analysis to Resistance Breeding. Rice Blast. IRRI. P 267-292.

Identification of Rice Lines with Tolerance Response to *Rhizoctonia solani*, Causal Agent of Sheath Blight

G. Prado, G. Aricapa, E. Torres, M. Agredo

Introduction

Rice sheath blight is produced by the fungus *Rhizoctonia solani* (Telemrph: *Tanatephorus cucumeris*) is becoming a major problem on rice in USA and Asia for the last 15 years. This problem is also affecting rice fields in Latin America where the *R. solani* AG-1 IA specie is the most common whereas *R. oryzae* is found mainly in temperate zone of South America. Disease incidence and severity is in continuous increase what has made fungicide application the control measure of choice. This implies a negative impact for the environment. In 2000 the rice program of CIAT implemented an inoculation and evaluation method under greenhouse conditions. This methodology allows selecting for materials that could tolerate the disease. Evaluated material includes Asian and United States cultivars that have shown tolerance levels, CIAT and FLAR advanced lines, commercial varieties from Colombia and Venezuela, and local varieties from Colombia.

Materials and Methods

Rice materials used in this study include: Five commercial varieties from USA, 17 lines from CIAT, 21 lines from FLAR, 8 local varieties of Colombia, 2 commercial varieties from Brazil, 3 commercial varieties from Venezuela, and 5 commercial varieties from Colombia.

Response of each rice material to *R. solani* was evaluated by inoculating a highly pathogenic isolate from the fungal collection of CIAT Rice Pathology Program.

Each inoculation event was performed on 5 plants grown in 6 inches pots and repeated 4 times through the time. After inoculation pots were distributed randomly on a growing chamber under optimal conditions of humidity and temperature for fungal infection. Plants were evaluated 10 days after inoculation for percentage of the main tiller affected by the disease over the total high of the plant. Score for each plant material was calculated by the mean of four measures obtained from each replication. Materials showing less than 50% of total area affected were scored as tolerant compared to the susceptible and tolerant controls

evaluated at the same time. Different values were assign to affected leaves and stem as follows: first leaf 30%; second leaf 15%; third leaf 15%; fourth leaf 10%; fifth leaf 10%; and sheath 20%. Susceptible and controls were always included as reference material.

Results

The use of screening methodology allows us to identify different responses type of rice material against *R. solani* infection under greenhouse conditions. The susceptible control Lemont showed reproducibility in our evaluations; on the other hand Araure and Palmar, from Venezuela, keep showing tolerance through the time. Interestingly, two lines from CIAT shown to be more susceptible than the control Lemont. Besides, Tres Marias variety from Brazil, which is interesting for its resistance to *Pyricularia grisea*, also showed high level of tolerance to *R. solani*.

In addition to all the materials previously described, local material like Ligerito Playero, Fedearroz 174, two lines from FLAR and one from CIAT (shown on table 1) should be further evaluated under field conditions to confirm their response to *R. solani* obtained on greenhouse.

Future activities

Greenhouse screening for sheath blight response should be maintained because it helps to narrow down the number of materials that should be evaluated on the field. In this way the experimental design can be more robust because this pathogen does not spread evenly on the ground. This is a major concern when the planting area is very big since low concentration or absence of pathogen can generate inaccurate germplasm characterization.

Due to the good performance of Tres Marias variety against sheath blight, this material could be used in genetic studies to characterize its tolerance response and see how it is related with the resistance to *Pyricularia grisea*.

References

Correa-Victoria, F. 1992. Foro nacional de Rhizoctonia en Arroz, Bogotá, Abril 9-10 FEDEARROZ

Jia, Y., Correa-Victoria, F.J., McClung, A., Zhu, L., Wamisha, Y., Xie, J., Marchetti, M., Pinson, S., Rutger, N., and Correll, J. 2006. Rapad determination of rice cultivar

responses to the Sheat blight pathogen *Rhizoctonia solani* using micro-chamber screening method. Plant Disease. 91: 485-489

Prado, G.A., Correa, F., Aricapa, M.G., Escobar, F. 2001. Caracterización preliminar de la resistencia de germoplasma de arroz al añublo de la vaina (*Rhizoctonia solani* Kunh). FORO arrocero Latinoamericano. Vol. 7 (13): 8-11

Table 1. *R. solani* susceptible and tolerant cultivars selected under greenhouse conditions.

Cultivar	Plant Affected Area (%)
Tolerants	
Tres Marías	48
Ligerito Playero	47
FL00147-8P-6-15P-M	49
FL03187-16P-11-2P-3P-M-1P-M	51
Fedearroz 174	47
Highly susceptible	
CT17238-1-1-1-2-1-7-1	92
CT17238-1-1-1-2-4-3-3	97
Tolerant control	
Araure Tres	39
Palmar	49
Susceptible control	
Lemont	86

Improving the Molecular Detection Method of *Burkholderia glumae* on Rice Seeds

P. Fory, G. Prado and Mosquera. G (CIAT)

Source of funding: FONTAGRO, and CIAT CORE

Introduction

Burkholderia glumae is the causal agent of panicle rot on rice and also can produce rot on seedlings when contaminated seeds are used for planting. The bacteria invade plant tissue through natural openings like stomata and also injures. This disease has been widely reported in United States (Shahjahan et al., 2000 y Sayler et al., 2006), Japan, Korea and Panama (Nandakumar et al, 2007). The bacteria was reported in Colombia for the first time in 1989 (Zeigler and Alvarez, 1989), but is just in 2007 when typical disease symptoms were seen more frequently on field.

A detection method using the Polymerase Change Reaction (PCR) method was developed in Rice Pathology Laboratory and used to screen field infected material (Annual Report 2007). The methodology was standardized to detect the bacteria on rice seeds heavily infected with *B. glumae*. These seeds are completely empty and are not included as planting material. What is really a threat for disease dissemination are the apparently healthy seeds coming from infected panicles. It rises up some questions like can the bacteria be present in symptomless seeds; what is the bacterial population in these seeds; and finally, is the PCR technique able to detect the bacteria present in healthy seeds?

In order to report and transfer an accurate detection method to other laboratories in Latin America, we extended the research to refine the technique in terms of sensitivity and specificity.

Materials and Methods

Plant Material

Naturally infected material collected at Monteria (Colombia) was used in this study. Panicles of Fedearroz 50 variety showed typical disease symptoms like seed discoloration and most of them were empty.

Seed Processing

Material was processed as groups of 5 seeds and sorted according to location in the panicle (up and down) and grade of infection (empty and filled) (Figure 1). Samples were washed under running water for 30 minutes then rinsed with sterile destilled water. Washed seeds were ground under germ-free conditions using sterile mortars. The powder was resuspended into 5 ml of destilled water (1ml/seed) and incubated for 20 minutes at room temperature on a shaker.

Seed suspensions were serially diluted until 10^{-4} using sterile water. Stock solutions and their dilutions were subject of two different studies: first, bacterial counting using media plates and second, bacterial detection by PCR method. For bacterial counting 20ul of each dilution were plated on King B media with 3 replications. Plates were incubated at 28°C for 48 hours and colonies from each dilution were counted. For PCR analysis, five hundred microliters of each concentrated suspension and serial dilutions were denatured for 8 minutes at 95°C. Then seed debris was precipitated by spinning the samples at 10,00RPM for 3 seconds. Three microliters of the supernatant were used as template for PCR reaction using primers designed from the spacer ribosomal region of *B. glumae*.

Results and Discussion

Natural Bacterial Population on Seeds

Infected panicles where bacteria colonized under natural conditions were subject of study, first to investigate what is the number of bacteria present on naturally infected tissue, and second to correlate the sensitivity of a technique previously described for heavily infected samples. If bacteria affecting panicle are coming from foliar infection or infected seeds is unknown. Other interesting question is if the panicle gets infected during flowering stage the upper part of the panicle will get more disease than the lower part because of the emerging process. To have clues about this questions we decided to inspect seeds from symptomatic panicles for number of bacteria and if their concentration vary along the panicle (figure 1).

The bacterial population on empty seeds located on the upper part of the panicle was 4.4 $x10^4$ (CFU). On the other hand, the population on filled seeds taken from the same part of the panicle was 1.0 $x10^3$. In contrast, when empty and filled seeds from the bottom of the panicle were used, bacterial population was similar in both 10 x 10^3 and 6.4 x 10^3 (FCU) respectively.

Bacteria detection on naturally infected seeds

The PCR method previously described (Annual report 2007) showed a detection sensitivity of 100 bacteria per reaction when a pure culture of bacteria was used. The objective of the present study was to test if the same method is able to detect bacteria present in low concentration, which would be the case of filled seeds. For this purpose we used the same samples used for bacterial counting.

PCR analysis showed an expected band on both, empty and filled seeds (figure 2). All the samples coming from empty seeds showed amplification. This is expected because lack of filling could be due to the high concentration of bacteria in this tissue. Surprisingly, no amplification was obtained from concentrated suspension or 10^{-1} dilution of filled seeds (10^{o} and 10^{-1}fig. 2) even though it was observed on 10^{-2} dilution. This could be due to the presence of PCR inhibitors coming from the seed grain. This is not an issue in empty seed samples because there is no grain inside. The processed material in this case is just the hulk. Other analysis done on symptomless filled seeds show absence of PCR amplification (Data not shown). We are predicting that the number of bacteria present on this type of samples should be lower than 100 CFU that is the limit of detection. Apparently bacteria are more concentrated in the upper part of the panicle compared with the lower part that last in emerge. We do not know if this difference is meaningful or not because this is a preliminary result. If it is the case, we could speculate that the *B. glumae* infection affecting the panicle takes place during the flowering stage. Otherwise the bacterial population would be homogeneous along the panicle. Other possibility would be that the bacteria migrate along the plant tissue that germinates from an infected seed. If this is true, the plant response would be predicted to be heterogeneous along the panicle parts. This would explain the variation of symptoms and bacterial number inside the same panicle. More experiments are in progress to rule out these possibilities.

Taken together, these results demonstrate that the grade symptoms on rice seed infected by *B. glumae* are related with the number of bacteria. Filled seeds showed lower concentration of bacteria than empty seeds. Another important finding was that PCR method is able to detect the bacteria on filled seeds showing subtle symptoms but not in completely healthy seeds.

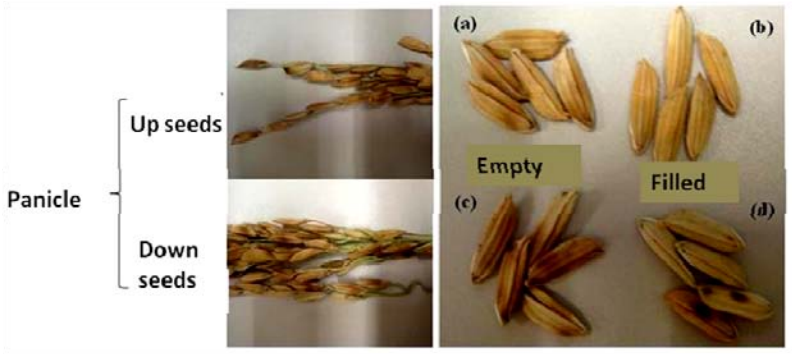


Figure 1. *B. glumae* Infected Panicle (Fed - 733). Up seeds, tip of the panicle. Down seeds, bottom of the panicle. a) Empty seeds from the tip. b) Filled seeds from the tip. c) Empty seeds from the bottom. d) Filled seeds from the bottom.

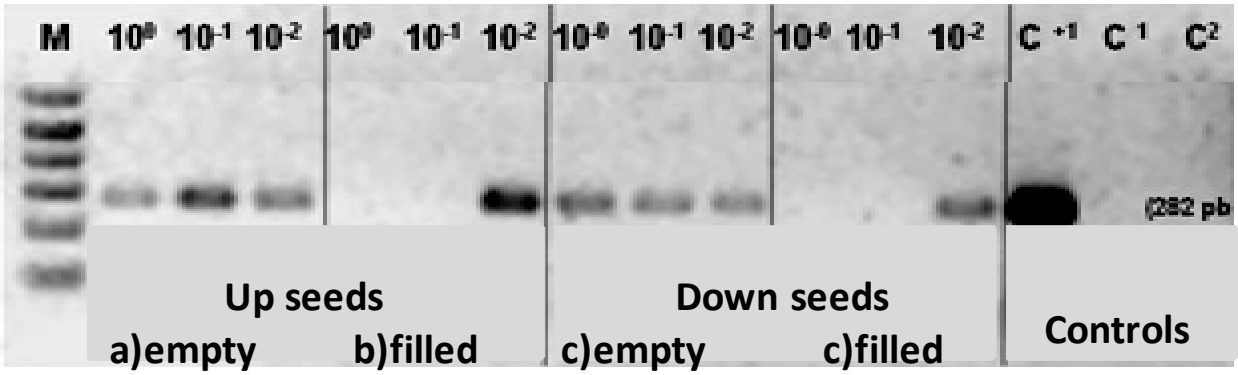


Figura 1. PCR detection of *B. glumae* infected seeds.

M, 1Kb ladder. Up seeds, tip of the panicle. Down seeds, bottom of the panicle. a) Empty seeds from the tip. b) Filled seeds from the tip. c) Empty seeds from the bottom. d) Filled seeds from the bottom. Controls, DNA of *B. glumae* , DNA from non related bacteria, and PCR reaction without DNA.

References

CIAT, 2007. Annual Report 2007 SBA-4: Rice. CIAT. Cali. Colombia.

King, E. O.; M. K. Ward; D. E. Raney: 1954. Two Simple Media for the Demonstration of Pyocianine and Fluorescin, *J. Lab. Clin. Med*. 44:301-307.

Nandakumar, R. Rush, M. C. Correa, F. 2007. Association of Burkholderia glumae and B. gladioli with Panicle Blight Symptoms on Rice in Panama. Plant Disease. 91:6.

Luo, J. G. Xie, B. Li, and X. Lihui. 2007. First Report of *Burkholderia glumae* Isolated from Symptomless Rice Seeds in China. Plant Disease. 91:1363.

Sayler, R.J., Cartwright, R.D., and Yang, Y. 2006. Genetic characterization and real-timePCR detection of *Burkholderia glumae,* a newly emerging bacterial pathogen of rice in the United States. Plant Disease 90: 603-610.

Shahjahan, A.K.M., M.C. Rush, D.E. Groth, and C.A. Clark. 2000. Panicle blight. Rice J. 103:26-28.

Zeigler, R. S., and Alvarez, E. 1989. Grain discoloration of rice caused by *Pseudomonas glumae* in Latin America. Plant Disease. 73:368.

Zhu Bo, LOU Miao-miao, Huai Yan, Xie Guan-lin, Luo Jin-yan and Xu Li-hui. 2008. Isolation and Identification of *Burkholderia glumae* from Symptomless Rice Seeds Rice Science, 2008, 15: 145–149.

2B RICE VIROLOGY

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

L.E. Romero, L. Jéhin, E. Torres, R. Perafan, C. P. Martinez and M. Lorieux

Partners:
IRD-UMR5096
Fedearroz

Project funded by:
The Colombian Ministry of Agriculture and Rural Development
Fedearroz
IRD

CIAT

Introduction

Resistance to Rice Hoja Blanca Virus (RHBV) is a difficult, laborious and expensive trait to study: (i) maintenance of colonies of its vector, *Tagosodes orizicolus*, is time-consuming and complex, (ii) evaluation of the resistance/tolerance of germplasm is highly dependent on the vector generation, number of insects per plants and other parameters that are difficult to control, including GxE interactions. This makes evaluations of the materials in the field very expensive, due in particular to GxE interactions, and the number of replications, needed to reach an acceptable statistical robustness.

We propose to use Molecular-Assisted Backcrossing (MAB), in order to speed up and facilitate the identification and selection of resistant candidates, and to increase the efficiency of the breeding process.

In a previous study carried out at CIAT by L. Calvert's team, three QTLs that contribute to RHBV resistance were identified, using two F2/F3 populations derived from the crosses between the highly susceptible line WC366 and the varieties Fedearroz 2000 (FD2000) and Fedearroz 50 (FD50) that show different levels of resistance to RHBV and *T. orizicolus* (Calvert et al 2006). The objective of the present project is to (i) better localize and estimate the resistance QTLs

parameters in both populations, (ii) introgress the RHBV resistance QTLs into two susceptible elite materials through MAB and (iii) fine map the most important QTLs in order to optimize future marker-based selection strategies.

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

Materials and methods

Several elite lines that are coming out from the FLAR and CIAT breeding programs are very promising for the Colombian rice sector due to their high yield, resistance to biotic and abiotic stresses and good grain quality. However, their susceptibility to RHBV discard them as final candidates for variety release. These lines are thus excellent candidates as genetic background for introgression of resistance QTLs to RHBV.

In order to select the best two elite susceptible lines, a set of ten elite lines from the breeding programs of CIAT and FLAR was selected: Fedearroz 60 selección 132, Fedearroz 60 selección 154, Fedearroz 174 (FD174), Fedearroz 369 selection 23, Fedearroz 369 selection 67, Centauro, CT18685-10-3-1-2-2-M, CT18244-7-5-2-3-1-5-M, CT18245-11-6-2-2-2-2-M and WC366. These lines were sown at the greenhouse and RHBV resistance was evaluated using CIAT's standard protocol. Collection of plant tissue (100mg) was done before the vector infestation and DNA was obtained. DNA was quantified in a Hoefer (DyNA QUANT 200) fluorometer and separated on 0.8 % agarose gels.

An evaluation of the DNA polymorphism between the susceptible lines and the two sources of resistance, Fedearroz 2000 and Fedearroz 50, was performed by screening 320 SSRs distributed along the 12 rice chromosomes. The microsatellites that were previously reported as associated to RHBV and *T. orizicolus* resistance (Calvert et al 2006) were included in the analysis. The samples were separated on 4% acrylamide gels.

Results and discussion

Polymorphism survey

As a preliminary result, a total of 310 out of 320 SSRs showed detectable polymorphism between elite lines and the donor of resistance Fedearroz 2000. The genotypes with the highest percentage of polymorphism were CT18685-10-3-1-2-2-M (26%) and Fedearroz 174 (30 %). Based on the RHBV susceptibility and

other agronomic traits, these two elite lines were selected. Molecular data also revealed that the selected progenitors are closely related, which is shown by low levels of polymorphism compared to the reported data for *indica* x *japonica* crosses (45.82%) (Orjuela, 2006). This result is congruent with the pedigree analysis, which shows that these progenitors have many common ancestors.

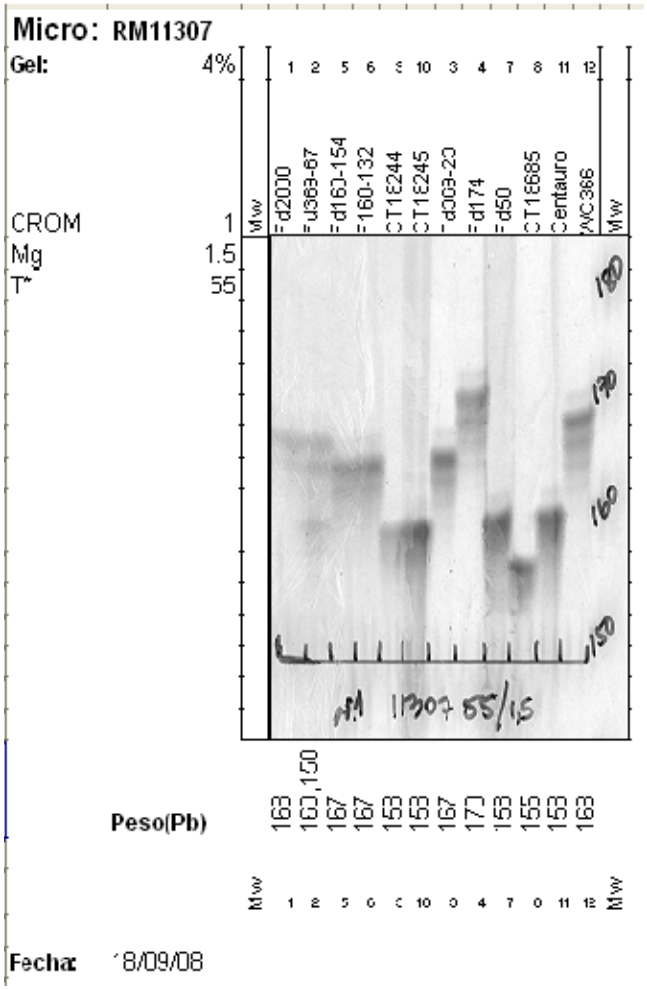
Validation of F1 hybrids

Two SSR markers, RM16459 and RM5633, were used to select the true F1 plants derived from the performed hybridizations. Out of 80 plants, 71 (88.7 %) were confirmed as F1s, and 9 as female parent selfings.

Conclusion

On the basis of accumulated results, we can now start the Marker-Assisted Backcrossing process itself. A total of 200 BC1F1 individuals will be produced for each population, and will be scanned for presence of the three resistance QTLs previously detected. The plants that combine the favourable alleles at the resistance QTLs will be selected for further backcrossing.

Figure 1. Detection of polymorphisms between eleven candidate elite lines and Fedearroz 2000 using SSR markers.



References

Orjuela, J. et al (2009). A Universal Core Genetic Map for Rice. Manuscript submitted for publication.

Reyes, M. Humberto (2000). A Model for Marker-Based Selection in Gene Introgression Breeding Programs. Crop Sci. 40:91–98

Calvert L.A., Lozano I., Villareal N., Romero L.E., Lorieux M., Martinez C., Garavito A. (2006). *Molecular analysis of the genetics of resistance to rice hoja blanca virus and its vector Tagosodes orizicolus*. APS Annual Meeting, July 31-August 3, 2006, Quebec, Canada. Phytopathology 96:S18.

Calvert L.A., Lozano I., Villareal N., Romero L.E., Lorieux M., Garavito A., Duque M.C., and Martínez C. *Molecular analysis of the genetics of resistance to rice hoja blanca virus and its vector Tagosodes orizicolus*. 2006. Annual Report. CIAT.

Output 3: Intensification and diversification of rice cropping systems for small farmers

3A. Participatory breeding of upland rice in Nicaragua

3A.1. Fitomejoramiento Participativo en arroz de secano en Nicaragua

Zildghean Chow W.[1], G.Trouche[2], Lázaro Narváez R.[3], José Corrales B.[4], Sergio Larios T.[5], Leonel Ramírez Ch.[6]

Resumen

El CIAT y CIRAD implementaron durante el período mayo 2002-junio 2008 el proyecto Fitomejoramiento Participativo para los sistemas de arroz de secano, siendo los objetivos principales: desarrollar y seleccionar germoplasma que se adaptaran a los diferentes sistemas de cultivos de los pequeños y medianos productores de Nicaragua y otros países de Centro América. Este proyecto integró a la institución nacional de investigación (INTA), a diferentes ONG's y organizaciones de productores. El proyecto desarrolló dos estrategias, con resultado a corto plazo la Selección Participativa de Variedades (PVS), utilizando por su diversidad genética y fenotípica el germoplasma generado por CIAT y CIRAD. Y a mediano y largo plazo la estrategia Creación Participativa de Líneas (PPB), que incluyó la introducción de poblaciones, las cruzas de PCT-18 con líneas promisorias o variedades comerciales y criollas, y la formación de poblaciones sintéticas para sitios específicos, constituyendo la fuente de diversidad genética para el desarrollo de líneas a través de la selección recurrente. La primera estrategia permitió la liberación de las variedades: INTA Kilambé (IRAT 364) e INTA Flora (IRAT 366) para el sistema de bajos insumos y condiciones favorables de la zona noroeste de Jinotega; la línea precoz WAB758-1-1-HB-4 recomendada para el sistema de bajos insumos y condiciones menos

[1] Asistente de Investigación CIAT-Nicaragua
[2] Coordinador Proyecto FP Arroz y Sorgo CIAT-CIRAD
[3] Coordinador Arroz INTA
[4] Investigador Regional INTA
[5] Coordinador INTA Siuna
[6] Coordinador UCM Siuna

favorecidas de la zona del pacífico y CT15679-17-1-1-4 para los sistemas tecnificados en condiciones favorables serán inscritas por INTA en el primer semestre del 2009. Los primeros resultados de los ensayos preliminares y avanzados de las líneas seleccionadas con la estrategia descentralizada PPB son muy promisorios, principalmente en los sistemas manuales.

Introducción

En el marco de las relaciones institucionales el CIAT-CIO, el CIRAD y CIAT ejecutaron un proyecto de investigación en Nicaragua, iniciando en mayo 2002. El propósito del proyecto fue desarrollar y aplicar metodologías de fitomejoramiento participativo en dos cultivos: arroz y sorgo para pequeños y medianos productores en sistemas de producción de bajos insumos, pero con la finalidad de la intensificación de los cultivos y mayor acceso a los mercados.

Los objetivos específicos del proyecto fueron los siguientes:

- Desarrollar y aplicar nuevos métodos de Selección Participativa de Variedades (PVS) y Creación Participativa de Líneas (PPB) utilizando poblaciones y selección recurrente.
- Identificar y desarrollar nuevo germoplasma que se adaptara a los sistemas de cultivo de los pequeños y medianos productores.
- Fortalecer las capacidades de los socios en el enfoque y metodología de fitomejoramiento participativo.

El arroz de secano conocido como arroz aeróbico es un importante cultivo y alimento en América Central. El área total de siembra, riego y secano en América Central es 225, 000 ha con una producción en arroz granza de aproximadamente 850,000 toneladas [1]. En Nicaragua el área de siembra de arroz de secano es 55, 000 ha, representando el 66% del área total [2].

Los principales problemas del cultivo de arroz en América Central son:

- La limitada disponibilidad de variedades mejoradas adaptadas a los diversos sistemas de producción, particularmente para las condiciones menos favorecidas y bajos insumos en los sistemas manuales
- Deficiente control de malezas
- Suelos ácidos y baja radiación solar en la región del atlántico
- Plagas y enfermedades principalmente piricularia, manchado de grano y el complejo de ácaro
- Calidad industrial inferior al arroz importado

El proyecto finalizó en junio 2008, INTA continuó las actividades como institución nacional agropecuaria y principal socio.

Materiales y Métodos

El proyecto Fitomejoramiento Participativo (FP) utilizó los recursos genéticos de CIAT-CIRAD, así como nuevo germoplasma desarrollado en Nicaragua (líneas y poblaciones sintéticas para sitios específicos utilizando el gen de esterilidad de la PCT-18).

En el ciclo agrícola 2008 las actividades de FP continuaron sólo en Siuna con el apoyo de la Unión de Cooperativas Multisectoriales (UCM) e INTA. En Chinandega la selección de líneas fue realizada por los investigadores y técnicos.

En el informe 2007 se presentan las estrategias y actividades realizadas en el proyecto [3].

1.1 Desarrollo de nuevas líneas

En el Centro Experimental de Occidente (CEO), Posoltega, Chinandega y en la finca de la UCM, Siuna se estableció un vivero de 90 y 191 líneas, respectivamente.

1.2 Evaluaciones de nuevas líneas en ensayos preliminares y avanzados

En 2008 se evaluaron 111 líneas en ensayos preliminares y 61 en ensayos avanzados, utilizando como testigos variedades comerciales y líneas promisorias, también se establecieron diez áreas de validación.

Diseño experimental y manejo agronómico

Los viveros y los ensayos fueron manejados según las prácticas comunes de los sistemas de producción predominantes en los sitios de Chinandega y Siuna. En los ensayos preliminares en los que se evalúan un mayor número de líneas el diseño utilizado fue bloques incompletos Federer y en los ensayos avanzados bloques completos al azar con tres ó cuatro repeticiones.

Resultados

En el CEO, Posoltega, la preparación de suelo se realizó tarde, por problemas de coordinación de INTA, los ensayos se establecieron hasta el 08 de agosto, afectando su desarrollo y rendimiento.

1.1 Desarrollo de nuevas líneas

En el CEO, Posoltega, de las 90 progenies S_4-S_6 derivadas de la población introducida PCT-4, del cruce PCT-18/INTA N-1, y de la población de sitio-específico PCTNic-1 (secano favorecido y sistema tecnificado) se seleccionaron 39 progenies S_5-S_7, es importante señalar que el 79% pertenecen a PCT-4 y PCTNic-

1. Estas progenies presentaron adecuado porte de planta, resistencia a las principales enfermedades del follaje, manchado de grano y aceptable tipo de grano.

En Siuna se evaluaron 191 progenies derivadas principalmente de la población PCT-11, PCT-4 y PCT-18; seleccionando los investigadores, técnicos y productores un total de 60 progenies con excelente tipo de grano entre otras características.

1.2 Ensayos preliminares y avanzados

Sistema de producción tecnificado en condiciones favorables (medianos y pequeños productores)

En la zona de Chinandega se evaluaron 15 nuevas líneas procedentes de la selección en las poblaciones introducidas PCT-4 y PCT-18, éstas son resultados del trabajo PPB manejados con un grupo de productores de Chinandega desde 2003 [4]. Los análisis de varianza de tres localidades indican diferencias altamente significativas para genotipo, ambiente e interacción genotipo ambiente para rendimiento de grano, días a floración y altura; excepto en la segunda variable que no fue significativa para ambiente. Es importante señalar que cuatro líneas PCT-4 presentaron rendimientos similares o superiores al promedio de las variedades comerciales INTA N-1 e INTA Chinandega (4208 kg/ha) (Cuadro 1). Estos ensayos fueron establecidos en fincas de productores colaboradores y sus siembras se realizaron en fechas más o menos normales (22-28 julio) y el manejo de los mismos fue adecuado.

En el CEO se evaluaron 27 líneas, la mayoría procedentes de PCT-11 (progenies seleccionadas en el taller de selección Villavicencio 2003 y luego desarrolladas por mejoramiento convencional en el CEO desde 2004). Tres líneas superaron a la variedad comercial INTA N-1 (2309 kg/ha) y diez a INTA Chinandega (1993 kg/ha) (Cuadro 2).

En el mismo sitio se evaluaron 18 líneas procedentes de los viveros riego CIAT 2006 (280 entradas) y LR IR64 x Azucena (173 entradas) enviado por CIRAD en el mismo año. A pesar de los bajos rendimientos obtenidos, se seleccionaron cinco líneas: dos líneas WARDA, dos procedentes del BC3 O. glaberrima/ Caiapo y la otra del cruce Azucena/IR64. Sólo una línea superó ligeramente a la variedad comercial INTA N-1.

En otro ensayo se confirmó el buen comportamiento de CT15679-17-1-1-1-4-M, esta línea se liberará en el ciclo agrícola 2009, se cuenta con la descripción varietal y progenies; y la producción de semilla se realiza en verano con el objetivo de garantizar la semilla para el establecimiento de las parcelas de difusión o

demostrativas. Esta línea también fue preferida por los productores de la zona de Siuna, Bonanza y Rosita (RAAN).

Sistema de producción de bajos insumos en condiciones favorables

En la zona noroeste de Jinotega las líneas de mejor comportamiento y preferencia por los productores fueron: IRAT 364 e IRAT 366. En el 2008 se realizó la presentación de las variedades con los nombres de INTA Kilambé e INTA Flora, respectivamente. A pesar de ser aceptadas por el Consejo Nacional de Semillas (CONASEM), no se registraron porque SERVITECA, socia del proyecto ha solicitado a INTA una reunión para discutir sobre el nombre de estas variedades, sin embargo, no ha sido posible realizar este encuentro con la nueva dirección de la institución.

En la finca de la Unión de Cooperativas Multisectoriales (UCM), Siuna, se evaluaron 34 líneas derivadas de los cruces PCT-18 con las variedades criollas Raizora Amarillo y Criolla Siuna. Trece líneas alcanzaron altos rendimientos (6401-4157 kg/ha), superando a la variedad local Raizora Amarillo (2222 kg/ha) (Cuadro 3). Los productores y fitomejoradores seleccionaron siete y cuatro líneas, respectivamente, siendo dos de éstas comunes.

En el mismo sitio, se evaluaron 50 progenies S_3 de la población sintética de sitio-específico PCTNic-3 (secano para sistemas manuales de bajos insumos). Los productores y técnicos seleccionaron un total de nueve progenies, las que se evaluaran en ensayos de rendimiento.

Además se establecieron 15 líneas seleccionadas en las poblaciones introducidas PCT-4 y PCT-18, dos líneas superaron en 56 y 20 % en rendimiento al testigo de la zona Raizora Amarillo (3154 kg/ha), Cuadro 4.

Sistema de producción de bajos insumos y condiciones menos favorecidas (zonas con problemas de sequía)

En algunas zonas planas de la región del pacífico, las variedades muy precoces (90 días o menos a madurez fisiológica del grano) son de gran interés, porque podrían alcanzar su ciclo de cultivo durante el período lluvioso de la época de siembra de primera (mayo-julio) ó postrera (15 agosto a finales de noviembre), y escapar del estrés hídrico debido a la "canícula" ó período seco (15 de julio-15 de agosto); es una nueva opción de cultivo, en comparación a las variedades de ciclo intermedio, sembradas en junio.

En 2003-2005 se establecieron los ensayos preliminares y avanzados; y en 2005-2008 las parcelas de validación, en los dos últimos ciclos se evaluaron las dos mejores líneas en tres zonas con condiciones climáticas menos favorecidas. La línea WAB 758-1-1-HB-4 fue la mejor en rendimiento y calidad industrial de grano, en 2008 se realizaron tres días de campo para ser presentada a

productores e instituciones. La semilla genética se obtendrá en verano de este ciclo agrícola, el informe técnico y los descriptores varietales se entregaron al INTA y esta previsto su presentación e inscripción en CONASEM en el primer semestre del 2009.

Entrega de semilla

Al finalizar el proyecto en julio de 2008 se distribuyó semilla de las progenies (398), poblaciones desarrolladas en Nicaragua (7), cruzas (4) y líneas avanzadas (63) a CIAT-CIRAD, CIRAD-Francia e INTA.

Conclusión

El proyecto utilizó la diversidad genética del germoplasma generado por CIAT y CIRAD y con la implementación de las estrategias PVS y PPB logró desarrollar nuevas variedades y líneas adaptadas a los diferentes sistemas de producción de secano existentes en Nicaragua y Centro América. La primera estrategia permitió la liberación de las variedades: INTA Kilambé (IRAT 364) e INTA Flora (IRAT 366) para el sistema de bajos insumos y condiciones favorables de la zona noroeste de Jinotega; la línea precoz WAB758-1-1-HB-4 recomendada para el sistema de bajos insumos y condiciones menos favorecidas de la zona del pacífico y CT15679-17-1-1-4 para los sistemas tecnificados en condiciones favorables serán inscritas por INTA en el primer semestre del 2009. Los primeros resultados de los ensayos preliminares y avanzados de las líneas seleccionadas con la estrategia descentralizada PPB son muy promisorios, principalmente en los sistemas manuales.

Referencias

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

[2] MAGFOR 2002. Estadísticas de producción de granos básicos de Nicaragua.

[3] Trouche G.; Chow Wong Z. 2008. Annual report 2007. Activity title: Participatory breeding of upland rice in Nicaragua. 12 p.

[4] Trouche G., Chow Z., Châtel M., Martínez C., Narváez L. Obregón J.R. 2008. Participatory breeding of upland rice in Nicaragua: matching the needs of small rice producers. Poster presentado durante el CIAT Knowledge Sharing and BOT Weeks, 7-19 abril de 2008, Cali, Colombia.

Cuadro 1. Características agronómicas de líneas promisorias desarrolladas por PPB para sistemas tecnificados en condiciones favorables, Posoltega, Chinandega, 2008.

Pedigree	Días a floración	Altura de planta (cm)	Reacción piricularia hoja	Reacción piricularia cuello	Acame	Aceptabilidad fenotípica	Rend promedio 3 sitios (kg/ha)
PCT-4>LM4-2G-1F-1F-M	79	130	1.0	1.4	3.4	3.0	4808
PCT-4>LM4-2G-2F-1F-M	80	116	1.0	1.2	1.2	1.0	4769
PCT-4>LM4-2G-3F-M-M	80	113	1.0	1.7	1.4	1.7	4370
PCT-4>SG9-1G-2P-M-M	80	113	1.0	1.2	1.7	1.7	4260
INTA Chinandega (v. comercial)	75	101	1.0	1.2	1.2	1.9	4240
INTA N-1 (variedad comercial)	79	103	1.9	2.3	1.9	2.6	4176
PCT-18>LM8-2P-2F-M-M	76	116	1.0	2.3	2.8	2.6	4148
PCT-4>LM4-2G-2F-2F-M	80	111	1.0	2.3	1.7	2.8	4043
PCT-4>SG9-1G-2P-4P-M	82	126	1.0	3.0	6.1	4.3	3738
PCT-4>LM4-2G-3F-2F-M	84	114	1.0	1.7	1.7	2.6	3514
PCT-4>SG9-1G-2P-2P-M	80	118	1.0	1.7	4.6	4.1	3389
POBL1-38	80	105	1.0	1.9	2.6	3.0	3369
PCT-18>SG4-1G-2F-M	78	103	1.0	1.7	1.2	2.3	3192
PCT-4>SG9-1G-2P-1P-M	82	118	1.0	1.9	6.3	4.8	3167
PCT-4>BE8-1P-2F-M-M	73	117	1.0	2.6	4.8	5.0	3122
PCT-18>SG5-1G-2F-M-M	77	124	1.0	2.6	6.8	5.4	3106
PCT-4>SG9-1G-2P-3P-M	82	120	1.0	2.1	3.4	2.3	3105
PCT-18>SG5-1G-2F-M-M	85	120	1.0	1.0	1.4	1.4	2667
Promedio	80	115	1.0	1.9	3.0	2.9	3733
CV (%)	1.8	4.9					18.0
F genotipo	**	**					**
F ambiente	n.s	**					**
F genotipo x ambiente	**	**					**
DMS	1.7	7.0					827.3

Cuadro 2. Características agronómicas de líneas de arroz para sistemas tecnificados en condiciones favorables, Posoltega, Chinandega, 2008.

Pedigree	Días a floración	Altura de planta (cm)	Reacción piriculari a hoja	Reacción piriculari a cuello	Manchado de grano	Acame	Rend (kg/ha)[1]
PCT-18\ 0\0\0>SD10-1-1-M-M-1F	83	80	1	1	1	1	3537
PCT-11\0\0\2,Bo\2\1>39-M-4-2-6-1	77	84	1	1	1	1	2877
PCT-11\0\0\2,Bo\2\1>61-M-2-1-1-1	64	94	1	1	1	1	2495
INTA N-1 (variedad comercial)	81	85	3	3	3	2	2309
PCT-11\0\0\2,Bo\2\1>36-M-2-3-1-1	79	86	3	1	1	1	2197
PCT-11\0\0\3>1479-M-1-2-M-2-3-1F	77	107	3	3	3	3	2157
PCT-11\0\0\2>Bo\2>5-1-M-5F-1F	82	88	3	1	5	1	2095
PCT-11\0\0\2,Bo\2\1>110-M-1-2-1-2	77	82	3	3	3	3	2074
PCT-11\0\0\2>Bo\2>69-2-M-1P-1F	77	82	3	1	1	1	2054
PCT-11\0\0\2,Bo\2\1>36-M-2-1-5-1	78	90	1	1	1	1	2036
PCT-11\0\0\2>Bo\2>5-1-M-2P-1F	79	81	3	3	3	3	2027
INTA Chinandega (variedad comercial)	76	80	3	2	3	2	1993

[1]Rendimiento no ajustado

Cuadro 3. Características agronómicas de líneas de arroz derivadas del cruce con variedades criollas, Siuna, 2008.

Pedigree	Días a floración	Altura de planta (cm)	Reacción piricularia en hoja	Reacción piricularia en cuello	Reacción a helm	Reacción a escaldado	Acame	Rend (kg/ha)[1]
(PCT-18\CS)-76M-1P-1F-1F	85	139	0	0	1	3	1	6401
(PCT-18\RA)-36H-1P-1F-1P	91	133	1	1	1	3	1	5666
(PCT-18\RA)-59H-2P-1P-1P	82	147	1	1	3	3	1	5463
(PCT-18\CS)-62M-1P-1F-1P	79	112	1	1	1	3	1	5397
(PCT-18\CS)-90M-3P-1P-2P	85	132	1	1	1	3	1	5381
(PCT-18\CS)-83M-1P-1F-1P	85	139	0	0	1	3	1	5072
(PCT-18\CS)-90M-3P-1P-1P	82	145	1	1	1	3	1	5032
(PCT-18\CS)-106G-1G-2F-1F	90	135	1	1	3	3	1	4897
(PCT-18\RA)-30H-2P-1P-1F	84	128	0	0	1	3	1	4721
(PCT-18\CS)-106G-1G-1F-1F	81	105	0	0	1	3	1	4652
(PCT-18\CS)-64M-1P-1P-1P	82	150	1	1	1	3	3	4312
(PCT-18\RA)-4H-2P-1P-1P	88	130	0	0	3	3	1	4227
(PCT-18\CS)-106G-1G-1P-1P	82	110	1	1	1	3	1	4188
(PCT-18\CS)-83M-2P-1P-2P	85	122	1	1	1	3	1	4157
(PCT-18\CS)-83M-2P-1P-1P	85	145	1	1	1	3	1	3987
CT15944-10-4-3-3 (línea testigo!)	85	128	0	0	2	4	1	3856
(PCT-18\CS)-106G-1G-1F-1P	82	120	0	0	3	3	1	3688
(PCT-18\RA)-108G-1P-1P-1P	92	127	1	1	1	3	1	3687
(PCT-18\RA)-45H-3P-1P-1P	88	126	1	1	1	3	1	3685
(PCT-18\CS)-90M-1P-1P-1F	82	120	1	1	1	5	1	3242
CIRAD 401 (línea testigo!)	85	125	1	1	2	4	1	3234
(PCT-18\CS)-20H-1T-1P-1F	90	138	0	0	1	3	1	2779
Raizora Amarillo (variedad criolla)	92	135	1	1	2	3	1	2222

[1]Rendimiento no ajustado ! mejores lineas fase 2004-2006, en fase de validación en 2007-2008

Cuadro 4. Características agronómicas de líneas desarrolladas por PPB y seleccionadas por los productores y fitomejoradores, Siuna, 2008.

Pedigree	Días a floración	Altura de planta (cm)	Reacción helm	Reacción escaldado	Acame	Rend (kg/ha)[1]
PCT-18>LM7-1P-2P-1F-1P	77	105	1	3	1	4912
CT15944-10-4-3-3 (línea testigo!)	79	116	1	3	3	3858
PCT-18>SI14-2G-2P-1F-1F	79	105	1	3	1	3774
Raizora Amarillo (variedad criolla)	92	170	3	3	1	3154
PCT-18>SI14-2G-1P-1F-1P	81	98	1	3	1	2796
CIRAD 401 (línea testigo!)	80	111	1	3	1	2093
PCT-4>LM12-1P-1P-1F-1F	79	94	1	3	1	nc
PCT-4>LM12-1P-1P-1F-1P	80	98	1	3	1	nc

[1]Rendimiento no ajustado ! mejores líneas fase 2004-2006, en fase de validacion en 2007-2008 nc: no cosechadas pero observadas como buenas por los fitomejoradores.