Current Status of Rice Interspecific Hybridization at CIAT[1]

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Introduction

Rice forms the nutritional basis for much of the world's population. This cereal is planted on 148 million hectares worldwide and is the world's most important food crop; globally rice provides 20% of energy and 15% of per capita protein. It is estimated that by the year 2025 some 8.3 billions people will live on earth and that 50% of them will he rice eaters. Therefore, current world rice production (approx. 575 million tons) must be increased by 70% to meet this demand. More than 90% of the world's rice is grown and consumed in Asia, while Latin America's rice production represents 3.5% of the total; over 70% of rice production in Latin America (LAC) comes from irrigated and rainfed lowland ecosystems. Rice production in LAC increased from 9.9 to 18.8 million tons from 1966 to 1994, while modern semidwarf varieties combined with appropriate management practices caused 76% regional average yield increase for the irrigated and rainfed lowland sectors from 2.5 to 4.4 t/ha.

Rapid population growth in Asia and Latin America puts increasing pressure on the already strained food-producing resources of these regions. By the year 2010, a food deficit in excess of 100 million tons each year is predicted unless the world agricultural community is able to develop crop varieties and farming methods that yield far more than is currently produced. In addition, long-term viability of our agricultural system and the resource base upon which they depend must be protected. New paradigms of international, regional and interinstitutional cooperation and new strategies for plant improvement are needed. Highly productive, nutritious, and resource-efficient crop varieties must be developed.

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Ongoing plant improvement efforts must be streamlined using a mixture of biotechnological and classical approaches. Familiarity with a variey of approaches to genetic enhancement of crop plants will be increasingly essential to those involved in agricultural research.

Early plant domestication by man followed by modern intensive breeding of crop varieties by plant breeders narrowed down the genetic base in many crops (Simmonds 1976; Ladizinsky 1985; Tanksley and Nelson 1996). This problem is more critical in self-pollinated crops, like rice (Wang et al. 1992). The reduced genetic variation among improved commercial cultivars make them more vulnerable to biotic and abiotic stresses, and could explain the already observed slower rate of genetic progress achieved by plant breeders (Tanksley and Nelson 1996). This problem is specially critical as it relates to yield, and has culminated in the phenomenon known as the "yield barrier".

There is an urgent need to increase rice production in a sustainable way but the reduced genetic variation in rice works again it. In LAC, irrigated rice breeding has depended on a genetic core of 12 landraces (Cuevas, 1993), while in Colombia average yield of irrigated rice has not changed since 1970 (Fedearroz, 1993). Numerous studies have indicated that after initial improvements obtained by using this core of germplasm, followed by the introduction of a set of plant traits necessary for, local adaptation, yield potential per se of irrigated rice in Latin America has reached a plateau (CIAT medium term plan, 1993 - 1998). At IRRI, the last major advance in rice yield potential was obtained with the development of IR8, and for the last 30 years, breeding efforts have been largely devoted to improving pest and disease resistance, quality, and general adaptation while maintaining yields at constant levels. A new plant type has been developed at IRRI (Peng et al. 1994) with an increased yield potential. However, it is not yet ready for commercial planting and more breeding work is needed to improve it's insect and disease resistance and the grain quality.

Fortunately, genetic variation is abundant in nature; the wild ancestors and related land races of most crop species can be still found in situ and/or in germplasm banks around the world. According to Tanksley and Nelson (1996), plant scientists have been unable to exploit the majority of this genetic potential because of two reasons:

i) difficulty in identifying genes for yield and quality in wild germplasm and ii) linkage drag. In the case of rice (Vaughan and Sitch. 1991), the genus *Oryza* consists of approx. 20 wild species and two cultivated species *(O. sativa and O. glaberrima)*. Occasionally, some of these wild species have been used in breeding programs, specially at IRRI, but most of the time as sources of genes for disease and insect resistance. On the other hand, two well-saturated maps of rice are available (Causse et al. 1994; Kurata et al. 1994). These maps contain closely linked, codominant loci that can be monitored for linkage to genes controlling traits of economic importance (Tanksley et el. 1989); Paterson et al. 1991). These maps if used in conjunction with traditional breeding methods, can allow breeders to locate and selectively transfer genes that will improve yield, quality, and adaptability to different production constraints (Xiao et al. 1995; Ahn et al. 1993; Nakamura et al. 1994; McCouch and Doerge, 1995). More recently, Tanksley and Nelson, 1996 proposed a new method called "advanced backcross QTL analysis" for combining QTL analysis with variety development. Plant breeders, have at hands a powerful tool to enhance their gene pools. The purpose of this paper is to present the status of interspecific hybridization at CIAT.

Materials 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. Single crosses were obtained and grown in the greenhouse at CIAT in early 1994. Three F1 hybrid plants were backcrossed to the improved cultivar, using the latter one as the female parent; approx. 100-180 BC1F1 seeds were obtained per cross combination. The resulting BC1F1 plants were transplanted (30x50 cm) and evaluated based on phenotype; negative phenotype 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 BC1 individual was back crossed again to the recurrent parent and approx. 30 BC2F1 seeds were produced; 20 BC2 seeds from each of the selected BC1 plant were sown in wooden

trays in the screenhouse and later on transplanted (30x40 cm) under irrigated conditions. A negative phenotypic selection was applied again and best individuals per cross were selected and harvested individually to generate BC2F2 seed; approx. 220-300 BC2F1 plants were selected per cross combination for field testing. Each selected BC2F1 plant was evaluated for 12 agronomic traits including days to heading and maturity, plant height, panicle length, panicles per plant, spikelets per panicle, grains per panicle, seed set rate, spikelets 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* ,O. *Llanos 5/ O. rufipogon*, and *Caiapo/O. rufipogon* were chosen for field testing.

Field trials (BC2F2 families)

The 300 BC2F2 families derived from the crosses Bg-90-2/ *O.rufipogon* and *Caiapo/O. rufipogon,* and the 220 families from the cross *O. 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 under irrigated/rainfed conditions. 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/familiy were taken.

Molecular characterization

Parental surveys filters containing *O. rufipogon,* Fanny, O. Llanos 5, Bg90-2, Caiapo and, O. Sabana 6, and the corresponding F1's were prepared using four 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 have 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 O. Llanos 5 and Bg90-2. Screening of BC2F2 families with polymorphic RFLP clones and microsatellites is underway.

Population development

A total of 36 crosses (Table 2) 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 rounds of backcrossing to the recurrent parent. Although all of the donor wild rices belong to the some 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.* Failure of embryo development was observed 10-15 days after pollination. Therefore, embryo rescue was used successfully to overcome this problem; hybrid plants recovered through embryo rescue were also used in the backcrossing scheme if they had a desirable phenotype.

Yield Trials

These evaluations were conducted during June-October 1996. 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 38 BC2F2 families (Bg90-2/*O. rufipogon*) chosen in the field based on phenotype and derived from plot yields consisting of 40 plants (20 plants/row x 2row) averaged over two replications is illustrated in Fig. 2. Transgressive segregation can be observed, with several lines having between 5 and 15% higher yield than the recurrent parent Bg90-2. 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 yields in elite rice cultivars. Furthermore, data from China (McCouch, S. 1995) suggest that two QTLs found in chromosomes 1 and 2 of *O. rufipogon* are responsible for this yield increase.

Further work at CIAT will indicate 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.

On the other hand, 220 BC2F2 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 symptom; 9=>70% diseased plants) is presented in Fig. 3. Transgressive segregation for rice hoja blanca virus resistance can be observed, with approx. 50% of the families falling in the categories 1 and 3. This preliminary data from an un-replicated experiment seem to suggest that positive alleles for rice hoja blanca virus from *O. rufipogon* could be contributing to increased resistance to this particular virus disease. More testing is needed to confirm this result.

In summary, 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.

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Table 1. Plant materials used in interspecific hybridization at CIAT

Parent	Accession No.	Source	Origin	Notes
Donor				
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. sativa*
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

Table 2. Inter-specific crosses made between several improved irrigated and upland rice cultivars, and three wild species of rice.

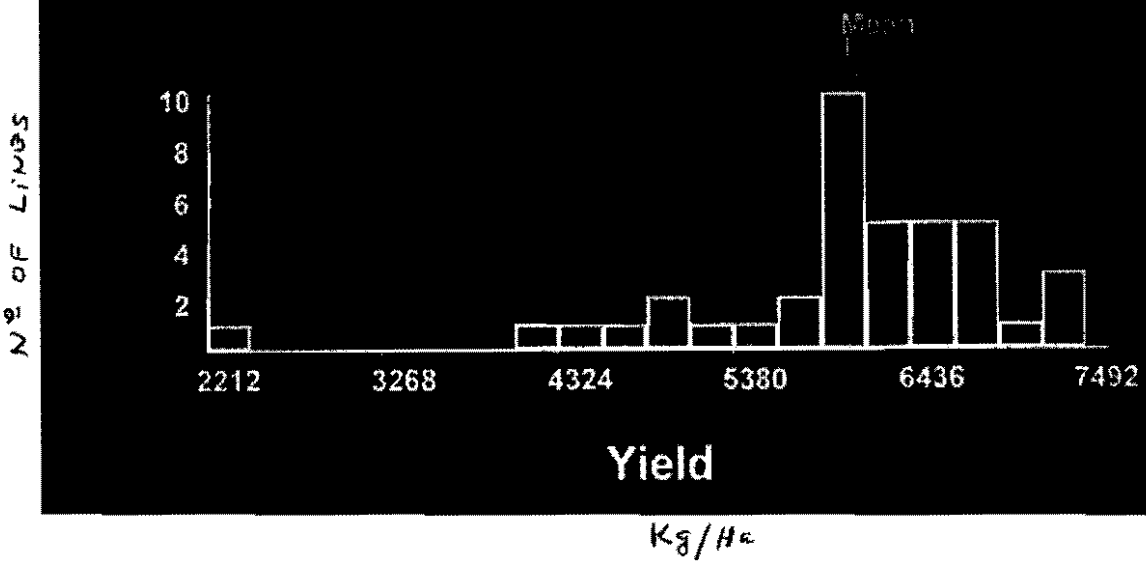
O. rufipogon /	BG90-2 // 2 * BG90-2 (3)
	MORELOS A88 // 2* MORELOS A88 (3)
	ORYZICA 3 // 2* ORYZICA 3 (3)
	ORYZICA LLANOS 5 // 2* ORYZICA LLANOS 5 (3)
	LEMONT // LEMONT (2)
	RU94030006 // 2* RU94030006 (3)
	CYPRESS // 2* CYPRESS (3)
	ORYZICA SABANA 6 // 2 * ORYZICA SABANA 6 (3)
	ORYZICA TURIPANA 7 (1)
	PROGRESSO (1)
	CT6196-33-11-1-3 (1)
	CAIAPO // 2* CAIAPO (3)
O. barthii /	BG90-2 // BG90-2 (2)
	MORELOS A88 // 2* MORELOS A88 (3)
	ORYZICA 3 // 2 * ORYZICA 3 (3)
	ORYZICA LLANOS 5 // ORYZICA LLANOS 5 (2)
	LEMONT // LEMONT (2)
	RU94030006 // RU94030006 (2)
	CYPRESS // CYPRESS (2)
	ORYZICA SABANA 6 // ORYZICA SABANA 6 (2)
	ORYZICA TURIPANA 7 // ORYZICA TURIPANA 7 (2)
	PROGRESSO (1)
	CT6196-33-11-1-3 // CT6196-33-11-1-3 (2)
	CAIAPO (1)

	BG90-2 // BG90-2 (2)
	MORELOS A88 (1)
	ORYZICA 3 // ORYZICA 3 (2)
	ORYZICA LLANOS 5 (1)
	LEMONT (1)
O. glaberrima /	RU94030006 //2* RU94030006 (3)
	CYPRESS (1)
	ORYZICA SABANA 6 // ORYZICA SABANA 6 (2)
	ORYZICA TURIPANA 7 // ORYZICA TURIPANA 7 (2)
	PROGRESSO (1)
	CT6196-33-11-1-3 (1)
	CAIAPO (1)

Remarks: (1) = SINGLE CROSS MADE
(2) = FIRST BACKCROSS MADE; IN SOME CASES SECOND BACKCROSS UNDERWAY
(3) = SECOND BACK CROSS MADE; IN SOME CASES REPLICATED YIELD TRIALS WITH F2BC2 PROGENIES UNDERWAY

Transfer of wild genes from wild Oryza

Yield of *O. rufipogon*, BG-90 and selected BC2F2 families



BG90-2/O.rufipogon
O. LLANOS 5/O. rufipogon
CAIAPO/O.rufipogon

MOLECULAR CHARACTERIZATION:
PARENTS, F1 : DONE
F2BC2 FAMILIES : UNDERWAY
QTL ANALYSIS : BEST FAMILIES -> 3er. BC -> NILS

100-180 F1BC1 seeds

↓

NEGATIVE SELECTION

↓

Best 40-50 plants

↓

F1BC2 : 900-1000 seeds

↓

NEGATIVE SELECTION

↓

300 F2 BC2 FAMILIES

↓

YIELD TRIAL (4 sites)

↓

2- ROW PLOTS, 2 REP.
(WEED COMPETITION)

↓

DATA ON 12 AGRONOMIC TRAITS

FLOW CHART FOLLOWED AT CIAT

Inter-specific crosses made between improved irrigated and upland rice cultivars, and wild species of rice

O. rufipogon /

- BG90-2 // 2 * BG90-2 (3)
- MORELOS A88 // 2* MORELOS A88 (3)
- ORYZICA 3 // 2* ORYZICA 3 (3)
- ORYZICA LLANOS 5 // 2* ORYZICA LLANOS 5 (3)
- LEMONT // LEMONT (3)
- RU94030006 // RU94030006 (3)
- CYPRESS // CYPRESS (3)
- ORYZICA SABANA 6 // 2 * ORYZICA SABANA 6 (3)
- ORYZICA TURIPANA 7 (1)
- PROGRESSO (1)
- CT6196-33-11-1-3 (1)
- CAIAPO // 2* CAIAPO (3)

O. glaberrima /

- BG90-2 // BG90-2 (2)
- MORELOS A88 (1)
- ORYZICA 3 // ORYZICA 3 (2)
- ORYZICA LLANOS 5 (1)
- LEMONT (2)
- RU94030006 // RU94030006 (2)
- CYPRESS (1)
- ORYZICA SABANA 6 // ORYZICA SABANA 6 (2)
- ORYZICA TURIPANA 7 // ORYZICA TURIPANA 7 (2)
- PROGRESSO (1)
- CT6196-33-11-1-3 (1)
- CAIAPO (1)

Remarks: (1) = SINGLE CROSS MADE
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(3) = SECOND BACK CROSS MADE; IN SOME CASES REPLICATED YIELD TRIALS WITH F2BC2 PROGENIES UNDERWAY

Fig. 2 . Evaluación virus hoja blanca F2RC2-Oryzica Llanos 5/O. rufipogon