

Breeding Rice Cultivars with Durable Blast Resistance in Colombia

Fernando Correa Victoria and Cesar Martinez

Abstract Rice blast disease (*Pyricularia grisea*) is the most important rice production constraint in Latin America. One strategy to improve the durability of blast resistance is to pyramid resistance genes. To do this, we have conducted extensive studies on the genetic structure of blast pathogen populations in Colombia and Latin America; determined composition, distribution and frequency of the avirulences that underlie race variation; identified and incorporated resistance gene combinations into commercial rice cultivars using genetic markers; and continuously evaluated and selected breeding lines under high disease pressure and pathogen diversity. Rice differentials with known blast resistance genes have been used to study avirulence gene composition and frequency in the blast pathogen and to identify relevant resistance genes. The combination of the blast resistance genes (*Pi-1*, *Pi-2*, *Pi-33*) for which their corresponding avirulence genes are highly conserved in blast pathogen populations in Colombia has proven to confer stable blast resistance after several years of testing under high blast pressure in the field and greenhouse inoculations. Additional pathogen characterization of spontaneous mutations of the blast pathogen allowed the identification of the blast resistance genes *Pi-b*, *Pi-9* and *Pi-ta²*, which will be needed for protecting rice cultivars from potential future changes in the avirulence/virulence genes in the blast pathogen population. Microsatellite markers highly linked to these blast resistance genes have been found from public databases facilitating the introgression and pyramiding of each of these six blast resistance genes into Latin American rice cultivars and elite lines derived from rice breeding programs aiming at developing rice cultivars with durable blast resistance.

Keywords Durable resistance · Rice blast · Avirulence/virulence · Resistance genes

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1 Introduction

Rice blast caused by *Pyricularia grisea* (Cooke) Sacc., is the most limiting biotic factor of rice production in the world. The use of resistant cultivars is the most effective and economical way of controlling the blast disease, therefore, breeding efforts for developing resistant cultivars continue to be a priority of all rice breeding programs worldwide. Rice is normally grown under irrigation or flooded conditions characterized by low blast pressure or under rainfed upland conditions characterized by a high blast pressure (Bonman and Mackill 1988, Bonman et al. 1991). Development of durable blast resistance for these two environments should be possible if breeding programs are based on a complete understanding of pathogen diversity in the target area (Correa-Victoria et al. 2004). The Rice Project at the International Center for Tropical Agriculture, CIAT, has been developing a breeding strategy for the development of durable blast resistance. This strategy is based on studies on the composition and frequency of avirulence genes of the pathogen, characterization of the genetic structure, identification and incorporation of resistance gene combinations into commercial rice cultivars effective against populations of each genetic family of the pathogen, and the continuous evaluation and selection of breeding lines under a high disease pressure and pathogen diversity (Correa-Victoria et al. 2004).

2 Breeding Strategies for Durable Rice Blast Resistance

2.1 Irrigated Rice Production System Under Low Blast Pressure

CIAT's Rice Project develops its breeding activities on resistance to *P. grisea* under favored upland conditions in the experiment station "Santa Rosa" from FEDEARROZ in the Department of Meta, Colombia. This "hot spot" site is characterized by high blast disease pressure and pathogen diversity, which is maintained in the breeding plots during the entire crop cycle using spreader rows composed of a mixture of commercial rice cultivars susceptible to the different genetic lineages of the pathogen (Correa-Victoria et al. 2004). Under this condition of evaluation and selection, we have found that the resistance selected is more stable and durable than when resistance is selected under conditions of less blast pressure. Using this site and studies on the virulence diversity of the fungus, we have identified specific resistance gene combinations which explain the resistance stability of certain lines under irrigated conditions, indicating that pyramiding non-allelic major genes may be used to develop durable resistance to blast. The isogenic lines CT 13432-68, CT 13432-54, and CT 13432-55 carry the resistance genes *Pi-1*, *Pi-2*, and *Pi-33* respectively. These complementary resistance genes, in combination in these lines or when pyramided in the isogenic line CT 13432-107, confer resistance to all the main isolates representing the most common genetic families of the blast fungus in Colombia (Table 1). Our results have demonstrated that the combination of these three major blast resistance genes is highly effective for breeding and development of rice cultivars with durable resistance to the blast pathogen in Colombia. The basis

Table 1 Resistance genes relevant to genetic lineages of *Pyricularia grisea* in Colombia

Cultivar	Resistance Gene	Isolate/Genetic Lineage (SRL)								
		1 L6-1	2 L6-2	3 L6-3	4 L4-1	5 L4-2	6 L4-3	7 L5-1	8 L5-2	9 L5-3
CT 13432-68	Pi-1	S ¹	S	R	R	R	R	S	S	S
CT 13432-54	Pi-2	S	S	S	S	S	S	R	R	R
CT 13432-55	Pi-33	R ¹	R	R	S	S	S	S	S	S
CT 13432-107	Pi-1 + 2 + 33	R	R	R	R	R	R	R	R	R

¹ S=susceptible interaction; R=resistant interaction

of this resistance comes from our studies indicating that the frequency of the avirulence genes in the pathogen can be used as an indicator to predict the durability of a resistance gene. In other words, those resistance genes corresponding to avirulence genes whose loss during resistance breakdown causes a deleterious effect on the pathogen will probably be durable (Correa-Victoria et al. 2004).

The great pathogenic diversity observed in *P. grisea* is considered the main cause of resistance loss in newly released cultivars. All breeding programs aimed at developing rice cultivars with durable resistance should be based on the study and understanding of the genetic diversity of the pathogen, virulence diversity, and early detection of possible changes in avirulence/virulence during evolution of the fungus. We have conducted continuous monitoring of the blast pathogen population in Colombia to identify possible spontaneous mutants able to breakdown the three gene combination, in order to identify potential resistance genes effective against these mutations before these isolates increase in frequency and become predominant within the fungus population. Analysis of several hundred isolates demonstrated the effectiveness and stability of the three resistance gene combination; however, three isolates (Table 2) defeated the combination of the resistance genes in the isolate CT 13432-107 as well as each individual gene (isolates 1, 2 and 3, Table 2) in greenhouse inoculations. Although these isolates were detected in very low frequency and it is not known their potential fitness to become predominant in nature, we used them for inoculating rice differentials with known blast resistance genes to identify potential resistance genes effective against these three isolates. These inoculations allowed the early identification of the resistance genes *Pi-9*, *Pi-b*, and *Pi-ta²*, which

Table 2 Identification of resistance genes to blast isolates compatible with the resistance genes Pi-1, Pi-2 and Pi-33

Rice Differential	Resistance Gene	Isolates							Avirulence Frequency
		1	2	3	4	5	6	7	
CT 13432-68	Pi-1	S	S	S	S	R	S	S	0.53
CT 13432-267	Pi-2	S	S	S	S	S	R	R	0.16
CT 13432-33	Pi-33	S	S	S	R	S	S	S	0.18
F 145	Pi-b	S	R	S	S	S	S	S	0.40
F 128-1	Pi-ta ²	S	S	R	S	S	S	R	0.15
75-1-127	Pi-9	R	R	R	R	R	R	S	0.98

01 are effective against the blast isolate mutants 1, 2, 3, respectively. Therefore, the
 02 newly identified resistance genes should also be incorporated into future rice cul-
 03 tivars in order to remain ahead of possible changes in virulence within the blast
 04 pathogen population.

05 The resistance gene *Pi-9* derived from the wild species *Oryza minuta* was found
 06 to be effective against the isolates 1, 2, and 3 as well as the isolates 4, 5 and 6. These
 07 last three isolates represent the genetic lineages SRL-6, SRL-4, and SRL-5, the pre-
 08 dominant genetic lineages from Colombia, respectively. The resistance genes *Pi-ta*²
 09 and *Pi-b* are not effective against isolates 4, 5 and 6. Although the resistance gene
 10 *Pi-9* is effective against the isolate mutants 1, 2 and 3, and the isolates representing
 11 the most common lineages of the pathogen, this gene should not be used as a single
 12 gene because of the high risk of a rapid breakdown. The avirulence frequency of
 13 0.98 for the resistance gene *Pi-9* (Table 2) is considerable high compared to the
 14 frequencies found for the other resistance genes (Table 2). However, a few isolates
 15 were found to be fully compatible with this gene. Fortunately, the resistance genes
 16 *Pi-ta*² and *Pi-2* are effective against this pathotype (isolate 7, Table 2). *Pi-9* has
 17 been reported to exhibit a broad spectrum of resistance to many blast populations of
 18 the world (Qu et al. 2006); however, this gene should be used in combination with
 19 other resistance genes to avoid a rapid breakdown of the resistance. It is interesting
 20 to note that although the resistance genes *Pi-2* and *Pi-9* are in the same gene cluster,
 21 isolates compatible with *Pi-2* do not infect *Pi-9*, and the isolate compatible with
 22 *Pi-9* do not infect *Pi-2*. In summary, development of durable blast resistance for
 23 the irrigated rice ecosystem in Colombia should be based on the combination of the
 24 resistance genes *Pi-1*, *Pi-2*, *Pi-33*, *Pi-b*, *Pi-ta*², and *Pi-9*, which in combination are
 25 effective against the present blast populations.

26 With the objective of identifying sources of the six blast resistance genes of inter-
 27 est, more than 200 commercial rice cultivars from Latin America were inoculated
 28 with different blast isolates carrying the corresponding avirulence gene to each one
 29 of the six blast resistance genes. The presence of the genes and their frequency in
 30 the germplasm evaluated were inferred from the phenotypic evaluations (Table 3)

31
32
33 **Table 3** Frequency of blast resistance genes in 211 commercial rice cultivars from Latin America

34 Resistance Gene	Cultivars No.	Frequency	35 Resistance Gene	Cultivars No.	Frequency
36 Pi-9	12	0.06	Pi-4b	69	0.33
37 Pi-k ^a	35	0.17	Pi-3	83	0.39
38 Pi-t	46	0.22	Pi-ta	94	0.45
39 Pi-z ^f	48	0.23	Pi-k	99	0.47
40 Pi-k ^h	55	0.26	Pi-b	112	0.53
41 Pi-k ^m	55	0.26	Pi-i	117	0.55
42 Pi-z	62	0.29	Pi-4a	125	0.59
43 Pi-k ^p	64	0.30	Pi-ta ²	137	0.65
44 Pi-1	64	0.30	Pi-k ^s	158	0.75
45 Pi-33	64	0.30	Pi-sh	178	0.84
	Pi-2	68			

01 and will be confirmed by the use of molecular markers. Sources of each gene to
02 be used in genetic crosses were found among the cultivars evaluated being already
03 present in backgrounds with other desirable agronomic traits. The frequency of the
04 six genes of interest was between 6 (*Pi-9*) and 65 (*Pi-ta²*) percent. Pyramiding blast
05 resistance genes in a common background can be assisted by the use of molecular
06 markers linked to the resistance genes. Several microsatellite markers associated to
07 these six blast resistance genes have been reported and are currently used at the rice
08 breeding program at CIAT (Correa-Victoria et al. 2006, Fuentes et al. 2007) with
09 the objective of developing rice cultivars with durable blast resistance for irrigated
10 rice ecosystems.

11 12 13 14 **2.2 Favored Upland Rice Production System** 15 **Under High Blast Pressure**

16
17 Rice blast is a major constraint to rice production, particularly under the rainfed con-
18 ditions prevalent in Latin America. Since many rice growers in developing countries
19 do not have access to fungicides, cultivar resistance has been the preferred means
20 of controlling the disease. Developing durably resistant lines, therefore, is a high
21 priority for most rice-breeding programs working for this upland environment. As
22 indicated above, the CIAT Rice Project develops its breeding activities on resistance
23 to *P. grisea* under favored upland conditions in the Santa Rosa experiment station.
24 This site is characterized for having high blast disease pressure and pathogen diver-
25 sity. Correa-Victoria and Zeigler (1995), concluded that high levels of resistance
26 selected from rice populations with diverse combinations of resistance genes in the
27 presence of diverse and abundant pathogen population may yield lines with stable
28 blast resistance.

29 Oryzica Llanos 5 was released from the CIAT Rice Breeding Program in 1989 as
30 a modern, high yielding, and blast resistant cultivar for the very blast-prone eastern
31 plains of Colombia. It was widely grown in this region and remains highly resis-
32 tant after more than 15 years. This cultivar has shown a remarkable durability in
33 resistance over space as well as over time. It had a virtually unmatched degree of
34 resistance when evaluated in several highly blast-conducive sites in Asia (Correa-
35 Victoria and Zeigler 1995). At that time, it was presumed that the resistance in
36 the rice cultivar Oryzica Llanos 5 was most probably multigenic, because all its
37 ancestors were susceptible to isolates from our breeding experiment station.

38 It has been suggested by many rice blast researchers that in environments highly
39 conducive to the development of severe blast epidemics, such as favored uplands,
40 partial resistance may offer adequate protection from the blast pathogen. However,
41 selection for partial or quantitative resistance to rice blast is difficult because of its
42 genetic nature where several minor genes are controlling it. Consequently, breeding
43 for partial resistance usually involves simply selecting plants showing interme-
44 diate disease levels over several generations, discarding both highly susceptible and
45 highly resistant plants. In addition, Correa-Victoria and Zeigler (1995) concluded

01 that rice lines selected with complete resistance at this experiment station were
 02 more stable than rice lines selected as partially resistant. They also concluded that
 03 developing high levels of multigenic resistance for disease-prone environments is
 04 an attractive and achievable alternative to partial resistance.

05 The genetic basis of the high level of durable resistance to rice blast in the cultivar
 06 *Oryzica Llanos 5* was characterized in two recombinant inbred line (RILs) popula-
 07 tions from a cross between the susceptible cultivar *Fanny* and *Oryzica Llanos 5*
 08 (Lopez-Gerena 2006, Lopez-Gerena et al. 2004). The number and chromosomal
 09 location of quantitative trait loci (QTL) conferring resistance against eight iso-
 10 lates of the blast fungus representing different genetic lineages were tested in these
 11 two populations and a linkage map was constructed using 350 molecular markers.
 12 Twenty-one QTL were detected and associated with the resistant traits, disease leaf
 13 area and lesion type, on nine chromosomes. Most but not all of the QTL occurred in
 14 the same genomic regions as either genes with major race-specific effects or other
 15 resistance QTL that had been described in previous experiments. Most of the QTL
 16 appeared to be race-specific in their effects but it is possible that some QTL with
 17 smaller effects were nonspecific. Three QTL affected resistance to one blast iso-
 18 late, which causes limited disease on *Oryzica Llanos 5* in the greenhouse and was
 19 probably virulent on most or all of the major genes from the cultivar. As a whole,
 20 the observed durable resistance in the rice cultivar *Oryzica Llanos 5* seems to be
 21 the result from a combination of quantitative and qualitative resistance genes. The infor-
 22 mation from these studies is being used for the development of improved lines with
 23 *Oryzica Llanos 5* derived QTL for resistance. The growing number of mapped minor
 24 and major resistance genes and development of marker-aided selection suggests that
 25 quantitative blast-resistance genes may be efficiently combined with major genes.

26 Despite all the efforts made to improve the efficiency for selecting and developing
 27 rice cultivars with durable blast resistance for the favored upland rice ecosystem,
 28 blast resistance is continuously being lost in breeding lines after the F_4 or later gen-
 29 erations. A long-term study (Tables 4 and 5) was conducted by selecting resistant
 30 plants in the F_2 populations of year 2000 at our experiment station to associate the
 31 stability of blast resistance in advanced generations and the reaction of the F_2 popu-
 32 lations. All crosses were developed from progenitors which exhibited a stable blast
 33 resistance at the experiment station. F_7 lines derived from F_2 resistant plants selected
 34

35 **Table 4** Long term study on the stability of rice blast resistance under upland conditions and high
 36 blast pressure in Colombia (F_2 – F_3 generation)

Population	Crosses No.	Families No.	Resistant F_2 plants Selected 2000	Resistant F_3 lines 2001		Resistant F_3 plants Selected 2001
				No.	%	
F_2 Family Field Reaction						
Susceptible	69	100	194	39	20	661
Segregating	72	96	171	53	31	698
Resistant	54	78	155	80	52	644
Total	156	274	520			2003

Table 5 Long term study on the stability of rice blast resistance under upland conditions and high blast pressure in Colombia (F₄–F₇ generation)

Population	Resistant F ₃ plants Selected	Resistant F ₄ lines 2002		Resistant F ₅ lines 2003		Resistant F ₆ lines 2004		Resistant F ₇ lines 2005	
		No.	%	No.	%	No.	%	No.	%
F₂ Family Field Reaction									
Susceptible	661	89	13	5	1	0	0	0	0
Segregating	698	166	24	36	5	25	4	22	3
Resistant	644	126	20	30	5	25	4	22	3
Total	2003	371	19	71	4	50	3	44	2

in 2000 were evaluated for several seasons. A high decline in the number of resistant lines was observed (Table 4). No line derived from F₂ families with a predominant reaction of susceptible plants (more than 50% susceptible plants) remained with stable blast resistance at the F₇ generation (Table 5). Although F₂ families with predominant segregation (half of the F₂ plants are resistant and half susceptible) or resistant plants (more than 50% of the F₂ plants are resistant) yielded stable blast resistant lines, this percentage was very low (3%) for both cases (Table 5). This percentage is considered significantly low given that the breeders normally have to evaluate several thousand lines in the F₃ generation. A total of 44 rice lines (Table 5) exhibited a stable blast resistant reaction over the years and these lines are being used for distribution in the region, as progenitors in future crosses, and for evaluation studies of the stability of the resistance on a yearly basis in replicated trials.

Our hypothesis in this study was to demonstrate that those lines originating in crosses, where the F₂ families show a higher number of blast resistant plants, and which showed a higher number of resistant sister lines, would give origin to more stable resistant lines in the advanced generations. In addition, those advanced resistant lines originating from F₂ resistant plants selected within crosses where F₂ susceptible plants predominate, would be less stable. Our reasoning behind this hypothesis is that F₂ populations exhibiting a predominant number of resistant plants carry a larger number of different resistant genes including major and minor genes. Advanced resistant lines originating in these populations have a greater probability to carry a larger number of these resistance genes and therefore would be more stable. Those families with few F₂ resistant plants would probably have fewer resistance genes, and these would be easily defeated by the pathogen in early generations. If this hypothesis is correct, breeders should rate the F₂ populations and eliminate those crosses where the susceptible plants predominate allowing breeder's efforts to be concentrated on those crosses where there is a greater probability of selecting stable blast resistant rice lines. On the basis of our results in the F₆ and F₇ generations, our hypothesis seems to be true in relation to the development of stable blast resistant lines originating from F₂ populations where resistant plants predominate.

We should consider the important concern arising from the large decline in the number of resistant lines from one generation to the other. Our studies on the parents involved in the crosses evaluated suggest that some parents are better than others for potentially giving origin to stable blast resistance. We recommend that before incorporating new parents as sources of stable blast resistance in a breeding program, a careful evaluation over time and under high blast pressure be conducted. We also recommend selection of individual resistant plants in the F_3 generation. Plant selection in the F_2 generation is not an accurate indicator of resistance, because it is based on a single plant and not on a line composed of several plants that would better represent the range of resistance genes in the original F_2 plant.

Several potential sources of stable blast resistance (32 F_7 resistant lines) were selected from our long-term study to be involved in the development of a modified recurrent selection program aimed at accumulating the different resistance genes controlling their stable resistance. A total of 32 progenitors were selected to initiate the development of the F_1 of 16 single crosses; these F_1 were used for the development of double crosses (2×2) repeating the process for the development of 4×4 , 8×8 , and 16×16 populations. Besides the stable blast resistance exhibited by each progenitor, other criteria such as yield potential, grain quality, adaptation to the upland ecosystem and tolerance to other diseases was considered. All crosses are being developed manually controlling the involvement of all 32 parents in the last cross (16×16). Resistant plants are being selected and advanced expecting to generate large numbers of stable resistant lines that could become commercial rice cultivars in the near future. We expect through recurrent selection to be able to accumulate both major and minor blast resistance genes conferring durable blast resistance. We expect from our other studies to use molecular markers associated with major genes to assure that selected lines carry them and expect in the near future to identify markers associated to relevant minor genes to assure that selected lines do not carry only major resistance genes.

3 Conclusions: Breeding Strategies for Durable Rice Blast Resistance

3.1 Irrigated Rice Production System Under Low Blast Pressure

- Pyramiding of major rice blast resistance genes based on targeted blast populations
- Breeding assisted by molecular markers and pathogen characterization (avirulence frequencies)

3.2 Upland Rice Production System Under High Blast Pressure

- Evaluation and selection of potential sources of stable blast resistance over time under blast hot spot conditions. Crosses among progenitors following a recurrent selection procedure

- 01 • Evaluation and selection of segregating populations under high blast pressure
- 02 and pathogenic variation (hot spots)
- 03 • Selection of advanced rice lines exhibiting stable blast resistance over time
- 04 combined with desired agronomic traits
- 05 • Marker assisted selection for major blast R genes
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