Strategies and methodologies for improving blast resistance in rice in the U.S.

Marchetti M.A.

Strategies and methodologies for improving blast resistance in rice in the U.S.

M.A. Marchetti
USDA-ARS, Texas Agricultural Experiment Station, Beaumont (USA)

Abstract. The discovery of pathogenic specialization in the blast fungus, *Pyricularia grisea*, led to intensified interest in this disease in the U.S. in the early 1960s. The traditionally close working relationship among rice breeders and pathologists in the southern U.S. provided an excellent environment for characterizing U.S. pathotypes, determining inheritance of resistance and identifying sources of race-specific resistance.

Consequently, a fairly broad knowledge base was developed rapidly, perhaps disproportionately broad given the relatively small acreage of rice in the U.S. (currently around 1.5 million ha) and the sporadic importance of the disease at that time.

Strategies for using this information evolved slowly. First of all, the main objectives of U.S. varietal improvement were to increase yield potential and maintain or improve the milling and cooking quality for which U.S. rices were noted. Therefore, disease resistance was a distant third in importance. Secondly, we were slow to recognize the concept and importance of partial resistance in blast management, at least partly because simply inherited race-specific resistance seemed to provide adequate control of blast. In time, we recognized that many U.S. cultivars possessed levels of partial or field resistance effective in our environment. In fact, no U.S. cultivar was ever completely resistant to all known U.S. pathotypes even before its release.

Consequently, there were subtle changes in selection philosophy, e.g. eliminate the worst materials rather than save only the most resistant. As other researchers have pointed out, by saving only the most resistant, one may eliminate important sources of partial resistance, whose absence or presence would be masked by race-specific resistance in the absence of compatible (virulent) pathotypes. In the U.S. program, most early-generation evaluation is conducted in specially constructed upland nurseries, in which breeding lines are challenged in polycyclic exposures to five broadly pathogenic pathotypes under conditions favorable to disease development. A level of acceptable resistance can be set based on performance relative to that of acceptable commercial cultivars. We are refining our evaluation further by comparing resistances under both upland and flooded conditions, since all U.S. commercial rice is irrigated. Rice under flooded culture is more resistant than upland rice, but rice lines vary in the degree of improved resistance. Race-specific resistance has not been abandoned as we seek to improve the more durable partial resistance. There is strong evidence that certain race-specific resistance (Pi-) genes provide some measure of residual resistance or are closely linked to genes which provide partial resistance to compatible pathotypes. The most advanced lines are screened with eight or more distinct pathotypes to characterize them as to the presence of certain Pi-genes. Pyramiding of Pi-genes is also an effective strategy. In dealing with this highly variable pathogen, we are utilizing both race-specific and general sources of resistance. Each appears to perform more effectively in the presence of the other.

The discovery of pathogenic specialization in the rice blast fungus, *Pyricularia grisea* (Cooke) Sacc. [teleomorph, *Magnaporthe grisea* (Hebert) Barr] (Latterell *et al.*, 1960) intensified interest in rice blast in the U.S. during the 1960s. Subsequently, a cooperative U.S.-Japan project led to the establishment of international differentials for identifying blast fungus races (Atkins *et al.*, 1967). Within a few years they were either supplemented with local varieties or abandoned as inadequate in separating pathotypes in most countries including Japan. They seemed to provide adequate differentiation among pathotypes in the U.S. until recently. Since 1953, some 25 pathotypes have been identified in the southern U.S., though rarely do more than three or four appear in any one year. Early inheritance studies revealed simply inherited race-specific resistance to blast in several U.S. cultivars (Atkins and Johnston, 1965). Kiyosawa in Japan (1967, 1974) identified other resistance (Pi-) genes in U.S. rice cultivars. This knowledge base provided a starting point for formulating a strategy to improve blast resistance in U.S. rices.

Much of the strategy and methodology discussed here is effective because of broad collaboration among the southern states with active rice-breeding programs: Arkansas, Louisiana, Mississippi, and Texas. (California, though a major rice-producing state, is free of blast and therefore not included in this discussion.) Each state has its own breeding program, independent of the others. Close cooperation exists bet-

ween breeders and pathologists, both among and within programs, as well as with researchers in other disciplines such as entomology, plant nutrition, and weed management. Regional yield trials provide opportunities to evaluate and compare germplasm and advanced lines from each program in all states. Breeding lines from all states are screened in disease nurseries in each state. These multiple-location tests give a more reliable indication of varietal adaptation and stability. Information is shared freely among the cooperators, and germplasm is exchanged among breeders. Cooperation among pathologists includes region-wide collections of diseased specimens and disease loss assessment, as well as sharing field observations and data from disease nurseries.

The strategy for improving blast resistance is simply stated, if more difficultly implemented. First of all, know the pathogen. Secondly, identify sources of resistance. Finally, incorporate the desired resistance into agronomically superior rice cultivars.

The pathogen. As mentioned previously, some 25 U.S. pathotypes of *P. grisea* have been identified since 1953. Pathotype prevalence is influenced strongly by rice varieties being grown. As new cultivars gain in hectares planted, pathotypes virulent on the new cultivars become more prevalent and the aviru-lent pathotypes disappear (Marchetti 1994). We maintain an active collection, dating back to 1959, of over 1000 isolates of *P. grisea*, each characterized on the international differentials in greenhouse inocu-lations. As new pathotypes appear, they are used to challenge breeding lines, cultivars, and exotic germ-plasm, not only to identify potential sources of resistance, but also to further characterize the isolates. The culture collection also has proved useful in comparisons of old and new isolates for benomyl fungici-de resistance, and in the first successful DNA fingerprinting of *P. grisea* pathotypes (Levy *et al.*, 1991).

It is important to determine which avirulence genes are present in the pathogen population. In our case, it is necessary to assess the population of the entire southern U.S. rice area, since many cultivars are grown in states other than the state of origin. Each year, with the assistance of county extension agents, farmers, and farm consultants, the rice pathologists collect blast specimens, with emphasis on fields of new cultivars and resistant cultivars. In this way, we continually monitor for shifts in prevalence and the emergence of new or rarely found pathotypes. We can also associate changes in pathotype population with cultural changes, most notably the widespread adoption of new rice cultivars. We believe this infor-mation is essential to the success of our blast resistance breeding program.

Presently we are including three U.S. cultivars, 'Katy', Lebonnet', and Mars', in each planting of interna-tional differentials. Because of several pathotypic anomalies in DNA fingerprint lineages (i.e., some pathotypes were being grouped in atypical lineages according to DNA fingerprints), we speculated that the international differentials were not always differentiating along relevant pathogenic lines. In fact, this was the case. For example, we identified both virulent and avirulent isolates of race IC-17 on the cultivar Lebonnet, and both avirulent and virulent isolates of IE-1 on Katy. These are significant discoveries in that they involve two important resistance genes, Pi-kh and Pi-ta2. Consequently, we have begun to develop a set of near-isogenic lines better suited to differentiate among U.S. pathotypes of *P. grisea*.

I – Sources of resistance

The main objectives of most rice breeding programs are to improve yield, milling, and cooking quality. Without adequacy in those traits, improved disease resistance is of little consequence. Therefore, domestic sources of resistance are sought first, which obviate the need to eliminate other undesirable agronomic traits such as photoperiod sensitivity, tallness, hairiness, leafiness, etc., which one is more likely to encounter in exotic germplasm. The existence of four independently operating breeding pro-grams in the U.S. provides a potentially rich source of disease resistance, in that rice breeders are intro-ducing different germplasm into their respective programs from different sources and for various reasons.

Breeders have been quick to take cidvantage of improved lines incorporating genes from exotic germ-plasm, in order to avoid having to repeat the "cleaning up" process. For example, the blast-resistant culti-var Katy was developed in Arkansas from a cross including the Indochinese variety Tetep', which contri-buted the gene Pi-ta2 to Katy (Moldenhauer *et al.*, 1990). Now Katy, rather than the agronomically unacceptable Tetep, is being used in all programs as the source of the Pi-ta2 gene in efforts to combine

Pi-ta2 with Pi-z and Pi-kh in more broadly resistant cultivars. Considerable effort also goes into developing "elite" germplasm, lines which are not commercially acceptable, but which combine several desirable traits into agronomically acceptable and adapted phenotypes.

We have not had to resort to interspecific crosses to improve blast resistance. As molecular biological techniques and genome mapping progress, it is almost certain that these technologies will play an ever increasing role in varietal improvement and make interspecific gene transfer routine and increasingly useful.

II – Incorporation of blast resistance into agronomically superior cultivars

The chances for success are enhanced greatly if the breeder has a clear idea of the objective(s) of a hybridization (in this case better blast resistance), knows what traits must be preserved (e.g. semidwarfism, grain shape, cooking quality traits), and then chooses the parents which give the best chance of combining desired traits. Obviously, the more desirable traits shared among parents, the fewer there will be to select for. One of the major contributions of the pathologist is to identify sources of resistance in local lines, whenever possible, to give the breeder better choices regarding the preservation of essential traits.

In most cases, breeders will have done some early generation culling of progeny based on visible traits such as height, maturity, hairiness, plant type and the like. Depending upon the cross itself and other objectives of the cross, lines may be evaluated for disease resistance as early as the F2, but usually in the F5 to F7 generation.

Breeding lines are evaluated in two ways: in greenhouse inoculations and in outdoor nurseries. The greenhouse tests are monocyclic: inoculate, incubate in a dew chamber for 16-20 hours, followed by 5-7 days in the greenhouse until symptoms develop. Then the plants are rated using a standard scale (IRRI, 1975) with susceptible cultivars as checks. The rice lines are challenged with many individual pathotypes to determine the absence of certain Pi-genes and the likely presence of others, based primarily on similarity to other lines whose Pi-genes are known, and on ancestry if known. Greenhouse tests are valuable for characterizing pathotype-specific resistance, which usually is of a hypersensitive type. Because of their monocyclic nature and highly variable temperature-light-moisture conditions, greenhouse tests are not reliable indicators of partial resistance.

Outdoor nurseries provide opportunities to observe disease development over time with polycyclic exposure of test lines to infection. These tests give a relative measure of field or partial resistance to leaf blast, assuming a compatible host-pathogen combination. In the absence of a virulent pathotype, there can be no disease development. At the beginning of the nursery season, some 20 greenhouse flats of susceptible rice seedlings are inoculated each with one of five test pathotypes in the greenhouse. After symptoms develop, the flats are transferred to the windward-most bed of the blast nursrey, which is planted to a universally susceptible cultivar such as Calrose' from California. This bed, planted two weeks before the first test material, is the primary source of inocula of the five test pathotypes. No attempt is made to exclude invasion of the nursery by wild strains, since it is neither desirable nor possible.

Nursery beds are one meter wide and 15.75 meters long. Test lines are planted in 60-cm rows, 2 g of seed per row, with 10 cm between rows. Every third row is planted to a spreader (e.g. Calrose), as are the six rows at either end of each bed. A single row of spreader is planted the length of the windward side of each bed. Usually a test takes 5-6 weeks from planting of test lines to rating, thereby allowing us to replant the beds for a second series of evaluations if needed.

The blast nurseries at Beaumont, Texas (there are two, each with 16 beds) are equipped with automatic misting systems, with clocks to control when the systems are active, and cycle timers to determine the duration of each misting and the interval between mistings. Usually they are set to mist for six seconds every six minutes from 1630 to 2000 hours and from 900 to 1000 hours. Without this supplementary leaf wetness, dew periods here are usually insufficient to allow epidemic development of leaf blast.

Serial ratings every 3-5 days over a two week or longer period provide data to plot disease progress curves. We have done this for detailed comparisons among limited numbers of lines (Marchetti 1983a, 1983b). But for rapid screening of large numbers of breeding lines, a single evaluation, recorded when the spreaders are killed by leaf blast, provides an effective comparison of field resistance among lines from the same cross and between experimental lines and commercial cultivar standards (Marchetti, 1994). At Beaumont, we screen up to 4000 advanced breeding lines annually from all four breeding programs. These data are computerized and forwarded to the respective breeders as quickly as possible. Timely blast nursery data often facilitate subsequent work in that field plots of unacceptable lines can be culled prior to harvest.

The breeder may choose not to advance any lines with a disease rating higher than that of some acceptable cultivar, or to advance only those with a rating of 4 or less (IRRI, 1975). Obviously, the criteria can vary according to the primary goal of the cross. For instance, if the cross was made for the purpose of improving processing quality, then a lower resistance may be accepted in order to increase the numbers from which to select for better processing quality. However, the breeder tries to maintain or improve disease resistance with every selection, since the new cultivar must be competitive with existing commercial cultivars.

We feel that we have a sound approach toward blast resistance breeding, but it is far from perfect. One of our weaknesses is the fact that our blast nurseries are grown as upland rice, but all U.S. commercial rice is grown in flood culture. In 1995 we modified several pairs of upland beds to permit flooding. In side-by-side upland and flooded beds, we demonstrated that the increase in leaf blast resistance caused by flooding varies markedly among cultivars. This disadvantage is offset by numerous advantages of screening for leaf blast resistance in upland beds, e.g., more flexible planting schedules, supplementary leaf wetting, fewer weather-related interruptions, easier access to electrical power, to name a few. Therefore, we will continue to use upland nurseries, being aware that sometimes results may lead to erroneous conclusions. In the meanwhile, we will work on methodology for efficient supplementary leaf-wetting under field conditions, which will also facilitate screening for resistance to panicle blast. Although we will not have the resources to screen as large numbers of lines in a flooded field nursery, screening of the most advanced 200 lines in duplicate is a realistic goal.

In closing, I wish to state that most of the strategies and procedures summarized here are not original. We have drawn heavily on the world literature and on the experiences and ideas of fellow researchers throughout the world. While the strategies will be slow to change, we are constantly in search of better methodology to improve efficiency and reliability. Most important to our success, I believe, has been the close collaboration, comradery and shared sense of purpose among the rice researchers in the southern U.S.

References

• **Atkins J.G., Johnston T.H.** (1965). Inheritance in rice of reactions to races 1 and 6 of *Piricularia oryzae*. *Phytopathology*. 55: 993-995.

• **Atkins J.G., Robert A.L., Adair C.R., Goto K., Kozaka T., Yanagida R., Yamada M., Matsumoto S.** (1967). An international set of varieties for differentiating races of *Piricularia oryzae*. *Phytopathology*, 57: 297-301.

• **IRRI** (1975). *Standard evaluation system for rice. International Rice Research Institute*. PO Box 933, Manila, Philippines, 64 p.

• **Kiyosawa S.** (1967). Inheritance of blast resistance in Zenith type rice varieties. *Ikushugaku Zasshi,* 17: 25-35.

• **—** (1974). Inheritance of blast resistance of a US rice variety, Dawn. *Ikushugaku Zasshi*, 24: 117-124.

• **Latterell F.M., Tullis E.C., Collier J.W.** (1960). Physiologic races of *Piricularia oryzae* Cav. *USDA Plant Disease Reporter*, 44: 679-683.

• **Marchetti M.A.** (1983a). Dilatory resistance to rice blast in USA rice. *Phytopathology*, 73: 645-649.

• **—** (1983b). Dilatory blast resistance in rice lines exotic to the southern United States. *Plant Disease*, 67: 1362-1364.

• **—** (1994). Race-specific and rate-reducing resistance to rice blast in US rice cultivars. In : *Rice Blast Disease*. RS Zeigler, SA leong, PS Teng, eds. CAB International, Wallingford, UK, in association with IRRI, pp. 231-244.

• **Moldenhauer K.A.K., Lee F.N., Norman R.J., Helms R.S., Wells B.R., Dilday R.H., Rohman P.L., Marchetti M.A.** (1990). Registration of Katy' rice. *Crop Science,* 30: 747-748.