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Inheritance of blast resistance in rice to two *Pyricularia grisea* races, IB-1 and IB-9*

Marta C. de Filippi[1] *and A.S. Prabhu*[2]

ABSTRACT

Seven sources of resistance to the two predominant races IB-1 and IB-9 of the rice blast pathogen *Pyricularia grisea* were selected based on leaf blast reaction in tests conducted under controlled greenhouse conditions. Crosses involving resistant and susceptible parents were made to study the inheritance of the disease reaction for different sources of resistance. The F_1 and F_2 progenies of all crosses, including backcrosses to resistant and susceptible parents, were tested for reaction to leaf blast. The data showed that resistance is controlled by one to three genes that segregate independently in most of the donors. Non-allelic interaction among resistance genes, including dominant epistasis, was identified.

INTRODUCTION

Rice blast caused by *Pyricularia grisea* (Cooke) Sacc., earlier referred to as *Pyricularia oryzae* Cav., is worldwide in distribution (Ou, 1985). In Brazil it is the major yield-limiting factor both in irrigated and upland ecosystems. This disease can be controlled effectively by the utilization of resistant varieties. A large number of cultivars with different degrees of resistance have remained useful only for a limited period of time due to the great variability of the blast pathogen (Ou, 1985; Bonman *et al.*, 1987; Correa-Victoria and Zeigler, 1993).

Many physiological races have been reported in different states of Brazil (Amaral *et al.*, 1979; Bedendo *et al.* 1979; Tanaka, 1986; Ribeiro and Terres, 1987). In a recent physiological race survey conducted in west central Brazil, it was shown that 92 isolates collected from different upland rice cultivars in different locations belonged to 27 races. The most predominant races were IB-1, IB-9, IB-13 and IB-41 (Prabhu and Filippi, 1989). Several sources of broad spectrum resistance to blast were identified based on tests in a uniform blast nursery, conducted in Brazil (Prabhu *et al.* 1982). Some of these sources are being widely used in current breeding programs. Knowledge on the inheritance of disease resistance would facilitate the adoption of appropriate breeding strategies and improve the efficiency of selection procedures. Earlier studies demonstrated the applicability of the gene for gene hypothesis (Flor, 1955). Kiyosawa (1981) conducted extensive studies on inheritance of blast resistance using Japanese races and identified 13 dominant resistance genes at eight different loci. Padmanabhan *et al.* (1967) identified three and four gene pairs in cultivars S67 and CL 5309, respectively. The genetic studies utilizing different races showed that resistance is controlled by one or two dominant genes (Yu *et al.*, 1987). Atkins and Johnston (1965) demonstrated two independent genes for races 1 and 6 in crosses utilizing cultivars Zenith and Gulfrose.

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[1] Bolsista, Convênio: Comunidade Européia, EMBRAPA/Centro Nacional de Pesquisa de Arroz e Feijão (CNPAF), Caixa Postal 179, 74001-970 Goiânia, GO, Brasil. Send correspondence to M.C.F.

[2] Pesquisador, EMBRAPA/CNPAF, Goiânia, GO, Brasil.

Although vertical resistance is considered monogenic or oligogenic in nature, Goto (1970) reported continuous distribution in a segregating population, and identified three gene pairs with additive effects in the cultivars Sensho and Imochi-Shirazu. He and Shen (1990) studied the inheritance of resistance in cultivars against two races of *P. grisea*, and identified 11 dominant genes. Expression of resistance in some of them was altered by modifiers or multiple alleles.

There is very little information on the inheritance and nature of resistance utilizing tropical isolates of *P. grisea*. In Brazil, Tanaka (1986), utilizing upland rice cultivars IAC 47 and IAC 25, and three races of rice blast, demonstrated the presence of two or three independent dominant genes. Recently, an analysis of near isogeneic lines of rice with single resistance genes showed that blast resistance is controlled by independent dominant genes (Mackill & Bonman, 1992). Divergent results on the nature of resistance have been attributed to the genetic material utilized, different methods of inoculation, and evaluation criteria for classifying plants into categories of resistance and susceptibility. The differential reaction of varieties to the different races suggests that many more genes would be identified if more races of the fungus were used (Ou, 1985).

The present study reports the mode of inheritance for seven sources of resistance to two prevalent races, IB-1 and IB-9.

MATERIAL AND METHODS

The experiments were conducted under controlled greenhouse conditions at the National Rice and Bean Research Center (CNPAF/EMBRAPA) during 1990-92. The isolate Py-Dawn, obtained from the cultivar Dawn in the uniform rice blast nursery, and ECG4P[1]89, a field isolate obtained from cultivar IAC 165 from Goiania, were identified as belonging to races IB-1 and IB-9, respectively, based on the international key for the identification of races. Seven highly resistant (Araguaia, Basmati-370, Carreon, Dawn, Huan-Sen-Goo, Ramtulasi, Três Marias) and two susceptible (Bluebelle and IAC-47) cultivars to the isolates IB-1 and IB-9, according to previous greenhouse inoculation tests (Filippi, 1993), were selected and used for crosses in the present study.

Both resistant and susceptible parents were transplanted to 6-kg pots for seed multiplication. The seed multiplied in the greenhouse was utilized for the crosses. All crosses were performed in the greenhouse using susceptible cultivars as female and resistant donors as male parents, utilizing the method of Coffman and Herrera (1980), modified by Neves and Taillebois (1990). The F_1, F_2 and backcross progenies of these crosses were tested for their reaction to the two races IB-1 and IB-9 to determine the inheritance of disease reaction. The F_2 and backcross progenies were classified as resistant or susceptible on an individual seedling basis, and F_1 and parents on a row basis. Disease assessment was made on 30-day old seedlings raised in trays.

The test material for inoculations was planted in plastic trays (15 x 10 x 30 cm), filled with 6 kg of soil fertilized with 5 g of NPK 5-30-15 + Zn, in addition to 2 g of ammonium sulfate and 1 g of zinc sulfate. A top dressing with 3 g of ammonium sulfate was given 15 days after seeding.

Inoculations of the progenies were made separately for each isolate, at the third leaf stage, with 30 ml of the spore suspensions (10^5 spores/ml). Twenty plants of each parent, as well as F_1 progenies, 200 plants of F_2 progenies, and 100 plants each of BC_1 and BC_2, were inoculated separately with the rice blast races IB-1 and IB-9. Soon after inoculation the trays were incubated for 24 h in humid chambers and later transferred to greenhouse benches. They were maintained under high humidity (70-90%) with an average temperature ranging from 26°C to 30°C.

Evaluations were made nine to 12 days after inoculation, using a 0 to 9 scale in which 0 = no evidence of infection; 1 = few to many non-sporulating brown spots; 3 = round to elliptical lesions less than 3 mm in diameter with gray center and brown margins; 5 = typical spindle shaped isolated lesions 3 mm or more longer; 7 = many spindle shaped lesions often coalescing; 9 = many coalescing lesions resulting in wilt or death of the leaf (Leung *et al.*, 1988). The disease ratings 0 to 3 were considered resistant (incompatible) and 5 to 9 susceptible (compatible). Unless otherwise mentioned the same inoculation and evaluation procedure was followed throughout this study.

Chi-square tests for goodness of fit were used to analyze all F_2 and backcross data. The test was conducted with one degree of freedom, utilizing the Yates correction factor, according to Strickberger (1971).

RESULTS AND DISCUSSION

The results of the inoculation tests against race IB-1 and IB-9 are presented in Tables I and II, respectively. The F_2 populations from each of the crosses IAC-47 (S) x Ramtulasi (R) inoculated with race

Table I - Segregation of F_2, BC_1 and BC_2 populations of crosses involving susceptible (S) and resistant (R) parents to race IB-1 of *Pyricularia grisea*.

Parentage	Parent reaction	Generation	Observed data		Expected* ratio	χ^2	Probability
			R	S			
Bluebelle x Ramtulasi	S x R	F_2	125	6	15:1	0.91	0.50-0.30
		BC_1**	80	21	3:1	1.08	0.30-0.20
		BC_2***	96	1	1:0	0.02	0.90
IAC-47 x Ramtulasi	S x R	F_2	194	6	63:1	0.05	0.90-0.80
		BC_1	85	6	7:1	3.33	0.10-0.05
		BC_2	98	0	1:0	0.001	> 0.99
Bluebelle x Araguaia	S x R	F_2	194	4	63:1	0.05	0.90-0.80
		BC_1	51	8	7:1	0.03	0.90-0.80
		BC_2	100	0	1:0	0.001	> 0.99
Bluebelle x Carreon	S x R	F_2	194	6	15:1	3.07	0.10-0.05
		BC_1	80	15	3:1	3.83	0.10-0.05
		BC_2	55	0	1:0	0.001	> 0.99
IAC-47 x Carreon	S x R	F_2	186	14	15:1	0.10	0.80-0.70
		BC_1	79	21	3:1	0.97	0.50-0.30
		BC_2	100	0	1:0	0.001	> 0.99
Bluebelle x Huan-Sen-Goo	S x R	F_2	144	57	3:1	1.13	0.30-0.20
		BC_1	54	43	1:1	1.26	0.30-0.20
		BC_2	94	2	1:0	0.07	0.80-0.70
IAC-47 x Huan-Sen-Goo	S x R	F_2	149	53	3:1	0.14	0.80-0.70
		BC_1	52	43	1:1	0.86	0.50-0.30
		BC_2	99	0	1:0	0.001	> 0.99
Bluebelle x Dawn	S x R	F_2	184	13	15:1	0.01	0.95-0.90
		BC_1	76	22	3:1	0.42	0.70-0.50
		BC_2	78	21	1:0	4.67	0.05-0.01
IAC-47 x Dawn	S x R	F_2	193	11	15:1	0.04	0.90-0.80
		BC_1	85	12	3:1	8.60	0.01-0.001
		BC_2	97	3	1:0	0.12	0.80-0.70
Bluebelle x Três Marias	S x R	F_2	192	9	15:1	1.36	0.30-0.20
		BC_1	71	25	3:1	0.04	0.90-0.80
		BC_2	98	0	1:0	0.001	> 0.99
IAC-47 x Três Marias	S x R	F_2	195	5	63:1	0.63	0.50-0.30
		BC_1	88	11	7:1	0.29	0.70-0.50
		BC_2	99	0	1:0	0.001	> 0.99
IAC-47 x Basmati-370	S x R	F_2	205	13	15:1	0.09	0.50-0.30
		BC_1	88	12	3:1	9.37	0.01-0.001
		BC_2	85	9	1:0	0.96	0.50-0.30

*Ratio = Resistant to susceptible.
**BC_1 = Backcrossed to susceptible parent.
***BC_2 = Backcrossed to resistant parent.

Table II - Segregation of F_2, BC_1 and BC_2 populations of crosses involving susceptible (S) and resistant (R) parents to race IB-9 of *Pyricularia grisea*.

Parentage	Parent reaction	Generation	Observed data		Expected* ratio	χ^2	Probability
			R	S			
Bluebelle x Ramtulasi	S x R	F_2	93	1	63:1	0.64	0.50-0.30
		BC_1**	101	10	7:1	1.50	0.30-0.20
		BC_2***	101	0	1:0	0.001	> 0.99
IAC-47 x Ramtulasi	S x R	F_2	114	0	63:1	0.05	0.90-0.80
		BC_1	96	11	7:1	0.66	0.50-0.30
		BC_2	98	0	1:0	0.001	> 0.99
Bluebelle x Araguaia	S x R	F_2	211	15	15:1	0.02	0.90-0.70
		BC_1	63	20	3:1	0.08	0.80-0.70
		BC_2	109	0	1:0	0.001	> 0.99
IAC-47 x Araguaia	S x R	F_2	223	5	15:1	7.03	0.01-0.001
		BC_1	74	27	3:1	0.13	0.80-0.70
		BC_2	118	0	1:0	0.001	> 0.99
Bluebelle x Carreon	S x R	F_2	205	2	63:1	0.94	0.50-0.30
		BC_1	68	13	7:1	0.55	0.50-0.30
		BC_2	83	0	1:0	0.001	> 0.99
IAC-47 x Carreon	S x R	F_2	224	4	63:1	0.001	> 0.99
		BC_1	105	15	7:1	0.001	> 0.99
		BC_2	115	1	1:0	0.001	> 0.99
IAC-47 x Huan-Sen-Goo	S x R	F_2	181	28	15:1	17.17	< 0.001
		BC_1	83	32	3:1	0.43	0.70-0.50
		BC_2	101	0	1:0	0.001	> 0.99
Bluebelle x Dawn	S x R	F_2	178	17	15:1	1.68	0.20-0.10
		BC_1	81	31	3:1	0.37	0.70-0.50
		BC_2	65	2	1:0	0.09	0.90- 0.80
IAC-47 x Dawn	S x R	F_2	202	14	15:1	0.00	> 0.995
		BC_1	85	37	3:1	1.72	0.30-0.20
		BC_2	94	5	1:0	0.31	0.70-0.50
Bluebelle x Três Marias	S x R	F_2	228	11	15:1	1.37	0.30-0.20
		BC_1	80	22	3:1	0.75	0.50-0.30
		BC_2	119	0	1:0	0.001	> 0.99
IAC-47 x Três Marias	S x R	F_2	230	10	15:1	2.11	0.20-0.10
		BC_1	104	14	3:1	11.22	< 0.001
		BC_2	54	0	1:0	0.001	> 0.99
Bluebelle x Basmati-370	S x R	F_2	205	5	63:1	0.48	0.50-0.30
		BC_1	105	5	7:1	0.01	> 0.99
		BC_2	115	0	1:0	0.001	> 0.99

*Ratio = Resistant to susceptible.
**BC_1 = Backcrossed to susceptible parent.
***BC_2 = Backcrossed to resistant parent.

IB-1 (Table I) and Bluebelle (S) x Ramtulasi (R), and IAC-47 (S) x Ramtulasi (R) inoculated with race IB-9 (Table II) segregated at a ratio of 63 resistant to one susceptible plants indicating that resistance in cultivar Ramtulasi is governed by three dominant gene pairs.

The F_2 population from the cross Bluebelle (S) x Ramtulasi (R) segregated at a ratio of 15:1 to race IB-1 indicating duplicate dominant epistasis. In practice it is difficult to differentiate segregation ratios of 15:1 and 63:1, when a limited number of plants is utilized. The use of a large segregating population would permit a critical analysis (Tanaka, 1986).

In the crosses in which Araguaia was involved as the donor, three and two genes governed the resistance to races IB-1 and IB-9, respectively (Tables I and II). The inheritance of disease reaction differs for the different races of the pathogen (Flores, 1981). However, the resistance in many cases is conditioned by dominant genes (Yu *et al.*, 1987).

The F_2 population segregated at a ratio of 15 resistant and one susceptible to race IB-1, indicating two pairs of genes (Table I). Similar results were obtained in inoculations of cultivar Carreon with PO6-6 and IK81-3 (Yu *et al.*, 1987). Also the F_2 populations from the cross IAC-47 with IAC-25 segregated in a similar manner to the three distinct races indicating that each one of the parents possesses different genes for resistance (Tanaka, 1986). The cultivar Carreon possesses three gene pairs for resistance to race IB-9. According to Kiyosawa (1981) the resistance is determined by one, two or three pairs of genes, depending upon the race utilized.

The F_2 population from the crosses involving cultivar Huan-Sen-Goo indicated that resistance is controlled by a single dominant gene to race IB-1 and two genes to race IB-9.

The crosses of cultivar Dawn with IAC-47 and Bluebelle indicated the presence of two genes for resistance to both races. The crosses of Três Marias with IAC-47 and Bluebelle showed the presence of two pairs of genes to race IB-9 (Table II), with duplicate dominant epistasis. The F_2 progeny from the cross IAC-47 with Três Marias segregated at a ratio of 63 resistant plants to one susceptible to the same race. The resistance in cultivar Basmati 370 is controlled by two gene pairs to race IB-1 and three pairs to race IB-9, as is evident from the ratios 15:1 and 63:1, respectively (Tables I and II).

The present study showed that resistance to *P. grisea* is controlled by one, two or three genes. Other investigators have observed that resistance is also controlled by recessive genes in some cultivars (Yu *et al.*, 1987). Isogenic lines with different known resistance genes constitute a more accurate method for determining the number and distribution of resistance genes (Flor, 1955; Mackill and Bonman, 1992). Most of the crosses in the present study showed the expected duplicate 15:1 ratio for two independently inherited dominant resistance genes. These results are in conformity with those obtained using near isogenic lines and a set of Philippine isolates (Mackill and Bonman, 1992). Genetic studies on rice blast are often complicated due to the existence of different reaction types (Ou, 1985; Mackill and Bonman, 1992). Several crosses segregated for infection type 3, which was considered a resistant reaction. Some of the parents considered to be resistant, such as Dawn and Basmati-370, also showed this infection type, and the F_2 population gave a good fit for the expected 15:1 ratio. Thus, the disease ratings 3 and 5 separate the incompatible and compatible reaction classes. However, further studies are required to determine the inheritance of reaction type 3.

RESUMO

Selecionaram-se sete fontes de resistência de arroz (*Oryza sativa*) às raças IB-1 e IB-9 do patógeno *Pyricularia grisea*, baseando-se na reação de brusone nas folhas, em testes realizados sob condições controladas de casa de vegetação.

Foram feitos cruzamentos, envolvendo pais resistentes e suscetíveis, para estudar a herança da reação à doença, das diferentes fontes de resistência. As progênies F_1 e F_2 de todos os cruzamentos, incluindo retrocruzamentos com o pai suscetível (RC_1) e com o pai resistente (RC_2), foram avaliadas para a reação de brusone nas folhas.

Os dados mostraram que a resistência é controlada por um, dois ou três pares de gene, que segregam independentemente, na maioria dos doadores. Também, em alguns casos, foi identificada a interação não alélica, como a epístasia dominante.

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