RICE PATHOLOGY

Activity 1. Characterization of Blast Pathogen Populations. Monitoring the Evolution in the Genetic and Virulence Diversity of the Blast Pathogen over time.

Abstract

Rice blast, the most important rice disease worldwide can be managed through genetic resistance. Continuous monitoring of the evolution leading to important changes in the genetic structure and virulence spectrum of the pathogen is very important for the identification of resistance genes and their combinations to resist those pathogenic changes and preventing resistance breakdown. Understanding this pathogen-host interaction can attain development of suitable breeding strategies for a more stable blast resistance.

Introduction

Rice blast caused by *Pyricularia grisea* Sacc. is the most important disease worldwide. Genetic resistance is the most effective way to control the disease but resistance is defeated by the pathogen shortly after cultivar release with the exceptions of the Colombian commercial cultivars Oryzica Llanos 5 and Fedearroz 50. This breakdown is mainly due to the continuous changes and evolution of the pathogen, which gives origin to new pathotypes compatible with the new rice cultivars. Continuous monitoring of blast pathogen populations in breeders fields is needed to detect recent changes in pathogen virulence. New pathotypes detected are used to identify resistance genes that can be introgressed into new genetic material before there is an increase in frequency of these new isolates, and therefore reducing the risks of resistance breakdown.

Materials and Methods: Rice leaves and panicles with typical blast symptoms are continuously collected from different rice lines in the pathology and breeder's plots at Santa Rosa experiment station. Blast isolates recovered from the infected samples in the laboratory are inoculated on a set of differential rice lines with different resistance genes to identify potential sources of resistance to new pathotypes. The same sample of isolates is used for determining their genetic structure using the Pot-2 PCR fingerprinting technique. More than 100 blast isolates recovered from several rice lines were analyzed in 2003 and new pathotypes are reported in this chapter.

Results and Discussion: All blast isolates analyzed belonged to the known genetic groups SRL-6, SRL-5, SRL-4 and SRL-2 already identified in Colombia. The most common pathotypes found **(Table 1)** were #1 (SRL-4) and # 2 (SRL-5). These isolates were recovered from several cultivars. Pathotypes identified as #1 induced in 2003 more typical blast lesion on cultivars Fedearroz 50 and Oryzica Llanos 5. This susceptible reaction was also observed in greenhouse inoculations, although the disease severity observed was around 10% of leaf area affected. Resistance genes effective against this pathotype are Pi-1 and Pi-K^h as had been reported in year 2002. The resistance gene Pi-1 is being incorporated in the commercial cultivar Fedearroz 50 through marker assisted selection, greenhouse inoculations and field evaluations.

In our efforts to detect new changes in virulence in the pathogen population, few blast lesions observed in the highly resistant line FL 00147-8P-6-15P were collected and analyzed in the laboratory. All four isolates retrieved turned to be lineage SRL-4, however three of them belonged to a different pathotype identified as # 3 **(Table 1)**, and the fourth isolate as pathotype #1. Greenhouse inoculations of pathotype #3 indicated for the first time in many years the ability of an isolate to potentially defeat the three resistance genes Pi-1, Pi-2, and Pi-33. This pathotype was recovered from only one cultivar, indicating that its frequency is very low. Besides, the cultivar giving origin to this pathotype exhibits a highly resistant reaction to blast. We don't know at this point the relevance of this pathotype and the role it can play in breaking down the resistance conferred by the combination of these three genes as we have demonstrated in previous years. It is interesting to observe, however, that in order to lose the three avirulence genes avr-Pi-1, avr-Pi-2, and avr-Pi-33, this pathotype had to maintain the avirulence gene for Pi-ta^2 **(Table 1)**. The corresponding resistance gene Pi-ta^2 present in the differential F 128-1 confers resistance to this isolate **(Table 1)**. These results indicate that the resistance gene Pi-ta^2 will probably have to join the combination of the three resistance genes Pi-1, Pi-2, and Pi-33 to prevent a potential breakdown of the resistance genes. The presence of the Pi-ta^2 gene in the cultivars Oryzica Llanos 5 and Fedearroz 50 explains why this isolate did not infect severely these cultivars in greenhouse inoculations **(Table 1)**. We are in the process of analyzing more blast samples collected in 2003, both, in terms of genetic structure and virulence spectrum, to determine if this new pathotype can be recovered from other cultivars, including few lesions observed in the near isogenic lines from the cross CT 13432 carrying the three resistance genes Pi-1, Pi-2 and Pi-33.

Our results from the last several years, and of this particular year, suggest that in order to develop a more stable blast resistance, a combination of several resistance genes is needed to resist the potential changes in virulence of the rice blast pathogen. The combination of several major resistance genes will probably have to be accompanied of some important minor or quantitative trait loci, as will be discussed later in this chapter in the analysis of the stable resistance of the cultivar Oryzica Llanos 5.

As we can see **(Table 1)**, few gene combinations would confer resistance to the blast population present in the upland environment of the Llanos Orientales from Colombia. We see the urgent need to identify new genes, probably present in other rice species. We have tested for two years the resistance gene Pi-9 present in the line 75-1-127 and derived from *Oryza minuta*, finding that the gene confers complete resistance in greenhouse inoculations as well as field evaluations **(Table 1**). We have observed high levels of field resistance in the species *O. glaberrima* that deserve attention to identify potential new resistance genes. Once more, we see the importance of having a "hot spot" site with high blast pressure and pathogen diversity, to identify the best resistance gene combinations, and to detect in advance potential changes in genetic structure and virulence in the pathogen population that could threaten cultivar resistance.

Future Activities: Blast populations will continue being analyzed for their genetic structure and virulence spectrum to determine the potential changes of the pathogen that would lead to resistance breakdown. New resistance genes and proper combinations will be identified in the cultivated as well as wild species of rice to be incorporated in our breeding program. We will analyze the importance and potential role of the new pathotypes identified in 2003 and to

determine the effectiveness of resistance genes effective against those isolates. The potential importance of the resistance gene Pi-9 will be evaluated again in 2003 under field conditions and greenhouse inoculations.

Table 1. Virulence Spectrum and Frequency of Rice Blast Pathotypes Detected at the Santa Rosa Experiment Station in 2003.

Rice Line	Resistance Gene	Pathotypes (frequency %)							
		1 (60)	2 (23)	3 (5)	4 (4)	5 (2)	6 (2)	7 (2)	8 (2)
C 104 LAC	Pi-1		+++	+++	+	+++			
C 101 A51	Pi-2	+++		+++		+++		+++	+++
C 101 LAC	Pi-1+Pi-33		+++	+++		+++			
CT 13432-33	Pi-33	+++	+++	+++	+	+++	+++		+++
CT 13432-34	Pi-1+Pi-2+Pi-33			+++		++			
C 104 PKT	Pi-3	+++	+++	+++		+++			+++
C 101 PKT	Pi-4a	+++	+++	+++	+++	+++			+++
C 105 TTP4 (L23)	Pi-4b	+++		+++	+++	+++			+++
F 124-1	Pi-ta	+++	+++	+++	+++	+++	+++	+++	+++
F 128-1	Pi-ta^2	+++	+++		++		++	+	
F 80-1	Pi-k	+++	+++	+++	+	+++	++		+
F 98-7	Pi-k^m	+++	+++	+++	+++	++	+++	+++	+++
F 129-1	Pi-k^p	+++	+++	+++	+++	+++	+++	+++	+++
F 145-2	Pi-b	+++	+++	+++			+++	+	+
Aichi Asahi	Pi-a	+++	+++	+++	+++	+++	+++	+++	+++
K 3	Pi-k^h		+++	+++		+			
K 59	Pi-t	+++	+++	+++	+		+++		
Rico 1	Pi-k^s	+++	+	+++	+++	++	+++		+++
Norin 2	Pi-sh	+++	+	+	+++	++	++	+	++
Nato	Pi-I	+++	+++	+++	+++	++	+++		+++
Ou 244	Pi-z	+++		+++		++	++		+++
Toride 1	Pi-z^t	+++		+++		++		+	+++
Commercial Cultivars									
Fanny		+++	+++	+++	+++	+++	+++	+	+++
Metica 1		+++		+++	+++	+++			+++
Oryzica 1		+++		+++	+++	++		+	+++
Oryzica 2				+++		+			
Oryzica 3		+++		+++		+			
Cica 7		+++		+++	+++	+++			+++
Cica 8				+++		+			
Cica 9		+++		+++		+		+++	
IR 22		+++	++	+++	+++	++	+++		
Tetep				+++		+			
Ceysvoni		++		+++		++	+++		
O. Llanos 5		+		+					+
Línea 2 (Semillano)		++		+++		+		+++	
O. Llanos 4		+	-/+	+++					+
O. Caribe 8		+++		+++		+++			+++
O. Yacu 9		+++		+++		+++			+
Fedearroz 50		++		+		+			
75-1-127	Pi-9								

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Activity 2. Selection of Rice Blast Resistance Sources to Different Genetic Lineages of the Blast Pathogen. Development of a Blast Nursery with Potential Sources of Resistance.

Abstract

The frequency of blast resistant plants in F2 populations is highly dependent on the blast reaction and stability of the parents used for the development of these populations. We initiated in year 2000 the development of a nursery with potential sources of durable blast resistance. Advanced rice lines are being evaluated for at least seven seasons under high disease pressure and only highly and durable resistant lines will be maintained into the nursery. This nursery will be tested under different conditions in several countries and used as a source of parents for breeding programs in Latin America.

Introduction

The frequency of blast resistant plants observed in F2 populations in the field is highly dependent on the blast reaction and stability of this reaction of the parents used for the development of these populations. An increase in the number of susceptible F2 plants and F4 lines found in the past years in different breeding materials from CIAT and FLAR at the Santa Rosa experiment station has been observed and will be discussed later in this chapter. This has been related probably to the low stability of the blast resistance of the parents used in the corresponding breeding programs. We have initiated the blast evaluation over time in the field and greenhouse of several hundred advanced as well as segregating lines exhibiting desired agronomic traits to identify potential sources of blast resistance. We are developing a nursery of potential sources of blast resistance to be used as parents, and will distribute them to partners in Latin America for testing and use in their breeding programs. Materials and Methods were followed according to those described in the Annual Report of the Rice Project for 2001.

Results: A total of 418 advanced rice lines from different sources described in last year report were evaluated and selected at the Santa Rosa field experiment station in 2003. The most resistant lines over the last three years are shown in Table 1. Most of the resistant lines with a blast score 0-3 belong to the Germplasm Bank of CIAT-FLAR **(Table 1**). Several of these lines have already been used in different crosses and yielded rice lines with potential stability of their blast resistance in advance generations as will be shown later in this chapter. These results indicate the importance of evaluating the potential donors of blast resistance for several semesters before their inclusion in a breeding program. Selected lines with a blast score of 0-3 as well as those with an intermediate reaction with a score of 4 will be evaluated again in replicated trials in year 2004 for their inclusion in a nursery as potential donors of stable blast resistance. We had already reported in previous years the high resistant reaction of the japonica lines to the grain discoloration pathogens. It should also be noted the resistant reaction of the rice cultivars from Surinam such as Ciwini and Eloni which can be incorporated in our breeding programs as they might help to broaden the genetic diversity of our rice germplasm.

Discussion: Durability of blast resistance is in general associated with the period of time that a cultivar remains as resistant after being exposed to a targeted pathogen. Field studies conducted

by CIAT at Santa Rosa demonstrated that stable blast resistance could only be identified if the lines were evaluated through the F6-F7 generation. It is possible that only after several generations of exposure that the most effective resistance genes and their combinations can be identified. These genes at the same time should correspond to those avirulence genes highly conserved in the pathogen population with lower rates of change or mutation. In order to identify resistance genes associated with durability, it is necessary to evaluate and confirm the stable resistance of the potential donors for at least seven generations. We are in the process of developing a nursery with potential donors of resistance to different pathogens. Therefore, these nurseries will be evaluated continuously for several seasons under high disease pressure in the field to assure that the resistance selected is not a escape to infection and that the lines retain their durable resistance.

Future Activities: The evaluations of advanced breeding lines will be an annual activity to assure that the selected sources retain their stable resistance to the different pathogens. The search for new blast resistance genes will continue. The pathogen population will be monitored on these resistant lines to identify changes leading to a potential breakdown of the resistance. An analysis of the parents used in the genetic crosses giving origin to rice lines with stable and durable resistance will be initiated. Genetic crosses giving origin to rice lines with potentially durable resistance will be developed on the basis of the information generated since year 2000.

Table 1. Potential Progenitors for Stable Blast Resistance Exhibiting Blast Scores 1-3 in Santa Rosa Field Evaluations during Four Cycles, Santa Rosa 2000 - 2003.

Pedigree	Pedigree
1. FL 00478-29P-23-3P	22. FL00478-29P-5-1P-M
2. FL 00518-16P-8-2P	23. FL00518-14P-15-3P-M
3. CNAx5013-13-2-2-4-B	24. FL00518-23P-11-2P-M
4. CT11275-3-F4-8P-2	25. FL00530-29P-4-2P
5. CT11280-2-F4-12P-5	26. FL00530-7P-7-1P-M
6. CT11891-2-2-7-M	27. FL00535-21P-4-3P-M
7. CT13394-5-6-M-M-1	28. FL00542-45P-8-2P-M
8. CT13449-M-3-1-M	29. FL00585-26P-1-2P-M
9. CT13449-M-8-2-M	30. FL00595-12P-10-4P-M
10. CT13458-M-3-2-M-M	31. FL00595-25P-9-3P-M
11. CT13458-M-3-3-M-M	32. FL00837-8P-5-2P-M
12. CT13458-M-3-4-M-M	33. FL00871-1P-3-1P-M
13. CT13464-M-10-1-M-M	34. FL00871-1P-5-2P-M
14. CT13937-16-1-M-M-2	35. HUALLAGA INIA
15. CT13937-16-2-M-M-2	36. IRAK 13
16. CT13937-16-2-M-M-3	37. LINEA 30
17. CT13937-16-3-M-M-4	38. PROGRESO
18. CT13941-11-1-M-M-4	39. PURG-2\0\0\1>27-1
19. CT13943-10-2-M-M-3	40. RIO PARAGUAY
20. CT8222-7-6-2P-1X	41. SAN MARTÍN 83
21. FL00447-35P-4-2P-M	42. TRES MARIAS

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Activity 3. Identification of Molecular Markers Associated with the Blast Resistance Genes Pi-1, Pi-2, Pi-33 and their Incorporation into Commercial Rice Varieties Through Backcrossing and Marker Assisted Selection (MAS).

Abstract

Farmers often choose blast susceptible varieties because they have high yields and grain quality. To sustain these characteristics, farmers have to spray fungicides, including several applications per planting season, increasing their production costs and the possibilities of contaminating the environment and/or affecting the human health. Incorporation of blast resistance genes to these varieties would make them more cost effective and ecologically sustainable. The combination of the blast resistance genes Pi-1, Pi-2, and Pi-33 confers resistance to all known blast pathogen populations of Colombia. We are identifying molecular markers associated with different resistance genes and initiated a backcrossing program assisted by these markers to introduce these resistance genes into several popular Latin American rice varieties.

Introduction

Farmers adopt faster rice varieties that have high yields and excellent grain quality. The characteristic such as resistance to diseases is highly desirable but not enough to make a variety successful. Inconsistent yields because of diseases are enough to cause varieties to be discarded by farmers. Varieties without durable blast resistance become more susceptible every year, and they need more applications of fungicides. In seasons favorable for rice blast, even fungicides may not be sufficient to prevent substantial losses. Farmers would like these varieties to be blast resistant.

We have initiated a backcrossing program in order to introduce blast resistance genes into some of those susceptible rice cultivars, which still play an important role in the economy of many rice farmers and regions of Latin America. The resistance genes being incorporated into the commercial varieties are Pi-1, Pi-2, and Pi-33 as they confer resistance to all the pathogen population in Colombia and probably the Latin America region based on their reaction to other blast populations of the region. The BC1F1 and BC1F2 breeding populations derived from the crosses between several Latin American rice varieties and four rice lines used as sources of the three resistance genes have been developed for the identification of heterozygous lines carrying the three resistance genes. Materials and Methods were described in the 2001 Annual Report of the Rice Project. Greenhouse inoculations with appropriate avirulence genes were performed in 2003 for the selection of resistant plants to perform the BC2. Additionally, BC1F2 populations were planted at the Santa Rosa experiment station for selection of resistant plants and backcrossed to the corresponding recurrent parents. A total of 56 microsatellites or PCR based markers relatively close to the resistance genes Pi-1, Pi-2 and Pi-3 were identified from different publications or databases and tested for their association with these genes.

Results: Near isogenic lines carrying any of the three resistance genes, combination of any two genes, and combination of the three resistance genes Pi-1, Pi-2, and Pi-33 were developed based on controlled inoculations in the greenhouse and evaluations under field conditions in 2002 in our Santa Rosa experiment station. The only isogenic lines exhibiting a leaf and panicle blast

resistance in 2002 and 2003 were the four lines carrying the three resistance genes Pi-1, Pi-2, and Pi-33. However, some plants within these lines exhibited few blast samples in 2003, which are being used in the laboratory for pathogen isolation and for further analysis of the genetic structure and virulence spectrum of the pathogen. Most of the lines carrying one or two genes died before panicle development both in 2002 and 2003.

The four isogenic lines carrying the three resistance genes Pi-1, Pi-2, and Pi-33 with a high leaf and panicle blast resistance were used for the development of the BC1F1 populations of fourteen Latin American rice varieties and the BC2F1 of four of them following the procedure described in the Rice Annual Report of year 2002. Greenhouse inoculations of the BC1F1 and the BC2F1 were performed with particular blast isolates carrying the corresponding avirulence genes and resistant plants selected for the production of the BC2 and BC3 populations. Field evaluations of the BC1F2 at the Santa Rosa experiment station were also performed under field conditions and resistant plants selected to perform the BC2F1. A total of 56 molecular markers (Gene Bank web site) close to the three blast resistance genes (37 for Pi-1, 11 for Pi-2, 8 for Pi-33) were identified and tested for their gene association in near isogenic lines carrying single or combinations of the three genes. Six markers were identified highly associated with the presence of the resistance gene Pi-1, while one marker was associated with the absence of Pi-1 exhibiting a band only in the susceptible lines; three markers were associated with Pi-33, and one with Pi-2 **(Table 1)**. Most of the other markers did not amplify at the conditions tested of 50C and 55C for the annealing temperature and will be tested under different conditions. The eleven markers associated with the three resistance genes were tested in replicated trials on 38 isogenic lines carrying different combinations of the three resistance genes and on 19 rice cultivars including those Latin American varieties used in our backcrossing program. The markers associated with Pi-1 and Pi-2 seem to be suitable to follow the introgression of these genes into the commercial cultivars, while the markers for Pi-33 failed in discriminating the presence or absence of the gene in susceptible and resistant cultivars according to greenhouse inoculations. Efforts will be made to develop closer markers associated with the three genes. The number of backcrosses will depend on the recovery of the desired agronomic traits of the recurrent rice varieties.

Discussion: The combination of the three resistance genes Pi-1, Pi-2, and Pi-33 in a single near isogenic line exhibited in years 2002 and 2003 a high level of leaf and neck blast resistance when exposed under high blast pressure in a blast pathogen population with pathotypes compatible with the combination of any two of these three resistance genes. It seems then that the blast pathogen is able to lose any of the three avirulence genes or any combination of two avirulence genes, but not the three of them in a single isolate. It is possible that losing avirulence genes for one of these three resistance genes might affect some fitness parameters such as competitiveness among pathotypes with different avirulence gene composition, and therefore this parameter needs to be measure under natural conditions of infection by collecting blast samples from different near isogenic lines carrying different combinations of the resistance genes. Few blast samples collected from lines carrying the three resistance genes were collected in 2003 and will be analyzed for their genetic and virulence structure to determine its potential importance.

Future Activities: Evaluation and selection of the BC2F1 populations and development of the BC3F1 of several backcrosses between Latin American rice varieties and near isogenic lines carrying the resistance genes Pi-1, Pi-2, and Pi-33. Molecular markers as well as inoculations

with appropriate blast isolates will be used for the identification of the rice lines carrying the three resistance genes. Field studies will be carried out to determine the possible association of the lost of an avirulence gene with parameters of pathogenic fitness such as pathogen competitiveness. These activities will be carried out by collecting blast isolates from near isogenic lines with different combinations of the three blast resistance genes and comparing the frequencies of the different avirulence gene combinations present in the pathogen population with the expected frequencies based on the resistance genes of each line. Genetic distances between the molecular markers and the resistance genes will be determined using a set of 283 near isogenic lines.

Table 1. Identification of Microsatellite Markers Associated with the Resistance Genes Pi-1, Pi-2, and Pi-33 using a set of Near Isogenic Lines.

Marker Identification		Resistance Gene	Primer Sequence
RM 1233*I	Forward	Pi-1	TTCGTTTTCCTTGGTTAGTG
	Reverse		ATTGGCTCCTGAAGAAGG
RM 7654*A	Forward	Pi-1	CAAAAGTCTGACCGTTTACC
	Reverse		TAAGAGACGGAAGAGTGAGC
RM7654*H	Forward	Pi-1	CTCATGGTTGTGTCGTGGTC
	Reverse		GTGCAGTGCCAGTGGTACG
RM 7654-2	Forward	Pi-1	GTGTCGTGGTCGTAACTTG
	Reverse		TAAGAGACGGAAGAGTGAGC
RM 6094	Forward	Pi-1	TGCTTGATCTGTGTTCGTCC
	Reverse		TAGCAGCACCAGCATGAAAG
RM 5926	Forward	Pi-1	ATATACTGTAGGTCCATCCA
	Reverse		AGATAGTATAGCGTAGCAGC
RM 224	Forward	Pi-1	GATCGATCGATCTTCACGAGG
	Reverse		TGCTATAAAAGGCATTCGGG
RM 527	Forward	Pi-2	GGCTCGATCTAGAAAATCCG
	Reverse		TTGCACAGGTTGCGATAGAG
RM 409	Forward	Pi-33	CCAATCATTAACCCCTGAGC
	Reverse		GCCTTCATGCTTCAGAAGAC
RM 483	Forward	Pi-33	CTTCCACCATAAAACCGGAG
	Reverse		ACACCGGTGATCTTGTAGCC
RM 72	Forward	Pi-33	CCGGCGATAAAACAATGAG
	Reverse		GCATCGGTCCTAACTAAGGG

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