

# INDUCED MUTATIONS TO DEVELOP SOURCES OF RESISTANCE TO RICE BLAST, *Pyricularia Grisea* Sacc.

F.J. CORREA-VICTORIA  
International Center for Tropical Agriculture (CIAT),  
Cali, Colombia



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## Abstract

Rice blast caused by *Pyricularia grisea* is the most important disease limiting yields worldwide. The pathogen has many virulent forms or pathotypes, hence durable blast resistance is lacking. Studies on strategy to develop durable blast resistance based on defining the genetic structure of the population, using DNA-fingerprinting, and virulence diversity are described. This strategy is leading to the identification of resistance genes/sources against all isolates within a genetic family of the pathogen. Combinations of genes showing complementary resistance to different genetic families of the fungus exclude any compatible interaction with a blast isolate. Identification of complementary resistance genes is based on detecting those virulence factors whose combinations in individual isolates within the pathogen population have a frequency near zero. Identifying and combining resistance genes to which combinations of corresponding virulence genes are absent in the pathogen population should confer more durable resistance than that previously obtained. The use of induced mutations in the development of resistance was limited, since in most cases single gene changes were responsible for the induced resistance against all the pathogen population. The main objective here is to develop many mutants, each with a gene resistant to just one or a few families of the blast pathogen; and crossing them can accumulate the different resistance genes. A total of 201 Latin American commercial cultivars, including Cuban, Brazilian and Venezuelan were analyzed with different genetic families of the blast pathogen to identify potential sources of resistance to blast and identify complementary resistance sources. Characterization of the resistance of 37 mutants of the Colombian rice cultivar Oryzica 1 was conducted in collaboration with the INEA in Colombia. Results suggested that mutations for resistance to genetic families to which Oryzica 1 is susceptible were induced, although one isolate in the most common genetic family (SRL-6) of the pathogen was compatible with all mutants. These results suggest that a larger population of mutants is needed to increase the probability of identifying a gene resistant to all members of this family; and that mutations in a variety, initially resistant to this genetic family (SRL-6) but susceptible to a different family, could have better probability of success. Seven additional commercial rice cultivars from Colombia were irradiated and selection of mutants, differing in their resistance to the different genetic families of the pathogen has been conducted during 1997 and 1998. Several different resistant mutants have been selected and will be used in crosses to combine the various resistance genes.

## 1. INTRODUCTION

Rice blast (*P. grisea* Sacc., the anamorph of *Maganaporthe grisea* (Hebert) Barr), is a major factor limiting yields of rice (*Oryza sativa* L.) worldwide, particularly under rainfed and upland conditions prevalent in Latin America. The pathogen produces necrotic lesions on the seedlings' leaves and on the leaves, nodes, necks and panicles of mature plants, causing severe yield losses. Development of resistant cultivars is the preferred means of controlling the disease, as fungicides are expensive and not environmentally friendly. However, resistance is overcome by the pathogen shortly after the resistant cultivars' release. Developing durably resistant cultivars is then a high priority at CIAT and where blast occurs.

The rice blast pathogen reproduces asexually and is noted for the large number of pathogenic races, this variability being cited as the main cause of the breakdowns in resistance. Nevertheless, it has been proposed that continuous challenge to resistant rice

breeding lines by a diverse pathogen population and detailed genetic information on the population structure to understand the virulence dynamics of the blast pathogen would reduce the risk of resistance breakdown and allow identification of more stable resistance.

In order to develop more sustainable production systems in agriculture through the development of more durable resistance to diseases, CIAT with funding from the Rockefeller Foundation, the Colombian Government, and in close collaboration with the IAEA, has been developing strategies directed to the protection of the rice crop. This is being achieved by understanding of the genetic structure of blast populations in Colombia, and thus developing a model that can be applied in all Latin American countries. Such studies are allowing understanding the pathogen changes and mechanisms leading to the breakdown of resistance. Finally, the resistance genes selected for developing a more durable blast resistance will depend on the genetic diversity and virulence composition of a pathogen population. This paper introduces the potential application of induced mutations in developing sources of resistance leading to a more durable blast resistance.

## 2. GENETIC AND VIRULENCE STRUCTURE OF THE RICE BLAST PATHOGEN IN COLOMBIA

The sustainability of agricultural production systems constantly threatened by the development of epidemics caused by different plant pathogens, can be maintained by developing breeding strategies that facilitate more durable and stable forms of resistance to these pathogens. Accomplishment of this objective is possible by understanding the genetic and virulence diversity of the pathogen. Controlled inoculations of the blast pathogen collected from different sources in Colombia have yielded more than 50 international races. Virulence factors compatible with at least 13 known resistance genes were detected in this population, with no cultivar susceptible to all isolates. Accumulation of a large number of virulence factors to most resistance genes in an individual isolate is common, making it more difficult to identify potential sources of resistance to the fungus. Virulence diversity studies suggest that new resistance genes, or combinations of resistance genes, that can be overcome only by part of the pathogen population, are needed to control rice blast. Combinations of resistance genes could be generated from crosses between complementary groups for which the pathogen does not accumulate the corresponding virulence genes. Long term observation of virulence/avirulence frequencies and accumulation of virulence factors would be needed to monitor this strategy. Absence of certain virulence combinations could be due to deleterious effect of such combinations on the pathogen. In other words, as virulence is expressed after the loss of an avirulence gene, there are apparently certain avirulence gene combinations that if lost are detrimental to the fungus. One of these effects could be a reduced fitness in the population.

A repeated DNA probe (MGR 586) obtained from a rice blast fungus genome was found to be useful for grouping blast populations in genetic lineages, each with a particular spectrum of virulence characteristics. The complex virulence structure in the blast fungus pathotypes found in Colombia was classified into six distinct genetic lineages (SRL1 to SRL-6) using the DNA-MGR 586 probe. The average similarity among all the lineages was 49%, while isolates within each lineage expressed very similar fingerprints, with similarities ranging from 92 to 98%. In general each genetic lineage was composed of several pathogenic races; one race could be present in different genetic lineages, and one lineage could be recovered from different cultivars. However, if *P. grisea* populations are generally composed of a limited number of lineages and if there is a close relationship between the lineage and virulence characteristics of the constituent individuals, population analysis at the lineage level

could aid in directing resistance breeding projects that target the pathogen population in question.

Characterization of the genetic lineage structure, together with the virulence spectrum and the virulence frequencies within the whole blast pathogen population, should provide a more reliable estimate of the durability of cultivar resistance than only consideration of virulence or lineage alone. Results indicate that a given resistance gene may be overcome by isolates from different genetic lineages. On the other hand, several virulence factors are shared among isolates within the same genetic lineage and may be accumulated in a single blast isolate, or are present in individual pathotypes that are a subset of virulence, corresponding to the full virulence spectrum of that lineage. Certain specific virulence-lineage-resistance gene interactions are observed where a resistance gene seems to be effective against all the individuals of entire lineages yet is susceptible to most individuals of other lineages. Although genetic lineage SRL-6 in Colombia exhibits a wide spectrum and accumulation of virulence genes to most commercial cultivars released in the past, a high specific interaction between certain rice cultivars and genetic lineages has been observed. Some commercial cultivars are resistant to all the isolates tested from some lineages, yet, they exhibit a susceptible reaction in the field. Greenhouse studies have demonstrated that in some cases such susceptibility is only to isolates from one genetic lineage, and therefore the cultivar carries resistance genes to other families of the pathogen. Durable blast resistance such as the one exhibited by the commercial cultivar Oryzica Llanos 5 in Colombia is apparently controlled by the combination of complementary resistance genes derived from parents which are susceptible to different genetic lineages of the blast pathogen. Combinations of genes showing complementary resistance to different genetic families of the fungus should exclude any compatible interaction with any blast isolate in any lineage.

The resistance in Oryzica Llanos 5 did not emanate from just one of its ancestors, since all were susceptible in the field and to at least some of the isolates to which this cultivar is resistant. None of the genetic lineages of the blast pathogen in Colombia exhibit compatibility with all the parental lines. Instead, complementary resistance to all the lineages is observed among the different parents. Thus, it seems that development of stable resistance is possible by combining different sources that exhibit susceptibility to some segment of the blast population. Useful resistance genes would be those for which combinations of the matching virulence factors are absent in the pathogen population. Therefore, identification of these potential gene combinations requires continuous characterization of the genetic structure and monitoring of the virulence frequencies of the blast pathogen as well as germplasm screening for the identification of complementary resistance genes.

### 3. POTENTIAL APPLICATION OF INDUCED MUTATIONS IN DEVELOPING DURABLE BLAST RESISTANCE

Although this technique has been successfully implemented in many cases in the identification of resistance to diseases, rapid breakdown of mutation-induced resistance has also been reported very often. One of the reasons for the failure of resistance obtained through mutation is that in most cases, a single gene change (monogenic resistance) is responsible for the induced resistance against all the pathogen population. In this research agreement we have considered the alternative of developing mutants which are resistant only to a few genetic lineages of the pathogen. The idea is to identify as many mutants as are needed so that targeted crosses between these mutants will facilitate combining different resistance genes to exclude all the genetic lineages of the blast pathogen. We believe that the possibility offered pathogen is a worthy alternative for developing a more stable resistance in rice.

We evaluated at CIAT the reaction to rice blast of 37 mutants of the commercial cultivar Oryzica 1 obtained by gamma ray irradiation of the seeds at the INEA (Instituto de Ciencias Nucleares y Energias Alternativas) in Colombia. The results demonstrated that mutants resistant to the isolates to which the non-irradiated cultivar was susceptible could be obtained. However, we detected in the most predominant genetic family of the pathogen (SRL-6) one isolate compatible with all the mutants. The results suggested that evaluating a larger population of mutants was needed to increase the probabilities of identifying a resistance gene to all members of this family. Results also suggested that mutations in a variety initially resistant to this genetic family (SRL-6) but susceptible to a different family could have better probabilities of success. Resistant mutants identified are being evaluated under field conditions for other traits and used in crosses with other rice cultivars.

A total of 201 commercial rice cultivars from Latin America were analyzed for their reaction to different genetic families of the blast pathogen to identify potential sources of resistance to blast and identify complementary resistance sources. Dr. Adolfo Alvarez irradiated seven of these rice commercial cultivars obtained from Colombia at the INEA institute with gamma rays (300 Gy). Selection of mutants differing in their resistance to different genetic lineages of the pathogen has been conducted during 1997 and 1998. Three of the cultivars, Oryzica Caribe 8 (susceptible only to lineage SRL-4), Cica 8 (susceptible only to lineage SRL-5), and Oryzica Llanos 5 (partially compatible with lineage SRL-4), exhibit resistance to genetic lineage SRL-6. Several mutants exhibiting resistance to every one of those lineages have been selected and will be used in crosses to generate segregating populations for selecting lines combining the corresponding complementary resistance genes. Selection within a larger sample of mutants in the cultivar Oryzica 1 yielded several mutants resistant to lineage SRL-6. The total number of resistant mutants selected were: 26 of Oryzica 1 with resistance to lineage SRL-6; 9 mutants from cultivar Oryzica Caribe 8 with resistance to lineage SRL-4; and 2 mutants from cultivar Cica 8 with resistance to lineage SRL-5.

The future objectives of this project are: 1) Corroborate resistance to blast in M<sub>4</sub>, M<sub>5</sub>, and M<sub>6</sub> lines of each commercial cultivar (Cica 8, Oryzica Caribe 8, Oryzica 1, and Oryzica Llanos 5). 2) Design crosses (simple, double, triple) among selected resistant mutants to combine complementary resistance genes excluding all pathogen lineage/virulence. 3) Plant segregating lines under field conditions for evaluation and selection of blast resistant lines. 4) Conduct genetic studies of resistance in selected mutants exhibiting blast resistance.

## REFERENCES

- [1] CORREA-VICTORIA, F.J. Induced mutations for developing sources of resistance to rice blast *Pyricularia grisea*. 2<sup>nd</sup> FAO/IAEA Research Coordination Meeting on Induced Mutations in Connection with Biotechnology for Crop Improvement in Latin America. Buenos Aires, Argentina, 21-25 October 1996.
- [2] CORREA-VICTORIA, F.J., MARTINEZ, C. Genetic structure and virulence diversity of *Pyricularia grisea* in breeding for rice blast resistance, In: Induced Mutations and Molecular Techniques for Crop Improvement, IAEA, Vienna (1995) pp. 133-145.
- [3] CORREA-VICTORIA, F.J., ZEIGLER, R.S. Pathogenic variability in *Pyricularia grisea* at a rice blast "hot spot" site. *Plant Dis.* **77** (1993) 1029-1034.
- [4] LEVY, M., CORREA-VICTORIA, F.J., ZEIGLER, R.S., XU, S., HAMER, J.E. Genetic diversity of the rice blast fungus in a disease nursery in Colombia. *Phytopath.* **83** (1993) 1427-1433.