VARIATION FOR RICE BLAST RESISTANCE IN EARLY SOMACLONAL GENERATIONS DERIVED FROM IMMATURE PANICLES[1]

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ABSTRACT - Somaclones derived from immature panicles of the rice cultivars Bluebelle and Maratelli susceptible to blast and their F_1's from crosses with resistant donors (Araguaia, Basmati-370 and Ramtulasi) were compared in the R_2 (plants from self-pollinated seed from primary R_1 regenerants) and R_3 generations in the greenhouse and field for blast resistance. In the greenhouse the R_2 plants and R_3 lines were inoculated with the races IB-1 and IB-9 of *Pyricularia grisea*. The frequency of plants resistant to both races ranged from 16 to 42% in the F_1 derived somaclones, whereas no resistant plant was recovered from the susceptible parents when inoculated with IB-1. The means of the leaf blast rating of R_2 and R_3 populations of F_1's were significantly lower than those derived from susceptible parents. Similar results were obtained in field trials. However, the performance of the F_1 derived somaclones varied depending on the resistant donor parent. In field trials, the mean visual ratings for leaf blast of regenerants from F_1 derived R_2 and R_3 populations were significantly lower than those regenerants derived from the parental genotypes Maratelli and Basmati-370.

Index terms: *Oryza sativa, Pyricularia grisea*, rice, tissue culture.

VARIAÇÃO SOMACLONAL DA RESISTÊNCIA À BRUSONE EM ARROZ NAS GERAÇÕES INICIAIS DERIVADAS DE PANÍCULAS IMATURAS

RESUMO - Somaclones derivados de panículas imaturas das cultivares de arroz Bluebelle e Maratelli suscetíveis à brusone, e seus respectivos F_1's de cruzamentos com doadores de resistência (Araguaia, Basmati-370 e Ramtulasi) foram comparados em R_2 (plantas obtidas de autofecundação de sementes de regenerantes primários) e na geração R_3, em casa de vegetação e no campo com relação à resistência à brusone. Em casa de vegetação, as plantas R_2 e linhas R_3 foram infectadas com as raças IB-1 e IB-9 de *Pyricularia grisea*. A freqüência de plantas resistentes a ambas raças variou de 16 a 42% nos somaclones derivados de F_1's, enquanto nenhuma planta resistente foi encontrada nos pais suscetíveis quando inoculados com a raça IB-1. As médias de notas de brusone nas folhas das populações R_2 e R_3 foram significativamente menores do que as derivadas dos pais suscetíveis. Resultados semelhantes foram obtidos nos experimentos de campo. Contudo, o comportamento dos somaclones derivados de F_1 variou dependendo do pai doador de resistência. Nos experimentos de campo, as médias de notas visuais de brusone nas folhas dos regenerantes derivados de F_1 das populações R_2 e R_3 foram significativamente menores do que os regenerantes derivados dos genótipos Maratelli e Basmati-370.

Termos para indexação: *Oryza sativa*, *Pyricularia grisea,* arroz, cultura de tecido.

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INTRODUCTION

Rice blast caused by *Pyricularia grisea* (Cooke) Saccardo (Syn *P. oryzae*) is one of the major yield constraints in both upland and irrigated rice in Brazil. Breeding for blast resistance is the most economical mean to reduce grain yield losses. Several cultivars with different degrees of resistance have been released but the durability of the resistance has been limited. Most of them have narrow genetic base (Cuevas-Perez et al., 1992) and sources of the resistance involved were restricted to a few genotypes. The resistance gene sources available in nature are being exhausted and there is a need for inducing genetic diversity in the commercial

varieties (Marshall, 1977). The induction of genetic variability and the selection for improved gene combination among variants are the important aspects of plant breeding.

Somaclonal variation for diverse agronomic characters including disease resistance has been shown to be heritable (Larkin & Scowcroft, 1981; Fukui, 1983). Homozygous stable variants occur both in polygenic and monogenic traits (Croughan, 1988; Oono, 1988; Adkins et al., 1990). A great number of resistant plants has been obtained from susceptible parents for other crop diseases (Daub, 1986; Cheng-Zhang et al., 1988; Pachón, 1989; Xie et al., 1990; Bouharmont et al., 1991). The frequency of variants depends upon the genotype utilized. However, not all genotypes show desirable variants in acceptable frequencies. One of the methods to increase frequency of variants for disease resistance is the use of the F_1 of the crosses between susceptible parents and resistant donors. The somaclonal variation for resistance to rice blast has not been adequately explored.

In the present paper, the frequency of somaclonal variants from immature panicles of F_1's and susceptible parents were compared in the R_2 and R_3 generations.

MATERIAL AND METHODS

Genotypes and isolates

The genotypes Bluebelle and Maratelli were utilized as susceptible parents in crosses made with four different sources of resistance (Araguaia, Basmati-370, Ramtulasi and Dawn). The donors are resistant to predominant races of *P. grisea* in Brazil and were widely utilized in the breeding program. Six parents and five F_1 progenies were used for generating somaclones. Single conidial isolate T_1 was established from the leaf lesions of the cultivar Tetep (race IB-1) in the rice blast nursery located at Goiânia, GO, Brazil, in 1990, and the isolate ECJ5P_1-88 (race IB-9) was obtained from the infected panicles of cultivar Guarani in the experimental fields at Jaciara (MT) in 1988. The physiologic races were identified based on the reaction on eight standard international differentials (Atkins et al., 1967). The cultivars Araguaia, Basmati-370 and Dawn are susceptible to race IB-1 and resistant to race IB-9, whereas Ramtulasi is resistant to both races.

Induction of callus and regeneration of plants

Immature panicles were used as explant source. The boots containing panicles were surface sterilized with 30% of commercial bleach containing 1% of sodium hypochlorite for 40 to 60 minutes. Panicles measuring 1 to 4 cm long were aseptically excised and placed on the MS (Murashige & Skoog, 1962) culture medium supplemented with 30 g/L of sucrose, 50 mg/L of casein hydrolysate and 4 mg/L of 2,4-D (Xie et al., 1990) and incubated in the dark for 20 to 30 days.

For plant regeneration, calli were incubated on MS culture medium supplemented with 0.5 mg/L of NAA and 3.0 mg/L of Kinetin at 26°C with a 16-hour day/8-hour night photoperiod and light intensity of 75 $\mu E.m^{-2}.s^{-1}$ and were subcultured to a fresh medium at intervals of 30 days. Regenerated green plantlets at one to two leaf stage were transplanted to pots containing 6 kg of soil fertilized with 2.5 g of NPK (4-30-16) and 0.5 g of micronutrients FTE-BR12 (Ferro Enamel do Brasil Ind. Com. Ltda, São Paulo, Brazil). These plants were grown to maturity under greenhouse conditions. Seed was harvested from individual plants. Regenerated plants here are referred to as R_1 generation and the subsequent generations as R_2, R_3, etc, according to the nomenclature of Yurkova et al. (1982).

Greenhouse experiments

Greenhouse experiments were conducted in plastic trays (30 x 10 x 15 cm) containing 6 kg of soil fertilized with 5 g of NPK (4-30-16), 1 g of zinc sulphate, 2 g of ammonium sulphate. Top dressing was made with 2 g of ammonium sulphate per tray 20 days after planting.

The genotypes of the R_2 population evaluated in this study included four parents (Bluebelle, Maratelli, Ramtulasi, Basmati-370) and five F_1's (F_1: Bluebelle x Araguaia; F_1: Bluebelle x Dawn; F_1: Maratelli x Basmati-370; F_1: Maratelli x Ramtulasi; F_1: Maratelli x Araguaia) in addition to their respective controls. The controls totalling nine were sown each in a separate tray in two replications.

Seeds harvested from R_1 plants of each genotype were sown in 10 rows per tray. Eleven to twelve seeds were sown per row and later were thinned to 10 plants. Twenty two days old R_2 plants, 100 per tray totalling 200 in two replications were inoculated with the aqueous suspension of spores of the *P. grisea* isolate T-1 belonging to race IB-1, at the concentration of $3x10^5$ spores/mL. The inoculations in plants were made using Devilbiss N° 15 (Health Care Division, Somerset, PA. 15501, USA), connected to a compressor maintaining uniform pressure of 11 g/sq.inch. The plants after inoculation were

incubated in the moist chamber for 24 hours before transferring them to greenhouse benches at temperature varying from 25-29°C.

Leaf blast evaluations were made seven to nine days after inoculation using a visual rating scale (0-9) according to Leung et al. (1988): 0 = immune; 1 = hypersensitive reaction or brown necrotic specks; 3 = few small spindle shaped sporulating lesions; 5 = several typical lesions often coalescing; 7 = many spindle shaped lesions coalescing ≥ 50% leaf area; 9 = many lesions coalescing causing partial or total death of plants. The disease severity ratings 0 to 1 represent complete or vertical resistance, and 3 to 9 susceptible reaction.

Seedlings showing susceptible reaction were removed from the trays and the remaining resistant seedlings (0-1) were inoculated with the isolate ECJ5P_1 belonging to race IB-9 when most of the seedlings had reached fourth leaf stage. Seedlings resistant to both races were transplanted to pots for harvesting the seed.

The R_3 lines derived from individual resistant plants of R_2 varying from 3 to 20 per genotype were inoculated with two races IB-1 and IB-9 using the same method described for evaluation of R_2 seedlings.

Field experiments

Assessment of R_2 populations

Seeds harvested from individual self fertilized R_1 plants were utilized to obtain the R_2 progeny in the field. Twenty-day-old seedlings, grown in the greenhouse in plastic trays containing sterilized vermiculite and supplemented with Hoegland's nutrient solution, were transplanted to a field under flooded conditions on December 7, 1993. The field was fertilized with 250 kg/ha (4-30-16) NPK and 125 kg/ha of N in the form of ammonium sulphate and 20 kg/ha of zinc sulphate before transplanting. An additional 100 kg/ha of N in the form of ammonium sulphate was applied as topdressing at the maximum tillering stage. The R_2 populations, totalling 10 consisted of four parents (Bluebelle, Maratelli, Ramtulasi and Basmati-370) and six F_1's (F_1: Bluebelle x Araguaia; F_1: Bluebelle x Dawn; F_1: Maratelli x Basmati-370; F_1: Maratelli x Araguaia; F_1: Maratelli x Ramtulasi e F_1: Maratelli x Dawn). Also, 14 controls including parental genotypes and F_1 progenies were evaluated in a randomized complete block design with two replications. The bulk R_2 population of each genotype totalling 222 seeds was derived from six R_1 plants each with 37 seeds with the exception of Ramtulasi. The R_2 population of Maratelli x Ramtulasi was composed from 42 R_1 plants. Each plot consisted of 10 rows of 1.0 m length. A three row spreader strip with susceptible cultivars (Bluebelle, BR-IRGA 409, Aliança and Cica-8) was established on both sides of the block 30 days prior to planting.

Leaf blast was assessed 48 days after transplanting in a total of 160 plants per genotype, 80 in each block, utilizing a visual rating scale of 0 to 9 where 0 and 1 represent complete resistance and 3 to 9 susceptible reaction. Also, elliptical susceptible lesions with white to grayish center were counted on all leaves of the main tiller. Panicle blast was evaluated based on the percentage of panicles infected per genotype 10 days before harvesting. At maturity, the height of the tallest tiller was determined based on measurements of five randomly selected plants per plot.

Assessment of R_3 population

Lines varying from 10 to 160 per genotype were assessed in the field for leaf and panicle blast. The field trial consisted of seven R_3 populations including four parents (Bluebelle, Maratelli, Ramtulasi, Basmati-370) and three F_1's (F_1: Bluebelle x Araguaia, F_1: Maratelli x Basmati-370, F_1 Maratelli x Ramtulasi) and their respective controls. Control material was harvested from both F_1 plants and parent genotypes in R_2 field trial. The plots consisting of one row of 2.0 m length and spaced 0.35 m between rows were direct-seeded and fertilized at the same rates as in R_2 trial. The rows were space planted with approximately 20 plants per row. The spreader rows were maintained as described in R_2 field trial. The test rows of each genotype were interspersed with one row of their respective controls (parents and R_2 progenies) after every 10 rows. The standard cultural practices were followed as in R_2, but the trial was replicated by planting at two different dates, one on 10 December 1994, and the other on 10 January 1995.

Leaf blast was evaluated using a visual rating scale as described for R_2 field trial, 50 days after planting. Disease score was given on a row basis. Panicle blast was also assessed 10 days before harvest on a row basis to determine the percentage of incidence for each genotype.

Individual plants, one from each row, were harvested except for lines which showed segregation for other morphological characters such as height, grain color, grain type etc. In segregating lines individual plants showing variation for different traits were harvested separately. The disease data from only the first planting was utilized for analysis because of severe and uniform blast incidence.

Analysis

The segregation ratios of R_2 plants inoculated with the race IB-1 was subjected to a chi-square test for goodness of fit. The means of the visual rating for leaf blast for regenerants derived from susceptible parental genotypes (Maratelli and Bluebelle) were compared with the means of their respective F_1 derived populations using the Student's t-test in both R_2 and R_3 generations of field and greenhouse tests. The field data was subjected to ANOVA for visual leaf blast ratings and lesion number in R_2 generation. Treatment comparisons of disease data were performed by orthogonal contrasts (Steel &

Torrie, 1980). The number of observations of leaf blast for Bluebelle and F_1 (Bluebelle x Dawn) was less than the rest of the genotypes and thus omitted from this analysis. Analysis of lesion number per plant was performed with transformed data $\sqrt{(X+1)}$ to improve the homogeneity of variance.

RESULTS AND DISCUSSION

Greenhouse assessment

Segregation was observed among R_2 plant populations of parental genotypes and F_1's for leaf blast reaction in artificial inoculation tests made with races IB-1 and IB-9 (Table 1). The R_2 plant population from cultivar Bluebelle segregated in the ratio of one resistant to three susceptible, thereby indicating that resistance in this cultivar is conferred by a single recessive gene to race IB-1. Control plants of Bluebelle were all susceptible. Of the 100 R_2 inoculated plant population of Maratelli, only one was resistant to race IB-1, which could be an escape whereas all control plants were susceptible. Both R_2 and control plant population of Ramtulasi were resistant to race IB-1 indicating no breakdown of resistance in cell culture. The segregation of R_2 plants of Basmati-370 did not closely fit the expected ratio of 1:3 (Table 1).

TABLE 1. Segregation of R_2 plants resistant to physiologic race IB-1 of *Pyricularia grisea* and the reaction of the resistant variants selected for IB-1 to race IB-9.

Genotype	Number of R_2 plants					
	IB-1				IB-9[1]	
	Resistant	Susceptible	Ratio	Chi-Square	Resistant	Susceptible
Bluebelle (RG)[2]	26	74	1:3	0.09	13	13
Bluebelle (NRG Control)[3]	0	100	S[4]	-	-	-
Maratelli (RG)	1	99	S	-	0	1
Maratelli (NRG Control)	0	100	S	-	-	-
Ramtulasi (RG)	100	0	R[5]	-	100	0
Ramtulasi (NRG Control)	100	0	R	-	100	0
Basmati-370 (RG)	13	87	1:3	7.68**	4	9
Basmati-370 (NRG Control)	0	100	S	-	-	-
Bluebelle x Dawn (RG)	25	75	1:3	0	15	10
Bluebelle x Dawn (F_1 Control)	0	100	S	-	-	-
Bluebelle x Araguaia (RG)	81	19	3:1	1.92	62	19
Bluebelle x Araguaia (F_1 Control)	100	0	R	-	75	25
Maratelli x Basmati-370 (RG)	38	62	1:3	8.92**	17	21
Maratelli x Basmati-370 (F_1 Control)	0	100	S	-	-	-
Maratelli x Ramtulasi (RG)	64	36	3:1	6.45*	46	18
Maratelli x Ramtulasi (F_1 Control)	100	0	R	-	0	100
Maratelli x Araguaia (RG)	46	64	1:3	19.25**	20	26
Maratelli x Araguaia (F_1 Control)	0	100	S	-	-	-

[1] The number of plants resistant and susceptible was based on total number of resistant plants to IB-1.
[2] Regenerated genotypes.
[3] Nonregenerated parental control.
[4] Susceptible.
[5] Resistant.
* $P < 0.05$.
** $P < 0.01$.

Segregation of R_2 regenerated plants from F_1 progenies from the crosses of Bluebelle x Araguaia, Bluebelle x Dawn, fitted the Mendelian ratios of 3:1 and 1:3, respectively. On the other hand the F_1 progenies of crosses Maratelli x Basmati-370 and Maratelli x Araguaia did not agree with the ratios of one resistant and three susceptible plants expected for a single recessive gene control of resistance to race IB-1. The corresponding F_1 progenies utilized as control were susceptible. The Chi-square value for 1:3 segregation in these crosses was significant and requires confirmation in R_3 families before drawing any conclusions regarding the genetic nature of this trait.

The proportion of resistant and susceptible plants in R_2 population derived from F_1 progeny of cross Maratelli x Ramtulasi did not closely follow the 3:1 ratio expected for monogenic control of resistance, while the corresponding F_1 control was resistant, showing the dominant nature of resistance to race IB-1 (Table 1).

The R_2 plants that were resistant to race IB-1 again segregated into resistant and susceptible when inoculated with race IB-9. Out of 26 R_2 plants of Bluebelle, 13 were resistant and 13 were susceptible whereas the control plants were all susceptible indicating that the recessive genes controlling reaction for these two races probably are different, and require further confirmation (Table 1).

The only one resistant R_2 plant of Maratelli became susceptible to race IB-9. Out of 13 R_2 resistant plants of Basmati-370, nine showed susceptible reaction. The R_2 plants derived from F_1's (Bluebelle x Araguaia, Bluebelle x Dawn, Maratelli x Basmati-370, Maratelli x Araguaia, Maratelli x Ramtulasi) segregated into different proportions of resistant and susceptible plants (Table 1).

The results of inoculation tests of R_3 population obtained from phenotypic resistant variants selected in R_2 generation are shown in Table 2. The R_3 plants of Bluebelle and Maratelli were all susceptible to race IB-1 whereas all plants of Ramtulasi remained resistant. The resistant plants to both races were recovered from R_3 plants of F_1's (Bluebelle x Araguaia, Bluebelle x Dawn, Maratelli x Basmati-370, Maratelli x Araguaia, Maratelli x Ramtulasi). The frequency of resistant plants varied from 16 to 34% whereas the respective controls corresponding to F_2 progenies showed no segregation for resistance.

TABLE 2. The stability of resistant variants selected in R_2 generation to race IB-1 and IB-9 of *Pyricularia grisea* in R_3 generation.

Genotype	Number of plants				
	IB-1		IB-9		
	Resistant	Susceptible	Resistant	Susceptible	% plants resistant
Bluebelle (RG)[1]	0	160	-	-	0
Bluebelle (NRG Control)[2]	0	60	-	-	0
Maratelli (RG)	-	-	-	-	0
Maratelli (NRG Control)	0	60	-	-	0
Ramtulasi (RG)	100	0	100	0	100
Ramtulasi (NRG Control)	100	0	100	0	100
Basmati-370 (RG)	0	3	-	-	0
Basmati-370 (NRG Control)	-	-	-	-	0
Bluebelle x Dawn (RG)	17	45	17	0	27.4
Bluebelle x Dawn (F_1 Control)	0	60	-	-	0
Bluebelle x Araguaia (RG)	75	25	34	41	34.0
Bluebelle x Araguaia (F_1 Control)	76	24	42	34	42.0
Maratelli x Basmati-370 (RG)	87	39	47	40	37.3
Maratelli x Basmati-370 (F_1 Control)	0	60	-	-	
Maratelli x Ramtulasi (RG)	98	203	47	51	15.6
Maratelli x Ramtulasi (F_1 Control)	0	60	-	-	0
Maratelli x Araguaia (RG)	189	212	106	83	26.4
Maratelli x Araguaia (F_1 Control)	0	60	-	-	0

[1] Regenerated genotypes.
[2] Nonregenerated parental control.

Significant differences were obtained between susceptible parental genotypes Bluebelle and Maratelli and F_1's in relation to mean of the leaf blast score, both in R_2 and R_3 populations inoculated with race IB-1 (Table 3). Even though the difference in disease score of R_2 population of F_1 cross Bluebelle x Dawn and Bluebelle was not statistically significant, the F_1 derived population was relatively superior. The R_3 population of F_1 from Bluebelle x Araguaia had mean disease score of 2.8 compared to 5.3 for Bluebelle, showing thereby a high degree of resistance. The R_3 population of F_1's from Maratelli x Basmati-370 and Maratelli x Araguaia exhibited relatively lower mean for leaf blast ratings than the R_3 population of Maratelli x Ramtulasi.

TABLE 3. Comparison of means of the visual rating of leaf blast between susceptible parental genotypes and F_1's derived from immature panicles of rice in R_2 and R_3 populations inoculated with race IB-1 in the greenhouse.

Genotype	Means of the visual rating (R_2 population)					Means of the visual rating (R_3 population)				
	n[1]	$\overline{X}_1$	n	$\overline{X}_2$	t	n	$\overline{X}_1$	n	$\overline{X}_2$	t
Bluebelle$_{(X1)}$ vs Bluebelle x Araguaia$_{(X2)}$	100	4.1	100	1.1	8.3**	160	5.3	184	2.8	13.15**
Bluebelle$_{(X1)}$ vs Bluebelle x Dawn$_{(X2)}$	100	4.1	100	3.9	0.5ns	160	5.3	62	4.4	2.72**
Maratelli$_{(X1)}$ vs Maratelli x Basmati-370$_{(X2)}$	100	7.82	100	3.5	12.6**	0	-	126	2.1	-
Maratelli$_{(X1)}$ vs Maratelli x Ramtulasi$_{(X2)}$	100	7.82	100	1.6	22.8**	0	-	301	3.8	-
Maratelli$_{(X1)}$ vs Maratelli x Araguaia$_{(X2)}$	100	7.82	100	2.8	15.0**	0	-	401	2.6	-

[1] Number of test plants.
** $P < 0.01$.
ns Non-significant.

Field assessment

A highly significant variation was observed, both among somaclones and between somaclones and controls for visual leaf blast ratings as well as lesion number in field assessment of R_2 populations (Table 4). The control x somaclones interaction was also significant. Orthogonal contrasts using means of the disease ratings and lesion number showed significant ($P<0.05$) differences between control Maratelli (CM) and the somaclones from its F_1's (Maratelli x Basmati-370, Maratelli x Araguaia, Maratelli x Ramtulasi, Maratelli x Dawn) as well as somaclones of Maratelli (SM) and those derived from F_1's.

TABLE 4. Analysis of variance for leaf blast visual ratings and lesion number in eight R_2 populations derived from immature panicles of rice and controls under field conditions.

Source of variation[1]	DF	Mean square value	
		Visual ratings	Lesion/number/plant[2]
Block	1	28.094**	158.572**
Between somaclones and controls (C)	1	27.309*	71.633*
Among somaclones (SC)	7	2590.196**	1029.335**
C x SC	7	407.863**	253.264**
Experimental error	15	5.841**	14.619**
Sampling error (Residual)	2528	0.697	0.666
Controls (Total)	2559	-	-
CM vs SF_1	1	2808.751**	1083.120**
SM vs SF_1	1	2646.281**	898.676**

[1] C: controls (Maratelli, Basmati-370, Ramtulasi, F_1: Maratelli x Araguaia, F_1: Maratelli x Basmati-370, F_1 Maratelli x Ramtulasi, F_1: Maratelli x Dawn, F_1: Bluebelle x Araguaia); CM: control Maratelli; SM: somaclones derived from Maratelli; SF_1: somaclones derived from F_1's (Maratelli x Araguaia, Maratelli x Ramtulasi, Maratelli x Basmati-370 Maratelli x Dawn).
[2] Data were transformed to $\sqrt{x+1}$ for analysis.
* $P < 0.05$.
** $P < 0.01$.

Leaf blast assessment of eight R_2 populations in the field showed significant differences among the regenerants and their controls in relation to mean visual ratings and lesion numbers (Table 5). The mean of the leaf blast score as well as lesion numbers of somaclones from F_1 (Maratelli x Basmati-370) and Basmati-370 were significantly lower than their respective controls. On the other hand, the somaclones from F_1's (Maratelli x Araguaia, Maratelli x Ramtulasi) had significantly higher mean of the leaf blast rating and lesion numbers compared to their controls. The R_2 population of F_1 from Bluebelle x Araguaia showed significantly

higher leaf blast score than the control but did not differ in lesion number. The somaclones and controls of Maratelli were highly susceptible whereas Ramtulasi were highly resistant in the field.

TABLE 5. Means of the visual rating and lesion number of leaf blast in eight R_2 populations derived from immature panicles of rice and their controls under field conditions[1].

Genotype	Means of the visual rating		Mean lesion number/plant	
	Population R_2	Control[2]	Population R_2	Control
Maratelli	8.86a	9.00a	6.12a	6.38a
F_1 : Maratelli x Dawn	6.63b	6.68b	5.37*a	3.19c
F_1 : Maratelli x Araguaia	6.58*b	4.36c	5.43*a	2.22b
F_1 : Maratelli x Ramtulasi	3.02*cd	1.00d	1.79*b	0d
F_1 : Maratelli x Basmati-370	1.01*e	5.17ce	1.29*b	3.81ce
F_1 : Bluebelle x Araguaia	3.88*c	1.00d	1.47b	1.24d
Basmati-370	3.55*c	4.68c	2.10*b	3.05c
Ramtulasi	0f	0f	0b	0d

[1] Means followed by the same letter in a column do not differ significantly according to Tukey's test at the 0.05 probability level; means followed by the asterisk differ significantly from the control according to Tukey's test at the 0.05 probability level.
[2] Nonregenerated

The comparison of means of R_2 and R_3 populations of F_1 (Bluebelle x Araguaia) showed significantly lower leaf blast ratings than the mean of regenerants from the susceptible parent Bluebelle, under field conditions (Table 6). Similar results were obtained for Maratelli in comparison with the F_1's (Maratelli x Basmati-370, Maratelli x Ramtulasi).

The frequency distribution of leaf blast ratings in populations derived from F_1's were compared with their parental genotypes and their respective controls (Figs. 1 to 3). Resistance and susceptibility of R_2 populations of Araguaia and Bluebelle, respectively were maintained in R_3 (Fig. 1). While the R_2 population from Bluebelle x Araguaia was highly susceptible few resistant lines were recovered in R_3. The F_1 nonregenerated control was resistant and F_2 showed susceptible leaf blast scores of 5 and 7. A high frequency of resistant R_3 lines derived from Basmati-370 exhibited resistant reaction whereas both regenerants and nonregenerants of Maratelli were susceptible (Fig. 2). The distribution of population from Maratelli x Basmati-370 skewed more towards resistance both in R_2 and R_3 generations relative to F_1 and F_2 controls. The regenerants from Ramtulasi remained resistant in R_2 and R_3 generations (Fig. 3). The frequency of resistant plants of R_2 derived from Maratelli x Ramtulasi was not greatly altered in R_3. The distribution of R_3 population was nearly normal and wider than the F_2 control.

TABLE 6. Comparison of means of the visual rating of leaf blast between susceptible genotype and F_1's derived from immature panicles of rice in R_2 and R_3 generations under field conditions.

Genotype	R_2 population				R_3 population				
	n^1	$\overline{X}_1$	$\overline{X}_2$	t	n^2	$\overline{X}_1$	n^2	$\overline{X}_2$	t
$Bluebelle_{(X1)}$ vs F_1: Bluebelle x $Araguaia_{(X2)}$	160	6.0	3.8	16.0**	35	5.4	90	3.5	7.6**
$Maratelli_{(X1)}$ vs F_1: Maratelli x $Basmati\text{-}370_{(X2)}$	160	8.9	1.0	37.2**	10	9.0	149	1.8	14.1**
$Maratelli_{(X1)}$ vs F_1: Maratelli x $Ramtulasi_{(X2)}$	160	8.9	3.0	24.2**	10	9.0	127	3.4	8.8**

[1] Number of R_2 plants.
[2] Number of R_3 lines.
** $P < 0.01$.

Field incidence of panicle blast in R_2 and R_3 populations derived from Bluebelle, Maratelli, F_1's of Bluebelle x Araguaia, Maratelli x Basmati-370 and Maratelli x Ramtulasi and the controls is illustrated in Fig. 4. Panicle blast incidence was 100% both in Bluebelle and Araguaia in R_2 and R_3 generations. Furthermore, it was low in R_2 populations from Bluebelle x Araguaia and did not differ from its F_1 nonregenerated control.

The incidence in the population from Maratelli x Ramtulasi was negligible. A high percentage of R_3 lines from Bluebelle x Araguaia, Maratelli x Basmati-370 and Maratelli x Ramtulasi showed resistance to panicle blast as compared to their controls and these results are consistent with those obtained for leaf blast.

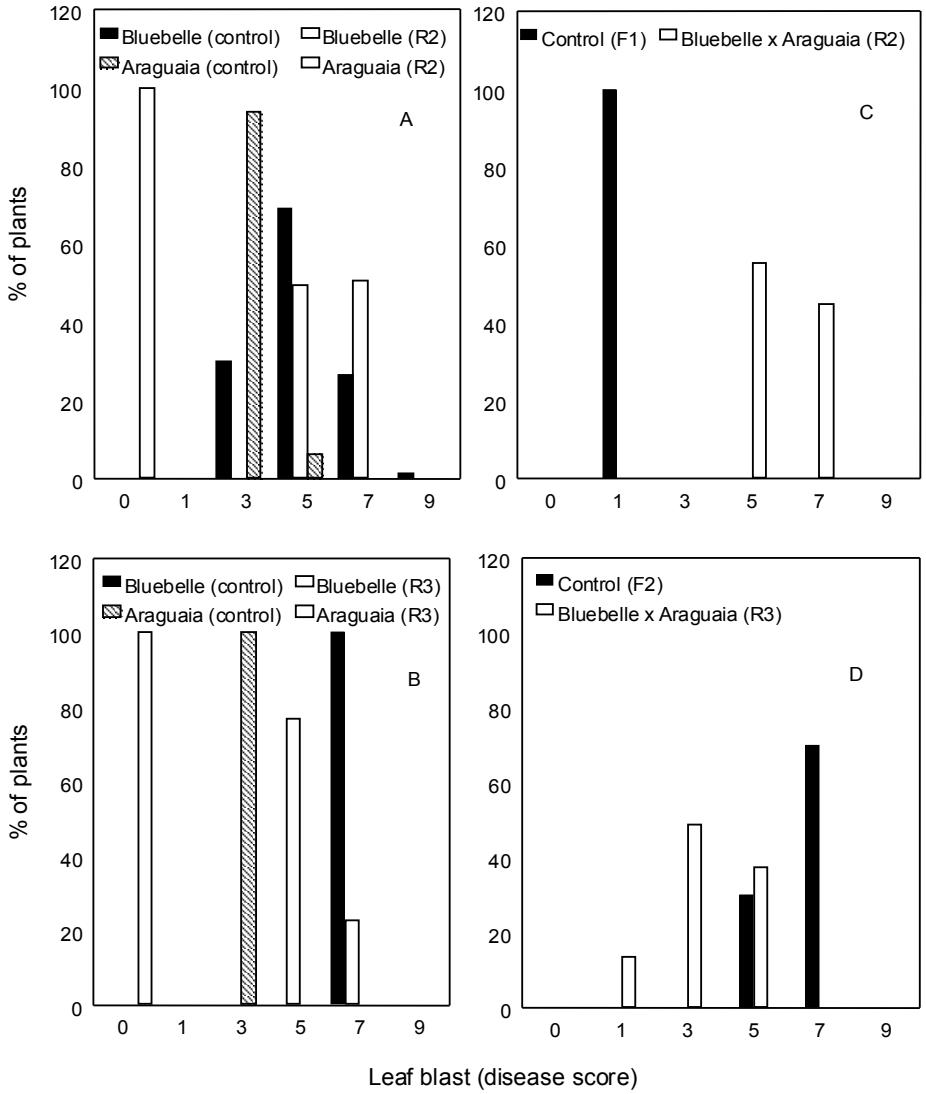

FIG. 1. Frequency distribution of somaclones according to leaf blast rating in the field. A: R_2 population derived from Bluebelle and Araguaia and their nonregenerated controls; B: R_3 population of Bluebelle and Araguaia and their nonregenerated controls; C: R_2 populations derived from F_1: Bluebelle x Araguaia and the nonregenerated F_1 control; D: R_3 populations derived from F_1: Bluebelle x Araguaia and nonregenerated F_2 control.

The R_2 and R_3 generations of rice cultivars and F_1's that had been regenerated *in vitro* from immature panicles were screened for blast resistance in the greenhouse under artificial inoculation conditions and in field trials for over a period of two years. The inoculations of R_2 and R_3 populations with races IB-1 and IB-9 showed that the leaf blast reaction of the highly susceptible cultivar Maratelli and the resistant donor parent Ramtulasi utilized in the crosses, did not differ from their respective controls. Similar results were obtained in field assessment of R_2 and R_3 populations. The cultivars Maratelli and Ramtulasi also did not show any variation for other morphological and agronomic characters in the field. These results are in accord with the observations of Xie et al. (1990) that some rice cultivars such as Tetep and Taipei 309 are tissue culture stable

and seldom produce variants. According to Evans & Gamborg (1982), callus was cultured only for 30 to 45 days in induction medium to obtain a limited change for one trait such as disease resistance because much of the variation is proportional to the duration of culture.

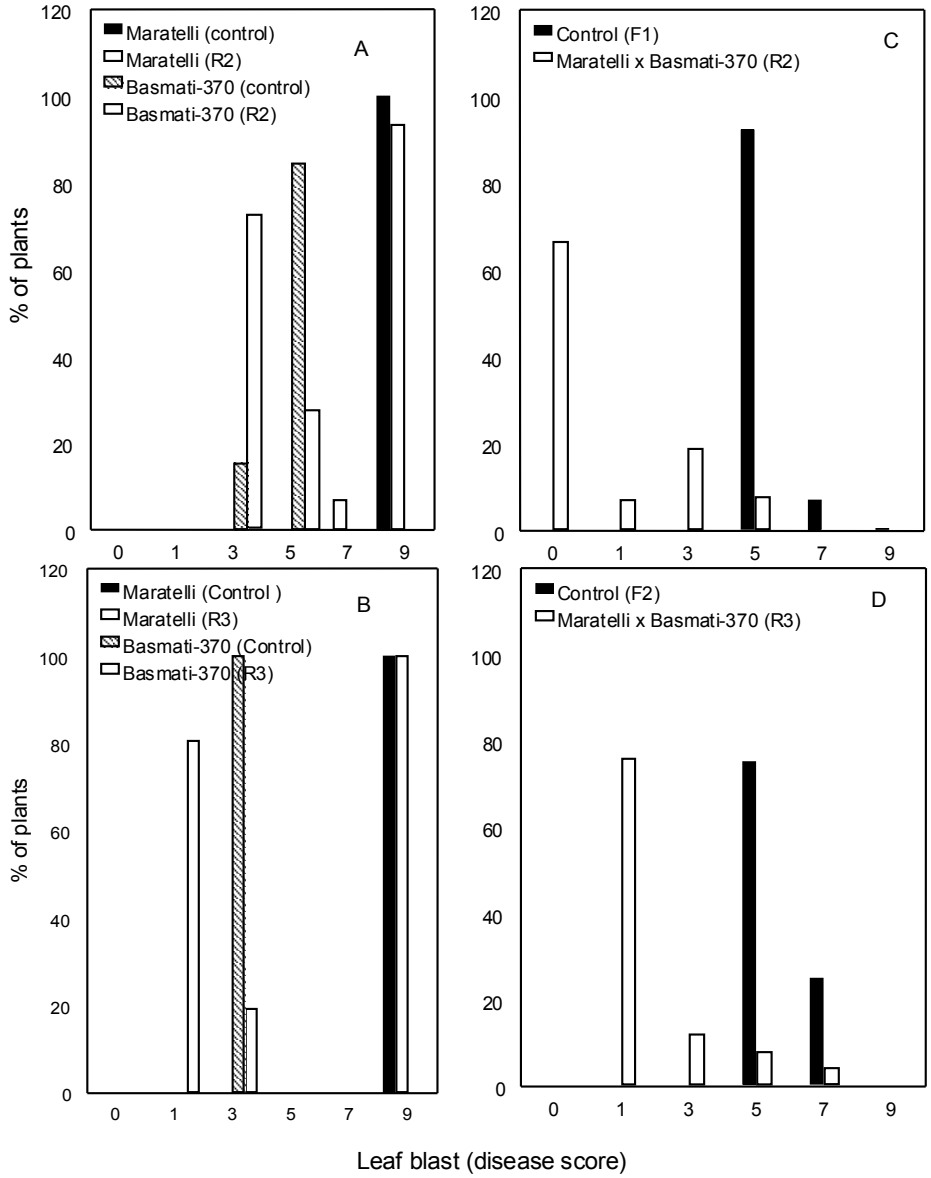

FIG. 2. Frequency distribution of somaclones according to leaf blast rating in the field. A: R_2 population derived from Maratelli and Basmati-370 and their nonregenerated controls; B: R_3 population of Maratelli and Basmati-370 and their nonregenerated controls; C: R_2 populations derived from F_1: Maratelli x Basmati-370 and nonregenerated F_1 control; D: R_3 populations derived from F_1: Maratelli x Basmati-370 and nonregenerated F_2 control.

The R_2 regenerants derived from F_1 plants segregated while the nonregenerated F_1's utilized as controls were either uniformly resistant or susceptible to race IB-1. The plants resistant to race IB-1 segregated again when inoculated with race IB-9. The aberrant ratios to race IB-1 could be attributed to the cytogenetic abnormalities or disturbed Mendelian segregation resulting from culture medium (Larkin & Scowcroft, 1981). The R_3 population derived from resistant plants of R_2 segregated again for those two races. A high percentage of resistant plants were recovered in R_3 only from regenerants derived from R_2's while the F_2 progeny

maintained as controls were all susceptible (Table 2). Genetic variation in the regenerated plants appears to have resulted from the gene heterozygous for blast resistance in the F_1 progeny from which they were derived. However, genetic stability has to be further tested in advanced generations.

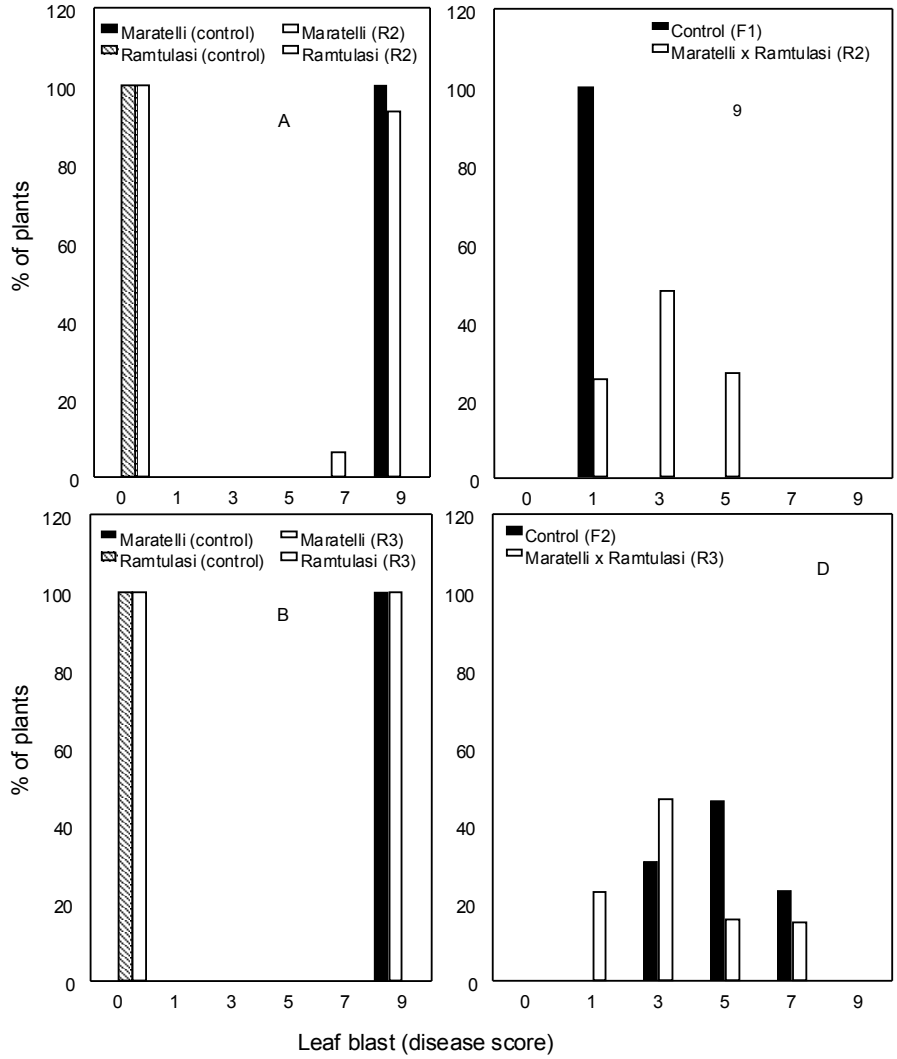

FIG. 3. Frequency distribution of somaclones according to leaf blast rating in the field. A: R_2 population derived from Maratelli and Ramtulasi and their nonregenerated controls; B: R_3 population of Maratelli and Ramtulasi and their nonregenerated controls; C: R_2 populations derived from F_1: Maratelli x Ramtulasi and nonregenerated F_1 control; D: R_3 populations derived from F_1: Maratelli x Ramtulasi and nonregenerated F_2 control.

The results of field tests are consistent with those obtained in the greenhouse. Among the regenerants derived from F_1 plants, only the populations derived from Maratelli x Basmati-370 and Basmati-370 showed high degree of leaf blast resistance as compared to their nonregenerated controls. These results indicated that the frequency of stable variants depends on the genotype utilized in the cross.

Variations which are not included in this paper were found for other traits such as plant height, heading date, grain color, apiculus, awn length, etc, in R_2 and R_3 generations. A high frequency of somaclonal variation have been reported in rice in earlier studies (Kucherenko, 1979; Fukui, 1983; Oono, 1983; Sun et al., 1983). The fertility of the regenerants derived from F_1 crosses was greater in R_3 than their F_2 segregating populations utilized as controls. Some of the lines in R_3 field trial showed uniform blast reaction suggesting a

significant change in initial regenerants and this would reduce the number of generations to produce fixed blast resistant lines.

In the field, leaf blast was uniform and severe in both years as compared to the panicle blast incidence. However, the performance of R_2 and R_3 populations in relation to panicle blast incidence was similar.

The identification of variants for blast resistance depends greatly on the screening and selection procedures. Minor variations and mutations for blast resistance cannot be distinguished under field conditions. The segregation of R_2 populations for resistance to races IB-1 and IB-9 and their stability in R_3 indicate the potential for obtaining somaclones resistant to specific races. The mechanism by which the variation occurred has not been elucidated in this study.

Attempts to select variants in tissue culture for resistance to rice blast are very few and the results are conflicting (Pachón, 1989; Xie et al., 1990).

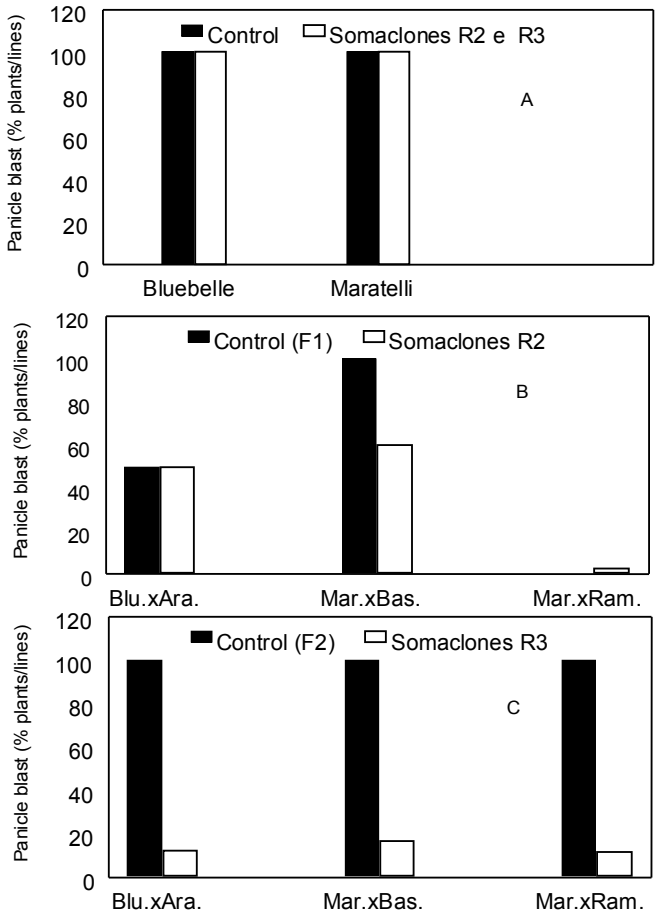

FIG. 4. Incidence of panicle blast. A: R_2 and R_3 populations derived from Bluebelle (Blu.) and Maratelli (Mar.) and their nonregenerated controls; B: R_2 populations derived from F_1: Bluebelle x Araguaia (Ara.), Maratelli x Basmati-370, Maratelli x Ramtulasi (Ram.) and their nonregenerated F_1 controls; C: R_3 populations derived from F_1: Bluebelle x Araguaia, Maratelli x Basmati-370, Maratelli x Ramtulasi and their nonregenerated F_2 controls under field conditions (percentages were based on plants in R_2 and lines in R_3).

CONCLUSIONS

1. Somaclones derived from F_1 crosses using resistance gene donors and commercial cultivars as parents are useful for obtaining a high frequency of resistant, fertile and uniform lines in the early R_3 generation.

2. Somaclones produced in this study may be of value for basic studies and for their use in crosses for resistance breeding.

REFERENCES

ADKINS, S.W.; SHIRAISHI, T.; McCOMB, J.A.; RATANOPOL, S.; KUPKANCHANAKUL, T.; ARMSTRONG, L.J.; SCHULTZ, A.L. Somaclonal variation in rice submergence tolerance and other agronomic characters. **Physiologia Plantarum**, Copenhagen, v.80, p.647-654, 1990.

ATKINS, J.G.; ROBERT, A.L.; ADAIR, C.R.; GOTO, K.; KOZOKA, T.; KANOGITA, R.; YAMADA, M.; MATSUMOTO, S. An international set of rice varieties for differentiating races of *Pyricularia oryzae.* **Phytopathology**, St Paul, v.57, p.297-301, 1967.

BOUHARMONT, J.; DEKEYSER, A.; SINT JAN, V. van; DOGBE, Y.S. Application of somaclonal variation and in vitro selection to rice improvement. In: INTERNATIONAL RICE RESEARCH INSTITUTE. **Rice genetics II.** Manila, 1991. p.271-277.

CHENG-ZHANG, Z.; KANGE-LE, Z.; ZONG-XIU, S.; XIU-FANG, Q. Somaclonal variation and rice improvement. In: INTERNATIONAL RESEACH INSTITUTE. **Genetic manipulation in crops.** London: Cassel Tycooly, 1988. p.115-116.

CROUGHAN, T.P. Tissue culture of US rice varieties: development of short-stature variants. In: INTERNATIONAL RESEARCH RICE INSTITUTE. **Genetic manipulation in crops.** London: Cassel Tycooly, 1988. p.119-120.

CUEVAS-PEREZ, F.E.; GUIMARÃES, E.P.; BERRIO, L.E.; GONZÁLES, D.I. Genetic base of irrigated rice in Latin America and the Caribbean, 1971 to 1989. **Crop Science**, Madison, v.32, n.4, p.1054-1059, 1992.

DAUB, M.E. Tissue culture and the selection of resistance to pathogens. **Annual Review of Phytopathology**, Palo Alto, v.24, p.159-186, 1986.

EVANS, D.A.; GAMBORG, O.L. Chromosome stability of cell suspension culture of *Nicotiana* spp. **Plant Cell Reports**, Berlin, v.1, p.104-107, 1982.

FUKUI, K. Sequential occurrence of mutation in a growing rice callus. **Theoretical and Applied Genetics**, Berlin, v.65, p.225-230, 1983.

KUCHERENKO, L.A. Tissue culture in rice improvement: experiences in the USSR. In: INTERNATIONAL RICE RESEARCH INSTITUTE. **Innovative approaches to rice breeding.** Manila, 1979. p.93-102.

LARKIN, P.J.; SCOWCROFT, W.R. Somaclonal variation a novel source of variability from cell cultures for plant improvement. **Theoretical and Applied Genetics**, Berlin, v.60, p.197-214, 1981.

LEUNG, H.; BORROMEO, E.S.; BERNARDO, M.A.; NOTTEGHEM, J.L. Genetic analysis of virulence in the blast fungus *Magnoporthe griseae*. **Phytopathology**, St. Paul, v.78, p.1227-1233, 1988.

MARSHALL, D.R. The advantages and hazards of genetic homogeneity. In: DAY, R. (Ed.). **The genetic basis of epidemics in agriculture**. New York: New York Academy of Sciences, 1977. p.1-20. (Annals of the New York Academy of Sciences, 287).

MURASHIGE, T.Y.; SKOOG, F. A revised medium for rapid growth and bioassays tobacco tissue culture. **Physiologia Plantarum**, Copenhagen, v.15, p.473-479, 1962.

OONO, K. Genetic variability in rice plants regenerated from cell cultures. In: CELL AND TISSUE CULTURE TECHNIQUES FOR CEREAL CROP IMPROVEMENT, 1981, Beijing. **Proceedings**... Manila: IRRI, 1983. p.95-104.

OONO, K. Somatic mutation in rice tissue culture. In: SEMINAR CELL AND TISSUE CULTURE IN FIELD CROP IMPROVEMENT, 1987, Tsukuba. **Proceedings**... Taiwan: FFTC, 1988. p.108-112.

PACHÓN, J.G. **Evaluación del uso potencial de la variación somaclonal en el mejoramiento de algunos caracteres de importancia económica en el arroz (*Oryza sativa* L.)**. Bogotá: Pontificia Universidad Javeriana, 1989. 94p. Tese de Graduação.

STEEL, R.G.D.; TORRIE, J.H. **Principles and procedures of statistics.** 2.ed. New York: McGraw-Hill, 1980. 633p.

SUN, Z.X.; ZHAO, C.Z.; ZHENG, K.L.; QI, X.F.; FU, Y.P. Somaclonal genetics of rice (*Oryza sativa* L.). **Theoretical and Applied Genetics**, Berlin, v.67, p.67-73, 1983.

XIE, Q.J.; RUSH, M.C.; CAO, J. Somaclonal variation for a disease resistance in rice (*Oryza sativa* L.) In: GRAYSON, B.T.; GREEN, M.B.; COPPING, L.G. (Eds.). **Pest management on rice**. London: Elsevier Applied Science, 1990. p.491-509.

YURKOVA, G.N.; LEVENKO, B.A.; NOVOZHJLOV, P.V. Plant regeneration in wheat tissue culture. **Biochemie und Physiologie der Pflanzen**, v.177, p.337-344, 1982.