

GC-91, a broad-based rice synthetic population for blast (*Pyricularia grisea* Sacc.) resistance

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ABSTRACT

Population improvement methods used in cross-pollinated crops have not been extensively applied to rice (*Oryza sativa* L.). This article describes the formation of and selection within a genetically broad-based gene pool for resistance to rice blast (*Pyricularia grisea* Sacc.), and progress during one generation of selection. Thirty rice lines of diverse origin were chosen as parents based upon stability of the reaction of the lines to a range of leaf (BL) and panicle (NBL) blast races during eight growing seasons at a location favorable for disease development ("hot spot" site). Each parent was crossed to five other parents and each F_1 to three other F_1 's to ensure recombination. The resulting seeds were bulked to form the GC-91 gene pool (C0P0). Genetic diversity, as measured by geographical origin of the germplasm, was higher in the GC-91 than in the "genetic core" of rice varieties grown in Latin America and the Caribbean. Three groups were selected from GC-91, based upon three different objectives of selection for blast: a) rapid fixation of major genes for high levels of resistance (population C1P1), b) gradual accumulation of polygenes for partial resistance (population C1P2), and c) a combination of objectives a and b (population C1P3). Lines selected had greater resistance to BL and NBL in populations C1P1 and C1P3 compared to C0P0, while there was no evidence of successful selection for C1P2.

INTRODUCTION

Rice (*Oryza sativa* L.) breeding programs have used pedigree selection to develop germplasm resistant to blast (*Pyricularia grisea* Sacc.). However, resistance usually breaks down shortly after varietal release (Crill *et al.*, 1981; Horino *et al.*, 1990). Few rice varieties grown commercially have exhibited stable blast resistance for more than five years over a wide range of environments (Notteghem, 1981; Correa-Victoria and Zeigler, 1993a).

Genetic studies on blast resistance using specific races of the pathogen indicate that inheritance of this trait is mainly under simple "gene-for-gene" control (Ou, 1985). There have been at least 13

dominant genes at eight loci for blast resistance identified (Kiyosama, 1972, 1981). However, when different isolates are used to test lines with previously identified genes, evidence for additional genes is typically found (Kiyosama, 1972). Partial or horizontal resistance controlled by numerous genes has also been reported in rice blast resistance (Notteghem, 1981; Ahn and Ou, 1982; Marchetti, 1983). Studies on the expression and inheritance of partial resistance to *P. grisea* indicate that its heritability is relatively low and that selection for resistance should be effective only if resistance levels can be assessed accurately (Wang *et al.*, 1989). Partial resistance cultivars considered to have durable blast resistance adequate for irrigated lowland rice areas have proved highly susceptible under high blast pressure (Bonman *et al.*, 1991). In view of the great virulence diversity reported by Correa-Victoria and

Zeigler (1993b), a range of parents with complete and partial resistance should be included in breeding for durable blast resistance (Correa-Victoria and Zeigler, 1993a).

Population improvement methods normally employed for quantitative traits have not been used for rice. One reason is the labor requirement for manual cross-pollination of rice. A male-sterile gene, produced in the variety IR36 (Singh and Ikehashi, 1981) has been used by the "Centro Nacional de Pesquisa Agropecuaria de Arroz e Feijão" (CNPAP/EMBRAPA), Brazil, to enhance cross-pollination (Neves *et al.*, 1992). The limitations of using male-sterility are: it does not allow precise disease evaluation on male-sterile plants (percentage of sterile grains is used to evaluate NBI, and male-sterility mimics this symptom); much time and effort are required to transfer such genes into adapted genetic backgrounds before starting recombination and selection; and since recombination is uncontrolled, there is no way to determine the gene frequency of each parent in the new population, an important objective in directional selection and in maintaining diversity. Recently, a simple manual-crossing technique for rice was developed at the "Centro Internacional de Agricultura Tropical" (CIAT), which permits the production of three times as many crosses with the same amount of labor and increases the feasibility of a recurrent selection program based on hand crossing (Sarkarung, 1991). Instead of planting parents in the greenhouse or bringing the whole plant from the field for emasculation and pollinization, the new methodology uses only a single tiller, detached from each plant, for these operations.

The literature shows several examples of the risk involved in reducing genetic diversity (NAS, 1972). In the case of rice, the development and rapid adoption of semidwarf germplasm have played a major role in reducing genetic diversity (Hargrove, 1979). Cuevas-Perez *et al.* (1992) studied the contribution of land races to irrigated rice varieties in Latin America and the Caribbean and found that 69.2% of the gene composition of the region's released cultivars came from just 14 land races, mainly from Asia. A single germplasm source, the variety IR8, accounted for 39% of the genes currently in commercial use over the entire irrigated area of the region. IR8 is derived from just three land races (Dee Geo Woo Gen, Cina, and Lati Sail).

The objectives of this work are: a) to describe the methodology used by CIAT to develop a genetically broad-based synthetic population containing high levels of blast resistance; b) to compare the genetic diversity of the population to the diversity currently utilized in the region, and c) to document progress

made after one generation of selection in three populations extracted from the GC-91.

MATERIAL AND METHODS

The GC-91 rice synthetic population was created by intermating 30 rice breeding lines (Table I) chosen from germplasm evaluated by CIAT's rice program at Santa Rosa Experimental Station (SRES), located at Villavicencio, Colombia (333 m elevation, 4°5' latitude N, 73°40' longitude W). This upland rainfed site has favorable environmental conditions for the development of diseases, particularly Bl and NBI, i.e., it is a "hot spot". SRES also contains an unusually diverse range of blast pathotypes (Correa-Victoria and Zeigler, 1993b). Mean maximum and minimum temperatures are 30 and 21°C, respectively; the average relative humidity is above 80%, and the 2700 mm of annual rainfall are well distributed. Soils are classified as Ustropept Isohyperthermic Silty over Mixed Sandy Skeletic.

The parents were chosen based on blast reaction over at least eight growing seasons at SRES (Correa-Victoria and Zeigler, 1993a), with the exception of the cultivar Araguaia, which was evaluated at Goiânia, a blast-prone area in Brazil (Guimarães *et al.*, 1992). These lines were selected on the basis of four criteria. The first was stability of blast resistance. Selected lines exhibited either complete or partial resistance to blast with a stable reaction over time. Reactions were subsequently confirmed using controlled inoculations under greenhouse conditions. The second criterion was the specific case of cultivar 'CICA 9'. The genetic diversity of the fungus at SRES has been classified into six genetic "lineages" or families. Blast isolates collected from 'CICA 9', a cultivar highly susceptible in the field, belong to only one genetic lineage (Levy *et al.*, 1993). This cultivar is resistant to all other *P. grisea* lineages at the site (Correa-Victoria and Zeigler, 1993b). The intent, then, was to use 'CICA 9' as a donor of resistance genes against all other lineages of the pathogen (Correa-Victoria and Zeigler, 1993b). The third criterion consisted of agronomic traits. Some lines were chosen because of high yields and good grain quality, with only a reasonable level of blast resistance (scores 4 or 5, in a 0 to 9 scale). The fourth criterion was the genetic background of the line. To assure wide genetic diversity in the population, parents were traced back to their original land races, and sources of resistance were identified. Germplasm from the two major sub-groups

Table I - Parents, crosses, and gene frequency in the GC-91 rice gene pool.

Parent	Cross	Population ^a			
		GC-91	C1P1	C1P2	C1P3
P 5589-1-1-3P-4-MP	Carolino/TOx 1785-19-18//Colombia 1/TOx 1011-4-1	2.76	0.29	0.13	0.38
CT6196-33-11-1-3-M	Col 1 X M312A-74-2-8-8/IRAT 124//RHS 107-2-1-2TB-1JM	3.96	0.73	0.95	0.89
CT6261-5-7-2P-5-1P	Camponi/Col 1 X M312A-74-2-8-8//TOx 1785-19-18	3.24	0.44	0.27	0.64
CT7242-16-9-2-M	TOx 1780-2-1-1P-3/Col 1 X M312A-74-2-8-8//TOx 718-AL-27-1CM-1JN	4.80	1.32	0.68	1.15
Ceysvoni	SML 997/Aiwini	4.02	0.29	0.81	0.51
Ecia 122-J8-1-2-1	Rustic//7399/IRAT 13	3.12	0.14	1.35	0.38
Ecia 24	IR 1529-430/VNI IR3223	3.84	0.88	0.68	0.76
C 48CU76-3-2-1-4-5M	IR2003/IR1615	3.24	0.15	0.68	0.13
P 4076F3-2-2-4	P 1386-6-8M-1-3M-1//P 1482-8-9M-1-2M-1B/Camponi	4.98	0.15	1.08	0.25
P 2851F4-145-9-58-1B-10	S 7-6//IR11-452-1-1/Camponi	3.30	0.44	3.11	0.38
P 3055F4-3-4P-1P-1B	S 5-11//Camponi/K 8	3.06	0.59	1.08	0.51
P 4725F2-9-6-1X	P 2026F4-49-5-5//IR5533-13-1-1/Oryzica 1	3.00	0.29	1.08	0.38
TOx 1859-102-6M-3	TOx 95/Multiple Parent 109	3.24	2.35	0.68	2.67
P 4743F2-80-2-1X	P 1274-6-8M-1-3M-1//P 1386-6-8M-1-3M-1/Metica 1	2.64	1.61	1.62	1.40
CT6278-3-7-4P-1	P 2062F4-17-33-1/IRAT 120// Col 1 X M312A-74-2-8-8	4.02	2.20	6.22	2.16
CT6393-M-9-2-5-M	TOx 503-1-52-1/Suakoko//P 3085F4-59-1/Colombia 1	2.16	0.29	2.84	0.25
P 3621F2-1-2-8-1B	Metica 1//Suakoko/Ceysvoni	3.18	1.76	1.76	1.66
Araguaia	IAC 47//TOs 2578-7-4-2-3-B2	4.08	0.44	5.28	0.51
IRAT 144	IRAT 110/IRAT 13	3.54	1.17	3.92	1.27
IRAT 146	IRAT 13/Dourado Precoce	2.58	1.61	1.89	1.53
IR35353-94-2-1-3	IR17494/IR18272//IR19661	2.64	0.00	1.76	0.00
IR35410-16-3-2-2-2-2	IR2307/IR15689//IR4744	3.24	4.41	3.52	3.82
TOx 340-1-7-1	Tchenchouai//TN1/1031 1716-2-3-10	2.58	0.15	5.28	0.25
CICA 9	IR665/23/3/1//IR841-63-5-104-1B/C 46-15	0.90	3.23	2.71	3.69
Oryzica 2	BG90-2//CICA 8/CICA 7	3.90	1.76	10.28	1.78
CT6113-8-9-7-M	TOx 95/Oryzica 2//CICA 8	3.12	5.77	4.33	6.11
CT6458-9-3-6-M	P 3919F4-45-5/IRAT 120//IR4568-225-3-2/IR5657-33-2-2-3	4.80	7.05	8.93	7.64
CT5756-3-5-1-M	P 1274-6-8M-1-3M-1//P 5227//Campeche A-80	3.54	27.75	6.36	26.37
P 5446-9-4-4-M	TOx 1011-4-1/Oryzica 2//Ceysvoni/IRAT 122	3.00	15.71	10.28	15.92
CT6240-12-2-2-1-1P	Ngovie/IRAT 124//Col 1 X M312A-74-2-8-8	3.60	17.03	10.15	16.56

^aGC-91, C1P1, C1P2 and C1P3 refer to original gene pool and populations 1, 2, and 3, respectively.

of *Oryza sativa*, namely indica and japonica, were included, representing fundamental dimensions of genetic diversity.

The GC-91 gene pool was formed using two intermatings. The first involved crossing each of the 30 parents to five other parents, for a total of 150 single crosses. The second mating involved crossing each F₁ with three other F₁'s, avoiding the combination of F₁'s that had the same progenitor. All crosses were made manually as described by Sarkarung (1991). The crosses were made with the intent of keeping a similar contribution of each parent in the population (Table I). Seed of 417 double crosses (DC), of all 450 possible combinations, were obtained. Approximately 50 seeds

from each DC were planted and individually harvested. A bulk sample of equal numbers of seeds per plant was made of all DCs to produce the GC-91 rice synthetic population (S₀ population). For the purpose of the present study, however, all DCs and their progeny were also planted individually in order to track parental contribution during subsequent selection, as described below.

The recurrent selection scheme employed was based on S₂ line evaluation. The S₀ population was planted at CIAT-Palmira, a blast free site, for seed multiplication and selection for highly heritable agronomic traits. The S₁ and S₂ generations were grown at SRES, where blast pressure is high. Twenty-five

plants that traced back to individual S_0 were evaluated per DC, for a total of 10,425 S_1 plants. Their blast reaction distribution curves and the average were used to represent that cross. Individual plants included for S_2 evaluation were selected based on minimum performance criteria for the DC average and S_1 plant data. In the S_2 generation, 10 plants were evaluated per each of the 580 S_1 -derived line, and superior S_2 lines were selected based on their performance as parents for the next cycle. Approximately 60 lines were chosen and recombined for each population; each line was crossed to three other lines, using three plants within a line to represent the variability of that line.

In the development of these populations, Bl was scored using the Standard Evaluation System for Rice published by IRRI (1988), which follows a 0 to 9 scale, where 0 means no infection and 9 highly susceptible. Bl was scored three times, first when the infection was great enough to distinguish genotypes (around 22 days after planting) and twice more at seven-day intervals. Flowering date (50% anthesis) was noted, and NBl evaluations were made 28 and 35 days after flowering.

During the first generation of selection, information was recorded for individual crosses, rather than on lines from the GC-91 bulk, so that the contribution of each progenitor could be monitored. Three populations were extracted from GC-91 to test different selection strategies: a) rapid fixation of major genes for high levels of resistance (population C1P1), b) gradual accumulation of polygenes for partial resistance (population C1P2), and c) a combination of objectives a and b (population C1P3). Table II shows the criteria used in the S_1 generation to select plants used to produce these populations. Similar criteria were adopted in the S_2 generation, but changes were made for the DC average. For C1P1 the point of truncation was lowered for Bl; for C1P2 it was kept the same; and for C1P3 selection pressure was reduced for Bl and NBl.

As a result of these selection strategies, 58 rice lines were chosen as parents for the next cycle of population C1P1, 51 for population C1P2, and 58 for population C1P3 (the recombination of these lines to generate the new cycle of selection was done as described above). The Bl and NBl data for these three groups were compared to those for the GC-91 gene pool to provide results on selection progress with the three strategies. The 167 selected lines, in addition to the 30 parents, were planted in a randomized complete block design with three replications using two planting dates 15 days apart (May 20 and June 3, 1993). Spreader rows composed of a mixture of commercial susceptible varieties were used to maintain blast pathogen diversity and to increase the frequency of all pathotypes and thus maximize the probability of encounter between a breeding line and a compatible race, as proposed by Correa-Victoria and Zeigler (1993b).

To assess genetic progress in each plot, five plants were tagged and all evaluations made on them. Bl was evaluated at three dates (30, 37, and 45 days after planting) and NBl at two (28 and 35 days after flowering). To evaluate the effect of selection for blast on some agronomic traits, the number of days to flowering (Fl) and plant height (Ht) were measured.

For statistical analysis only the highest score per S_1 plant and per plant within S_2 line was considered for Bl and NBl. The disease reaction of each entry was placed in one of three categories: resistant (R) ≤ 3 , intermediate (I) 4 or 5, or susceptible (S) ≥ 6 . The disease was analyzed following the methodology proposed by Grizzle *et al.* (1969), using the PROC CATMOD, for categorical data (SAS, 1988), applying a logit model where the response functions were $\log(\Pi_R/\Pi_S)$ and $\log(\Pi_I/\Pi_S)$, where Π_R , Π_I , and Π_S correspond to the proportions of resistant, intermediate, and susceptible lines. For Fl and Ht analyses of variance were calculated.

RESULTS

The GC-91 rice gene pool was created to bring together diverse blast resistance sources in a broad-based genetic background. The germplasm base of GC-91 was analyzed through a genealogical study, tracing each line back to its original land race parents. The results show that 22.4% of the germplasm is of African origin and that the contribution of the Latin American and the Caribbean "genetic core" is reduced (Table III). The 30 parents used for crossing (Table IV) combine both indica and japonica sources (63.3% and 36.7%, respectively), representing a mixing of the major

Table II - Selection criteria employed in the S_1 generation to derive three populations from the GC-91 gene pool.

Population	S_1 cross average		Individual S_1 plant data		S_2 cross average	
	Bl ^a	NBl ^a	Bl	NBl	Bl	NBl
C1P1	≤ 2.0	≤ 2.5	≤ 3	≤ 3	≤ 1.5	≤ 2.5
C1P2	-	-	4 or 5	≤ 5	-	-
C1P3	≤ 2.0	≤ 2.5	≤ 5	≤ 5	≤ 4.5	≤ 3.0

^aBl and NBl refer to leaf and panicle blast rating, respectively.

diversity in *Oryza sativa*, which is also the predominant distinction between current lowland (indica) and upland (japonica) rice germplasm. By mixing upland with lowland groups, it is hoped that desirable traits of each can be interchanged (Lentini and Martinez, 1992), thereby obtaining novel transgressive types, particularly for blast but also for other adaptive characters, such as the yield potential of lowland rice (Kabaki, 1993) and the root system (Hasegawa *et al.*, 1991) and stress resistance of upland rice.

Studies by Levy *et al.* (1993) and Correa-Victoria and Zeigler (1993a) were used to classify the reaction to BL and NBL of the 30 parents used to form the GC-91 gene pool (Table IV). The most prevalent lineage of the pathogen was SRL-6; however, nine lines were not susceptible to any of the lineages belonging to the six groups of pathogens identified through inoculations under greenhouse conditions. In terms of field reactions at SRES (i.e., disregarding isolates per se), 15 parents showed complete resistance,

Table III - Land races and their origins and percent contribution to the GC-91 gene pool as compared to the region's commercial "genetic core"¹.

Percentage ² Land race	Origin	Participation	
		Genetic core	GC-91
Blue Rose	USA	2.11	1.28
Bruinmissie	USA	0.26	0.01
Cina	China	11.96	4.49
Dee Geo Woo Gen	China	14.93	10.45
Hill Selection	USA	0.42	-
I Geo Tze	China	4.80	0.15
Khao Dawk Mali 105	Thailand	1.78	0.10
Lati Sail	India	11.98	4.44
Marong Paroc	Philippines	5.41	3.61
Mong Chim Vang A	Vietnam	4.86	0.15
Pa Chiam	China	3.74	1.31
Sinawpagh	Philippines	0.52	12.95
Tadukan	Philippines	5.42	1.62
Tsai Yuan Chon	China	0.90	4.35
560	Liberia	-	6.67
63-105	Ivory Coast	-	6.29
M 312A	Ivory Coast	-	0.43
Makalioka	Madagascar	-	0.30
Multiple Parents 109	Nigeria	-	0.20
Ngovie	Sierra Leone	-	0.11
O.S.6	Congo	-	1.71
Variété du Zaire	Zaire	-	6.67
Total	-	69.19	67.29

¹Cuevas-Perez *et al.* (1992).

²Refers to the percent contribution of each parental line to the total.

13 partial, and two were susceptible. Greenhouse inoculations indicated that some lines exhibiting complete resistance in the field (e.g., 'P

Table IV - Leaf blast reactions of 30 parents used to form the GC-91 gene pool.

Parents	Greenhouse ^a	Field ^b	Morphological classification
	Susceptible to lineages	Level of resistance	
P 5589-1-1-3P-4-MP ^c	6	Complete	Japonica
CT6196-33-11-1-3-M	6	Complete	Japonica
CT6261-5-7-2P-5-1P ^c	None	Complete	Japonica
CT7242-16-9-2-M	None	Complete	Japonica
Ceysvoni ^c	6	Partial	Japonica
Ecia 122-J8-1-2-1	6	Partial	Indica
Ecia 24	None	Partial	Indica
C 48CU76-3-2-1-4-5M	6	Partial	Indica
P 4076-F3-2-2-4	6	Partial	Indica
P 2851F4-145-9-58-1B-10	2	Partial	Indica
P 3055F4-3-4P-1P-1B	None	Partial	Indica
P 4725F2-9-6-1X	5,6	Partial	Indica
TOx 1859-102-6M-3	6	Partial	Japonica
P 4743F2-80-2-1X	5,6	Partial	Indica
CT6278-3-7-4P-1	6	Partial	indica
CT6393-M-9-2-5-M	5	Partial	Indica
P 3621F2-1-2-8-1B	None	Partial	Indica
Araguaia	None	Complete	Japonica
IRAT 144	None	Complete	Japonica
IRAT 146	6	Complete	Japonica
IR35353-94-2-1-3	5,6	Complete	Indica
IR35410-10-16-3-2-2-2-2	6	Complete	Indica
TOx 340-1-7-1	5,6	Complete	Japonica
CICA 9	1,2	Susceptible	Indica
Oryzica 2	5,6	Susceptible	Indica
CT6113-8-9-7-M	6	Complete	Indica
CT6458-9-3-6-M	None	Complete	Indica
CT5756-3-5-1-M	None	Complete	Indica
P 5446-9-4-4-M	6	Complete	Indica
CT6240-12-2-2-1-1P	6	Complete	Japonica

^aSusceptibility under greenhouse inoculation with *Pyricularia grisea* isolates representing different genetic lineages (Levy *et al.*, 1993) at Santa Rosa Experimental Station in Colombia.

^bLevel of field resistance at Santa Rosa Experimental Station, Colombia, and stability for eight seasons or more. Complete = no symptoms or presence of non-sporulating lesions; Partial = presence of typical susceptible lesions covering less than 10% of leaf area or panicle blast reaction ≤ 5 ; Susceptible = presence of typical susceptible lesions covering more than 10% of leaf area or panicle blast reaction > 5 , according to the Standard Evaluation System for Rice (IRRI, 1988).

^cThese lines were also classified using isozyme analyses of subtype of *Oryza sativa*.

5589-1-1-3P-4-MP' and 'CT6196-33-11-1-3-M') may become partially infected at the seedling stage by some isolates of the pathogen.

One generation of selection affected the percentage of contribution of different parents in the populations, compared to the original gene pool (Table I). Most parents had decreasing contributions to the selected population. The most significant changes were observed in the following lines: 'IR35353-94-2-1-3', which disappeared from C1P1 and C1P3; 'Oryzica 2' and 'P 5446-9-4-4-M', which showed the highest frequency in C1P2 and decreased in C1P1 and C1P3; and 'CT5756-3-5-1-M', 'CT6240-12-2-21-1P', and 'P 5446-9-4-4-M', which increased to the extent that together they contributed 60 and 59% of the germplasm present in populations C1P1 and C1P3, respectively.

'IR35410-16-3-2-2-2' and 'CICA 9' were the only lines whose frequency was very similar in all populations and around the equi-proportional value (3.33%) of GC-91.

The variance analysis showed that planting date did not significantly affect Fl or Ht. The effect of genotype was significant at the 5% level for Fl, probably because of the difference detected between populations C0P0 and C1P2 (Table V). These results suggest that selection for blast had little indirect effect on Fl and Ht.

For Bl the logit model analysis showed a highly significant effect for the intercept, block within planting date, and population (Table VI). The highly significant estimate for the intercept (-2.3534 for Π_R/Π_S and 0.2725 for Π_I/Π_S) indicates that $\log \Pi_R/\Pi_S$ or Π_I/Π_S is different

Table V - Variance analysis for days to flowering (Fl) and plant height (Ht).

SV	DF	Fl		Ht	
		SS	Prob > F	SS	Prob > F
Planting date (D)	1	2772.48	0.1464 ns	15.87	0.9266 ns
Block within D	4	856.36	0.2047 ns	1652.09	0.6686 ns
Genotype (G)	199	1454.31	0.0001*	2982.08	0.2687 ns
C0P0 vs C1P1	1		0.8179 ns		0.2499 ns
C0P0 vs C1P2	1		0.0493*		0.1360 ns
C0P0 vs C1P3	1		0.2720 ns		0.5170 ns
C0P1 vs C1P3	1		0.2930 ns		0.5400 ns
Residual	195				
G x D	199	564.25	0.5669	2796.52	0.4840 ns
Error	403	1012.14		2869.89	
CV (%)			23.7		50.0

ns = not significant.

* = significant at 5% level.

Table VI - Logit model results for leaf (Bl) and panicle (NBl) blast, using categorical variables with three levels (R, I, and S).

SV	DF	Chi-square		Probability F	
		Bl	NBl	Bl	NBl
Intercept	2	156.62	317.98	< 0.0000**	< 0.0000**
Planting date (D)	2	5.33	129.57	0.0698 ns	< 0.0000**
Block within D	8	34.77	12.69	< 0.0000**	0.1230 ns
Population (P)	6	37.50	21.70	< 0.0000**	0.0014**
P x D	6	3.64	1.08	0.7248 ns	0.9824 ns
Residual	24	16.20	17.80		

ns = not significant.

** = significant at 1% level.

from zero, or that the overall proportions of R or I were significantly lower than S lines in the whole data set. The significance between populations was caused by the difference detected between all relationships Π_R/Π_S and Π_I/Π_S for all comparisons C0P0 vs C1P1, C0P0 vs C1P2, and C0P0 vs C1P3 (data not showed). The percentages of lines R, I, and S, presented in Table VII, show the differences detected in the above mentioned comparisons.

Highly significant effects were observed for the intercept, planting date, and population for the variable NBI (Table VI). The same explanation presented for BI can be used for NBI. The literature reports that this variable seems to be more affected by planting date than BI (Ou, 1985). The first date had more pressure than the second (data not shown), nevertheless, there was no interaction between planting date and populations. The significance observed for the source population was due to differences in the proportions Π_R/Π_S between C0P0 vs C1P1 and Π_I/Π_S between C0P0 vs C1P3 (Table VII).

Table VII - Percentage of lines resistant (R), intermediate (I), and susceptible (S) to BI and NBI for the original population and the populations developed following the three selection criteria. Data pooled across planting date.

Population ^a	BI			NBI		
	R	I	S	R	I	S
C0P0	5.56	48.33	46.11	21.11	8.89	70.00
C1P1	8.05	58.62	33.33	36.78	8.33	54.89
C1P2	0.65	47.88	51.47	27.69	10.75	61.65
C1P3	8.91	56.90	34.20	28.74	14.66	56.61

^aC0P0, C1P1, C1P2, and C1P3 refer to original gene pool and populations 1, 2, and 3, respectively.

DISCUSSION

In the formation of GC-91, crosses were made to keep the contribution of all parents similar, i.e., at 3.33% (Table I). With the exception of 'CICA 9', which contributed only 0.90%, values close to that target were achieved for all parents. During selection the contribution of line 'CT5756-3-5-1-M' had the greatest increase in contribution for populations C1P1 and C1P3. This change suggests that 'CT5756-3-5-1-M' has a strong major gene or genes for blast resistance. The same can be said for lines 'CT6240-12-2-2-1-1P' and 'P 5446-9-4-4M'. The increased percentage in R and I reactions to blast of population C1P1 (Table VII)

indicates that selection was successful. This may be attributed to the three lines mentioned above, which have no common parent (Table I). They combine germplasm from Latin America, Africa, and Asia, including both indica and japonica groups.

The cultivar 'CICA 9' contributed a similar but slightly increased (relative to GC-91) germplasm percentage to all three selected populations. The slightly increased contribution of 'CICA 9' suggests that major resistance genes are not involved, since selection for major gene effects, as practiced in population C1P1 and C1P3, did not skew the contribution of this parent. This is in agreement with the inference of genetic control suggested by Correa-Victoria and Zeigler (1993b).

Based on the study of Cuevas-Perez *et al.* (1992), it is possible to compare the genetic base of GC-91 with germplasm released as commercial varieties in Latin America and the Caribbean. The land races identified by Cuevas-Perez *et al.* (1992) as the region's "genetic core" contributed 44.9% of the genes to GC-91 (Table III), which is 35% less than that represented in the region's current irrigated cultivars. African germplasm provided 22.4% of GC-91's germplasm, and the remaining 32.7% came from more than 50 additional lines of diverse origins. Furthermore, GC-91 brings together germplasm from both indica and japonica groups, hopefully increasing recombination among them. GC-91 thus offers possibilities for extracting recombinants that include contributions from diverse sources not present in current Latin American and Caribbean commercial varieties. This increased genetic variability should thus contribute to a decrease in genetic uniformity in the region and improve the possibilities for achieving novel character combinations.

The 30 parents used to form the GC-91 rice gene pool were classified according to leaf blast susceptibility relative to the six lineage groups of the pathogen identified by Levy *et al.* (1993). All resistant parents were stable under field conditions for at least eight growing seasons (Correa-Victoria and Zeigler, 1993b). Nine parents were resistant to all six groups, but the majority showed susceptible reactions to lineage 6 under greenhouse conditions (*Pyricularia* races belonging to this lineage are prevalent at SRES). The presence of lines showing resistant reactions to different lineages suggests that broader-spectrum resistance might be achieved through their recombination, as recommended by Levy *et al.* (1993). Recurrent selection could be an appropriate breeding method for attaining this goal, since it facilitates the convergence of multiple resistance gene sources.

Leaf blast differences were observed for all relationships between the proportions Π_R/Π_S and Π_I/Π_S . This means that the proportions of R and I lines were statistically superior for populations C1P1 (8.05%) and C1P3 (8.91%) and less for C1P2 (0.65%) than for C0P0 (5.56%). Increases were evident in resistance to Bl (selection progress), possibly due to expression of genes with major effects present in the original parents. In population C1P2, because of the milder selection criteria used (Table II), improvement (increase in percentage of R and I lines) during one generation of selection was not observed. This is not surprising since the objective of the population is to gradually accumulate genes with minor effects, creating a germplasm source with stable, partial resistance. The population C1P3 is intended to combine both major and minor gene effects. After one generation of selection, similar progress was made in comparison to C1P1, as might be expected given the impossibility of identifying major and minor genes effects with the field experimental design used in this study.

Genetic improvement for NBl was clearly observed in the population C1P1; the number of R lines from C1P1 (36.78%) was statistically superior to the number of lines from C0P0 (21.11%). Some improvement was achieved within C1P3; the number of R lines (28.74%) was statistically similar between C0P0 and C1P3, but C1P3 showed significantly more I lines (14.66%) than C0P0 (8.89%). No statistical differences were observed between C1P2 and C0P0. These results demonstrate that progress was made when $NBl \leq 3$ or ≥ 6 was used as a selection criterion, but not when only plants and lines with scores equal to 4 or 5 were selected. That was as expected, given the impossibility of testing for major and minor genes in these trials.

Greenhouse work is under way to characterize blast reaction to the different genetic lineages of the pathogen and to identify the type of gene action predominant in populations C1P1, C1P2, and C1P3. Characterizing this resistance is more important than the progress made in increasing the percentage of R and I lines or decreasing the average value for Bl and NBl reactions for populations C1P2 and C1P3, since the objective of these populations is to incorporate as many minor or partial resistance genes as possible. Selection of highly resistant lines could mask the presence of minor genes. Variability for Bl and NBl reactions in population C1P1 indicates that further genetic improvement for resistance can be made through continuous selection, whereas for populations C1P2 and C1P3, progress will be better evaluated when the resistance spectrum has been assessed.

During selection blast was the main factor used to choose a S₁ plant and a S₂ line. Nevertheless, other agronomic traits were taken into consideration in selecting between lines showing similar reaction to the disease. The statistical analysis indicated that these strategies had little indirect effect on Fl and Ht, allowing genetic variability for these agronomic traits to be present in the selected populations. The main objective of the rice breeding program at CIAT is to develop improved populations for specific traits, while maintaining variability for other traits, so that national partners can carry out further selection (CIAT, 1991).

Even though the proposed recurrent selection for resistance to Bl and NBl is in its initial stages, and the results of the recombined S₂ selected lines are not available, the results of one generation of selection indicate that progress is being achieved. However, the change in parent frequency favoring three lines in C1P1 and C1P3 warrants concern, since it may result in narrowing the genetic base of these populations.

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RESUMO

Os métodos de melhoramento populacional usados nas culturas de polinização cruzada não têm sido aplicados de maneira extensiva ao arroz (*Oryza sativa* L.). Este artigo descreve a formação e o melhoramento de um pool gênico de base genética ampla para resistência a brusone do arroz (*Pyricularia grisea* Sacc.), e o progresso obtido com a primeira etapa de seleção. Trinta linhas de arroz de origem geográfica diversa foram selecionadas como progenitores, baseado na estabilidade de suas reações a uma gama de raças de brusone nas folhas (Bl) e na panícula (NBl) durante oito plantios em um local altamente favorável ao desenvolvimento da doença. Cada progenitor foi cruzado com outros cinco progenitores e cada F₁ com outras três F₁s buscando assegurar um bom nível de recombinação. As sementes produzidas por esses cruzamentos foram misturadas para formar a população sintética GC-91 (C0P0). A diversidade genética foi maior na GC-91 quando comparada à "base genética" das variedades plantadas na América Latina e no Caribe. Três populações foram obtidas da GC-91 com diferentes objetivos de seleção para brusone almejando alcançar: a) uma rápida fixação de genes maiores para desenvolver níveis elevados de resistência (população C1P1), b) um acúmulo gradual de poligenes para resistência parcial (população C1P2) e c) a

combinação dos objectivos a e b (população C1P3). Linhagens parentais seleccionadas para iniciar o próximo ciclo de seleção apresentaram maior nível de resistência a BI e NBI nas populações C1P1 e C1P3 quando comparadas com a C0P0 e não foi observado ganho na população C1P2.

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