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Comparison of rice lines derived through anther culture and the pedigree method in relation to blast (*Pyricularia grisea* Sacc.) resistance

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Abstract Crosses were made between Fanny (highly susceptible to blast) and 11 cultivars differing in blast resistance. Using the pedigree method (PM) segregating generations were evaluated and selected for blast resistance. Via anther culture (AC), doubled-haploids were obtained from F_1 plants and from F_2 blast-susceptible plants. Pedigree and anther culture-derived lines were planted together and evaluated for blast resistance under rainfed conditions at the Santa Rosa Experiment Station, Villavicencio, Colombia. The principal objective was to compare PM and AC in terms of their efficiency in producing rice lines resistant to blast. Results of a stratified analysis showed an association between method and blast resistance. Results of the logit-model analysis showed that AC produced a significantly ($P=0.0001$) higher proportion of lines with initial blast resistance (leaf- and neck-blast reaction ≤4) than did PM across all cross types. Stable blast resistance was assessed based on field performance over 3 years. AC was superior to PM in generating stable resistance for only some cross types. Consequently, with a few exceptions, AC can be used as effectively as PM to develop rice cultivars resistant to blast, with savings in time and labor. Additionally, blast-resistant lines were obtained either by the pedigree method or by anther culture from crosses between blast-susceptible cultivars (Fanny/CICA4 and Fanny/Colombia1). This excludes somaclonal variation as a possible mechanism responsible for this resistance and suggests that a recombination of minor genes could have occurred and was fixed through either method. However, the stability of the resistance was greater in pedigree-derived lines. The implications of these findings for rice blast-resistance breeding are discussed.

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Introduction

Rice blast, caused by *Pyricularia grisea* Sacc., is considered to be the single most important disease of rice world wide. Development of resistant cultivars has been the preferred method to control this disease (Rosero 1979; Weeraratne et al. 1981; Roumen 1992; Correa Victoria and Zeigler 1993a, b), and many rice breeding programs have durable blast resistance as an important objective. The pedigree method (PM) has been extensively used to combine blast resistance from various donors (Cuevas Pérez et al. 1992). While there has been some success in controlling the disease through PM breeding (Leal et al. 1989; Correa Victoria and Zeigler 1995), in many instances the resistance has not been durable. New resistant cultivars often showed levels of susceptibility similar to the cultivars they replaced in a relatively short period of time (Weeraratne et al. 1981; Correa Victoria and Zeigler 1993a, 1993b). This resistance "breakdown" has been attributed either to increased prevalence of previously rare pathotypes (Buddenhagen 1983) or to the development of novel pathotypes (Ou 1980, 1985).

Levy et al. (1991, 1993) suggested new approaches for analyzing the genetic diversity among rice-blast isolates using DNA fingerprinting to characterize the genetic structure of the pathogen population. Based on apparent differences in the virulence spectrum of *P. grisea* subpopulations, or lineages (Correa Victoria et al. 1994; Zeigler et al. 1995), a breeding strategy for developing durable blast resistance was proposed to combine resistance genes showing complementary resistance to all lineages in a given population (Zeigler et al. 1994). However, as blast resistance is now known to be genetically complex, involving a number of major and minor genes (Wang et al. 1994), efficiently manipulating this complexity in a large breeding program is a major challenge confronting rice breeders.

The production of doubled-haploids (DHs) through anther culture (AC) has been proposed as an effective, efficient, and economic breeding tool (Chu 1982; Baenziger and Schaeffer 1983; Caligari et al. 1987; Baenziger et al. 1989; Lynch et al. 1991; Sanint et al. 1996), and may offer the means to efficiently combine several major and minor blast resistance genes. Several studies in different crop species (Friedt et al. 1986; Huang et al. 1988; Pauk et al. 1988; Picard et al. 1988) compared the performance of anther culture-derived lines with other breeding procedures in terms of yield and other characters. Although most of these studies showed that the AC method was easier, faster, and produced the same genetic variability as PM, disease resistance was not considered in the comparisons.

The objective of the present study was to compare the efficiency of pedigree and anther-culture methods with respect to fixing blast resistance in the progeny of a range of crosses between resistant and susceptible parents. Blast resistance may be complex and involve numerous quantitative trait loci (QTLs) and/or recessive genes (Wang et al. 1994) that could be inadvertently discarded during generation advance using PM. Therefore, a secondary objective was to determine if, by selecting susceptible plants in the F_2 for subsequent processing via anther culture, stable blast resistance could still be obtained.

Materials and methods

Genetic material, field techniques and experimental design

The field experiments were conducted at the Santa Rosa Experiment Station, a rice blast-disease "hot spot" (Correa Victoria and Zeigler 1993a, b) located near Villavicencio, Colombia. The pathogen population at this site is composed of six genetic lineages (Levy et al. 1993; CIAT unpublished) with different virulence spectra (Correa Victoria et al. 1994). Frequencies of avirulence genes have been quantified in Santa Rosa (Correa Victoria and Zeigler 1993b), but they are not fixed, as introduced resistant cultivars can show extreme susceptibility after only two or three seasons in small plots (Correa Victoria and Zeigler 1995). High rice-blast levels were maintained in breeding plots by planting spreader rows prior to sowing breeding populations under conditions conducive to blast development (high seeding density, 3.0 g/m, and high nitrogen level, 150–200 Kg/ha). Spreader rows were composed of cultivars with complementing susceptibility to all local pathotypes. Abundant naturally occurring inoculum was supplemented with chopped diseased leaves harvested from pure plots of all spreader components, air dried, mixed, and added to the spreader rows early in the season. Three check cultivars (CICA8, Oryzica1 and Ceysvoni) were planted every 20 rows. Additionally, a set of 45 rice cultivars with different resistance genes were planted monthly at different sites of the station to monitor shifts in the pathogen population. During the study period, susceptible checks had, on average, a disease rating of 7–8, while resistant ones had a 1–3 rating suggesting no major changes in the virulence frequencies of the pathogen population. Rice-blast severity in breeding lines was evaluated twice in the leaf stage at approximately 30 and 45 days after seeding while neck-blast incidence was evaluated only once at the late dough stage (25 days after heading) using the standard evaluation system for rice (IRRI 1988).

Following the method described by Sarkarung (1991), three types of crosses (Table 1) were made between the rice cultivar Fanny [Japonica, and highly susceptible to local components of the *P. grisea* population (Correa Victoria and Zeigler 1993a; Correa Victoria et al. 1994)] and 11 cultivars (either Japonica or Indica) with different degrees of blast susceptibility. All the cultivars are known to have one or more major blast-resistance genes (Mackill and Bonman 1992; Correa Victoria and Zeigler 1993a; IRRI, CIAT, unpublished), and those that have been studied in some detail (e.g. Ceysvoni and IAC165) have a complex of major and minor resistance genes (IRRI, CIAT unpublished) similar to that described for Moroberekan (Wang et al. 1994). F_2-, F_3-, F_4-, F_5- and F_6-pedigree generations were evaluated, and selected for blast resistance. Beginning in 1988, approximately 1500 plants/cross (17 917 F_2 plants) were planted and evaluated for leaf and neck blast. Thereafter, segregating generations were planted in two 5-m rows/line. Individual resistant plants [blast scores ≤4 according to the standard evaluation system for rice (IRRI 1988)] were selected and harvested within those lines. Based on the lines' initial reaction to blast, a total of 681 F_4 blast-resistant lines were obtained and subjected to a three-semester evaluation cycle at Santa Rosa to determine the stability of blast resistance (see Table 2) under high disease-pressure conditions using standard Santa Rosa field methods (Correa Victoria and Zeigler 1993b).

Table 1 Type of cross and parents used to generate the genetic material for this study

Cross type[a] and cross identification	Parents
Japonica/Japonica – S×R[b]	
2 CT5782	Fanny/IRAT13
3 CT8813	Fanny/TOX1011-4-1
6 CT8816	Fanny/OS6
7 CT8817	Fanny/LAC23
9 CT8819	Fanny/IAC165
10 CT8820	Fanny/ITA235
Japonica/Indica – S×R	
4 CT8814	Fanny/Ceysvoni
8 CT8818	Fanny/Carreon
Japonica/Indica – S×S	
1 CT5780	Fanny/CICA4
11 CT8821	Fanny/Colombia1
5 CT8815	Fanny/Tetep

[a] Classification based on isoenzyme analysis
[b] Based on susceptibility (S) resistance (R) reaction to blast under Santa Rosa Experiment Station's conditions

Panicles collected from F_1 and F_2 blast-susceptible plants from each cross were used to produce the AC-R_2 and AC-F_2 populations, respectively, via anther culture according to the methodology described by Nuñez et al. (1989). A liquid potato medium (Chuang et al. 1978) was used for callus induction while a solid MS medium (Murashige and Skoog 1962) was used for plant regeneration. Regenerated plantlets from F_1 plants were transplanted (30 × 25 cm) and grown under irrigated conditions; fertile plants were harvested individually and the seed (AC-R_2) used for blast evaluations in the field. Blast-susceptible F_2 plants were identified at the seedling stage in the field from each cross. Plants were transferred to the greenhouse where they were treated with fungicide and allowed to recover fully. Several panicles were collected from each recovered susceptible plant and processed through AC to regenerate AC-F_2 populations. Thus 441 AC-F_2 and 740 AC-R_2 DHs were produced (see Table 2). These DHs were planted at Santa Rosa in 1990 and evaluated for their leaf- and neck-blast reactions yielding 64 AC-F_2 and 67 AC-R_2 lines resistant at leaf and neck stages (scores ≤4) (Table 2). These lines were subsequently evaluated during three consecutive semesters to determine stability of blast resistance. In this way, three different sets of blast-resistant lines (F_4, AC-R_2 and AC-F_2) were planted together, for comparison, during three semesters, and evaluated for the stability of blast resistance under upland conditions. "Resistance stability" was defined as the percentage of rice resistant lines which remained resistant (leaf- and neck-blast reactions ≤4) through the three-semester evaluation cycle. Previous studies (Correa Victoria and Zeigler 1993a) have shown that most resis-

tant cultivars that continue to show resistance after three susccessive seasons of cultivation at the Santa Rosa Experiment Station maintain their resistance for at least another five seasons. They concluded that, due to the pressure and high frequency of compatible races, stable resistance can be selected at this site for most of the resistance genes described in the literature. (Correa Victoria and Zeigler 1993a, 1995). The rice cultivar Oryzica Llanos 5, selected at this hot spot and commercially grown in Colombia since 1989, shows a remarkable durability in resistance over space as well as over time (Correa Victoria and Zeigler 1995).

Statistical analysis

Blast reaction was recorded under a 0–9 discrete scale according to the International System for Rice Evaluation (IRRI 1988). As these are non-equally distant levels, categorical data-analysis methods were used for the statistical analysis. The statistical-analysis methodology was divided into two steps:

(1) Descriptive analysis for overall blast-resistance performance of genetic populations generated by each of the three methods (PM, AC-F_2, AC-R_2).
(2) Inferential statistical analysis to assess the effect of method, cross type, and their interactions, on blast resistance and resistance stability.

For descriptive statistical analysis blast reaction was analyzed as a nine-level categorical variable following a multinominal distribution. For each population generated by each method (PM, AC-F_2 and AC-R_2), the following descriptive parameters were calculated:

(1) Initial number of lines generated.
(2) Number and percentage of blast-resistant lines in the initial population.
(3) Mean score, skewness, median, mode and range of blast-reaction score in the initial population.
(4) Number and percentage of blast-resistant lines at the beginning of the evaluation cycle for resistance stability.
(5) Number and percentage of stable resistant lines.

The skewness coefficient is defined as $S_k=M_3/(s^2)^{3/2}$; the S_k value has full meaning under a full value range (i.e. when values from 0 to 9 are present). If $S_k \geq 0.5$, the distribution is left-oriented indicating a higher proportion of resistant lines; if $-0.5<S_k<0.5$, the distribution is symmetric; and if $S_k \leq -0.5$, the distribution is right oriented, indicating a higher proportion of susceptible lines. The mode, and the median, served as descriptive statistics.

For inferential statistical analysis two response variables were analyzed: initial reaction to blast expressed for each line as the maximum leaf- or neck-blast score. A line was considered 'resistant to blast' when its blast reaction score was ≤4 within the 0–9 scale. A nine-level response variable was transformed into a binary variable, whose two levels were: 'resistant', with an initial blast level≤4, and 'non-resistant', with an initial blast level ≥5. The second response variable analyzed was resistance stability, based on the initial reaction to blast and expressed also as a binary variable with two levels: stable resistant, when the line maintained its resistance throughout the three-semester evaluation cycle, and non-resistant if otherwise.

A stratified analysis (Mantel and Haenszel 1959) using the Cochran-Mantel-Haenszel statistic (CMH) was performed to test the presence of a significant association between 'method' and resistance or 'resistance stability' across the three cross-types. As the CMH statistic was highly significant, a second analysis was performed for both response variables: a logit model to test the effect of 'method', 'cross type', and their interaction on the proportion of stable resistant lines. The logit model fits a linear model on a function of response frequencies – the logit function – in this case defined as log ($p_R/1-p_R$), where π_R is the proportion of resistant lines or stable resistant lines (p_{SR}). The model utilized was: log ($p_R/1-p_R$)=u+method(M)+cross type (C)+M×C.

All data processing and analysis was performed through SAS (1990) version 6.07. Procedures FREQ with a CMH option, and CATMOD were used.

Results

The overall percentage of lines initially resistant over the 11 crosses was lower in PM-derived lines than in AC-derived lines (Tables 2, 3). From the initial evaluation of the populations (Table 2) it can be observed that the three populations are similar in the sense that all are susceptible, as shown by the mean, median, and mode blast scores. However, the AC-R_2 population was more skewed towards susceptibility. Initially (F_2 population) PM provided a greater number (and %) of resistant plants (19.5 vs 14.5 and 9.1) (Table 2). However, the difference did not persist through the F_4 to the beginning of the three-season evaluation cycle for stable resistance, with PM producing a lower percentage of resistant lines (3.8) than AC-F_2 and AC-R_2 (14.5 and 9.1, respectively). After three semesters of evaluation for stable resistance, PM continued to produce a somewhat lower percentage (0.9) of resistant lines than AC-F_2(1.1) and AC-R_2(1.8).

The breakdown of resistance lines was consistent with prior experience and reflects the dynamic and complex nature of the Santa Rosa pathogen population (Correa Victoria and Zeigler 1993a, 1995). Those lines remaining resistant after three cycles of evaluation may be considered to be stable in Santa Rosa based on previous research (Correa Victoria and Zeigler 1995); however, no inference should be drawn as to the expected durability of this resistance if the lines were to be planted over large areas.

Differences were also observed, between methods and among crosses, regarding the number and percentage of blast-resistant lines and those that remained resistant after the three-semester evaluation cycle (Table 3). Some crosses did not yield any blast-resistant line (CT8815) while others did not produce any stable-resistant line (CT8816); others did not respond well to anther culture (CT8819).

A stratified analysis (Table 4) was performed to test the association between method, initial blast resistance and resistance stability, and across cross types. An independent Pearson-χ^2 test showed significance in all cross types, particularly in the case of initial blast resistance. This indicates that the percentage of resistant lines obtained depends on the method used. This was further confirmed by the significant value of the CMH statistic (112.3 with $P=0.0001$). In the case of stable resistance, the independent Pearson-χ^2 test was significant only for the cross type J×I–S×R, indicating that only in this cross type was AC-R_2 better than AC-F2 and PM. However, the general association test across cross types (CMH statistic 2.3) was non-significant suggesting that no generalization on the superiority of AC in terms of stable resistance can be made across cross types.

Based on the logit model there were highly significant differences between methods, cross types, and a significant method × cross-type interaction for both response variables (Table 5). This suggests that the effect of the method in generating resistant and stable resistant lines also depends on the cross type. Further analysis (Tables 6 and 7) showed a significant effect of method in favor of AC-F_2

Table 2 Overall performance of populations generated by each method across crosses in relation to initial blast resistance and stable resistance

Parameter	Pedigree	AC-F_2	AC-R_2
(1) Initial no. of lines generated	17917[a]	441[b]	740[b]
(2) Descriptive statistics for initial population[e]			
Mean score	6.0	6.6	7.3
Skewness	0.2	–0.2	–0.9
Median	6	7	8
Mode	6	7	9
Range	0–9	2–9	1–9
No. (and %) of resistant lines	3491 (19.5)	64 (14.5)	67 (9.1)
(3) No. (and %) of resistant lines at the beginning of evaluation cycle for stable resistance	683[c] (3.8)	64 (14.5)	67 (9.1)
(4) No. (and %) of lines with stable resistance	163[d] (0.9)	5 (1.1)	13 (1.8)

[a] F_2 Plants
[b] Doubled-haploids (DH) from F_2 susceptible plants (AC-F_2) and F_1(AC-R_2) plants
[c] F_4 lines
[d] F_6 lines
[e] standard evaluation system (SES), scale 1–9 where 1=highly resistant, 9=highly susceptible

Table 3 Performance of populations generated by three methods in relation to initial blast resistance (R) and stable resistance (SR) per individual cross

Cross	Pedigree			AC-F_2			AC-R_2		
	N[b]	R[c] (and %)	SR[d] (and%)	N[b]	R[c]	SR[d] (and%)	N[b]	R[c]	SR[d] (and%)
Cross type[a]*: J×J – S×R*									
2. CT5782	1291	170 (13.2)	79 (6.1)	207	38 (18.4)	2 (1.0)	42	6 (14.3)	1 (2.4)
3. CT8813	1899	125 (6.6)	51 (2.7)	47	8 (17.0)	0 (0.0)	55	12 (21.8)	6 (10.9)
10. CT8820	1465	74 (5.1)	6 (0.4)	–[e]	–	–	238	10 (4.2)	1 (0.4)
7. CT8817	1471	61 (4.2)	2 (0.1)	27	7 (25.9)	2 (7.4)	168	22 (13.1)	0 (0.0)
9. CT8819	1583	95 (6.0)	1 (0.06)	1	0 (0.0)	0 (0.0)	30	0 (0.0)	0 (0.0)
6. CT8816	1565	42 (2.7)	0 (0.0)	–[e]	–	–	16	2 (12.5)	0 (0.0)
Cross type: J×I – S×R									
4. CT8814	1404	6 (0.4)	1 (0.07)	104	5 (4.8)	1 (1.0)	101	7 (6.9)	4 (4.0)
8. CT8818	1480	50 (3.4)	0 (0.0)	–[e]	–	–	42	6 (14.3)	1 (2.4)
Cross type: J×I – S×S									
1. CT5780	1922	34 (1.8)	15 (0.8)	42	5 (11.9)	0 (0.0)	2	0 (0.0)	0 (0.0)
11. CT8821	2057	24 (1.2)	8 (0.4)	3	1 (33.3)	0 (0.0)	30	2 (6.7)	0 (0.0)
5. CT8815	1780	0 (0.0)	0 (0.0)	10	0 (0.0)	0 (0.0)	16	0 (0.0)	0 (0.0)
Total (%)	17917	681 (3.8)	163 (0.9)	441	64 (14.5)	5 (1.1)	740	67 (9.1)	13 (1.8)

[a] J=Japonica; I=Indica; S=susceptible; R=resistant
[b] N=initial no. of F_2 plants or DH generated
[c] R=number (and %) of resistant lines at the beginning of the evaluation cycle for stable resistance (blast score ≤4)
[d] SR=no. (and %) of stable resistant lines, at the end of the evaluation cycle (blast score ≤4)
[e] –=no response to anther culture

and AC-R_2 (14.5 and 9.1%, respectively) over PM (3.8%) in terms of initial blast resistance. However, there was no significant difference between AC-F_2 and AC-R_2.

For stable resistance, Table 7 shows that the across cross-type comparisons AC-F_2 vs PM and AC-R_2 vs PM were significant in favor of AC, suggesting that AC was again more efficient than PM in generating lines with stable resistance. However, in the cross type J×J–S×R there were no differences between methods while in J×I–S×S, PM was better. In this latter case it could be argued that problems in response resulted in AC failing to produce sufficiently large populations to give a reasonable chance of matching the rate of production achieved by PM. In summary, results indicate that AC, in general, was more efficient than PM not only in terms of generating initial blast resistance but also in terms of the stability of the resistance.

Analysis of the effect of cross type shows that crosses between susceptible and resistant Japonica cultivars (J×J–

Table 4 Stratified analysis for initial blast resistance (R) and stable resistance (SR) in rice

Method	Cross type[a]								
	J × J, S × R (6 crosses)			J × I, S × S (3 crosses)			J × I, S × R (2 crosses)		
	N[b]	R[c]	SR[d]	N[b]	R[c]	SR[d]	N[b]	R[c]	SR[d]
PM	9274	567 (6.1)	139 (1.5)	5759	58 (1.0)	23 (0.4)	2884	56 (1.9)	1 (0.03)
AC-F_2	282	53 (18.8)	4 (1.4)	55	6 (10.9)	0 (0.0)	104	5 (4.8)	1 (1.0)
AC-R_2	549	52 (9.5)	8 (1.5)	48	2 (4.2)	0 (0.0)	143	13 (9.1)	5 (3.5)
Total	10105	672 (6.7)	151 (1.5)	5862	66 (1.1)	23 (0.4)	3131	74 (2.4)	7 (0.22)
Pearson χ^2 test per cross type (prob. of signif.)		78.3 (0.0001)	0.02 (0.991)		52.0 (0.0001)	0.41 (0.813)		33.0 (0.0001)	75.8 (0.0001)
CMH Statistic across cross type (prob. of signif.)		112.3 (0.0001)	2.3 (0.315)						

[a] J=Japonica; I=Indica; S=susceptible and R=resistant
[b] N=initial no. of F_2 plants or DH generated
[c] R=number (and %) of resistant lines at the beginning of the evaluation cycle for stable resistance (blast score ≤4)
[d] SR=no. (and %) of stable resistant lines at the end of the evaluation cycle (blast score ≤4)

Table 5 Logit-model analysis. Effect of method and cross type on initial blast resistance and stable resistance to blast

Source	Initial blast resistance			Stable resistance[a]		
	df	Wald chi-quare statistic	*P*	*df*	Wald chi-square	*P*
Intercept	1	598.4	0.00001	1	313.34	0.00001
Method	2	63.2	0.00001	2	15.96	0.0003
Cross type	2	33.0	0.00001	1	4.13	0.0422
Method × cross type	4	19.2	0.0007	2	16.34	0.0003

[a] Cross type J×I–S×S was not included in this analysis as there were no stable resistant lines produced through anther culture

S×R) were more efficient than others in generating lines (Table 7) with initial blast resistance. This could be explained by the presence in this group of land races (OS6, LAC23) and cultivars (IRAT13, IAC165, and TOX 1011-4-1) known to have a broad spectrum of resistance to rice blast.

There was a significant interaction between method and cross type (Table 5). From the probability values in Table 7 the frequencies of initial blast resistance in AC-F_2 and AC-R_2 were higher than PM in all cross types. However, only in the cross type J×I–S×R, was stable blast resistance produced at higher frequencies in AC-F_2 and AC-R_2 than in PM. This confirms that AC was more efficient than PM in generating lines with higher initial levels of blast resistance; however, for producing stable resistance, AC was superior to PM only in certain cross types.

Discussion

AC has been proposed as an efficient tool to overcome the high costs in personnel and time of PM-based breeding programs. Our study shows that the percentages of blast resistance lines are equivalent, or slightly superior, to those obtained by PM in a blast "hot spot" using AC on the same crosses. As expected, there were differences in the number of lines produced. More lines were produced initially through PM, which could be due to the poor response of some genotypes to AC. It has been shown (Raina 1989) that response to AC in rice is genotype-dependent. However, this problem should become less of a constraint since the induction medium used for AC when this study was initiated has been greatly improved for Indica rice (Sanint et al. 1996).

Table 6 Logit-model analysis for initial blast resistance and stable resistance to rice blast. Parameter estimates

Parameter		Initial blast resistance (R)				Stable resistance[a] (SR)			
		N	Estimate	Std. error	p_R value (%)	N	Estimate	Std. error	P_{SR} value
Method (M)	AC-F_2	441	−1.8	0.14	14.5	386	−4.3	0.5	1.3
	AC-R_2	740	−2.3	0.13	9.1	692	−3.9	0.3	1.9
	PM	17917	−3.2	0.04	3.8	12158	−4.5	0.1	1.2
Cross type (C)	J×J–S×R	10105	−2.6	0.04	6.7	10105	−4.2	0.1	1.5
	J×I–S×R	3131	−3.7	0.12	2.4	3131	−6.1	0.4	0.2
	J×I–S×S	5862	−4.5	0.12	1.1	–	–	–	–
Cross type × method									
J×J–S×R	AC-F_2	282	−1.5	0.15	18.8	282	−4.2	0.5	1.4
	AC-R_2	549	−2.3	0.14	9.5	549	−4.2	0.4	1.5
	PM	9274	−2.7	0.04	6.1	9274	−4.2	0.1	1.5
J×I–S×R	AC-F_2	104	−3.0	0.46	4.8	104	−4.6	1.0	1.0
	AC-R_2	143	−2.3	0.29	9.1	143	−3.3	0.5	3.5
	PM	2884	−3.9	0.13	1.9	2884	−7.8	1.0	0.03
J×I–S×S	AC-F_2	55	−2.1	0.43	10.9	–	–	–	–
	AC-R_2	48	−3.1	0.72	4.2	–	–	–	–
	PM	5759	−4.6	0.13	1.0	–	–	–	–

[a] Cross type J×I–S×S was not included in this analysis as there were no stable resistant lines produced through anther culture

Table 7 Treatment comparisons following the logit-model analysis for initial blast resistance and stable resistance in rice crosses

Comparison		Initial blast resistance (R)			Stable resistance[a] (SR)	
		df	Chi-square	*P*	Chi-square	*P*
Method	AC-F_2 vs PM	1	48.1	0.00001	4.7	0.0296
	AC-R_2 vs PM	1	18.9	0.00001	15.9	0.0001
	AC-F_2 vs AC-R_2	1	1.3	0.2628	1.1	0.2875
Cross type	J×J–S×R vs J×I–S×R	1	21.2	0.00001	4.1	0.0422
	J×J–S×R vs J×I–S×S	1	14.7	0.0001	–[a]	–
	J×I–S×R vs J×I–S×S	1	0.4	0.5474	–	–
Method within Cross type						
J×J – S×R	AC-F_2 vs PM	1	64.0	0.00001	0.01	0.9128
	AC-R_2 vs PM	1	9.7	0.0018	0.01	0.9378
	AC-F_2 vs AC-R_2	1	14.2	0.0002	0.001	0.9646
J×I – S×R	AC-F_2 vs PM	1	3.8	0.0501	5.5	0.0188
	AC-R_2 vs PM	1	25.5	0.00001	17.9	0.00001
	AC-F_2 vs AC-R_2	1	1.6	0.2083	1.4	0.2326
J×I – S×S	AC-F_2 vs PM	1	30.3	0.00001	–[a]	–
	AC-R_2 vs PM	1	3.9	0.0479	–	–
	AC-F_2 vs AC-R_2	1	1.5	0.2187	–	–

[a] Cross type J×I–S×S was not included in this analysis as there were no stable resistant lines produced through anther culture

In some cases [e.g. cross CT5780 (Fanny/CICA4)], response to AC was better when panicles were collected from F_2 plants rather than from F_1 plants. Fanny and CICA4 are true Japonica and Indica types, respectively, and the F_1 was highly sterile. Since CICA4 is recalcitrant to AC while Fanny responds well, and AC is under genetic control (Miah et al. 1985; Quimio and Zapata 1990), in some F_2 plants some of the gene(s) responsible for the AC-response could already be fixed as compared to F_1 plants. Alternatively, since F_1 sterility may be due to non-viable pollen, one generation of selection could simply improve the probability of selecting viable microspores for processing through anther culture. In cases where response to AC is not good in the F_1 generation, the F_2 generation may yield more progeny.

Although the percentage of resistant lines in the initial population (Table 2) was significantly higher in PM than in AC-F_2 and AC-R_2, and a somewhat lower number of resistant lines was produced with a significantly lower amount of starting material, AC was more efficient in terms

of selection efficiency, considering the high number of F_2 plants (17 917) that had to be handled at the beginning of the process. At the beginning of the evaluation cycle for stable resistance, the situation changed in favor of AC with respect to initial blast-resistance. However, for stable resistance AC was better than PM only in some cross types, and there was an association between method and resistance. Consequently, there is no advantage to PM over AC in generating blast resistant lines, and the latter can be used effectively to develop rice cultivars resistant to rice blast. This will lead to savings in time and labor as shown by Sanint et al. (1996). Despite the overall slight advantage of AC over PM in terms of the numbers of blast-resistant progeny, more crosses generated stable resistant lines through PM (8) than AC (4–5) (Table 3). This result could simply be a reflection of the poor response to AC in several crosses. Until responsiveness can be assured, breeders must carefully select the crosses to be processed by AC.

A particularly intriguing result was the generation of blast-resistant progenies (Tables 2,3, and 4) produced by AC from F_2 blast-susceptible plants or through PM from crosses between blast-susceptible cultivars. Out of 441 DHs produced from F_2 blast-susceptible plants (Table 2) 64 were rated as resistant; these resistant DHs originated from all cross types (Table 3) but only some of those from crosses between resistant and susceptible parents showed stable resistance (Table 4). That resistant progenies were generated through either method from a susceptible genetic background excludes somaclonal variation as a cause, and suggests that genetic mechanisms such as transgressive segregation, complementary recessive resistance genes, or the segregation of supressor/inhibitor genes, may be involved. Such complementary resistance between field-susceptible parents was predicted by Correa Victoria and Zeigler (1993b), and forms the basis for a pathogen population-based blast-resistance breeding approach (Zeigler et al. 1994, 1995). That resistant lines were developed from crosses between susceptible cultivars challenges the general belief that such crosses should not be made. The data suggest that minor or major genes could have been involved and that recombination among them led to the development of resistant progenies.

It is possible that recombination of minor genes, complementation of recessive resistance genes, or segregation of suppressor/modifier genes occurred both in the F_2 plants in the case of AC and during the successive generations in PM. However, while none of the lines derived via AC remained resistant, 23 of the lines obtained through PM remained resistant over time (Table 4). Thus, in the latter method there may be greater opportunity for recombination from the F_2 to the F_4. This would be particularly important if multiple genes, particularly quantitative trait loci (QTLs), are involved in conferring stable blast resistance (McCouch et al. 1994; Wang et al. 1994). In this case, AC may be disadvantageous.

No stable resistant lines were obtained through either method in the Fanny/Tetep cross. Tetep is susceptible to blast in Santa Rosa, but has been widely used as a blast-resistance donor, since it shows a high level of resistance in many parts of the world. Tetep has been shown (Yu et al. 1991; Mackill and Bonman 1992) to carry several major genes for resistance to blast. That no stable resistant lines were obtained from this cross suggests that effective complementarity for blast resistance must be carefully examined before crossing field-susceptible parents. As suggested elsewhere (Zeigler et al. 1995), the resistance spectra of potential parents should be characterized with respect to the target population prior to crossing.

Several hundred F_2 populations from three-way and double crosses are grown out and evaluated by CIAT's Rice Program each year in Santa Rosa and susceptible populations are discarded based on leaf- and neck-blast data. It is argued that these susceptible populations should not be advanced because the probability of obtaining superior blast-resistant genotypes is very low or nil, and that more rapid progress can be made by selecting only among the resistant populations. However, the genetic behavior of the Fanny/CICA4 and Fanny/Colombia 1 crosses, as well as that of all resistant lines recovered by AC from F_2 susceptible plants, indicates that we should consider other factors before rejecting such populations. Pathogen population analysis and the corresponding resistance spectrum of parental lines may suggest which crosses yielding mostly susceptible progeny in the F_2 merit further attention and which are best discarded. The possibility arises that if the genetics of blast resistance and the interaction of resistance genes with pathogen populations are adequately understood, early steps in blast-resistance breeding may eventually be undertaken in blast-free environments. While true, the high selection pressure found in a blast-prone environment favors early identification of the most effective gene combinations. For PM this will reduce the number of breeding lines that must be advanced. If AC is the method chosen, then the R_2 lines should also be evaluated and selected for several seasons in a blast-prone environment to obtain better information on the stability of blast resistance.

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