RICE PATHOLOGY

Activity 1. Blast (*Pyricularia grisea*) and sheath blight (*Rhizoctonia solani)* diseases on rice.

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 (1 to 3 years) after cultivar release with the exceptions of the Colombian commercial cultivars Oryzica Llanos 5 and Fedearroz 50, whose resistance lasted for at least fifteen and six years, respectively. Compatible blast interactions with these two cultivars were observed in 2004 at the Santa Rosa experiment station in Villavicencio, Colombia. This breakdown is mainly due to the continuous changes and evolution of the pathogen, which gives origin to new pathotypes compatible with the resistant rice cultivars. Continuous monitoring of blast pathogen populations in breeders as well as commercial 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 and commercial cultivars in the pathology and breeder's plots at Santa Rosa experiment station. Blast isolates from the cultivars Oryzica Llanos 5 and Fedearroz 50 were recovered from infected samples in the laboratory and 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 was used for determining their genetic structure using the Pot-2 PCR fingerprinting technique. More than 50 blast isolates recovered from several rice breeding lines, the cultivars Oryzica Llanos 5 and Fedearroz 50, and the cultivar Bonanza, released by the private sector in 2004 were analyzed and new pathotypes are reported in this chapter. These studies also included some isolates from the commercial cultivar Cica 8, which is not planted any more by farmers due to its susceptibility to blast. However, this cultivar had a resistant reaction in Santa Rosa in most replications planted in 2004 in the station. Cica 8 is used as a component in the mixture of the spreader rows used in our field methodologies to increase the frequency of lineage SRL-5 compatible with this cultivar.

Results and Discussion: All blast isolates analyzed belonged mainly to the known genetic groups SRL-6, SRL-5, SRL-4 and SRL-2 already identified in Colombia. Blast isolates recovered from the cultivars Oryzica Llanos 5 and Fedearroz 50 were lineage SRL-4 and exhibited a similar and wide virulence spectrum defeating most known resistance genes present in the rice differentials used in this study and most commercial cultivars released in Colombia in the last 20 years **(Table 1)**. These isolates however, did not reinfect Oryzica Llanos 5 and Fedearroz 50 in controlled inoculations in the greenhouse **(Table 1)** exhibiting mainly small lesions and a low disease severity. Given the fact that many isolates were recovered from these two cultivars and that greenhouse inoculations gave similar results of a low compatible interaction, we can speculate that the resistance of these two cultivars will still be durable for a longer period of time under farmers fields. We are in the process of dissecting the resistance genes present in the cultivar Oryzica Llanos 5 and results suggest both the presence of major and minor genes. Greenhouse inoculations also indicate that the resistance genes Pi-1, Pi-k, Pi-k^m, and Pi-kh, all of which are in the same region of Chromosome 11, confer resistance to these pathotypes **(Table 1)**. We have initiated a backcrossing program to incorporate Pi-1 into these two cultivars using marker assisted selection, and greenhouse and field inoculations. The rice cultivars Oryzica 2 and Cica 8, and the rice accession Tetep were also resistant to these pathotypes, probably due to the presence of one of the genes located on Chromosome 11, or a different resistance gene. Analysis of the blast samples collected from the rice commercial cultivar Bonanza released by the private sector in 2004 exhibited a similar virulence spectrum to those pathotypes recovered from Oryzica Llanos 5 and Fedearroz 50 **(Table 1)**. The recent release by the private sector of several commercial cultivars susceptible mainly to the blast genetic lineage SRL-4, has allowed the increase of this lineage in frequency. It seems that the private sector is working probably with similar germplasm obtained from different nurseries obtained from CIAT and which lack resistance genes to lineage SRL-4 such as those located on Crhomosome 11 in the region of the Pi-k locus.

A new accession (75-1-127) carrying the resistance gene Pi-9 derived from the wild species *Oryza minuta* and located on Chromosome 6 also exhibited resistance to these as well as many other pathotypes **(Table 1)**. This line has exhibited also a complete resistant reaction under field conditions in Santa Rosa after three years of evaluation. We are in the process of conducting genetic studies to determine if this resistance is indeed controlled by only one resistance gene. Our experience for many years and controlled inoculations of several thousand of isolates indicate that there are no single genes within the cultivated species *Oryza sativae* effective against all pathotypes of the blast pathogen. This wide spectrum of resistance controlled by this single gene suggests that rice blast populations carry a common avirulence gene whose products interact with the products of the resistance gene conferring resistance. The durability of the resistance conferred by this gene still needs to be demonstrated. These results also suggest that it is worthwhile looking for new resistance genes in wild rice species, which may carry different resistance genes/alleles to those present in the cultivated species and which have no coevolved with the pathogen. We will also focus on wild species originating in Latin America such as *O. glumepatula*, or the cultivated species *O. glaberrima* originated in Africa as they may carry useful resistance genes that have not been exposed to blast populations for many years as they most probably evolved from weeds to rice in Asia.

In our efforts to detect new changes in virulence in the blast pathogen population, few blast lesions observed in 2003 on the highly resistant line CT 13432-34 carrying the resistance genes Pi-1, Pi-2, Pi-33 were collected and analyzed in the laboratory in 2004. All isolates retrieved turned to be lineage SRL-4 and exhibited at least two pathotypes **(Table 1)**. Greenhouse inoculations of these two pathotypes indicated their ability to potentially defeat the three resistance genes Pi-1, Pi-2, and Pi-33. This pathotype was not recovered from the same or any other cultivar in 2004, indicating that its frequency is very low and probably its fitness is weak compared to the well-established blast populations. This hypothesis will be studied closely monitoring blast isolates collected from rice lines carrying the three genes, however, it was not possible in 2004 as all lines planted in Santa Rosa with the three genes exhibited a complete resistance 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, these pathotypes had to maintain in one isolate the avirulence gene for Pi-ta^2 and in the other the avirulence gene for Pi-b **(Table 1)**, located on Chromosomes 12 and Chromosome 2, respectively. The corresponding resistance genes Pi-ta^2 and Pi-b present in the differentials F 128-1 and F 145-2 confer resistance to these isolates **(Table 1)**. These results indicate that these two resistance genes Pi-ta^2 and Pi-b 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 and Pi-b genes in the cultivars Oryzica Llanos 5 and Fedearroz 50 determined by us in other studies explains why these isolate did not infect severely these cultivars in greenhouse inoculations **(Table 1)** despite the fact that all other commercial cultivars were severely infected in the same test. We are in the process of analyzing more blast samples collected in 2004, both, in terms of genetic structure and virulence spectrum, to determine if this new pathotype can be recovered from other cultivars. It should be noted that these two pathotypes to defeat a large number of resistance genes had lost many avirulence genes, which may confer a deleterious effect on the pathogen affecting its fitness and establishment. This could explain the low frequency observed in 2003 and the possible absence in 2004.

Analysis of blast samples recovered from the cultivar Cica 8 yielded isolates within genetic lineage SRL-5 with a narrow spectrum of virulence **(Table 1)**. These isolates were highly specific on the cultivars Oryzica 3, Cica 8, and Tetep, which is the source of resistance of Cica 8. These isolates also defeated the resistance genes Pi-ta^2, Pi-b, and Pik^m. The low frequency observed in this lineage may be explained by the narrow spectrum of virulence present in these isolates. Cica 8 is not grown anymore commercially and this lineage is not compatible with all the actual commercial cultivars grown by farmers such as Fedearroz 50 and Bonanza. Pathogenicity tests indicate that this lineage carry many avirulence genes **(Table 1)**. In order to increase its frequency for next year evaluations of our breeding lines, it will be necessary to multiply this lineage previous to our planting and perform some artificial inoculations on the spreader rows which have Cica 8 as a component.

Our results from the last several years 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 it is being

indicated by our studies on the dissection and analysis of the stable resistance of the cultivar Oryzica Llanos 5.

As we can see from **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 three 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 2004 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 2005 under field conditions and greenhouse inoculations.

Table 1. Virulence Spectrum of Rice Blast Pathotypes Detected at the Santa Rosa Experiment Station in 2004.

Rice Line	Resistance Gene	Blast Isolate (Cultivar of Origin)							
		OLL5	OLL5	F 50	13432	13432	C 8	BZA	BZA
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	+++	+++	+++	+++	+++		+++	+++
K1	Pi-ta	+++	+++	+++	++	+++		++	+++
F 128-1	Pi-ta^2	+++	+++	+++		+++	+++		+++
Kanto 51	Pi-k				+++	+++			
Tsuyuake	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		+++	+++	+++	+++	+++		+++	+++

Rice Line	Resistance Gene	Blast Isolate (Cultivar of Origin)							
		OLL5	OLL5	F 50	13432	13432	C 8	BZA	BZA
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								

OLL5= Oryzica Llanos 5; F50= Fedearroz 50; 13432= CT 13432; C8= Cica 8; BZA= new commercial rice cultivar Bonanza.

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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 in the Rice Program Annual Report for 2004. 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 Reports of the Rice Project since 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 since year 2000. The most resistant lines over the last four 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)**. One line (CT 13937-16-1-M-M-2) exhibiting a stable blast resistance for all these years was derived from an interspecific cross with *Oryza rufipogon,* and two other lines which exhibit also a highly resistant reaction to grain discoloration come from the upland germplasm from Brazil (RIO PARAGUAY and TRES MARIAS). 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. These results indicate the importance of evaluating the potential donors of blast resistance for several semesters before including them 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 2005 for their inclusion in a nursery as potential donors of stable blast resistance, which will be multiplied and distributed to our partners in the Latin American region.

Table 1. Potential Progenitors for Stable Blast Resistance Exhibiting Blast Scores 1-3 in Santa Rosa Field Evaluations during Five Years, Santa Rosa 2000 - 2004.

Pedigree	Pedigree
1. FL 00518-1P-4-3P-M	10. FL00535-21P-4-3P-M
2. FL00518-14P-15-3P-M	11. FL00542-45P-8-2P-M
3. FL00518-16P-7-3P-M	12. FL00871-1P-3-1P-M
4. FL00518-23P-11-2P-M	13. CT11275-3-F4-8P-2
5. FL00447-35P-4-2P-M	14. CT11280-2-F4-12P-5
6. FL 00459-21P-2-2P-M	15. CT13937-16-1-M-M-2
7. FL 00459-21P-2-2P-M	16. RIO PARAGUAY
8. FL00530-7P-7-1P-M	17. TRES MARIAS
9. FL00530-7P-7-2P-M	

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.

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Acitivity 3. Identification of molecular markers associated with the durable blast resistance genes in the commercial rice cultivar Oryzica Llanos 5.

Abstract

The genetic basis of the high level of durable resistance to rice blast in the cultivar Oryzica Llanos 5 is being characterized in F7 Recombinant Inbred Lines (RILs) from a cross between the susceptible cultivar Fanny and O. Llanos 5. A linkage map was constructed using 250 molecular markers: SSR, RFLP and RGAs. Eleven loci, distributed on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 11, and 12, were associated with the resistance of the cultivar. As a whole, the observed durable resistance in Llanos 5 could be the result from a combination of quantitative and qualitative resistance genes.

Introduction

Blast resistance in the Colombian commercial rice varieties has been defeated in periods of 1-3 three years after cultivar release. However, the resistance of the cultivar Oryzica Llanos 5 has been durable and has remained stable under field conditions for more than 14 years. Genetic studies have indicated the presence of at least four major genes controlling the resistance to some blast isolates. Based on the presence of avirulence genes in our blast populations we have inferred that the cultivar O. Llanos 5 carries at least 8 major genes. Studies retrieving blast isolates from the immediate parents giving origin to this cultivar and characterizing their genetic structure and virulence composition suggest that the durable resistance is associated with the pyramiding of complementary resistance genes to the different lineages of the pathogen present in those parents. It is therefore important to understand the basis of the durable resistance of this rice cultivar in order to establish a breeding strategy based on the same principle. A study to identify and localize major and minor loci genes controlling the resistance in Oryzica Llanos 5 was initiated in year 2000 in collaboration with Kansas State University using a QTL (quantitative trait loci) detection approach with multiple rice blast isolates.

Materials and Methods: Recombinant inbred lines (RIL's) of the cross between the resistant indica cultivar Oryzica Llanos 5 and the japonica susceptible cultivar Fanny have been developed to a total of almost 1000 lines. An initial set of 120 lines was inoculated with different blast isolates representing the pathogen genetic lineages SRL-1 to SRL-6 from Colombia. Inoculations and evaluations were performed at the Rice Pathology greenhouse of CIAT according to the methodology described in other annual reports. Two evaluation methods, lesion type (LT) and disease leaf area (DLA) were used to score the blast resistance. One isolate named "killer", was recovered from O. Llanos 5 and observed to be highly aggressive and have a very broad virulence spectrum. This isolate has been used to detect minor resistance genes. DNA of each of the 120 lines was extracted at Kansas State University for molecular analysis and microsatellites were used as potential markers for the identification of the resistance genes present in Oryzica Llanos 5. Blast resistance genes to each genetic lineage of the pathogen are being identified based on the phenotypic reaction and located on the different chromosomes of the rice genome. The genetic linkage map constructed from the RIL mapping population contains 250 markers including simple sequence repeats, and RFLPs. The chromosomal locations of the markers were determined using the Mapmaker program Version 2.0. Both composite interval mapping (CIM) and multiple

interval mapping (MIM) techniques were used for QTL detection using QTL Cartographer package v2.0.

Results: QTLs associated with LT and DLA were detected for 7 of the 8 isolates on rice chromosomes 1,2,3,4,5,6,8,9, and 11. QTLs with the largest effects were on chromosomes 8, 6, and 11, which explained 28%, 72%, and 42% of the genetic variation in resistance to some isolates. Other QTL explained 2 to 16 % of the genetic variance. In some of the QTL locations there are blast resistance major genes that have been reported i.e. chromosome 4,6,5,8,9, and 11. However, some of the QTL have small effects, indicating the presence of minor genes. A number of these genes are located in areas previously identified to be associated with QTL with large effects. Chromosomes 1 and 8 were found to carry important resistance factors that are not associated with previously identified resistance genes.

Discussion: The durable broad-spectrum resistance in O. Llanos 5 is associated with multiple major genes that induce resistance to different blast isolates. All of the QTLs detected in this study for isolates other than killer were for lesion type blast resistance. LT is typically associated with QTLs with large effects (major, or "R" genes). In contrast all QTL identified by killer were for DLA. None of the major genes detected in Oryzica Llanos 5 are effective against the killer isolate, but O. Llanos 5 is still highly resistant. This resistance appears to be controlled by genes with small main effects. The killer isolate apparently allows these genes to be identified. As a whole, the observed durable resistance in O. Llanos 5 could be the result from a combination of quantitative and qualitative resistance genes.

Future Activities

Evaluate the reaction of more RIL's to the blast isolates already used in previous inoculations as well as new isolates exhibiting a compatible reaction with the cultivar O. Llanos 5. Continue analysis with microsatellite markers to identify and locate more blast resistance genes. Develop near isogenic lines with blast resistance genes present in Oryzica Llanos

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Activity 4. Identification of Resistant Lines to *Rhizoctonia solani* (Sheath Blight) and Development of an Evaluating Methodology.

Abstract

A suitable greenhouse screening method and evaluation scale has been developed for identification of tolerance to the sheath blight pathogen in rice. This method allows a better differentiation between tolerance and susceptibility. Tolerance to sheath blight present in the wild species *O. rufipogon* has been successfully transferred to the cultivated species. Unknown tolerance to sheath blight has also been identified in advance rice lines of *O. sativa* with the new screening and evaluation method, and both sources of tolerance can be used in our breeding program. Tolerance to sheath blight identified in the greenhouse has been corroborated under field conditions in several rice lines. Tolerance seems to be controlled by the action of several minor genes. Increasing resistance to sheath blight seems to be possible by crossing parents with high levels of tolerance.

Introduction

The filamentous basidiomycete *Thanatephorus cucumeris* (anamorph = *Rhizoctonia solani*) is the causal agent of sheath blight of rice. This disease has increased in economic importance in most Asian countries as well as in the USA in the last 10 years. The disease is also increasing in importance in most rice growing countries of tropical Latin America where the species *R. solani* AG-1 IA seems to be the most common while the species *R. oryza-sativae* seems to be the most common in the temperate areas of South America. The disease, which has increased in incidence and severity, is most frequently controlled with the use of fungicides, however this method of control has increased the production costs of many farmers. There are not well known sources of resistance to the pathogen. We initiated in year 2000 the evaluation in the greenhouse of different rice lines in order to identify potential sources of resistance to this pathogen. The evaluated germplasm includes Colombian commercial cultivars, wild rice species, Asian and USA reported sources of resistance, and advanced breeding lines of CIAT's and FLAR Rice Projects. The wild species *Oryza rufipogon* exhibited a resistant to intermediate reaction to more than 14 different isolates in trials conducted between 2000 and 2002, while the species *O. barthii* and *O. glaberrima* were susceptible to most isolates.

Materials and Methods: Several groups of rice lines including 523 advanced lines from the interspecific cross between *O. rufipogon* x Oryzica 3, advanced elite lines from FLAR, accessions from the rice germplasm bank, and a set of progenitors and populations developed by FEDEARROZ were evaluated in the greenhouse for their sheath blight tolerance in 2004. Tolerant lines were selected and their reaction corroborated in different and replicated trials. The best lines identified were planted in the field in collaboration with FEDEARROZ (Saldaña) for determining the correlation between the two screening sites. The genetics of sheath blight tolerance to determine the possible number of genes was evaluated in the F2 generation (70-80 plants) of six different crosses.

For greenhouse evaluations, each line is planted in three replications. Each replication consists of five plants sown in a pot. Disease development is favored by applying a high dose of nitrogen

divided in several applications. Inoculation is performed when the plants are 50 days old by placing at the base of the main stem of each plant a 5 mm plug of agar +mycelium of a *R. solani* isolate grown for 4-8 days on rice polish agar. Inoculated plants are incubated under high relative humidity in a growth chamber for 12-15 days. After this period of time, the pots are removed form the chamber and placed onto greenhouse benches for five days to reduce the stress conditions and allow a period of time for recovery and expression of potential tolerance. The pots and then moved back to the high humidity conditions for another period of 10 days before evaluating their reaction.

For evaluating the reaction to sheath blight, we are considering the percentage of plant area affected, by evaluating the main tiller of each plant. The reaction score of a line is considered as the average of the 15 plants evaluated in the three replications. Percentage of plant area affected is calculated by given the following maximum values to the different leaves and stem evaluated: First leaf (flag leaf): 30%; second leaf: 15%; third leaf: 15%; fourth leaf: 10%; fifth leaf: 10%; stem: 20%. Since disease development and severity observed depends on the effect of the environmental conditions in the greenhouse, tolerant and susceptible checks are always included in each replication and used for determining the level of tolerance of the lines being evaluated.

Results: The greenhouse screening methodology including the two periods of incubation in the growth chamber under high humidity with an intermediate period of plant recovery has been suitable to detect better differences in tolerance among the rice lines evaluated. Tolerance to sheath blight observed in the wild species *O. rufipogon* has been successfully transferred to the cultivar Oryzica 3. The tolerant reaction identified was corroborated in several replicated trials **(Table 1)** and the best lines have been planted for evaluation under field conditions. Tolerance to sheath blight has also been detected in different rice lines and populations including a set of progenitors being used by FEDEARROZ in its breeding program **(Table 2)**. This tolerance has also been confirmed in several replicated trials in the greenhouse and is being confirmed under field evaluations. The tolerant reaction of several rice lines from the interspecific cross between *O. rufipogon* x Oryzica 3 and rice lines from the germplasm bank observed under greenhouse conditions has been corroborated under field conditions in evaluations performed by FEDEARROZ at the Saldaña experiment station in 2003 and 2004. These lines could be used as potential donors of tolerance to this pathogen. Tolerance to sheath blight has also been identified in several advanced elite lines from FLAR **(Table 3)**. These lines are being used, as progenitors for other desired agronomic traits, or for potential release as commercial cultivars in different countries. The tolerance to sheath blight observed in these lines is a plus since they have never been selected for this trait.

Evaluation of the F2 generation in six different crosses do not suggest the presence of major dominant gene resistance as most F2 plants exhibited a susceptible or intermediate reaction to sheath blight. Tolerance seems to be controlled by the effect and accumulation of probably several minor genes. In all crosses, rice lines with better tolerance than the two parents were identified, suggesting the effect of transgressive segregation or accumulation of several genes. These lines were present in a group of 30-50% plant area affected. The highest number of susceptible F2 plants was observed in those crosses where the commercial cultivar Fedearroz 2000 was involved. However, tolerant lines better than the two parents were identified even in the cross between Fedearroz 2000 x Remadja. Although the cultivar Pankai exhibited a higher

tolerance to sheath blight than the cultivar Remadja, the tolerance of this last cultivar seems to be better inherited. Those crosses involving Remadja yielded more tolerant lines than those crosses with Pankai.

Table 1. Selection of the most tolerant lines to *Rhizoctonia solani* in the greenhouse evaluation of 523 advanced lines from the cross *Oryza rufipogon* x Oryzica 3.

Rice Line	Plant Area Affected %	Rice Line	Plant Area Affected %
CT14524-2-M-2-1	28.0	**Checks**	
CT14534-2-M-5-2	29.0	ORYZICA 3	58.0
CT14543-6-M-5-2	30.0	ORYZA RUFIPOGON	58.0
CT14543-10-M-3-1	25.0	PN 1	70.0
CT14544-1-M-4-3	20.0	ORYZICA 1	72.0
CT14544-12-M-4-3	28.0	FEDEARROZ 50	64.0
CT14547-6-M-4-2	27.0	COLOMBIA 1	89.0
CT14547-31-M-7-1	26.0	CT 6096-7-4-4-3-M	97.0
CT14548-34-M-2-2	28.0	PALMAR	41.0
CT14554-20-M-4-4	22.0	REMADJA	36.0
CT 14546-8-M-M-2-2-M	25.0	PANKAI	52.0
		AMAZONAS	41.0

Table 2. Selection of Tolerant Segregating Lines to *Rhizoctonia solani* in the Greenhouse Evaluation of 171 Crosses from FEDEARROZ.

Line Identification	Cross	Plant Area Affected %
FS1R007-2	Fs 261-8-1//Pankai/Fedearroz 50	19.0
FS1R010-9	Remadja / Fedearroz 50//Fedearroz 50	22.0
FS1R021-6	FOA-16-9/FL00809-26P-5-2-M	25.0
FS1R029-5	FL00809-26P-5-2-M/Fedearroz 50	25.0
FS1R029-6	FL00809-26P-5-2-M/Fedearroz 50	20.0
FS1R029-7	FL00809-26P-5-2-M/Fedearroz 50	14.0
FS1R029-13	FL00809-26P-5-2-M/Fedearroz 50	25.0
FS1R034-2	FSR1310-1-1-1/FL00984-10P-5-2P-M	25.0
FS1R034-6	FSR1310-1-1-1/FL00984-10P-5-2P-M	25.0
FS1R034-7	FSR1310-1-1-1/ FL00984-10P-5-2P-M	23.0
FS1R034-8	FSR1310-1-1-1/ FL00984-10P-5-2P-M	19.0
FS1R037-1	FSR 1185-1-1-1/ CT 13958-13-M-2-1-M-M	23.0
FS1R037-6	FSR 1185-1-1-1/ CT 13958-13-M-2-1-M-M	22.0
FS1R038-2	LV636-1-7-4-1/ FL00984-10P-5-1P-M	25.0
FS1R038-3	LV636-1-7-4-1/ FL00984-10P-5-1P-M	18.0
Checks		
LV636-1-7-4		22.0
FOA-16-9		27.0
ORYZICA 3		37.0
O .rufipogon		38.0
Fedearroz 50		41.0
PN-1		76.0
Colombia 1		64.0

Table 3. Elite Rice Lines from FLAR tolerant to *Rhizoctonia solani* in Greenhouse Evaluations in 2004.

Rice Line	Plant Area Affected %	Rice Line	Plant Area Affected %
FL03186-1P-7-3P-2P-M	30.3	**Checks**	
FL03197-22P-4-1P-2P-M	36.3	PALMAR	43.0
FL03186-1P-4-2P-1P-M	48.3	ORYZICA 3	48.3
FL03186-1P-4-3P-1P-M	46.7	REMADJA	50.7
FL03191-6P-9-2P-1P-M	48.7	*Oryza rufipogon*	53.0
FL03323-5P-21-1P-1P-M	44.8	PANKAI	58.3
FL03199-26P-3-1P-2P-M	50.0	ORYZICA 1	60.7
		COLOMBIA 1	66.3
		FEDEARROZ 50	67.0
		FEFFERSON	77.0
		FL03187-12P-5-2P-3P-M	87.3

Discussion: The new greenhouse screening method is suitable for selecting tolerant lines to sheath blight. It seems that the period of plant recovery after incubation for 15 consecutive days is allowing tolerant plants to express this reaction for a better differentiation with susceptibility. Modification in the evaluation scale is also allowing a better differentiation between tolerance and susceptibility as it considers the plant as a whole (plant area affected) and not the relative position reached by a single lesion (traditional scale). There is a better correlation between the percentage of plant area affected and the visual aspect of a diseased plant. The new method for screening and evaluation differentiates better between tolerance and susceptibility. This has permitted us to identify unknown tolerance present in the cultivated *O. sativa*. Genetic studies conducted in 2004 suggest that tolerance to sheath blight can still be increased by crossing tolerant lines. Our previous studies have suggested a possible isolate-cultivar interaction with the existence of races. We are reporting this year the characterization of the genetic structure of 140 isolates of the sheath blight pathogen from Colombia and have started pathogenicity tests of the same population.

Future Activities

Characterization of the genetic resistance present in the rice populations developed from the crosses between the wild species *Oryza rufipogon* and the commercial cultivars Oryzica 3 and BG 90-2 were initiated in 2003 and continued in 2004. More than 300 advanced lines have been evaluated for their sheath blight reaction and the resistance genes are expected to be located with the use of the microsatellite markers described in **Table 4**. Molecular markers associated with sheath blight resistance genes have been reported on different regions of Chromosomes 2, Chromosome 3, Chromosome 5, Chromosome 9, and Chromosome 11. Microsatellite markers saturating these regions have been identified and used in this study. Data is in the process of analysis and will be reported in 2005. These studies should allow us to develop a breeding strategy for incorporating tolerance to sheath blight in our breeding populations of the rice project.

Table 4. Rice Microsatellites used in the identification of Molecular Markers Associated with Tolerance to *Rhizoctonia solani* in advanced Populations of the crosses between *Oryza rufipogon* with the cultivars Oryzica 3 and BG 90-2.

Chromosome 1	**Chromosome 5**	**Chromosome 9**
Rm 323 1.0cM	Rm 164 78.7cM	Rm 316 1.8cM
Rm 272 37.3cM	Rm 146 78.7cM	Rm 285 1.8cM
Rm 24 78.4cM	Rm163 78.7cM	Rm 105 32.1cM
Rm 297 155.9cM	Rm 430 78.7cM	Rm 288 74.6cM
Rm 315 165.3cM	Rm 161 96.9 cM	Rm 278 77.5CcM
	Rm 173 99.8cM	
Chromosome 2	Rm 233B 110cM	**Chromosome 10**
Rm 154 4.8cM	Rm 87 129.2c	Rm 222 11.3cM
Rm 279 17.3cM		Rm 239 25.2cM
Rm 290 66.0cM	**Chromosome 6**	Rm 184 58.3cM
Rm 221 143.7cM	Rm 170 2.2cM	Rm 228 96.3cM
Rm 318 150.8cM	Rm 217 26.2cM	
Rm 6 154.7cM	Rm 121 43.8cM	**Chromosome 11**
	Rm 340 133.5cM	Rm 202 54.0cM
Chromosome 3	Rm 345 145.2cM	Rm 287 68.6cM
Rm 232 76.7cM		Rm 209 73.9cM
Rm 251 79.1cM	**Chromosome 7**	Rm 21 85.7cM
Rm 227 143.2cM	Rm 125 24.8cM	Rm 206 102.9cM
Rm 135 157.3cM	Rm 180 30.1cM	Rm 254 110.0cM
Rm 186 168.2cM	Rm 214 34.7cM	Rm 224 120.1cM
Rm 143 207.3cM	Rm 11 47.0cM	
Rm 114 208.2cM	Rm 346 47.0cM	**Chromosome 12**
	Rm 346 47.0cM	Rm 4 5.2cM
Chromosome 4		Rm 19 20.9cM
Rm 307 0.0cM	**Chromosome 8**	Rm 247 32.3cM
Rm 564 73.1cM	Rm 152 9.4cM	Rm 309 74.5cM
Rm 273 94.4cM	Rm 339 72.2cM	Rm 17 109.1cM
Rm317 118.3cM	Rm 210 90.3cM	
Rm 131 148.8cM	Rm 256 101.5cM	

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Activity 5. Characterization of the Genetic Structure of the Fungus *Rhizoctonia solani* Causal Agent of the Sheath Blight Disease of Rice.

Introduction

Sheath blight, caused by the anastomosis group AG1-IA of the complex *Rhizoctonia solani* is the second most important rice disease in the world. The increase in incidence of the disease is associated with modern techniques of exploitation of the crop that favor the pathogen such as planting high yielding semi-dwarf cultivars, use of high nitrogen applications, rotation with soybean, and high seeding rates among others (Marchetti, 1983). In Colombia, the disease became important since 1990 causing yield reductions up to 50% in the cultivar Oryzica 1 (Correa-Victroia, 1992). The genus *Rhizoctonia* has a wide number of species with characteristics of growth and plant symptoms similar to sheath blight, making it difficult any taxonomic classification, field identification, epidemiological studies, and management of the disease complex. This project aims at studying the genetic structure of the sheath blight pathogen in Colombia by using several molecular markers allowing establishing species, anastomosis groups and genetic diversity within pathogen populations. We expect to get a better understanding of this host-pathogen interaction to guide our work for developing resistant cultivars and appropriate disease management strategies.

Materials and Methods: A total of 140 isolates were studied including 130 isolates from the rice pathology CIAT's collection, two control isolates of *R. oryzae-sativae*, two *R. solani* AG1-IA from dry beans, and six isolates morphologically classified as *Sclerotium* spp. The isolates were collected from 24 rice cultivars representing 23 localities in the Departments of Tolima, Huila, Valle, Casanare, and Meta. The isolates were obtained from infected leaves, stems, or from soil (four isolates) and two isolates from the Brachiaria and pasto estrella pastures were included. The isolate collection extends from 1987 to 2004.

Each isolate was grown on rice polish agar for 24 hours and sub-cultivated from hyphal tips developing monothalic cultures equivalent to monosporic pure isolates. ADN was extracted from each isolate following the protocols of the CIAT's rice pathology lab (Escobar and Correa). Each DNA sample was amplified using the primer combination ITS 3/ ITS 4 of the Internal Transcribed Spacer regions of the fungus rDNA ribosomal genes (Liu and Sinclair, 1993; White *et al,* 1990), running the amplified fragments in agarose gels and staining with ethidium bromide. Amplified products were also digested with the restriction enzymes MBOI, HinfI, EcoRI, HaeIII and Taq I (15 enzyme units per 10ul of PCR product) and a total of 42 bans were determined. Similarity analysis (Dice index) and multiple correspondence analyses were performed for each individual enzyme and on the total PCR plus the five restriction enzyme data.

Five primer combinations with reported specificity for distinguishing the species *R solani*, *R. oryza-sativa, and R oryzae* (Johanson *et al*, 1998) were used for the identification or diagnostic of species. These primers were developed after secuencing the regions 5.8S of the rDNA ribosomal genes of each species. Genetic variability was preliminarily determined by using the RAPD primer 91300 (Lilja *et al*, 1997).

Results: Amplification with the primers ITS3/ITS4 with no digestion produced four fragments while digestion of the amplified products with the five different enzymes yielded a total of 38 bands. Similarity analysis of the no digested products revealed four genetic groups with one, three, 127 and nine isolates per group **(Table 1)**. Genetic group # 3 was the largest and included the *R. solani* isolates from beans and the *R. oryza-sativa* controls. Group one had only one isolate collected from Valle (Jamundí), which was classified with the species-specific primers as both *R. oryza-sativae and R. oryza.* Group three included three isolates that had some specific characteristics such as small and round sclerotia, black sclerotia collected from stems, or production of white mycelium on agar media. However, the three isolates were classified as *R. solani* by the species-specific primers. Pathogenicity and anastomosis tests will be conducted to determine their association with rice. Group four had nine isolates: six had morphological characteristics on agar media similar to *Sclerotium* with an intense black color. Three of them were not classified as any of the three species pathogenic on rice with the species-specific primers, two were classified as *R. solani*, and one classified as both *R. solani/R. oryza-sativae*; another isolate recovered from a soil sample was classified as *R. solani*, and the last two isolates classified as probably *R. oryzae* with also a weak band for *R. solani* and collected from the cultivar Oryzica 3 although there was no record if from leaves or from stems.

Table 1. Number of Genetic Groups in the Analyses of Sheath Blight Pathogen Populations using the Interspace ITS 3-ITS 4 Regions of the Ribosomal Genes Amplified and Digested with Five Enzymes.

Marker / Digestion	PCR ITS3-ITS4	Digestion MBOI	Digestion HinfI	Digestion EcoRI	Digestion HaeIII	Digestion Taq I	Digestion 5 Enzimas	PCR + Digestion 5 Enzymes
Groups	**4**	**7**	**8**	**5**	**6**	**5**	**14**	**14**
Number of	1	1	1	1	1	1	4	4
Isolates	3	32	2	32	31	1	2	2
Per	127	7	7		2	2	79	79
Genetic	9	1	87	3	1	127	9	9
Group		89	9	95	7	9	1	1
		8	1	9	4		1	1
		2	2		94		30	30
			31				1	1
							1	1
							1	1
							1	1
							1	1
							7	7
							2	2

Digestions of the PCR products with the five different enzymes yielded different number of genetic groups **(Table 1)**. Analysis of the data pooling results for the five restriction enzymes or adding the PCR products without digestions data to the digested data formed fourteen genetic groups **(Table 1)**. Isolates within groups 1, 2, and 4 of the PCR products without digestion were further separated into six genetic groups (Groups 6, 10, 11, 12, 13, 14, **Table 2**). Isolates classified as different to *R. solani* were left in different groups (group 6 and group 14); isolates named as Sclerotium were within group 13, which also included a soil isolate. The largest group 3 with 127 isolates was divided in 11 genetic groups. Some of the groups were formed by single isolates, which in general had some specific characteristics such as a specific site of origin,

collected from soil or stems, or originated from sclerotia. In general, the largest group 3 (after digestion) obtained after pooling all the data included isolates obtained from infected leaves and which were classified as *R solani* using specific primers **(Table 3)**. The second largest group 7 included many isolates classified as no *R. solani*, which originated mainly from infected stems of the cultivar Fedearroz 50 or from sclerotia **(Table 3)**. This group included two control isolates of *R. oryzae-sativa* obtained from Uruguay. Interestingly, these two isolates were not classified within any of the expected three species *R. oryzae, R. oryza-sativae, or R. oryza* by the species-specific primers. The two control isolates obtained from dry beans were classified within group 1 together with two isolates obtained from sclerotia or from the soil. The two bean isolates AG1-IA were classified as *R. solani* by the specific primers. Preliminary observations indicate that a single plant can be infected by more than one genetic group of the pathogen, however, it is possible that only one group, and most probably group 3 containing *R. solani* pathogenic rice is economically the most important. Studies using RAPDs also exhibited between 1 to 3 different haplotypes within a single genetic group **(Table 4)**. These variants will be evaluated in terms of their pathogenicity and aggressiveness in inoculation studies.

Table 2. Relationship between Genetic Groups Determined based on the Amplification and Digestion of the ITS 3/ITS 4 region of the Ribosomal Genes of the Rice Sheath Blight Pathogen.

Isolates	PCR No digest. 4 groups	Dx1 MBOI 7 groups	Dx 2 HinfI 8 groups	Dx 3 EcoRI 5 groups	Dx 4 Hae III 6 groups	Dx 5 Taq I 5 groups	Dx´s 1 to 5 14 groups	PCR + Dx´s 14 groups
VJD03A_2768_F2000	g1 (1)	g2 (1)	G1 (1)	g1 (1)	g1 (1)	g4 (1)	g6 (1)	g6 (1)
TCP88A-2063_01C	g2 (3)	g 4 (1)	g6 (1)	g4 (1)	g2 (1)	g3 (1)	g7 (1)	g10 (1)
XXX01B_2766_QR6		g 4 (1)	g6 (1)	g4 (1)	g2 (1)	g3 (1)	g8 (1)	g11 (1)
TSD04A-2854-TAI4		g 7 (1)	g 5 (1)	g4 (1)	g4 (1)	g5 (1)	g12 (1)	g12 (1)
VLCO2B_2756-01L	g3 (127)						g1 (1)	g1 (1)
Tpf00A_2793_F50							g1 (1)	g1 (1)
96B_2835_RhAG1-I-Col							g1 (1)	g1 (1)
M43 bean							g1 (1)	g1 (1)
96B_2836_RhAG1-I-C								
M43 bean								
VJD88A_1929-01M							g2 (1)	g2 (1)
VJD88A_1929-01M							g2 (1)	g2 (1)
XXX01A_2705_M F.VICT.1							g5 (1)	g5 (1)
TSD00A_2785-F50							g10 (1)	g8 (1)
TSD00A_2775-F50							g11 (1)	g9 (1)

Pathogenicity tests will be conducted by inoculating several rice cultivars with several isolates representing each one of the fourteen genetic groups and the different genetic variants (haplotypes) within a genetic group determined by RAPDs. These studies will let us know in more detail the symptomatology caused by them and their association with any part plant (leaves, stem). We will determine if the sheath blight disease observed in the fields could be a complex of several pathogens represented in the different genetic groups found, which could

infect simultaneously different plant parts. These isolates will also be grown under different artificial media to determine their phenotypic and morphological characteristics in terms of mycelia growth and sclerotia developed. We will also determine if there is any intereaction isolate-cultivar or differences in aggressiveness in different rice cultivars, which would have important implications in our breeding program for developing rice cultivars with tolerance to the sheath blight pathogen. It will be necessary to determine the best sample collection representing a wide genetic diversity of this pathosystem to conduct more detailed studies. These collections should include mainly different regions, different cultivars, different plant parts, different rice production systems (irrigated/upland), and different structures (sclerotia's size, color, shape) of the pathogen developed on the infected tissue. These studies should also include morphological and cytological observations as well as determination of the anastomosis groups and a detailed observation of the symptoms developed.

Table 3. Genetic Group and Species of some Isolates Associated with Rice Sheath Blight Symptoms.

Isolates	PCR 4 groups (No. Isolates)	Species (Johanson Markers)	Global: PCR + Digestions 14 Groups
R. oryza-.sativae			
2820 M	3 (127)	-	7
2821 M	3 (127)	-	7
Rhizoctonia Control causing sheath blight			
2399-1r	3	*R. solani*	3
2399-copy			
Oryzica Llanos 5-1			
2369-1r R. s.	3	-	3
Fedearroz 50			
2599	3	R. s.	3
2679-01	3	R. s.	3
2695	3	R. s.	3
2695-01	3	R. s.	3
2695-02	3	R. s.	3
2738-01	3	R. s.	3
2770 r3 new	3	R. s.	3
2826	3	R. s	3
2832 f	3	R. s.	3
2777	3	No R. s.	7
2778	3	R. s	7
2780 m	3	No R. s	7
2791	3	No R. s ¿?	7
2794	3	No R. s	7
2796	3	No R. s	7
2798	3	No R. s	7
2800	3	No R. s	7
2805	3	No R. s	7
2806	3	No R. s	7

Isolates	PCR 4 groups (No. Isolates)	Species (Johanson Markers)	Global: PCR + Digestions 14 Groups
2810	3	No R. s	7
2823 M	3	No R. s	7
2824	3	No R. s ¿?	7
2825	3	No R. s	7
2785	3	No R. s ¿?	8
2793	3	R. s.	1
2775	3	R. s	9
Fedearroz 2000			
2704	3	*R.s.*	3
2768	1	*R.o.s./R.o.*	6
2769	3	*R.s.*	3
Rhizoctonia Frijol			
XXX96B_2835_RhAG1-I-Col M43 frijol	3	*R.s.*	1
XXX96B_2836_RhAG1-I-C M43 frijol	3	*R.s.*	1
Rhizoctonia (Brachiaria)			
2744-01r	3	*R.s.*	3
Rhizoctonia (Pasto Estrella)			
2745	3	*R.s.*	3
Rhizoctonia (soil)			
2390	3	*R.s.*	3
2828 Saldaña	3	*No R.s.*	7
2830 campo alegre	3		7
NR1 (MER)	4		13
Sclerotium	4		13
2813		*R.s.*	
2814		*No R.s.*	
2816		*R.s.*	
2817		*No R.s.*	
2818		*R.s./R.o.s.*	
2819		*No R.s.*	

Table 4. Genetic variation (RAPD Patterns) within ITS Genetic Groups of some Isolates Associated to Sheath Blight Symptoms on Rice.

Isolate	Isolates per Plant	No. ITS Groups	RAPD Pattern No.	Cultivar	Date Collection	Site Collection	Zone
1700	2	1	2	Oryzica 3	88[a]	Jamundí	Valle
1929	2	1	1	Oryzica 3	88[a]	Jamundí	Valle
1953	8	1	2	Oryzica 1	88A	Saldaña	Tolima
1954	7	1	1	Cica 4	88[a]	Saldaña	Tolima
1957	3	1	1	F3	87B	Santa Rosa	Meta
1959	7	2	3	Esparcidor	87B	Santa Rosa	Meta
2052	2	1	2	-	88A	Tolima	Tolima
2054	2	1	2	-	88A	Salda	Tolima
2059	2	1	1	-	88A	Tolima	Tolima
2062-1r	5	1	1	Cica 4 ?	88A	Ambalema	Tolima
2237	2	1	1	-	89A	Agua Chica	Cesar
2399	2	1	1	Oryzica 1	93A	Tolima	Tolima

The correspondence multiple analyses (CMA) classified the pathogen population in five genetic groups **(Table 5)**, which in general coincides with the classification obtained in the similarity analyses. Group1 with 68% of the isolates (95) included most of the isolates classified as *R. solani.* This group includes the two isolates from pastures, which had also been included in the genetic group 3 in the similarity analyses and which were classified as *R. solani*. These isolates will probably be pathogenic on rice determining two potential hosts of the pathogen. The bean isolates were also included in this genetic group, however they appeared in a different group according to their ITS regions. Group two included the 9 isolates from groups 13 and 14 of the diversity analyses being equivalent to group 4 of the non-digested PCR products. Group 3 included 32 isolates, which are mainly non-*Rhizoctonia* according to the specific primers used. Once again, the two control isolates obtained as *R. oryzae-sativae* were grouped together with the non-*Rhizoctonia* isolates in this group 3. Groups 1 and 3 of the CMA have a similar structure to groups 3 and 7 of the diversity analysis, respectively.

Group 4 included three isolates (2063, 2766, 2768-01), which have specific characteristics (black sclerotia isolated from the sheath, small-round sclerotia, and Jamundí origin, respectively). The isolate 2768 was classified as *R. oryza/R. oryza-sativae* while the other two as *R. solani.* Group 5 included only the isolate 2854, which produces a white mycelium on artificial media and no-sclerotia. This isolate was collected from the cultivar Tailandia 4 in Saldaña-Tolima in 2004, however was classified as *R. solani* by the species-specific primers.

We used reported species-specific primers (Johansen and Tuerner, 1998) to diagnose the presence of the species *R. solani, R. oryza-sativae, and R. oryza.* In many cases, *R, solani* isolates were positively identified, however, there were several pathogenic isolates that were not identified with these markers, and in other cases, the isolates were classified in more than one

species **(Table 6)**. We need to elucidate if this is a problem with the specificity of the markers or to the presence of a different pathogen. Parmeter (1967) reports that from lesions similar to sheath blight it is possible to obtain not only different strains of basydiomycetes, ascomycetes, and deuteromycetes, but also different strains of *R.* solani. Turner *et al* (1999) using these species-specific primers detected the presence of at least two of the three species in a single field or in a single plant in India. Pathogenicity tests will also help to understand this pathogen-host interaction. There were clear associations however in the grouping of isolates both in the diversity analysis as well as the CMA with the classification of isolates as *R.* solani or as no *R. solani.* These results suggest that *R.* solani is more important than the other two species in the Rhizoctonia complex in Colombia. We will repeat the test with more isolates to determine the specificity and the real potential use of these markers in the identification of species. Isolates should be collected in similar numbers from different parts of the plant as in general, isolates collected from infected leaves correspond to *R. solani.*

Table 5. Genetic Groups of Fungal Isolates Associated with Sheath Blight Symptoms on Rice based on Multiple Correspondence Analysis (MCA).

MCA Groups	Isolates Number	%
1	95	67.86
2	9	6.43
3	32	22.86
4	3	2.14
5	1	0.71

Table 6. Rice Isolates Associated with Sheath Blight Symptoms Classified as more than one Species based on Species-Specific Markers developed by Johansen and Turner (1998).

Isolate	Host or Classification	Species		
		R. solani	***R. oryzae sativae***	***R. oryzae***
2818	Sclerotium	+	+	-
1959-3	Esparcidor	+	-	+
2064-5	Oryzica 1	+	-	+
2752-1	None	+	-	+
2768	Fedearroz 2.000	-	+	+

These studies will allow us to determine which species is the most important economically in rice farmers fields Colombia and if there are other rice pathogens causing similar symptoms to sheath blight on the plant leaves or the lower part of the stems.

Discussion and Future Work: Establishing the genetic structure and genetic relations of the sheath pathogens of rice using the interspace ITS genetic regions of the ribosomal RNA genes together with other molecular markers is necessary to understand this host-pathogen interaction in order to be able to develop breeding strategies focused on the development of resistant cultivars and to develop an integrated management of the disease. These studies will be complemented with pathogenicity tests of the populations studied as well as morphological characteristics and determination of anastomosis groups on artificial media in the laboratory during 2005.

We should explore the possibility to develop more specific primers for the identification of all the species and forms present in Colombia and other countries in the regions as the results obtained in this study suggest that the markers reported in the literature may not be adequate enough for much diverse pathogen populations. These results also suggest the need to develop a regional project to study the sheath pathogens associated with the disease as we may be facing different pathogens/species increasing in importance in each country. Collection of isolates should include infected samples from different countries, sites, cultivars, plant parts, etc.

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