Identification and utilization of genes from wild germplasm for the improvement of yield and stress resistance in rice[1].

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INTRODUCTION

Rapid population growth in Asia and Latin America is putting increasing pressure on the already strained food-producing resources of these regions. Besides, intensive breeding of crop varieties by man narrowed down the genetic base in many crops (Tanksley and Nelson, 1996), problem that is more critical in self-pollinated crops, like rice (Wang et al. 1992). This reduced genetic variation renders modern crop varieties more vulnerable to biotic and abiotic stresses, and could explained the already observed slower rate of genetic progress achived by plant breeding (Tanksley and Nelson, 1996). There is an urgent need to increase rice production in a sustainable manner. New paradigms of international, regional and interinstitutional cooperation and new strategies for crop improvement are needed. Ongoing plant improvement efforts must be streamlined using a mixture of biotechnological and classical approaches. The main objective of this project is to develop and implement a marker assisted breeding strategy that will lead to the development of improved cultivares and simultaneously broaden the genetic base of cultivated rice.

MATERIAL AND METHODS

Population development

Few plants (2-3) in each of the wild species *O. rufipogon, O. glaberrima, and O. barthii* were hybridized to several plants of each of the improved rice cultivars (recurrent parent) listed in Table 1.

Recurrent parents were choosen based on specific agronomic traits such as yield potential, grain quality, disease resistance, etc. Single crosses were obtained and grown in the greenhouse at CIAT in early 1994. Three F_1 hybrid plants were backcrossed to the improved cultivar, using the latter one as the female parent; approx. 100-180 BC_1F_1 seeds were obtained per cross combination. The resulting BC_1F_1 plants were transplanted (30x50 cm) and evaluated based on phenotype; negative phenotypic selection for undesirable agronomic traits (spreading plant type, excessive shattering, long awns, dark-color grains, high sterility, etc) was used to narrow the selection down to the best (40-50) individuals. Each selected BC_1 individual was back crossed again to the recurrent parent and approx. 30 BC_2F_1 seeds were produced; 20 BC_2 seeds from each of the selected BC_1 plant were sown in wooden trays in the screehouse and later on transplanted (30x40 cm) under irrigated conditions.

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Table 1. Plant material used in interspecific hybridization at CIAT

Parent	Accession No.	Source	Origin	Notes
O. rufipogon	105491	IRRI	Malaysia	Ancestor of *O. sativa*
O. glaberrima	103544	IRRI	Mali	Cultivated in Africa
O. barthii	104119	IRRI	Chad	Relative of *O.glaberrima*
Recipient (Recurrent parent)				
Cypress		Louisiana	USA	Tropical japonica, quality
Lemont		Texas	USA	Tropical japonica, quality
RU9403006 (Jefferson)		Texas	USA	Tropical japonica, quality
Oryzica Llanos 5		CIAT	Colombia	Indica; resistant to *P. Oryzae*
BG90-2		CIAT	Sri Lanka	Indica; high yield
Morelos A88		CIAT	Mexico	Good combining ability
Oryzica 3		CIAT	Colombia	Indica; high yield
O. Sabana 6		CIAT	Colombia	Tropical japonica; upland
O. Turipana 7		CIAT	Colombia	Tropical japonica; upland
Progresso		CIAT	Brasil	Tropical japonica; upland
CAIAPO		CIAT	Brasil	Tropical japonica; upland
CT6196-33-11-1-3		CIAT	Colombia	Tropical japonica; upland

A negative phenotypic selection was applied again and best individuals per cross were selected and harvested individually to generate BC_2F_2 seed; approx. 220-300 BC_2F_1 plants were selected per cross combination for field testing. Each selected BC_2F_1 plant was evaluated for 12 agronomic traits including days to heading and maturity, plant height, panicle length, panicles per plant, spikelests per panicle, grains per panicle, seed set rate, spikelests per plant, grains per plant, 1000-grain weight and yield per plant. Based on field observations and genetic potential only three populations (BG90-2/ *O. rufipogon,* Oryzica *Llanos* 5/ *O. rufipogon*, and *Caiapo/O. rufipogon* were chosen for field testing.

Field trials (BC_2F_2 and BC_2F_3 families)

The 300 BC_2F_2 families derived from the crosses BG-90-2/ *O.rufipogon* and *Caiapo/O. rufipogon,* and the 220 families from the cross *Oryzica Llanos 5*/O. *rufipogon* were planted in replicated yield trials in four sites in Colombia (CIAT-Palmira and CIAT-Santa Rosa, Villavicencio, La Libertad Exp. Station, Villavicencio, and Saldaña. Tolima). The *Caiapo/O. rufipogon* cross was planted under upland-savanna conditions, and the other ones under irrigated/rainfed conditions. In collaboration with EMBRAPA/CNPAF/CIRAD the Caipao/O. *rufipogon* cross was also evaluated in Goiania, Goias, Brazil. Transplanting (20x30cm) was used at CIAT-Palmira, while direct seeding was used elsewhere. A completely randomized design with two reps., 2 row-plot, 5 m. long. was used. Data on the 12 agronomic data described elsewhere, including plot yield/family were taken on 10 randomly selected plants/plot.

Based on yield potential and good agronomic traits, 38 BC_2F_2 families from the cross BG90-2/O. *rufipogon* were selected and further evaluated for grain yield; a completely randomized design with four reps, 4 row-plot, and 5m. long was used.

Molecular characterization

Parental surveys filters containing *O. rufipogon,* Fanny, O. Llanos 5, BG90-2, Caiapo and, O. Sabana 6, and the corresponding F_1's were prepared using five restriction enzymes (Eco RI, EcoRV, Hind III, and Dra I). Approximately 140 markers from the rice molecular framework linkage map were selected at 10-20 CM intervals throughout the genome. A set of 50 mapped rice microsatellite markers, which were developed at Cornell University, is also being used to complement the RFLPs in QTL analysis. DNA from each BC2F2 family of the BG90-2/ *O. rufipogon* cross has already been extracted.

RESULTS AND DISCUSSION

Probe selection

Data indicated that so far 90 probes out of 140 clones were polymorphic (64%); polymorphism was greater between *O. rufipogon* and the tropical japonica cultivars Fanny, O. Sabana 6 and Caiapo, compared to *O. rufipogon* and the indica cultivars *Oryzica Llanos* 5 and BG90-2. Screening of BC_2F_2 families with polymorphic RFLP clones and microsatellites is underway.

Population development

A total of 36 crosses was made using the parental lines listed in Table 1; population development from these crosses is underway but at different stages; the more advanced populations have gone through two-three rounds of backcrossing to the recurrent parent. Several populations involving crosses between Lemont, Cippress and Jefferson, *with O. rufipogon, O. barthii, and O. glaberrima* were sent to Cornell University for further evaluation and development. Although all of the donor wild rices belong to the same genome AA, as cultivated rice. and crossability should not be a problem, however there were some sterility problems and embryo abortion in some cases specially in crosses with *O. barthii* and *O. glaberrima.* Failure of embryo development was observed 10-15 days after pollination. Therefore, embryo rescue was used successfully to overcome this problem, although porcentage of recovery varied among cross combinations. Hybrid plants recovered through embryo rescue were also used in the backcrossing scheme if they had a desirable phenotype.

220 BC_2F_2 families derived from the cross Oryzica Llanos 5/O. rufipogon were evaluated under field conditions at CIAT-Palmira using a highly viruliferous insect colony of *Tagosodes oryzicola.* M. Distributions of disease incidence (%) based on a 0 - 9 scale (0=no disease symtom; 9=>70% diseased plants) (Fig. 1).

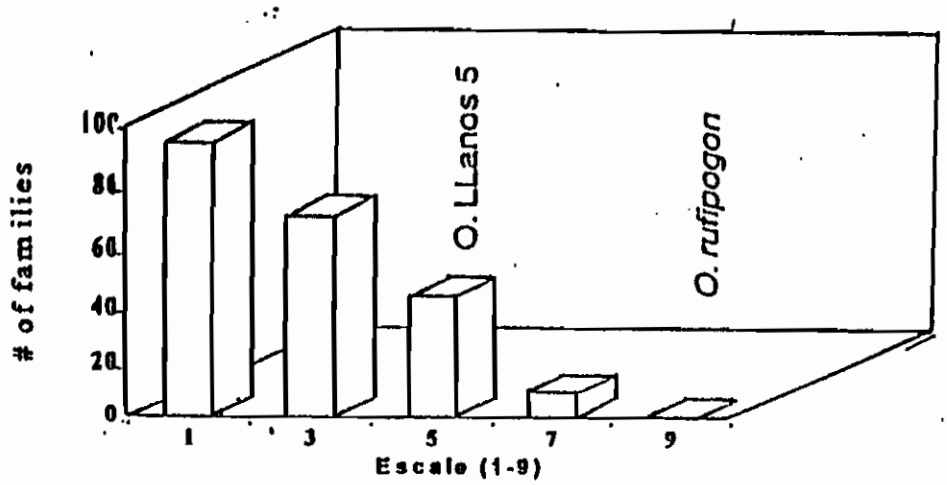


Fig. 1. Frequency distribution for rice Hoja Blanca virus disease in 200 BC2F2 Families of the Oryzica Llanos 5/O.rufipogon cross.

Yield Trials and linkage analysis

These evaluations were conducted during June-October 1996 and Dec/96-April/97. We are still processing and recording data taken on main agronomic traits and only partial information generated in CIAT-Palmira is presented. The distribution of grain yield (kg/ha) of 300 BC2F2 families (Bg90-2/*O. rufipogon*) derived from plot yields of 40

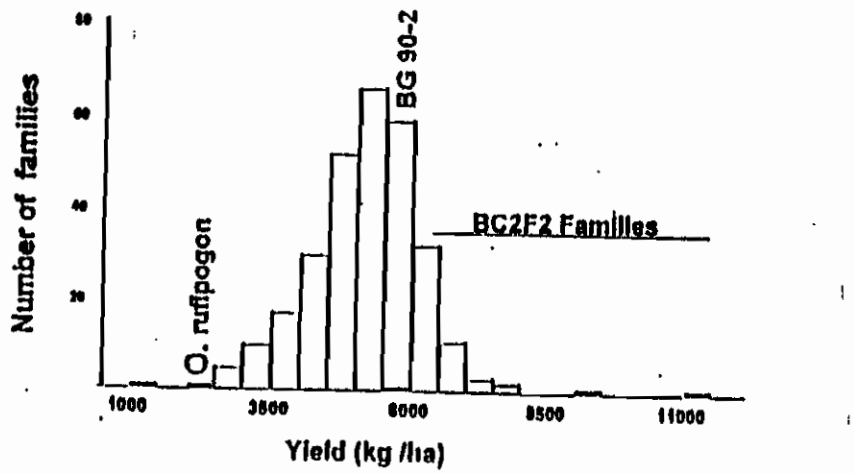


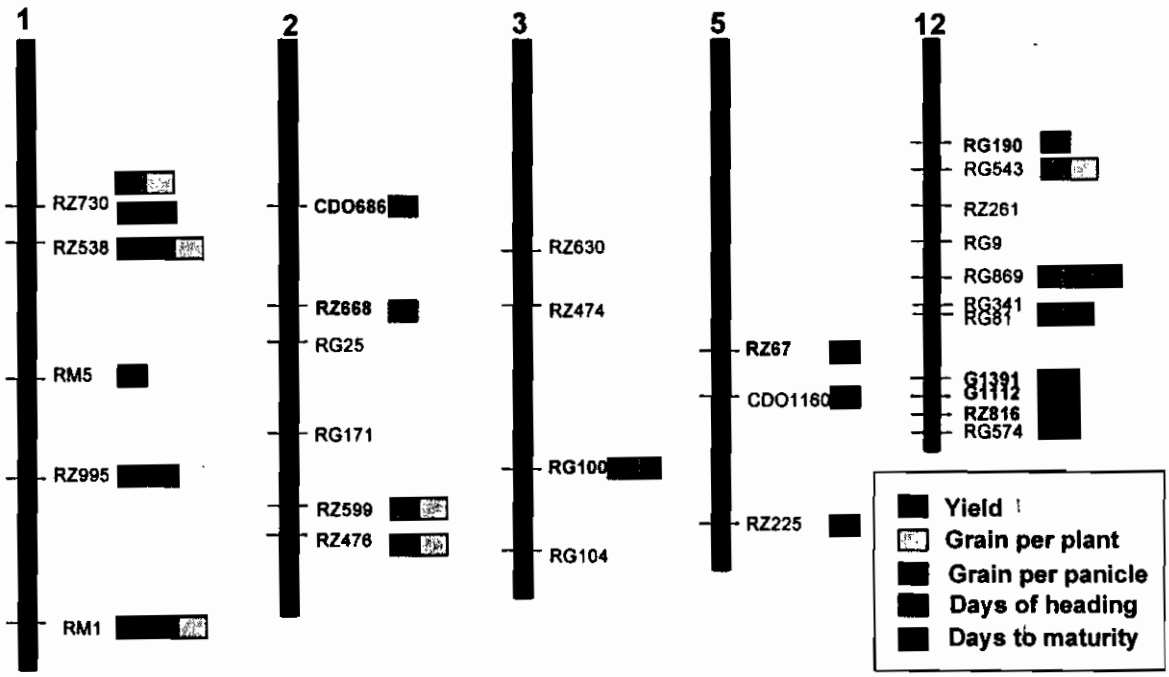
Fig. 2. Frequency distribution of grain yield of 300 BC2F2 Families of the BG90-2/*O. rufipogon* cross

plants (20 plants/row x 2row) averaged over two replications is illustrated in Fig2. Transgressive segregation can be observed, with several lines (11%) having between 5 and 25% higher yield than the recurrent parent Bg90-2. Transgressive segregation for other yield components was also observed. Grain yield data taken on 38 BC2F3 families

confirmed results obtained in the BC2F2 generation. This preliminary data is in agreement with reports coming from the work being conducted in China at the Hunan Hybrid Rice Research Center (McCouch, S. 1995) and in South Korea. These data from different groups working with diverse recurrent parents suggest that DNA introgressed from *O. rufipogon* can contribute positively to yield in elite rice cultivars. Evermore, data from China (Xiao et al, 1996) suggest that two QTLs found in chromosomes 1 and 2 of *O. rufipogon* are responsible for this yield increase.

Based on the 52 RFLP and 3 microsatellites from RF-Cornell framework map screened on 303 BC2F2 families putative linkage were identified with yield, yield components from replicated data available for the whole mapping population. Preliminary results using one way anova and t-test indicate associations between markers and yield on chromosome 2 similar to J.Xiao and S. McCouch results (Fig 3). No linkage for yield was detected so far on chromosome 1 as reported by J.Xiao and S. McCouch. Other associations were also identified for yield and the various yield components (Fig 3).

Fig 3. Preliminary results of positive QTL alleles for yield and yield components



Further work at CIAT will indicate more definitive identification of positive QTLs for yield and whether or not we are dealing with the same QTLs reported by the Chinese group; besides, QTL analysis performed in populations developed with *O. glaberrima* and *O. barthii* will show if they carry the same QTLs for yield found in *O. rufipogon*. If each wild rice posses specific positive alleles for yield, then rice breeders will have a tremendous breeding strategy for increasing yield in a systematic and pyramided manner, that is, in a step-wise process.

In sumary, preliminary data presented support the hypothesis that DNA introgressed from *O. rufipogon* can contribute positively not only to yield in elite rice cultivars but also in terms of stress resistance. This information also provides the basis for implementing the method proposed by Tanksley and Nelson 1996 referred to as "advanced backcross QTL analysis" for the simultaneous discovery and transfer of valuable QTLs from wild germplasm into elite breeding lines.

FUTURE ACTIVITIES

1. Complete agronomic and molecular characterization of progenies, and QTL analysis in the BG90-2/*O. rufipogon* cross to determine number of QTLs associated with yield increase and its expressions across environments using already identified 50 RFLP polymorphic clones and 30 microsatellites.

2. Analysis of other traits and determination of contribution for positive alleles of each of the parents.

3. Development of NiLs to be initiated based on QTL analysis carrying specific QTLs for use in breeding programs.

4. Start agronomic and molecular characterization of several other populations involving crosses with *O. barthii* and *O. glaberrima* to determine the presence of QTLs for yield increase.

5. Training of scientists from national programs in the area of marker-assisted breeding.

6. Estimate the cost of developing improved varieties through the use of molecular markers.

COLLABORATORS

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