Eduard C. Roumen

Partial resistance in rice to blast and how to select for it

Promotor : dr. ir. J.E. Parlevliet,
hoogleraar plantenveredeling

Eduard C. Roumen

Partial resistance in rice to blast and how to select for it

Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op maandag 19 april 1993
des namiddags te vier uur in de Aula
van de Landbouwuniversiteit te Wageningen.

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Roumen, Eduard C.

Partial resistance in rice to blast and how to select for it / Eduard C. Roumen. -[S.l.:s.n.]
Proefschrift Wageningen. -Met samenvatting in het Nederlands.
ISBN 90-5485-089-2
Trefw.: rijstbouw

The work reported in this publication resulted from a collaborative effort of the Plant Pathology Division of the International Rice Research Institute, Los Baños, the Philippines, the Dept. of Plant Breeding of the Wageningen Agricultural University, Wageningen, the Netherlands, and the section Research and Technology Transfer of the Netherlands' Ministry of International Development Cooperation (DGIS/DST), the Hague, the Netherlands. The research was partly financed by the Netherlands' Ministry of International Development Cooperation.

Printing of this thesis was partly financed by the Wageningen Agricultural University, and by the 'Landbouw Export Bureau' fund.

Responsibility for the contents and for the opinions expressed rests solely with the author; publication does not constitute an endorsement by the Netherlands' Minister for International Development Cooperation.

Stellingen behorend bij het proefschrift 'Partial resistance in rice to blast and how to select for it'

Partial resistance to blast in tropical lowland (Indica) rice genotypes is not fundamentally different from that in temperate (Japonica) or tropical upland (Javanica) rice genotypes.

Efficient screening and breeding for partial resistance to rice blast is not possible without a laboratory that can handle isolation, storage, and multiplication of *Magnaporthe grisea* isolates.

Projects aimed at developing breeding methodology for creating cultivars with durable resistance would benefit from collaboration with plant physiologists and/or molecular biologists.

Het verschil in betekenis tussen het Spaanse woord seguro (= zeker) en het daarvan afgeleide Pilipino woord siguro (= misschien) laat zien dat het geringe vertrouwen van de Filippijnse bevolking in de lokale machthebbers een lange geschiedenis heeft.

In de Filippijnen zal, bij ongewijzigd regeringsbeleid, een toename van het inkomen per hoofd van de bevolking eerst leiden tot een versnelde bevolkingsgroei en pas op de langere termijn tot een afname van die groei.

Uitzending van posts-docs i.p.v. universitair afgestudeerden binnen het zogn. research-BAD programma van DGIS (assistent-deskundigen die geplaatst worden op een van de CGIAR-insituten), zou beter aansluiten bij het beleid dat activiteiten in het kader van de ontwikkelingssamenwerking afgestemd moeten zijn op de behoeften en cultuur van de counterparts.

Het gedrag van weggebruikers in een land als Indonesie biedt geen ondersteuning aan de veel gehoorde bewering dat men zich in de westerse cultuur eerder bedient van een conflictmodel, terwijl men in de Zuid-Oost Aziatische cultuur een harmonie-model zou gebruiken.

De bewering dat de ontwikkeling en verpreiding van high-yielding-varieties (de zgn. groene revolutie) een gevolg is van problemen met de sustainability van (traditionele low external input) landbouwsystemen, is evengoed zo niet beter te verdedigen dan de bewering de groene revolutie de oorzaak van veel problemen met de sustainability van landbouwsystemen zou zijn.

Een van de belangrijkste knelpunten voor het ontwikkelen van rassen met duurzame resistentie (in tropische gewassen) binnen het door DGIS gefinancieerde programma, is het gebrek aan duurzaamheid van de afzonderlijke onderzoeksinspanningen op dat gebied.

Het gezegde "elk volk krijgt de leiders die het verdient", berust op een fictie.

Foreword

The present work was carried out at the International Rice Research Institute (IRRI) at los Baños, the Philippines, with inputs of the Netherlands Ministry of International Cooperation (DGIS) the Hague, the Netherlands, the Dept. of Plant Breeding, Wageningen Agricultural University, the Netherlands, and IRRI.

Finding my own way in these three organizations, each with its own, sometimes conflicting, objectives and each having a different organizational culture, was not so easy. Luckily, my Philippine colleagues in the Plant Pathology Division were usually able to help me out. Ellen Silab, Yollie Mendoza, Boyet Lazaro, Bennie Estrada, Joe Bandong, Fausto Nuque, Sonia Ebron, Tita Mew-Vergel Dios, Beth Guico, Zhe Flores, Mannie Tiongco, Yosie Mata, Nestor Fabellar, Rorie and Nong Calvero, Menchu Bernardo, Bennie Pangan, and many others, all contributed in one way or another to the realization of this thesis. I want to thank J.M. Bonman, under whose responsibility the work was carried out, for his trust, and for always having left me the freedom to carry out the research according to my own insights.

Special thanks are due to the "boys" of the greenhouse, who literally pour tons of sweat to the advance of rice research, but who usually don't get much credit in return. IRRI is lucky to have persons as Julio Banasihan, Ding Jordan, "mang Teme", Nido Angeles within their work force.

Walter de Boef, a student from Wageningen, took part in the work for six months, and will be able to recognize some of his work in chapter 3. During office hours he proved to be a serious worker, but this did not keep him from being one of the liveliest persons during evening hours.

I still much appreciate the hours of discussions with Henry Klein-Gebbink, my office mate during the last stretch, who also indulged in the joys and frustrations of working at IRRI on a difficult topic as resistance to blast. Many additional hours of fruitful discussions were spend with Ekkehart Kürschner and Michel Arraudeau. The enthousiasm of Rebecca Nelson was always very inspiring. A couple of times I was able to spend time working on other things than those presented in this thesis: Nathalie Boissot, Dr. Hisatochi Kaku, and Lammert Bastiaans, it was a pleasure working with you.

IRRI can stand for different names, but the International Rest and Recreation Institute isn't one of them. Luckily, the presence of many young, starting scientists from different countries saw to it that there were plenty of opportunities to relax and forget the IRRItations of daily life. Especially the creative energies of Caroline Begg and Marco Wopereis gave new meaning to the word party.

I am very much indebted to my promotor Jan Parlevliet. During the time that I was at IRRI, he somehow always came to visit when it was most needed, restoring confidence when spirits were low. Very valuable was also the moral support of Jean Loup Notteghem, who encouraged me to continue at a time that I was about to give up. Still, their support doesn't come close to that given by Joozee Schellekens. I am sure that I could not have done the same amount of work and conclude my assignment at IRRI if she had not been with me in the Philippines during the last year of my assignment. Finally, I like to thank Theo Jacobs for a year of good companionship during the time that I was writing this thesis at the Dept. of Plant Breeding of the Wageningen Agricultural University.

Contents

General introduction

Blast disease is one of the most widespread diseases of rice (*Oryza sativa* L.) and occurs in nearly all of the world's rice growing areas. The disease is caused by a haploid fungus belonging to the ascomycetes that is known under various names. The most commonly used names nowadays are *Magnaporthe grisea*, used to describe the teleomorph, and *Pyricularia oryzae* and *Pyricularia grisea*, used to describe the anamorph (Rossman et al., 1990). In older literature, the name for the anamorph is sometimes spelled as *Piricularia*, while the name *Ceratosphaeria grisea* has been used for the teleomorph (Ou, 1985).

Life cycle of the pathogen

The blast pathogen is heterothallic (Kato et al., 1976). The sexual stage of the fungus has not been encountered in nature. Laboratory studies indicate that sexual recombination among rice isolates is difficult because nearly all isolates show female sterility (Valent et al., 1986; Notteghem and Silue, 1992). However, the fungus easily propagates asexually through conidiospores. The life cycle of the fungus can start after conidia are deposited on a rice plant. All above ground plant parts can be infected, but the symptoms are most conspicuous when infection occurs in the leaf blades or the node just below the panicle. A certain minimum period of host surface wetness, such as a dew layer, is necessary for germination and a successful infection. This minimum period depends on the temperature, being between six to eight hours at a temperature of about 25 C, and longer for lower or higher temperatures (Barksdale and Jones, 1965; Kato, 1974). Each of the three cells in a conidium can form a germtube and, starting about six hours after germination, appressoria are formed at the end of germ tubes. The fungus penetrates the epidermal cell wall by forming a penetration hypha from underneath the appressorium. Within the host, hyphal growth is intracellular and the colony quickly enlarges once the hyphae grow beyond the initially infected epidermal cell into neighbouring cells. At 26-28 C, the first visible symptoms of infection in leaves are observed at four to five days after inoculation, but the incubation period is longer at lower temperatures (Hemmi et al., 1936, cited by Hashioka, 1965). In susceptible host genotypes, rapidly enlarging whitish or grey lesions emerge that often develop a brown margin in a later stage. When a brown margin develops the growth of the lesions is reduced or stopped. Sporulation occurs in the grey area of lesions, but only at a very high (>90%) relative air humidity (Hemmi and Imura, 1939; Heath et al., 1990). Conidia form on conidiophores which usually extrude through the stomata, but extrusion through the epidermal cell wall can also occur (Hashioka and Nakai, 1974). Depending on the temperature and the position of a lesion on the plant, lesions produce conidia for up to 20 days (Kato and Kozaka, 1974). Under natural conditions, the conidia are usually released during the night. After release, the conidia are dispersed by the wind or by splashing.

Host species specificity

Aside from rice, the blast pathogen has been isolated from a wide range of other grass species, including other important cultivated species as wheat, barley and sugarcane. Studies investigating the occurrence of cross infection between rice and other plant species have yielded contradictory results (Asuyama, 1965; Ou, 1985). The host range of individual isolates seems to be limited, at most a few species are attacked. Mackill and Bonman (1986) found that only certain isolates from rice are able to infect some of the grassy weed species in tropical rice fields and vice versa.

Unlike rice isolates, isolates from some of the other host species are highly fertile, and these isolates can be crossed to rice isolates with the latter as the male parent (Yaegashi and Nishihara, 1976; Valent et al., 1986). The pathogenicity towards rice of ascospore progenies resulting from such crosses is lower than that of the parental rice isolates (Leung and Williams, 1986). Valent et al. (1991) made a cross between an isolate both pathogenic to rice and weeping lovegrass, and one that was pathogenic to weeping lovegrass only. Just 6 of 59 tested ascospore progenies were pathogenic to the highly susceptible rice cultivar CO39 whereas all progenies were pathogenic to weeping lovegrass. Pathogenicity to rice was improved by backcrossing, suggesting polygenic control of pathogenicity towards rice.

Recent studies using molecular techniques support a restricted host range of rice pathogenic isolates. Regardless of their geographic origin, isolates from rice contain certain repetitive sequences in the genome that are not present in isolates from other host species, indicating that genetic exchange between rice pathogenic isolates and rice non-pathogenic isolates is limited (Hamer et al., 1989).

Importance in various rice cultivation systems

In tropical areas, the importance of the disease is largest when rice is grown as an upland crop and least when the crop is grown under irrigated (flooded) conditions. Drought has a marked predisposing effect on the plants towards increased susceptibility, but even without moisture stress, plants grown under upland- are more susceptible than plants grown under flooded conditions (Akai, 1939; Suzuki, 1935a and 1935b; El Refaei, 1977). Another factor that causes upland rice cultivations to be prone to blast is the usually longer dew period in this type of cultivation. In temperate rice growing areas, blast can also be important under irrigated conditions, especially when large amounts of nitrogen fertilizer are used. Increased supply of nitrogen fertilizer strongly favours blast development (Ou, 1985). Thus, in some of the irrigated rice areas in the tropics where cultivation is intensified to cope with the growing demand for food due to fast population growth, blast may become more important in the future.

Problems in breeding resistant cultivars

Blast can be effectively controlled if resistant cultivars are used. A highly resistant reaction of the plant to the

disease results when the plant has one or more effective hypersensitivity genes (Ohata et al., 1963; Kozaka, 1979). Because selection for these genes is relatively easy due to their "major" phenotypic effect, breeders have extensively used and are still using these genes for breeding resistant cultivars. Unfortunately, the hypersensitivity resistance is not effective to all genotypes of the fungus[1]. The chance that a cultivar possessing a new major gene comes into contact with a genotype of the pathogen (race) to which the major gene is inoperative, is sharply increased once that cultivar is grown on a large scale. A rapid multiplication of this race is usually the result, causing the cultivar to become susceptible after some time. In general, the effectiveness of major resistance genes is lost within a few years after the release of the new cultivars, in temperate as well as tropical countries (Ahn and Mukelar, 1986; Ezuka, 1972; Jeanguyot, 1983; Ou, 1985).

Meanwhile, in Japan, where the major genes were first utilized, some cultivars were observed to express some degree of resistance in the field even after the major genes became ineffective. This type of resistance, characterized by the presence of a susceptible infection type, fits the definition of partial resistance (PR) by Parlevliet and van Ommeren (1975). In Japan, PR seemed little influenced by the pathogen races that the cultivar was exposed to and it remained effective through time. In some cases, however, PR was found to behave similar as the complete resistance conferred by the major genes (Yunoki et al., 1970; Ezuka, 1972).

Because PR does not seem to rapidly become ineffective, Japanese researchers consider breeding for higher levels of PR important even though the resistance level is usually lower than when effective hypersensitivity resistance is present. In the tropics, from circa 1970 onwards, researchers of IRAT in Ivory Coast and Madagascar were among the first to put emphasis on breeding for PR after they discovered that some traditional cultivars with this type of resistance remained durably resistant (Jeanguyot, 1983). For small farmers in the tropics, durable resistance is of great importance since these farmers do not have the means to compensate for a lost crop and cannot easily switch from one cultivar to the other. In many countries there's no physical and organizational infrastructure to rapidly supply these farmers with seed of alternative rice cultivars to be grown as their next season's rice crop.

A problem in breeding for higher levels of PR is that a number of different pathogen races are usually present in the field. Since many rice cultivars carry one or more major resistance genes (Kiyosawa et al., 1986; Kozaka et al., 1970), accurate evaluation of PR is hindered due to the major genes' epistatic effects (Ezuka, 1972; Notteghem, 1989; Parlevliet, 1983). In addition, the expression of PR is

[1] Based on the observed differential interaction between host and pathogen genotypes, Yamasaki and Kiyosawa (1966) assumed that the major genes in rice interact in a gene-for-gene relationship with (a)virulence genes in the pathogen, similar as described by Flor (1956) for flax and flax rust. Recently, proof of a gene-for-gene relationship for one of these major genes was demonstrated by Silue et al. (1992).

strongly influenced by the environmental conditions. Breeding for PR to blast is thus no easy matter.

In the present work, a detailed study of various aspects of PR was made so that more efficient breeding technology for identifying and accumulating PR might be developed. The work was conducted at the International Rice Research Institute (IRRI), at Los Baños, the Philippines. It focused on tropical lowland rice cultivars because these can be easily grown at IRRI and because stable virulent isolates to these cultivars were available for the study of PR.

References

Ahn, S.W. and A. Mukelar, 1986. Rice blast management under upland conditions. In: F. Shideler and C.V. Mendoza (Eds), Progress in upland rice research, Proceedings of the 1985 Jakarta Conference, pp. 363-373. IRRI, Los Baños, Philippines.

Akai, S., 1939. On the ash figures of leaves of the rice plants transplanted from the different kinds of nursery beds and their susceptibilities to the blast disease. Ann. Phytopath. Soc. Japan 9:223-235.

Asuyama, H., 1965. Morphology, taxonomy, host range, and life cycle of *Piricularia oryzae*. In: IRRI, The rice blast disease, Proceedings of a symposium at the International Rice Research Institute, pp. 9-22. John Hopkins Press, Baltimore, Maryland, U.S.A.

Barksdale, T.H. and M.W. Jones, 1965. Minimum values of dew period and temperature required for infection by *Piricularia oryzae*. Phytopathology 55:503.

El Refaei, M.J., 1977. Epidemiology of rice blast disease in the tropics with special reference to the leaf wetness in relation to disease development. PhD-Thesis, faculty of the post-graduate school, Indian Agric. Res. Inst., New Delhi, India. 195 pp.

Ezuka, A., 1972. Field resistance of rice varieties to blast disease. Rev. Plant Prot. Res. 5:1-21.

Flor, H.H., 1956. The complementary genic systems in flax and flax rust. Advan. Genet. 8:29-54.

Hamer, J.E., L. Farral, M.J. Orbach, B. Valent and F.G. Chumley, 1989. Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. Proc. Natl. Acad. Sci. U.S.A. 86:9981-9985.

Hashioka, Y., 1965. Effects of environmental factors on development of causal fungus, infection, disease development, and epidemiology in rice blast disease. In: IRRI, The rice blast disease, Proceedings of a symposium at the International Rice Research Institute, pp. 153-161. John Hopkins Press, Baltimore, Maryland, U.S.A.

Hashioka, Y. and Y. Nakai, 1974. Fine structure of the rice blast. Outthrust of conidiophore cells of *Pyricularia oryzae*, etc., through an epidermal outer wall of host leaf. Res. Bull. Fac. Agric. Gifu Univ. Japan 36:9-18.

Heath, M.C., B. Valent, R.J. Howard and F.G. Chumley, 1990. Interactions of two strains of *Magnaporthe grisea* with rice, goosegrass and weeping lovegrass. Can. J. Bot. 68:1627-1637.

Hemmi, T. and J. Imura, 1939. On the relation of air humidity to conidial formation in the rice blast fungus, *Piricularia oryzae*, and the characteristics in the germination of conidia produced by the strains showing different pathogenicity. Ann. Phytopath. Soc. Japan 9:17-156.

Hemmi, T., T. Abe, J. Ikeya and Y. Inoue, 1936. Studies on the rice blast disease. 4. Relation of the environment to the development of blast disease and physiologic specialization in the rice blast fungus. Mater. Rural Improv. Dep. Agric. For. Japan 105:145.

Jeanguyot, M., 1983. Lutte contre la pyriculariose du riz. Mémoires et Travaux de l' IRAT 2. IRAT, Nogent-sur-Marne, France. 55 pp.

Kato, H., 1974. Epidemiology of rice blast disease. Rev. Plant Prot. Res. 7:1-20.

Kato, H. and T. Kozaka, 1974. Effect of temperature on lesion enlargement and sporulation of *Pyricularia oryzae* in rice leaves. Phytopathology 64:828-830.

Kato, H., T. Yamaguchi and N. Nishihara, 1976. The perfect stage of *Pyricularia oryzae* Cavara in culture. Ann. Phytopath. Soc. Japan 42:507-510.

Kiyosawa, S., D.J. Mackill, J.M. Bonman, Y. Tanaka and Z.Z. Ling, 1986. An attempt of classification of world's rice varieties based on reaction pattern to blast fungus strains. Bull. Natl. Inst. Agrobiol. Resour. Japan 2:13-37.

Kozaka, T., 1979. The nature of the blast fungus and varietal resistance in Japan. In: IRRI, Proceedings of the rice blast workshop, pp. 3-26. IRRI, Los Baños, Philippines.

Kozaka, T., S. Matsumoto and M. Yamada, 1970. Reactions of foreign rice varieties to major races of *Pyricularia oryzae* in Asia. Bull. Natl. Inst. Agric. Sci. Japan, Series C 24:113-152.

Leung, H. and P.H. Williams, 1986. Genetic and cytological characterization of the rice blast fungus, *Pyricularia oryzae*. In: Rice genetics, Proceedings of the international rice genetics symposium, pp. 491-500. IRRI, Manila, Philippines.

Mackill, A.O. and J.M. Bonman, 1986. New hosts of *Pyricularia oryzae*. Plant Disease 70:125-127.

Notteghem, J.L., 1989. La création de variétés résistantes: Cas du riz et de la pyriculariose. Colloque les apportes recents de la biologie végétale en regions tropicales. UPSA Orsay, 16-18 nov. 1989. Bulletin de la Société Botanique de France 136. Actualités Botaniques 3/4: 227-237.

Notteghem, J.L. and D. Silue, 1992. Distribution of the mating type alleles in *Magnaporthe grisea* populations pathogenic on rice. Phytopathology 82:421-424.

Ohata, K., K. Goto and T. Kozaka, 1963. Observations on the reaction of rice cells to the infection of different races of *Piricularia oryzae*. Ann. Phytopahol. Soc. Japan 28:24-30.

Ou, S.H., 1985. Rice diseases. 2nd edition. Commonwealth Mycological Institute. Kew, Surrey, England. 380 pp.

Parlevliet, J.E., 1983. Can horizontal resistance be recognized in the presence of vertical resistance in plants exposed to a mixture of pathogen races ? Phytopathology 73:379.

Parlevliet, J.E. and A. van Ommeren, 1975. Partial resistance of barley to leaf rust *Puccinia hordei*. II. Relationship between field trials, micro plot tests and latent period. Euphytica 24:293-303.

Rossman, A.Y., R.J. Howard and B. Valent, 1990. *Pyricularia grisea*, the correct name for the rice blast fungus. Mycologia 82:509-512.

Silue, D., J.L. Notteghem and D. Tharreau, 1992. Evidence of a gene-for-gene relationship in the *Oryza sativa - Magnaporthe grisea* pathosystem. Phytopathology 82:577-580.

Suzuki, H., 1935a. Studies on the influence of some environmental factors on the susceptibility of the rice plant to blast and *Helminthosporium* diseases and on the anatomical characters of the plant. II. Influence of differences in soil moisture and in the amount of nitrogenous fertilizer given. J. Coll. Agric. Tokyo Imp. Univ. Japan 13:235-276.

Suzuki, H., 1935b. Studies on the influence of some environmental factors on the susceptibility of the rice plant to blast and *Helminthosporium* diseases and on the anatomical characters of the plant. III. Influence of differences in soil moisture and in the amounts of fertilizer and silica given. J. Coll. Agric. Tokyo Imp. Univ. Japan 13:277-332.

Valent, B., M.S. Crawford, C.G. Weaver and F.G. Chumley, 1986. Genetic studies of fertility and pathogenicity in *Magnaporthe grisea* (*Pyricularia oryzae*). Iowa State J. Res. U.S.A. 60:569-59.

Valent, B., L. Farrall and F.G. Chumley, 1991. *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of backcrosses. Genetics 127:87-101.

Yaegashi, H. and N. Nishihara, 1976. Production of the perfect stage in *Pyricularia* isolates from cereals and grasses. Ann. Phytopath. Soc. Japan 42:511-515.

Yamasaki, Y. and S. Kiyosawa, 1966. Studies on inheritance of resistance of rice varieties to blast. 1. Inheritance of resistance of Japanese varieties to several strains of the fungus. Bull. Natl. Inst. Agric. Sci. Japan, Series D 14:39-69.

Yunoki, T., A. Ezuka, T. Morinaka, Y. Sakurai, H. Shinoda and K. Toriyama, 1970. Studies on the varietal resistance to rice blast. 4. Variation of field resistance due to fungus strains. Bull. Chukogu Agric. Exp. Stn. Japan, Series E 6:21-45.

Leaf age related partial resistance to *Pyricularia oryzae* in tropical lowland rice cultivars as measured by the number of sporulating lesions

E.C. Roumen, J.M. Bonman[1], and J.E. Parlevliet[2].

[1]former plant pathologist, IRRI; present address: Du Pont Co., P.O. Box 30, Newark, Delaware 19616, U.S.A. [2]professor of Plant Breeding, Wageningen Agricultural University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands.

Summary

To study the effects of leaf age on resistance, plants of four rice cultivars were inoculated with a virulent isolate of *Pyricularia oryzae*. Susceptibility of leaves declined rapidly with increasing leaf age. In older leaves, fewer sporulating lesions developed per unit of leaf area and eventually leaves became completely resistant. The period during which newly formed leaves were susceptible, as well as the initial level of susceptibility of new leaves, differed greatly between cultivars. Cultivars with high levels of partial resistance to leaf blast showed typical susceptible lesions, but the resistance in leaves rapidly increased with age and the initial level of susceptibility of new leaves was low.

Introduction

The number of sporulating lesions that develop after inoculation is an important parameter of partial resistance to blast disease on rice (*Oryza sativa* L.), caused by *Pyricularia oryzae* Cav., the imperfect stage of *Magnaporthe grisea* (Hebert) M.E. Barr (Toriyama, 1975; Yeh and Bonman, 1986; Yunoki et al., 1970). Resistance to blast is influenced by plant and leaf age. The infection frequency is less in older than in younger plants (Andersen et al., 1947; Kahn and Libby, 1958). In plants of the same age, it is less in older leaves, and after a certain time the resistance of leaves becomes virtually complete. This resistance appears to be common if not ubiquitous in rice cultivars and is expressed in various environments (Goto et al., 1961; Kahn and Libby, 1958; Notteghem and Andriatompo, 1979; Yeh and Bonman, 1986).

Although resistance to blast increases with leaf age, there is little information available concerning possible cultivar differences for the increase of this age-related resistance or for the period after which newly formed leaves become highly resistant. Cultivars that rapidly build up high resistance levels might be expected to develop fewer lesions after inoculation, because the susceptible leaf area is reduced. The experiments reported in this paper help determine the extent to which age-related resistance differs between rice cultivars.

Materials and methods

The research was done in a green-

house at the International Rice Research Institute (IRRI) at Los Baños, the Philippines. The tropical lowland rice cultivars CO39, IR50, IR66 and IR36 were used. CO39 lacks effective major gene resistance against many tropical blast isolates (Bonman et al., 1986; Mackill et al., 1988) and served as the susceptible check. The cultivars IR36 and IR50 were included to compare results with those obtained from earlier work (Yeh and Bonman, 1986). IR66 was included as a representative of a recently released Philippine cultivar.

Isolate P06-6 of *P. oryzae* was used for each inoculation. In preliminary tests, after inoculating plants with this isolate at the six- or seven-leaf stage, the four cultivars developed a susceptible infection type, according to the classification of Yamasaki and Kiyosawa (1966).

Three series of plants were grown with an interval of 1 month between the series from October to December 1987. To ensure the presence of all stages of leaf expansion at the time of inoculation, stagger planting was done on four consecutive days per series for IR36, IR50 and IR66 and on six consecutive days for CO39. Seeds were germinated in petri dishes and the seedlings were planted to 10 cm diameter plastic pots, with four plants per pot. Nine pots per day were planted for each cultivar, and these were equally divided over three 1.5 m^2 blocks. On completion of the planting of a series, each block contained 12 pots of IR36, IR50, and IR66 and 18 pots of CO39. Within the blocks, pots were completely randomized. A few days after planting, nitrogen fertilizer was added by the application of an ammonium sulfate solution to each pot at 0.157 g N per pot (equivalent to 20 g/m^2). The soil moisture in the pots was monitored twice daily and watering was done as necessary to avoid moisture stress. Care was taken to keep pots well drained, and plants were grown under non-flooded soil conditions.

We inoculated the three blocks of each planting series on different, usually consecutive days to spread the work load of the data collection. Expression of partial resistance to blast is known to be very sensitive to changes in environmental conditions (Ou, 1985). Thus a different predisposing effect on the plants, because of variations in weather conditions, may have occurred between the different days of inoculation. Furthermore, fresh inoculum was prepared for each block; each block (=inoculum day) was regarded as a separate test for a total of nine inoculation series.

On the day of inoculation, we made a small mark, with a felt-tip pen, at the point of emergence of the youngest leaf to determine the leaf stage of each plant. After the leaves were marked, the pots were again completely randomized within the block. Inoculum was produced as described by Bonman et al. (1986). The plants were inoculated just before sunset with 400 ml of a conidial suspension containing 3 x 10^4 conidia/ml. Inoculum was sprayed as a fine turbulent mist; a nozzle attached to a portable air compressor was used. To ensure uniform distribution of the inoculum, we directed the turbulent mist beam at all plants in the same manner in slow systematic movements, covering the plants from different directions. After inoculation, the plants were covered with a plastic cage and placed in a humid glasshouse room which was

kept at 25 C. The cage was removed the following morning, and the plants were kept in the same room until evaluation, 6 or 7 days later.

The number of sporulating lesions in each leaf of the main culm was counted, and the leaf length measured. Only lesions with a greyish centre were considered sporulating (Jeanguyot, 1983). Long narrow sporulating lesions growing along the leaf edge were excluded from the count because of their atypical shape. In each plant, the number of leaves on the main culm with at least one sporulating lesion and the total number of sporulating lesions in leaves of the main culm were counted. Leaf width was measured on a sample of 15 plants per cultivar in series 4, because it was observed that leaf width in CO39 was larger than in the other cultivars. Variation in leaf width between individual leaves within a genotype was small and the coefficient of variation for the samples varied between 4.2 and 5.8%. Leaf area (cm^2) was calculated as 0.7 x leaf width x leaf length. Lesion density was calculated by dividing the number of lesions per leaf by the leaf area. Adjustment for leaf width had little influence on relative differences between cultivars and did not affect cultivar ranking.

In series 6, only the presence or absence of sporulating lesions in leaves was scored. In the series 8 and 9, the lesion density on CO39 was too high to distinguish individual lesions, and assessment was hindered on CO39. Seed of IR66 used in the first planting appeared to be impure since two phenotypes could be distinguished, and one of these was completely resistant to isolate P06-6. Because of the possibility that there were other genotypes present in IR66 which could not be separated visually, the results of IR66 of the series 1-3 were excluded from the analysis. The seed of IR66 used in the remaining series was increased from a single panicle of the IR66 source that was used in the first planting.

Analysis of variance showed that residuals of the measurements were not normally distributed, and this could not be solved by transformation. Therefore, appropriate nonparametric tests were used (Siegel and Castellan, 1988). Analysis of the data for comparisons across series was done using the Wilcoxon ranked sign test with the treatment means per series as experimental unit, whereas comparisons within series were done using the Kruskal-Wallis test with the plant values as the experimental unit.

For the analysis of number of sporulating lesions per square centimetre of leaf area, plants were classified under three age categories based on leaf stage and leaf expansion. The first category had plants of the most common leaf stage with young expanding leaves of less than 15 cm in length; the second, leaves between 15 and 30 cm in length; and the third expanded or nearly expanded leaves exceeding 30 cm. The third group included leaves of plants on which the next leaf was already emerging, as long as this newly expanding leaf was shorter than 15 cm.

Results

At the time of scoring, sporulating lesions in leaves of IR36, IR50, and IR66 showed dark margins, but dark margins were not always observed

Table 1-1. Average number of leaves with sporulating lesions on the main culm of four rice cultivars inoculated with *Pyricularia oryzae* in nine series

	Cultivar			
Series	CO39	IR50	IR66	IR36
1	1.90	0.97	...	0.66
2	1.83	0.75	...	0.65
3	2.47	1.44	...	1.15
4	2.46	1.56	1.63	1.05
5	2.45	1.48	1.39	1.15
6	2.50	1.41	1.47	1.11
7	3.12	1.34	1.86	1.13
8	...	1.90	2.46	1.28
9	...	1.72	1.93	1.21
Weighed mean[1]	2.60 a	1.40 b	1.59 b	1.04 c
Relative to CO39	100%	54%	61%	40%

[1]Different letters indicate significantly different means (Wilcoxon signed rank test; pair wise comparison between cultivars at $\alpha=0.05$)

Table 1-2. Average number of sporulating lesions per plant on leaves of the main culm of four rice cultivars inoculated with *Pyricularia oryzae* in nine series

	Predominant	Cultivar			
Series	leaf stage	CO39	IR50	IR66	IR36
1	6	17.9 a[1]	4.2 b	...	1.3 c
2	7	14.9 a	1.6 b	...	1.0 b
3	7	32.2 a	6.3 b	...	5.0 b
4	7	22.0 a	8.0 b	6.7 bc	3.8 c
5	7	46.7 a	10.3 b	10.6 b	6.8 b
7	7	113.2 a	19.5 b	20.5 b	13.0 b
8	8	...	20.4 b	17.6 b	9.1 c
9	8	...	15.8 b	8.4 bc	6.1 c
Weighed means[2]		43.2 k	9.6 l	8.5 l	5.1 m
Relative to CO39		100%	22%	20%	12%

[1] Different letters indicate significantly different cultivar means within series according to the Kruskal-Wallis rank test; multiple comparison at $\alpha=0.05$.
[2] Different letters indicate significantly different cultivar means across series according to the Wilcoxon signed rank test; pairwise comparison of cultivars at $\alpha=0.05$

around sporulating lesions in CO39. Lesions in older leaves developed a dark margin faster than lesions in younger leaves. The dark margins surrounding sporulating lesions in leaves of IR66 were well developed and were darker than those in the other cultivars. Each of the cultivars also developed nonsporulating lesions, ranging from barely visible to larger (to 2-3 mm in diameter) reddish-brown flecks. These lesions were observed mostly in older leaves.

The grey centre of lesions appeared to be smaller in older leaf parts regardless of cultivar. However, in the series 7-9 the lesions of CO39 with the largest grey centres were seen in slightly older leaves. In these series, the density of sporulating lesions in young leaves of CO39 was high, and lesions began coalescing soon after appearance. In all cultivars, larger lesions developed in leaves of tillers than in leaves of the main culm.

In all series, the average number of leaves with sporulating lesions on the main culm was consistently highest for CO39 and lowest for IR36, whereas IR50 and IR66 had intermediate scores. The average number of leaves with lesions was generally lower in IR50 than in IR66, but the difference between these cultivars was not significant (Table 1-1). The number of leaves that developed lesions varied between series and increased in all cultivars when conditions favoured infection, as expressed by the total number of lesions per plant (Table 1-2). Relative differences between cultivars did not depend on the general infection level. The average Pearson correlation coefficient for the number of leaves with lesions between the cultivars over the series was 0.82.

The number of sporulating lesions that developed differed greatly among series, indicating a strong influence of environment on infection (Table 1-2). Between the series 2 and 7, the number of lesions on CO39 varied more than seven fold and the number of lesions on IR36 and IR50 varied more than 10-fold. Despite the large environmental effect, the average number of sporulating lesions per plant was consistently highest in CO39 and lowest in IR36. Averaged over all series, the number of sporulating lesions in CO39 was about eightfold that in IR36. The number of sporulating lesions on IR50 and IR66 differed little, except in series 9, in which the number of lesions per plant in IR66 was about half that of IR50. Both cultivars developed about one-fifth the number of lesions in CO39.

In all cultivars, most lesions were found in the youngest leaf closest to the top. At the same time, the proportional distribution of the lesions over the leaves varied between genotypes and series (Table 1-3). In IR36, nearly all lesions were located in the top leaf in all series (range 93-98%). Only a few lesions developed in the second youngest leaf and the third leaf from the top developed no lesions. The third leaf from the top was also completely resistant in IR50, but in this cultivar relatively more lesions developed in the second youngest leaf (mean of 12% over series). The proportion of lesions in the second youngest leaf was considerably higher in IR66 and CO39 (means of 39% and 33%, respectively). In these cultivars a small proportion of the lesions was found in the third youngest leaf. In CO39, in series 7, a few sporulating lesions were observed in the fourth leaf from the top.

Table 1-3. Percentage of sporulating lesions on the youngest (N), second youngest (N-1) and third youngest (N-2) leaves of the main culm of four rice cultivars inoculated with *Pyricularia oryzae*

	Cultivar											
	CO39			IR50			IR66			IR36		
Series	N	N-1	N-2	N	N-1	N-2	N	N-1	N-2	N	N-1	N-2
1	73	26	1	98	2	0	...	...	...	96	4	0
2	80	19	1	93	7	0	...	...	...	97	3	0
3	53	45	2	87	13	0	...	...	...	97	3	0
4	67	30	3	84	16	0	78	21	1	97	3	0
5	64	34	2	91	9	0	61	39	0	98	2	0
7	51	43	6	92	8	0	81	19	0	98	2	0
8	...	...	...	74	26	0	29	63	8	94	6	0
9	...	...	...	85	15	0	42	53	5	93	7	0
Mean	65	33	2	88	12	0	58	39	3	96	4	0

Table 1-4. The number of sporulating lesions in the seventh leaf per 100 cm² of leaf for three categories of leaf expansion of four rice cultivars inoculated with *Pyricularia oryzae* expressed relative to mean values in young leaves of CO39

		Cultivar[1]											
		CO39			IR50			IR66			IR36		
Series	Lesions[2]	1[3]	2	3	1	2	3	1	2	3	1	2	3
2	89	100 a	125 a	94 a	44 a	16 ab	8 b	...	...	...	24 a	9 ab	1 b
3	149	100 a	161 a	84 b	85 a	43 b	...	...	...	...	78 a	38 a	9 b
4	229	100 a	61 b	44 b	66 a	33 b	...	50 a	31 a	10 b	38 a	17 b	10 c
5	294	100 a	95 a	97 a	66 a	40 a	...	40 a	36 a	...	58 a	23 b	4 b
7	530	100 a	102 a	80 a	62 a	32 a	16 b	59 a	29 ab	21 b	49 a	32 a	3 b
Means		100	109	80	65	33	12	50	32	16	49	24	5

[1] Different letters indicate significant difference between leaf age categories within cultivars (Kruskal-Wallis; multiple comparison at $\alpha=0.05$).
[2] Mean number of lesions in young leaves of CO39 (equal to 100 %)
[3] Leaf expansion categories are 1) leaves shorter than 15 cm, 2) leaves between 15 and 30 cm and 3) leaves longer than 30 cm

The number of sporulating lesions per unit leaf area of expanding seventh leaves varied greatly between cultivars, between age categories and between series (Table 1-4). CO39 was the most susceptible cultivar, consistently showing the highest lesion number per unit leaf area despite large differences in lesion number between series. IR36 had the fewest lesions per unit leaf area.

Except in CO39, the number of lesions per unit leaf area decreased considerably with increasing leaf age at inoculation. For CO39, in some series, more lesions per unit leaf area occurred in leaves which were in the middle of leaf expansion than in leaves of the youngest age group.

The relative difference between cultivars increased when leaves matured. For example, the resistance of IR36, in terms of lesions per unit leaf area, was about twice that of CO39 in young, newly expanding leaves. But in fully expanded leaves, IR36 was 16 times more resistant (Table 1-4).

Discussion

Resistance to infection of rice leaves by *P. oryzae* was strongly dependent on age and stage of leaf expansion. Resistance rapidly increased in more expanded (older) leaves, resulting in a reduced number of sporulating lesions per leaf area.

Similar results were obtained in the United States, Japan and the Ivory Coast (Goto et al., 1961; Kahn and Libby, 1958; Notteghem and Andriatompo, 1979) for other cultivars and isolates. The rapid increase of resistance with increased leaf age appears to be a general phenomenon in rice which can be observed in diverse environments. More importantly, the present study shows that the increase in resistance with aging of leaves differs among cultivars.

Because leaves become increasingly resistant to infection with time, the total number of successful infections resulting in sporulating lesions for a certain amount of inoculum will depend on the initial level of susceptibility of newly emerging leaves and the rate of increase in resistance of aging leaves. Successive leaves from the top are increasingly older and thus, more leaves from the top will be infected in cultivars with leaves that remain susceptible during a longer time after appearance. New leaves of IR36 became completely resistant faster than new leaves of IR50 or IR66 which in turn became resistant faster than leaves of CO39. The rapid build up of age-related resistance in IR36 compared to the other cultivars was demonstrated by a consistently low number of infected leaves, by a high proportion of the total number of lesions which was located on the top, youngest leaf and by a relatively large increase in resistance of the topmost leaf during expansion. Cultivar differences also were found for the initial level of susceptibility of the newly emerging leaves.

Partial resistance has been defined as a reduced epidemic in the field despite a susceptible infection type (Parlevliet and van Ommeren, 1975). Components associated with higher levels of partial resistance are a reduced infection frequency, longer latent period and reduced sporulation capacity (Parlevliet, 1979). The infection frequency, or related parameters such as the infection efficiency, has

been reported to be an important component of partial resistance in rice to *P. oryzae* (Toriyama, 1975; Yeh and Bonman, 1986; Yunoki et al., 1970). Because a more rapid build up of resistance in young leaves over time causes a reduction in the total number of successful infections resulting in sporulating lesions, this characteristic should be associated with the partial resistance level of rice cultivars. A low initial level of susceptibility in young leaves would further add to the resistance. Based on the initial susceptibility of young leaves and subsequent rate of increase of resistance with aging among the tested cultivars, CO39 should have the lowest partial resistance level, followed by IR50 and IR66, whereas partial resistance is expected to be highest in IR36. These findings agree with data available from field studies (Bonman et al., 1989; Yeh and Bonman, 1986).

The time that newly emerged leaves remain susceptible and their initial level of susceptibility may be partly independent. For example, the initial level of susceptibility of new leaves of IR66 was similar to that of IR36, but additional resistance increased more slowly in IR66. In addition, the number of infected leaves per plant and the proportion of sporulating lesions on older leaves was not only higher on IR66 compared to IR36, but also compared to IR50.

The leaf age related resistance studied in this paper was partial in its effect since each of the rice genotypes showed a susceptible infection type after inoculation. Considering the inability of *P. oryzae* to infect old leaves, even of highly susceptible cultivars, it is postulated that cultivar resistance characterized by a susceptible infection type and a rapid increase of age-related resistance is less likely to be quickly overcome by new strains of the fungus than cultivar resistance based on a resistant infection type.

References

Andersen, A.L., B.W. Henry and E.C. Tullis, 1947. Factors affecting infectivity, spread and persistence of *Pyricularia oryzae* Cav.. Phytopathology 37:94-110.

Bonman, J.M., B.A. Estrada and J.M. Bandong, 1989. Leaf and neck blast resistance in tropical lowland rice cultivars. Plant Disease 73:388-390.

Bonman, J.M., T.I. Vergel de Dios and M.M. Khin, 1986. Physiologic specialization of *Pyricularia oryzae* in the Philippines. Plant Disease 70:767-769

Goto, K., K. Hirano and K. Ohata, 1961. Susceptibility of leaf of rice to blast in reference to leaf age and position. I. Variation of susceptibility among different leaf positions and grades of emergence of top leaf. Spec. Bull. Okayama Pref. Agric. Exp. Stn. Japan 58:77-88.

Jeanguyot, M., 1983. Lutte contre la pyriculariose du riz. Mémoires et Travaux de l' IRAT 2. IRAT, Nogent-sur-Marne, France. 55 pp.

Kahn, R.P. and J.L. Libby, 1958. The effect of environmental factors and plant age on the infection of rice by the blast fungus, *Piricularia oryzae*. Phytopathology 48:25-30.

Mackill, D.J., J.M. Bonman, P.D. Tenorio and T.I. Vergel de Dios, 1988. Near-isogenic Indica rice lines with blast resistance genes. Rice Genet. Newsl. 5:98-101.

Notteghem, J.L. et G.M. Andriatompo, 1979. Étude de quelques facteurs de la résistance horizontale du riz à la Pyriculariose. Agron. Trop. 34:180-195.

Ou, S.H., 1985. Rice Diseases. Second edition. Commonwealth Mycological Institute. Kew, Surrey, England. 380 pp.

Parlevliet, J.E., 1979. Components of resistance that reduce the rate of epidemic development. Annu. Rev. of Phytopathol. 17:203-222.

Parlevliet, J.E. and A. van Ommeren, 1975. Partial resistance of barley to leaf rust *Puccinia hordei*. II. Relationship between field trials, microplot tests and latent period. Euphytica 24:293-303.

Siegel, S. and N.J. Castellan, 1988. Nonparametric statistics for the behavioral sciences. Second edition. McGraw-Hill Book Company, New York, U.S.A. 399 pp.

Toriyama, K., 1975. Recent progress of studies on horizontal resistance in rice breeding for blast resistance in Japan. In: G.E. Gálvez (Ed), Proc. of the seminar on horizontal resistance to the blast disease of rice, series CE-No. 9, pp. 65-100. CIAT, Cali, Colombia.

Yamasaki, Y. and S. Kiyosawa, 1966. Studies on inheritance of resistance of rice varieties to blast. I. Inheritance of resistance of Japanese varieties to several strains of the fungus. Bull. Nat. Inst. Agric. Sci. Japan, Series D 14:39-69.

Yeh, W.H. and J.M. Bonman, 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathology 35:319-323.

Yunoki, T., A. Ezuka, Y. Sakurai, H. Shinoda and K. Toriyama, 1970. Studies on the varietal resistance to rice blast 3. Testing methods for field resistance on young seedlings grown in greenhouse. Bull. Chugoku Agr. Exp. Stn. Japan, Series E 6:1-19.

Effect of leaf age on components of partial resistance in rice to leaf blast

Summary

Ten rice genotypes were inoculated with a virulent isolate of *Magnaporthe grisea* to study the effect of leaf age on components of partial resistance, and evaluate associations between these components. The number of sporulating lesions per cm^2 leaf declined with increase of leaf age in all genotypes. The number of lesions per cm^2 leaf area in one week old leaves of the susceptible cultivar CO39 was about 25%, and that in the more resistant cultivars IR36, IR60 and IR62 was less than 2% of that in very young leaves of CO39. Large differences between genotypes were found for the number of sporulating lesions that developed, and this factor was closely related to the period that leaves remained susceptible after appearance. The number of lesions in the most susceptible cultivar CO39 was about 7 times that in the cultivars IR60 and IR64. Differences between genotypes were also found for lesion size. The effect of aging on average lesion size was less pronounced than on lesion density. Lesion size and lesion density were positively correlated, but a rapid decline of density was not necessarily accompanied by a rapid decline of size. No apparent differences between genotypes were observed for latent period. Genotypes with leaves that became highly resistant soon after appearance expressed higher levels of partial resistance in the field.

Introduction

Blast, caused by the imperfect state of *Magnaporthe grisea* (Hebert) M.E. Barr, usually referred to as *Pyricularia oryzae* Cav, is a major disease of rice. The disease occurs widespread and can cause serious damage, but planting resistant cultivars provides an effective control. Up to the present, most breeders concentrate on the development highly resistant cultivars. This resistance is usually characterized by a low infection type after exposure to the pathogen, race-specificity, and a simple inheritance. Unfortunately, in most cases this type of resistance is 'broken' soon after release of the cultivar (Ezuka, 1972; Jeanguyot, 1983; Ou, 1985).

Partial resistance (PR) has been defined by Parlevliet and van Ommeren (1975) as a reduced epidemic build-up in the field despite a susceptible infection type. PR to rice blast appears to be predominantly race non-specific and in some cultivars PR has been shown to be durable (Ezuka, 1972; Toriyama, 1975; Yeh and Bonman, 1986; Vales, 1987). Thus breeding for higher levels of PR may be the better alternative for developing blast resistant rice cultivars.

Selection for PR in the field in the presence of major genes that are effective to specific races of the pathogen population is difficult (Parlevliet, 1983a and 1983b; Parlevliet, 1989), and it may be more efficient to evaluate components of PR and select for

one of the components. Among the components associated with higher levels of PR are a reduced infection frequency, a longer latent period, and a reduced sporulation capacity (Parlevliet, 1979). Based on selection for a longer latent period, Parlevliet and Kuiper (1985) were able to efficiently improve PR to barley leaf rust. In the case of PR to leaf blast in rice, the number of sporulating lesions that develop after inoculation was found to be an important parameter (Sakurai and Toriyama, 1967; Yunoki et al., 1970). Villareal et al. (1981) and Yeh and Bonman (1986) found PR to be also associated with a reduced average lesion size. PR to leaf blast increases with aging of leaves (Kahn and Libby, 1958). Roumen et al. (1992) showed that cultivars differed for the increase of resistance with leaf age and these differences were associated with differences for the number of sporulating lesions per plant. Goto et al. (1961) and Notteghem and Andriatompo (1979) reported that leaf age also affects lesion size. Perhaps, analogous to lesion density, lesion size may decrease more rapidly in some cultivars than in others with increasing leaf age. Therefore, the effect of leaf aging on lesion density and lesion size was studied in ten rice genotypes to determine in more detail how leaf age affects the expression of PR to leaf blast.

Materials and methods

In general, the recommendations of Sakurai and Toriyama (1967) for plant cultivation and inoculation were followed. For testing 'field resistance' of rice genotypes in the greenhouse, these authors recommended the use of an isolate possessing virulence to all genotypes, to apply a large amount of nitrogen fertilizer, to inoculate in the 6th or 7th leaf stage of the plants using spray inoculation, and use a conidia suspension with 1 to 2.5 x 10^5 conidia/ml.

Rice genotypes and pathogen isolate. Eight tropical lowland cultivars and two breeding lines were used (Table 2-2). All genotypes were developed at the International Rice Research Institute (IRRI), except CO39, which is from India. Components of resistance of some of the genotypes were previously studied by Yeh and Bonman (1986) and Roumen et al. (1992). The two breeding lines IR37704-98-3-2-2 and IR29725-22-3-3-3 were abbreviated to IR37704 and IR29725. Isolate Po6-6 was used for inoculations. In preliminary tests, all genotypes appeared to develop a typical susceptible infection type, as defined by Yamasaki and Kiyosawa (1966), with this isolate.

Plant cultivation. The experiment was carried out three times (series) between May and December 1988 in a greenhouse. Plants were raised in plastic pots (12 cm diameter, 14 cm high) containing a clay soil. The soil was not puddled and was kept well drained. To obtain plants of all possible stages of leaf expansion at the moment of inoculation, germinated seeds were planted on day 1, 3 and 5 in each of the series. On each of the three planting days per series, five (May 1988) or six (Oct. and Dec. 1988) pots were planted per genotype. Seven seeds were planted per pot. The pots of each planting date were placed together in a block (150 x 90 cm^2) and

the genotypes were randomized over rows within the blocks. When the plants developed the second leaf on the main culm, nitrogen was applied at an equivalent of 5 g/m² (56.55 mg N/pot) by adding 40 ml of an ammoniumsulfate solution to each pot. Nitrogen was applied again when the plants reached the fourth leaf stage and again one day before inoculation.

Inoculation. Inoculation took place when most plants were in the sixth leaf stage with an optimal spread in leaf age (measured in days from leaf emergence). The optimal day for inoculation was determined by monitoring the day of emergence of the fifth and higher leaves daily in the late afternoon followed by immediate analysis of progress of plant development. On the day of the inoculation, the point of emergence of the youngest leaf on the main culm was marked with a water proof pen and all pots were completely randomized over three new blocks. The three blocks of each series were inoculated on the same day, shortly before sunset. Per block, 400 ml of a conidia suspension containing circa 5×10^4 conidia/ml was sprayed over the plants as a fine mist. Conidia were obtained from sporulating cultures on agar plates by scraping the plates with a rubber spatula in the presence of a little water, and inoculum was prepared by mixing 200 ml suspension adjusted to 1×10^5 conidia per ml with 200 ml water containing 1% gelatine. The plants were incubated overnight in a plastic cage. Leaf wetness duration was about 16 hours. Leaves were kept dry afterwards.

Measurements. The length and width of the upper three leaves on each main culm were measured following incubation, before appearance of symptoms. For the leaf width, the widest leaf part was measured. However, leaf width was measured at about three quarters distance from the tip in very young leaves with a triangular shape. The area of each leaf was estimated as 0.7 x length x width.

In the same leaves, sporulating lesions (showing a grey centre) were counted six days after inoculation, excluding those that started growing from leaf margins. The lesion density in number of sporulating lesions per cm² leaf area was calculated for each 6th leaf. The total number of sporulating lesions as well as the number of leaves with at least one sporulating lesion was calculated per plant. The size of the sporulating areas in the lesions was visually assessed using a sample of two pots per genotype. The genotypes were then ranked according to the visual impression of the lesion size in the topmost leaves on the main culm.

In the December series, the upper three leaves on the main culm were cut and fixed on paper sheets with transparent plastic tape seven days after inoculation to collect more precise data on the sporulating area of the lesions and distribution of these lesions over the leaf. The size of the sporulating areas was estimated using a scale (Fig. 2-1). The scale included different shapes of lesions of exactly the same size to avoid bias of the estimate because of shape. The position of each lesion was determined by measuring the distance (mm) between the centre of the lesion and the leaf base or the point of emergence of the leaf at the time of inoculation.

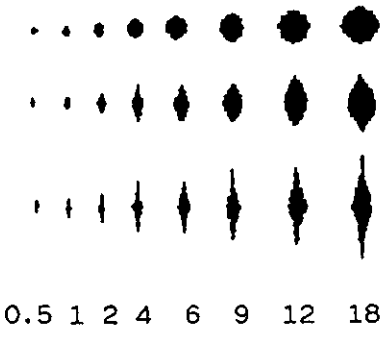

Fig. 2-1. Assessment key for estimating the size of the sporulating centre (mm²) in leaf blast lesions

Results and discussion

Effect of genotype and leaf age on the time of appearance of lesions. Sporulating lesions became first visible as very small depressed spots in the leaf with an ivory white or olive green colour. In the June and October series, the first signs of these lesions were visible in the morning of the fourth day after inoculation, while in the December series, the first signs were observed in the afternoon of that day. Many lesions became visible within a few hours without any distinct difference in time between the genotypes in any of the series. Neither was there any clear difference in time of appearance of these lesions between leaf parts of different ages. Depending on series and genotype, resistant type lesions also developed. These mostly appeared as minute dark brown spots or small dark stripes parallel to the veins and were always observed to appear several hours to half a day ahead of the sporulating lesions, similar as reported in Japan by Goto and Yamanaka (1968). The fact that the short time span between the time of appearance of the resistant lesion types and the sporulating lesions could be easily discerned, while there was no clear difference for the time of appearance of sporulating lesions between genotypes, indicates that differences for latent period between the rice genotypes were absent or less than a few hours.

Effect of genotype and leaf age on lesion density. With few exceptions, the number of sporulating lesions per cm² leaf area was highest in very young leaves and declined with increasing leaf age. The swiftness of this decline varied substantially between series. Averaged across genotypes, the sharpest decline was observed in June when the density of sporulating lesions for 7 or 8 day old leaves was only 4% of that for 1 or 2 day old leaves. The slowest decline occurred in October, with the lesion density for 7 or 8 day old leaves being 30% of that for 1 or 2 day old leaves (Table 2-1). Part of

Table 2-1. Number of sporulating blast lesions per cm² leaf at four leaf age categories relative to that of the youngest leaves in three series; average of ten rice genotypes

Series	Leaf age in days			
	1-2	3-4	5-6	7-8
June	100	66	26	4
Oct.	100	78	46	30
Dec.	100	65	33	18

100 equals 4.5, 4.7, and 5.0 lesions/cm² leaf in June, Oct., and Dec., respectively.

Table 2-2. Mean number of sporulating blast lesions per cm² inoculated leaf area for four leaf age categories (in days) of the sixth leaf of ten rice genotypes relative to the value obtained for 1 and 2 day old leaves of CO39 in three series

Geno-	June				Oct.				Dec.				Mean of 3 series[1]			
type	1-2	3-4	5-6	7-8	1-2	3-4	5-6	7-8	1-2	3-4	5-6	7-8	1-2	3-4	5-6	7-8
CO39	100[2]	71	47	7	100[2]	83	53	39	100[2]	69	47	28	100 a	74 a	49 a	25 a
IR29725	73	40	8	0	56	32	20	9	90	70	41	23	73 ab	47 ab	23 abc	11 abcd
IR37704	59	58	21	1	38	30	29	33	92	41	33	22	63 ab	43 ab	28 ab	19 abc
IR50	57	50	20	2	30	37	13	7	102	72	36	23	63 ab	53 a	23 ab	11 abc
IR52	47	30	15	9	34	32	22	10	91	77	35	21	57 abc	46 ab	24 ab	13 ab
IR36	50	29	9	1	26	17	7	2	96	81	21	3	57 abc	42 abc	12 bcd	2 bcd
IR66	55	21	14	2	22	24	12	8	53	34	29	14	43 bc	26 bc	18 abcd	8 abcd
IR62	35	25	4	0	27	17	6	1	89	40	9	2	50 bc	27 abc	6 cd	1 cd
IR60	37	17	3	0	23	9	3	1	76	36	13	1	45 bc	21 c	6 d	1 d
IR64	27	15	2	0	16	11	4	2	49	30	14	9	31 c	19 c	7 cd	4 bcd
Mean	54	36	14	2	37	29	17	11	84	55	28	15	58	40	20	9

[1] Within age categories, values followed by different letters indicate significant differences for the average lesion density between genotypes (Friedman's test; comparison of multiple contrasts at α=0.05). [2] 100%=8.3, 12.7, and 6.0 lesions per cm² leaf in the June, Oct., and Dec. series respectively.

the variation between the series may be explained by different weather conditions prior to and during incubation, resulting in different predisposing conditions of the plants. Incoming radiation, potential evapotranspiration, and temperature during plant cultivation were highest in June (Anonymous, 1989). Under such conditions, susceptibility of rice plants to blast is normally reduced (Kozaka, 1979; Hashioka, 1965; Kim and Crill, 1980).

The variation between series did not seem to affect the ranking order between host genotypes and/or leaf age categories within series. The Kendall coefficient of concordance (K_w) calculated over genotypes and leaf age categories was 0.88. Apparently, there were no important series x genotype interactions for the lesion density of the various leaf age categories.

The initial resistance level in terms of lesions per cm² leaf clearly varied between genotypes (Table 2-2). In June and October, lesion density for 1 or 2 day old leaves was highest for CO39 and lowest for IR64. In December, the lesion density in very young leaves was within close range for most genotypes, with IR50 having the highest density and CO39 the second highest. IR64 again had the lowest density. Averaged across the series, the lesion density in young leaves of IR64, IR66, IR60 and IR62 was less than 50 % of that in CO39 (Table 2-2). A low lesion density was reached soon after leaf appearance in IR36, IR60, IR62 and IR64 in all three series. On the other hand, older leaves of CO39 continued to develop a relative-

Table 2-3. Rank order of the size of the sporulating area of blast lesions (1=large, 6=small) in three series for ten rice genoypes based on visual assessment

Rank	Series		
	June	Oct.	Dec.
1	CO39	CO39	CO39
2	IR50 IR52 IR64	IR29725 IR50 IR52	IR50 IR52 IR64
3	IR29725 IR37704	IR37704 IR64	IR29725 IR37704
4	IR36 IR66	IR66	IR36 IR66
5	IR60 IR62	IR36	IR60 IR62
6		IR60 IR62	

ly large number of lesions per cm^2 leaf (Table 2-2). The genotype ranking for the number of lesions per cm^2 leaf over the leaf age categories was significantly ($P \leq 0.01$) associated in all three series (K_w was 0.77, 0.88 and 0.73 in June, October and December respectively), indicating that a lower lesion density in very young leaves usually concurs with a shorter period that new leaves remain susceptible after appearance. However, in IR66, lesion density in old leaves was higher than expected based on its density in young leaves (Table 2-2). This result agrees well with earlier measurements for this cultivar (Roumen et al., 1992) and shows that the initial resistance level of young leaves and the subsequent increase of the resistance with aging may be, at least partly, independent.

Effect of genotype and leaf age on the size of the sporulating area of lesions. The visual assessment of the size of the lesions' sporulating area showed consistent differences for this trait between genotypes. Mean size of the sporulating area was larger in CO39 than in any other genotype. Among the remaining genotypes, lesions developed a relatively large sporulating area in IR50, IR52 and IR64, and to a lesser degree in IR29725 and IR37704. The size of the sporulating area in IR36 and IR66 was again notably smaller than in afore mentioned genotypes. The sporulating area of lesions was smallest in IR60 and IR62 (Table 2-3). In June and October, dark margins in lesions developed sooner after appearance in genotypes with lesions having relatively small sporulating areas, such as IR36, IR66, IR62 and IR60 than in genotypes having lesions with larger sporulating areas, such as IR50, IR52 and CO39. Seven days after inoculation, dark margins were present in nearly all lesions in IR60 and IR62, but hardly any were observed in CO39. In general, dark margins tended to develop earlier in lesions in older leaf parts. In December, at the time of leaf sampling, no dark margins were observed in any of the genotypes. This may be related to the later time of appearance of lesions

Table 2-4. Average size of the sporulating area (mm^2) in blast lesions for four age categories of the sixth leaf of ten rice genotypes relative to the mean value for 1-2 day old CO39 leaves (Dec. series)

Genotype	Leaf age (days)[1]			
	1-2	3-4	5-6	7-8
CO39	100[2] a	106 a	100 a	92 a
IR52	97 ab	86 ab	64 bcd	46 bc
IR50	95 ab	85 a	81 ab	68 abc
IR29725	88 abc	84 a	80 ab	68 ab
IR36	85 bc	67 bc	60 cd	52 c
IR64	76 bcd	87 a	80 ab	73 abc
IR66	71 cd	65 bcd	68 bc	73 ab
IR37704	66 cd	70 b	69 bc	52 abc
IR62	56 d	53 cd	45 d	38 abc
IR60	56 d	38 d	42 d	47 c

[1] Within columns, values followed by the same letter are not significantly different (Kruskal-Wallis test, using the means of individual leaves as unit, and comparing multiple treatments at $\alpha=0.05$).
[2] 100% corresponds to a sporulating area of 2.5 mm^2

in this series and to variations in environmental factors (Ou, 1985).

The measurements of the December series showed that the average size of the lesions' sporulating area not only varied with genotype but also with leaf age (Table 2-4). In most genotypes, the largest mean size was found for 1 and 2 day old leaves. The mean size in the youngest leaf age category of IR60 and IR62 was 56% of that of CO39. Subsequent decrease of the size of the lesions' sporulating area with increase of leaf age depended strongly on the genotype. No or little decline occurred in CO39, IR64 and IR66, but a large (percentile) decline was observed in IR52 and IR36 (-53% and -39% respectively). On the whole, the effect of leaf aging was far less pronounced on size of the sporulating area of lesions than on lesion density. Nevertheless, the size of the sporulating area in some genotypes was smaller than in others regardless of leaf age, and thus may be an important component of PR to leaf blast (Table 2-4).

The genotype ranking based on the visual assessment of the size of the sporulating area of lesions in the December series did not always agree with that of the measurements. Large differences, such as between CO39 and IR60 or IR62 were easily observed. However, visual assessment apparently overestimated the size of the lesions' sporulating area in IR37704. Within each genotype, lesions of different type and size were interspersed. The size of the sporulating area in IR37704 did not decrease with leaf age, thus quite a number of large lesions were present. Probably, the visual judgement is biased by the amount and size of the largest sporulating lesions in the mixture. This might also explain why IR36 was judged to have small lesions, while the measurements showed that the average size of the sporulating area in lesions in the youngest leaves in this genotype was not that much smaller than in IR50 or IR64. Visually, lesions with very large sporulating areas were relatively rare in IR36.

Selection for a smaller lesion size resulting in choosing genotypes with a more resistant infection type should preferably be avoided. Jeanguyot (1983) reported that the resistance of genotypes that have an intermediate infection type and develop only small

Table 2-5. Mean percentage sporulating leaf area in four age categories of the sixth leaf for ten rice genotypes exposed to the blast pathogen

Genotype	Leaf age (days)[1]			
	1-2	3-4	5-6	7-8
CO39	14.7 a	11.2 a	7.5 a	3.7 a
IR50	14.1 a	9.5 ab	4.4 abc	3.6 ab
IR29725	14.0 ab	9.8 a	5.1 ab	2.6 ab
IR52	13.5 ab	9.4 ab	3.1 bcd	1.6 ab
IR36	12.7 abc	8.3 abc	2.2 ef	0.3 c
IR37704	8.0 bcd	3.7 bc	3.0 bcd	1.7 ab
IR62	7.7 cd	3.1 cd	0.6 f	0.0[2] c
IR66	6.8 cd	3.5 cd	2.7 cde	1.5 bc
IR60	6.0 cd	1.7 d	0.8 f	0.1 c
IR64	5.4 d	4.1 bc	1.8 ef	1.0 bc

[1] Within columns, values followed by the same letter are not significantly different (Kruskal-Wallis test, using means of individual leaves as unit, and comparing multiple means at $\alpha=0.05$). [2] trace

sporulating lesions behaves similar to complete resistance and usually is race-specific. The risks of selecting for smaller lesion size might be minimized by choosing genotypes such as IR36, with a rapidly declining lesion size with increase of leaf age, but with a relatively large sporulating area of lesions in the youngest leaf parts.

Association between lesion density and lesion size. The size of the sporulating area of lesions and lesion density in the youngest leaf age group were positively correlated (Spearman rank correlation $r_s = 0.60$; $P<0.05$). This suggests that there is no strong competition between lesions at the early stages of lesion development. The effect of leaf age on lesion size, however, did not necessarily closely correspond with that on lesion density. E.g., lesion density in IR64 rapidly declined with increase of leaf age without decline in size of the lesions' sporulating area. Selection for one of these components probably will not necessarily affect the other.

Relative importance of lesion density and size of the sporulating area per lesion. Using the results of lesion number and lesion size, the calculated fraction sporulating leaf area for the December series is shown in Table 2-5. The total sporulating leaf area may be assumed to be more closely associated to the spore production than lesion density or the size of the sporulating area of lesions alone. The fraction sporulating leaf area in 7 and 8 day old leaves was circa 25% of that of the youngest leaf group in CO39 and IR50. It was 2% or less in IR36, IR60 and IR62. The remarkable reduction in the latter cultivars is the result of the relatively large effect of increase of leaf age on both lesion density and

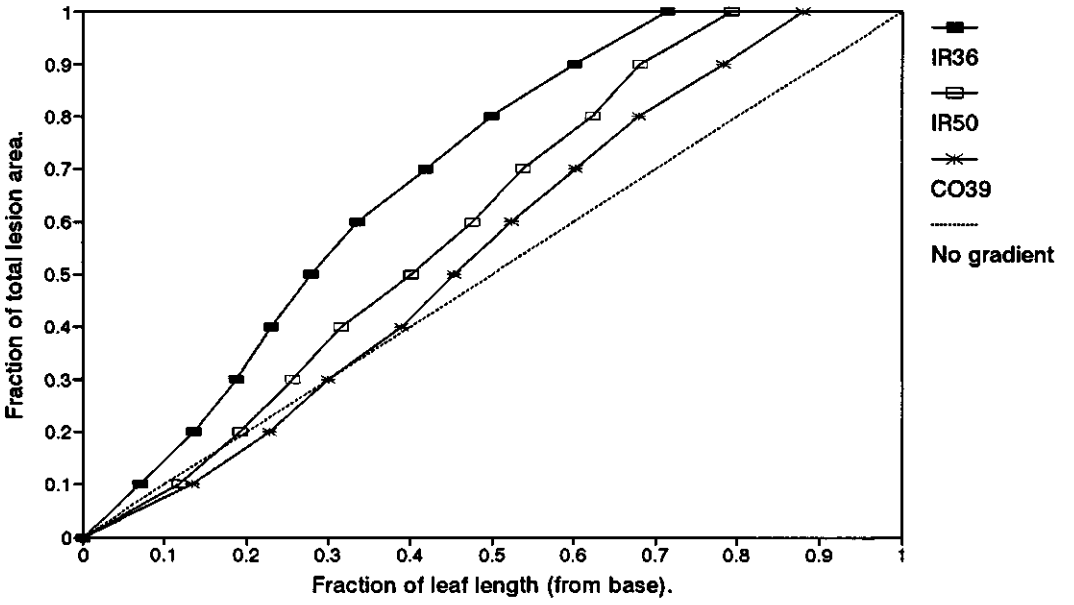

Fig. 2-2. Distribution of the sporulating area in 5 and 6 day old leaves of IR36, IR50 and CO39, averaged over a number of plants

size of the sporulating area per lesion. A few days after appearance, leaves of IR36, unlike leaves of e.g. CO39, even showed a substantial resistance gradient from the base to the top. Most of the sporulating area was found in the basal, youngest, leaf part (Fig. 2-2). The fraction sporulating area corresponded more closely with lesion density (r_s = 0.95) than with average lesion size (r_s = 0.75). This is not surprising considering the much larger effect of leaf age on lesion density and the larger differences between genotypes (especially in the older leaves). Also, when lesion density becomes very low, a larger sporulating area per lesion will hardly increase the ratio between the sporulating and the total leaf area.

Association between the time after appearance that newly formed leaves become highly resistant and the number of sporulating lesions that is formed. As a result of differences between genotypes for the lesion density in the youngest leaves and decline of this density with leaf age, the number of sporulating lesions in leaves of the main culm significantly varied between genotypes (Table 2-6). Averaged over the three series, the number of sporulating lesions in CO39 was about seven times that in IR64. The presence of considerable differences between genotypes for the number of sporulating lesions is in agreement with findings of other researchers (Villareal et al., 1981; Yeh and Bonman, 1986; Yunoki et al., 1970) and shows that the number of lesions is an important component of PR to leaf blast. The present data confirm earlier work

Table 2-6. Average number of sporulating blast lesions in leaves of the main culm for ten rice genotypes in three series, relative to CO39

Genotype	Series[1]			Mean
	June	Oct.	Dec.	
CO39	100[2] a	100[2] a	100[2] a	100 a
IR52	38 bc	30 b	80 a	49 b
IR37704	46 ab	36 b	64 ab	48 b
IR29725	37 bc	31 b	74 a	47 bc
IR50	28 bc	28 b	63 ab	40 bc
IR66	23 cd	18 c	47 bc	29 cde
IR36	19 de	12 cd	47 c	26 de
IR62	14 ef	10 d	32 cd	19 ef
IR60	11 f	9 d	23 d	14 f
IR64	9 f	8 d	26 d	14 f

[1] Within columns, different letters indicate significant differences between genotypes. Within series, genotypes were compared using Kruskal-Wallis' test for independent samples (total α=0.05); Scheffé's test was used for comparing means across series (α=0.05).
[2] 100%=60.3 for June, 153.2 for Oct., and 42.3 lesions/plant for the Dec. series.

Table 2-7. Average number of leaves on the main culm with sporulating blast lesions for ten rice genotypes in three series, relative to CO39

Genotype	Series[1]			Mean
	June	Oct.	Dec.	
CO39	100[2] a	100[2] a	100[2] a	100 a
IR37704	73 bc	81 b	84 bc	79 ab
IR66	63 bcde	70 bc	94 ab	76 b
IR52	79 ab	63 cd	82 bc	75 b
IR29725	59 cdef	63 cd	73 c	65 bc
IR50	63 bcd	55 de	72 c	63 bc
IR36	54 def	43 ef	52 d	50 c
IR64	46 ef	45 ef	58 d	50 c
IR62	52 def	42 ef	54 d	49 c
IR60	44 f	41 f	43 d	43 c

[1] Within columns, different letters indicate significant differences between genotypes. Within series, genotypes were compared using Kruskal-Wallis' test for independent samples (total α=0.05); Scheffé's test was used for comparing means across series (α=0.05).
[2] 100%=2.23 for June, 2.93 for Oct., and 2.67 lesions/plant for the Dec. series.

(Roumen et al., 1992), showing that fewer leaves on the main culm developed sporulating lesions the faster leaves of a genotype became highly resistant after appearance (Table 2-7). The average number of leaves with sporulating lesions on the main culm was highest in CO39, the genotype with the slowest build-up of resistance. Opposite results were found for IR36, IR64 and IR60. The genotype differences were highly consistent across series (K_w = 0.94). The correlation between the number of leaves with sporulating lesions and the total number of sporulating lesions in leaves of the main culm was found to be high (average Pearson correlation r_p=0.87), clearly demonstrating that a rapid increase to high resistance levels effectively reduced the relative infection efficiency.

Comparison with field data. The genotypes IR29725, IR36, IR37704, IR50, IR64 and IR66 were also included in a field experiment consisting of four trials by Bonman et al. (1989). Comparison of the results supported the assumption of Roumen et al. (1992) that a fast build-up to high resistance levels soon after appearance of leaves contributes to a higher resistance in the field. Among the six genotypes, IR36 and IR64, whose leaves became resistant fastest in the greenhouse (Table 2-2), also expressed the highest PR in the field. Likewise, the relatively low resistance in the field of IR37704, IR29725 and IR50 was in agreement with the observation that new leaves of these genotypes maintained a higher number of sporulating lesions during a longer period after appearance (Table 2-2).

The importance of a reduced number of sporulating lesions as a component of PR to blast (Yunoki et al., 1970; Yeh and Bonman, 1986; Sakurai and Toriyama, 1967) was supported by a perfect agreement between the rank orders of the six genotypes for average number of sporulating lesions in the three greenhouse series and the average amount of disease in the four field trials.

Resistance under field conditions could not be predicted from the average lesion size observed in the greenhouse. Although the average lesion size among the genotypes common to both studies was smallest in IR37704 (Table 2-4), this line was the most susceptible in the field. Conversely, lesion size in IR64, the most resistant genotype in the field, was relatively large. Apparently, with regard to the expression of PR in the field, genotype differences for lesion size are far less important than genotype differences for the number of sporulating lesions.

Because the genotypes showed large differences in PR in the field, without any notable differences for latent period in the greenhouse, it is concluded that latent period is probably of no importance as a component of PR to blast.

References

Anonymous, 1989. Compilation of weather data for 1988. Climate Unit. Multiple Cropping Department. IRRI.

Bonman, J.M., B.A. Estrada and J.M. Bandong, 1989. Leaf and neck blast resistance in tropical lowland rice cultivars. Plant Disease 73:388-390.

Ezuka, A., 1972. Field resistance of rice varieties to blast disease. Rev. Plant Prot. Res. 5:1-21.

Goto, K. and S. Yamanaka, 1968. Studies on the race of rice blast fungus. Bull. Coll. Agric. Utsunomiya Univ. Japan 7:21-71.

Goto, K., K. Hirano and K. Ohata, 1961. Susceptibility of leaf of rice to blast in relation to leaf age and position. 1. Variability of susceptibility among different leaf positions and grades of emergence of the top leaf. Spec. Bull. Okayama Pref. Agric. Exp. Stn. Japan 58:77-88.

Hashioka, Y., 1965. Effects of environmental factors on development of causal fungus, infection, disease development, and epidemiology in rice blast disease. In: International Rice Research Institute, Proceedings symposium on rice blast disease, pp. 153-161. John Hopkins Press, Baltimore, Maryland, U.S.A.

Jeanguyot, M., 1983. Lutte contre la pyriculariose du riz. IRAT. Mémoires et Travaux de l' IRAT 2. IRAT, Nogent-sur-Marne, France. 55 pp.

Kahn, R.P. and J.L. Libby, 1958. The effect of environmental factors and plant age on the infection of rice by the blast fungus, *Piricularia oryzae*. Phytopathology 48:25-30.

Kim, C.K. and P. Crill, 1980. Effect of predisposing temperatures on the histopathology of the rice blast fungus, *Pyricularia oryzae*. 2. Effect of four predisposing temperature regimes on the symptom development. Korean J. Pl. Protection 19:21-29.

Kozaka, T., 1979. The nature of the blast fungus and varietal resistance in Japan. In: IRRI, Proceedings of the rice blast workshop, pp. 3-25. Los Baños, Laguna, Philippines.

Notteghem, J.L. and G.M. Andriatompo, 1979. Étude de quelques facteurs de la résistance horizontale du riz à la Pyriculariose. Agron. Trop. 34:180-195.

Ou, S.H., 1985. Rice diseases. 2nd Edition. Commonwealth Mycological Institute. Kew, Surrey, England, 380 pp.

Parlevliet, J.E., 1979. Components of resistance that reduce the rate of epidemic development. Ann. Rev. Phytopathol. 17:203-222.

Parlevliet, J.E., 1983a. Can horizontal resistance be recognized in the presence of vertical resistance in plants exposed to a mixture of pathogen races ? Phytopathology 73:379.

Parlevliet, J.E., 1983b. Durable resistance in self fertilizing annuals. In: F. Lamberti, J.M. Waller and N.A. Van der Graaff (Eds), Durable resistance in crops, pp. 347-362. Plenum Publishing Corporation. New York, etc..

Parlevliet, J.E., 1989. Identification and evaluation of quantitative resistance. In: K.J. Leonard and W.E. Fry, (Eds), Plant disease epidemiology. Volume 2: Genetics, resistance and management, pp. 215-248. McGraw-Hill Publishing Company. New York, etc..

Parlevliet, J.E. and A. van Ommeren, 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, micro-plot tests and latent period. Euphytica 24:293-303.

Parlevliet, J.E. and H.J. Kuiper, 1985. Accumulating polygenes for partial resistance in barley to barley leaf rust, *Puccinia hordei*. I. Selection for increased latent period. Euphytica 34:7-13.

Roumen, E.C., J.M. Bonman and J.E. Parlevliet, 1992. Leaf-age related partial resistance to *Pyricularia oryzae* in tropical lowland rice cultivars as measured by the number of sporulating lesions. Phytopathology 82:1414-1417

Sakurai, Y. and K. Toriyama, 1967. Field resistance of the rice plant to *Piricularia oryzae* and its testing method. In: T. Ogura, (Ed), Rice diseases and their control by growing resistant varieties and other measures, pp. 123-135. Proc. Symp. Tropical Agriculture Researches. Ministry of Agriculture and Forestry, Tokyo, Japan.

Toriyama, K., 1975. Recent progress of studies on horizontal resistance in rice breeding for blast resistance in Japan. In: G.E. Gálvez (Ed), Proceedings of the seminar on horizontal resistance to the blast disease of rice, Series CE-no. 9, pp. 65-100. CIAT, Cali, Colombia.

Vales, M., 1987. La résistance durable: cas de la pyriculariose du riz. I. Les qualités associées à la résistance durable. Agron. Trop. 42:103-111.

Villareal, R.L., R.R. Nelson, D.R. Mackenzie and W.R. Coffman, 1981. Some components of slow blasting resistance in rice. Phytopathology 71:608-611.

Yamasaki, Y. and S. Kiyosawa, 1966. Studies on inheritance of resistance of rice varieties to blast. I. Inheritance of resistance of Japanese varieties to several strains of the fungus. Bull. Nat. Inst. Agric. Sci. Japan, Series D 14:39-69.

Yeh, W.H. and J.M. Bonman, 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathology 35:319-323.

Yunoki, T., A. Ezuka, Y. Sakurai, H. Shinoda and K. Toriyama, 1970. Studies on the varietal resistance to rice blast. 3. Testing methods for field resistance on young seedlings grown in greenhouse. Bull. Chugoku Agr. Exp. Stn. Japan, Series E 6:1-21.

Latent period to leaf blast in rice and its importance as a component of partial resistance

E.C. Roumen and W.S. de Boef[1].

[1] present address: Centre of Genetic Resources, at the Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands.

Summary

In many pathosystems, a prolonged latent period is an important component of partial resistance. Latent period in rice to leaf blast was assessed in cultivars representing a fairly wide range of partial resistance under various conditions that are known to influence the expression of partial resistance considerably. The latent period was slightly more than four days and varied only little between treatments, with a maximum difference of only eight hours between cultivars. The very small differences in latent period were not associated with differences in partial resistance due to cultivar, nitrogen, or leaf age effects. It was concluded that the latent period is of no importance as a component of partial resistance to leaf blast.

Introduction

Selection for higher levels of partial resistance (PR) to *Magnaporthe grisea* (anamorph *Pyricularia oryzae*) in tropical rice cultivars (*Oryza sativa*) is complicated. Many of these cultivars carry one or more effective major genes (Kiyosawa et al., 1986). In tropical fields, a mixture of races is usually present and efficient field evaluation of PR is hindered due to epistatic effects of the major genes (Ezuka, 1979; Notteghem, 1989; Parlevliet, 1983). In addition to the problem of epistatic effects of major genes, PR is very sensitive to changes in environmental conditions (Ou, 1985). Evaluation of components of PR and selection for one or more of the components might serve as an alternative, more efficient approach to improve PR. Among the components often associated with higher levels of PR are a reduced infection efficiency, a longer latent period, and a reduced sporulation capacity (Parlevliet, 1979).

Studies on latent period (LP) to leaf blast are relatively few, and the importance of LP as a component of PR to leaf blast is not clear. Some studies indicated substantial variation for LP among rice cultivars (Rodríguez and Gálvez, 1975; Brodni et al., 1988; Castaño et al., 1989), but other studies indicated little or no variation (Yeh and Bonman, 1986; Silue et al., 1992; Roumen, 1992). Under tropical conditions, the LP of leaf blast is relatively short, about 4 to 5 days (Ou, 1985). Leaf blast thus behaves as a compound interest disease, and even relatively small differences in LP might result in clear cultivar differences for PR (Zadoks, 1971; Zadoks and Schein, 1979). In the present paper, the importance of the LP as a component of PR in rice to leaf blast is investigated.

Material and methods

The isolate Po6-6 was used for inoculations. This isolate induces a susceptible infection type (large elliptical lesions with a grey centre) on the cultivars used in the experiments.

For assessing LP accurately, the beginning of sporulation of lesions has to be measured. For blast lesions, this cannot be done visually. However, sporulation is usually only observed in lesions with a grey centre (Jeanguyot, 1983). To investigate the relation between the start of sporulation and the development of grey centres, a new technique to detect the presence of spores on lesions was used (Experiment I). Three cultivars, IR50, IR64 and IR66, representing a fairly wide range of PR under field conditions (Bonman et al., 1989; Sah and Bonman, 1992) were assessed at several categories of leaf age, since leaf age markedly influences other components of PR to leaf blast in these cultivars (Roumen, 1992).

Experiment I was repeated three times (series) with intervals of four weeks. Per series and per cultivar, 20 pots were planted with seven germinated seeds each. The 60 pots were completely randomized and placed in a 1.5 m^2 block in a greenhouse. Plant cultivation, inoculation and incubation were as described previously (Roumen, 1992). Inoculation was done when most of the plants developed the seventh leaf on the main culm. The days of emergence of leave no. five, six and seven on the main culm were recorded for each plant. On the day of inoculation, the point of emergence of the youngest leaf was marked with a felt-tip water proof marker. In the first series, all plants were marked regardless of differences in stage of development between plants, but in the second and third series, subsets of at least 20 plants per genotype with the same day of emergence of leaf seven were selected. Up to nine well separated emerging lesions (visible as small white flecks) were monitored per plant. Per leaf, three emerging lesions were selected near the leaf tip, three in the middle section, and three near the leaf base. The time of first appearance of the lesions was recorded and the growth of grey centres was assessed using a key described previously (Roumen, 1992). The age of the leaf area for each lesion was estimated by interpolation using the time of leaf emergence.

To induce sporulation, a fine mist of water was sprayed over the plants shortly before sunset on day three after inoculation and onwards, until the leaves were covered with a barely visible layer of droplets. The plants were then covered with a plastic cage. Sporulation was measured each morning. Batches of two pots per cultivar were transferred from the cage to a wind-free room. There, the presence of spores was assessed using small agar disks that were stamped from a 2% water-agar layer of circa 1 mm thickness with a small plastic cylinder (3 mm in diameter). The disks were gently pressed on the lesions with a small spatula and were then transferred onto an object-glass. In a small preliminary test, the agar disks were found to be very efficient in removing spores from lesions without damaging these lesions.

The object glasses with the agar disks were stored in petri-dishes to prevent desiccation and contamination of the samples. The presence of spores

on the disks was assessed using a microscope (magnification: 100 x). The time between inoculation and the time that 50% of the lesions became visible as small white flecks (IC_{50}), and the time between inoculation and the time that 50% of the lesions started sporulating (LP_{50}) was calculated for each cultivar by linear interpolation of the daily measurements.

In experiment II, also carried out three times (series), cultivar differences for LP were assessed. The cultivar IR66 was replaced by the highly susceptible cultivar CO39. The three series were again planted with an interval of four weeks. Per series and per cultivar, 16 pots were planted with seven germinated seeds per pot. Two blocks were formed per series, each with eight pots per cultivar. The pots were completely randomized within blocks. Nitrogen fertilizer (ammoniumsulfate) was applied in three splits. Depending on the block, either 5-5-5, or 5-10-10 g/m² N was added to each pot at leaf stage two, at leaf stage four, and on the day before inoculation. The two nitrogen levels were included because increased supply of nitrogen markedly enhances the susceptibility of plant to blast (Kwon et al., 1974; Matsuyama, 1975; Tokunaga et al., 1966). Changes in the nitrogen supply thus are likely to also affect any important component of resistance.

From the fifth leaf onwards, the point of emergence of the topmost leaf was marked every other day, enabling the calculation of the age of each leaf part by interpolation. Inoculation and actions to induce sporulation were as in experiment I. Assessments were made on a sample of four (series 1 and 2) or five pots (series 3) per cultivar for each nitrogen application. The number of sporulating lesions that became visible as small white flecks was counted once daily in series 1 and twice daily in series 2 and 3 for each marked leaf segment until the number stopped increasing. The IC_{50} was calculated for each pot by linear interpolation, as the time (hours) from inoculation until 50% of the finally observed lesions in the leaves on the main culm of the seven plants were visible. In addition, the IC_{50} was calculated per cultivar and per nitrogen level for each of the marked leaf segments differing in age. In each series, at least 20 random lesions per cultivar were sampled with agar disks to estimate the LP_{50}.

Results

Experiment I. Non-sporulating lesions, which appeared mostly as minute dark spots, were observed to develop ahead of the sporulating lesions. Sporulating lesions emerged as minute white or grey flecks. At 71 hours after inoculation, no sporulating lesions were observed at all, but at 87 hours 60% (averaged across cultivars and series), and at 111 hours nearly all of the sporulating lesions were visible. The data confirmed earlier observations on these cultivars that most sporulating type lesions appear within a short time (Roumen, 1992).

The estimated period between inoculation and the time that 50% of the lesions became visible (IC_{50}) or started sporulating (LP_{50}) differed little, if at al, between the cultivars (Table 3-1). The LP_{50} was about 4 days, ranging from 91 to 106 hours depending on series and cultivar. The average difference between the IC_{50} and LP_{50} was 13

Table 3-1. Incubation and latent periods[1] (hours) in leaves of three rice cultivars after artificial inoculation to a virulent isolate of the blast pathogen in three series of experiment I

Cultivar	Incubation period				Latent period			
	series				series			
	1	2	3	Mean[2]	1	2	3	Mean[2]
IR50	83	87	82	84	100	91	98	96
IR66	94	82	81	86	103	90	106	100
IR64	88	87	85	87	99	99	105	101
Mean[2]	88	85	83		101	93	103	

[1] Period from inoculation until 50% of the selected lesions became visible and started sporulating, respectively, estimated by interpolation of data.
[2] Means were not significantly different (α=0.05) according to the F-test.

Table 3-2. LP_{50} in hours, lesion size (LS) in mm² at LP_{50}, and sample sizes (n) for three rice cultivars (Cult.) and four categories of leaf age (days) after inoculation with a virulent isolate of the blast pathogen in three series of experiment I

Cult.	Age	Series 1			Series 2			Series 3		
		LP50	LS	n	LP50	LS	n	LP50	LS	n
IR50	0-1	98	0.9	49	89	0.5	59	99	0.6	22
	2-3	98	0.9	56	88	0.3	56	92	0.4	38
	4-5	100	0.7	51	99	0.3	22	97	0.6	28
	6-7	104	0.7	34	111	0.4	12	113	0.7	16
IR66	0-1	100	0.9	33	89	0.3	59	102	0.3	27
	2-3	107	0.7	36	87	0.1	40	107	0.4	41
	4-5	104	0.8	50	92	0.2	35	...	...	0
	6-7	103	0.8	21	98	0.3	38	...	...	0
IR64	0-1	99	0.9	50	99	0.5	39	101	0.5	33
	2-3	99	0.7	47	97	0.4	31	109	0.5	43
	4-5	100	0.7	34	...	...	0	...	...	0
	6-7	...	...	0	...	...	0	...	...	0

hours. Breakdown of the LP_{50} by leaf age showed a slight tendency for the start of sporulation of lesions to be delayed in older leaves (Table 3-2). The average size of the grey centre in the visible lesions at the LP_{50} was very small, with a range of 0.1-0.9 mm². The data indicate that lesions were capable of sporulation shortly after appearance regardless of the age of the leaf area where it develops.

Experiment II. The agar disk samples showed that, in each series of the second experiment, lesions started sporulating very shortly (less than 10 hrs) after the time that the lesions became just barely visible as small white flecks. The LP_{50} was thus almost identical to the IC_{50} that was actually assessed (Tables 3-3 and 3-4), and treatment differences for IC were similar to those for LP. Visually, no differences were observed in time of appearance of lesions between cultivars.

Adding extra nitrogen did not influence the LP. Adding more nitrogen caused an increase of the number of sporulating lesions of 55% in CO39, 66% in IR50 and 118% in IR64, averaged over the three series of the experiment, whereas the LP_{50} was decreased with less than 0.2%. Similar as in experiment 1, the IC_{50} (LP_{50}) was about 4 days (Table 3-3). In each of the series, the incubation and latent periods were shortest in IR50 and longest in IR64, the average difference between these cultivars across series being about 8 hours (significant at α=0.05). No effect of leaf age on the IC_{50} (LP_{50}) was detected in any of the cultivars (Table 3-4).

Table 3-3. Incubation period[1] (hours) in leaves of three rice cultivars after inoculation to a virulent isolate of the blast pathogen in three series of experiment II

Cultivar	Series			Mean
	1	2	3	
IR50	88 a[2]	103 a	102 a	98 a
CO39	89 a	104 a	108 ab	100 a
IR64	98 b	107 a	112 b	106 b
Mean	92	105	107	101

[1] The incubation period was calculated as the time between inoculation and the time that 50% of the sporulating type lesions became visible. The latent period was nearly the same as the incubation period.

[2] Within colums, values followed by different letters indicate significant differences between cultivars at α=0.05 according to Bonferroni's test for inequalities.

Discussion

Since spores of the blast pathogen are small (19-23 x 7-9 μm) and lack a contrasting colour (Ou, 1985), their presence on blast lesions can only be detected using a microscope, making assessment of the LP a tedious operation. Direct observation of the leaves and lesions under the microscope was done by Rodríguez and Gálvez (1975), Brodni et al. (1988), and Castaño et al. (1989), strongly limiting the number of lesions per cultivar that can be measured. Besides, this method has the disadvantage that leaves are easily damaged, which is likely to influence the measurements. Excised lesions were observed by Yorinori and Thurston (1975), but such observations may not be representative since the authors mentioned that lesion development in detached leaves differed from that in intact leaves. In comparison, the sampling technique using agar

Table 3-4. Incubation period[1] (hours) in leaves of three rice cultivars for five categories of leaf age (days) after inoculation to a virulent isolate of the blast pathogen in three series of experiment II

Age	Cultivar									Mean
	CO39 Series			IR50 Series			IR64 Series			
	1	2	3	1	2	3	1	2	3	
0-1	89	105	107	88	105	103	103	106	112	102
2-3	88	105	107	86	102	102	85	105	113	99
4-5	86	102	108	86	92	101	87	112	114	99
6-7	88	108	109	86	94	105	...	...	...	
≥8	90	...	108	...	...	...	...	...	...	

[1] The incubation period was calculated as the time between inoculation and the time that 50% of the sporulating type lesions became visible. The latent period was nearly the same as the incubation period.

disks presented here is relatively fast. Some 200 lesions can be sampled per hour. Moreover, this method causes no or hardly any leaf damage and the flexibility of the experimental setup is improved since the plants don't have to be transferred to a microscope or vice versa.

The measurements of both experiments showed that lesions that developed grey centres began sporulating very shortly after these lesions became visible. The LP of a cultivar may therefore be estimated by measuring the far easier measurable incubation period, as was done by Yeh and Bonman (1986).

In contrast with the results of the present study, considerable cultivar differences for LP have been reported by others. Rodríguez and Gálvez (1975), reported that LP ranged from 5 to 9 days depending on the cultivar. However, examination of their results revealed that the isolates used by these authors appeared to be avirulent to most of the test cultivars. The LP increased when cultivars developed a more resistant infection type, but cultivar differences were not very clear when the isolate was virulent. Comparison of the present results with those of Brodni et al. (1988) and Castaño et al. (1989) is more difficult. Brodni et al. (1988) found a five day difference for LP among seven rice cultivars, but no information on the infection type was supplied. Castaño et al. (1989), studying a group of 69 cultivars, found a difference of about five days between the cultivars with the shortest and the longest LP, but also in this study the infection type of the cultivars was not clear. The cultivars were classified as medium resistant, medium susceptible, or susceptible, using a scale that mixes qualitative with quantitative criteria. Pooled across cultivars, the LP significantly increased with a more resistant classification, but whether significant differences between cultivars within each class were also present was not determined.

Generalizing the finding that sporu-

lating type lesions are able to start sporulating soon after appearance, studies that were restricted to cultivars with a susceptible infection type indicated little or no cultivar differences for LP (Yeh and Bonman, 1986; Roumen, 1992). Silue et al. (1992) did not notice clear genetic variation for incubation period among *Oryza sativa* while finding a six hours shorter period in *Oryza glaberrima*.

The results of the present study indicate that small cultivar differences for LP exist when a susceptible infection type is induced. However, these differences (up to 8%) are too small to be of use in breeding programs and the differences for LP were not clearly related to cultivar differences of PR measured under field conditions. Moreover, the LP was not affected by increased nitrogen supply although this is well known to increase blast susceptibility (Sakurai and Toriyama, 1967; Yunoki et al., 1970 cited in: Toriyama, 1975). Also leaf age, known to affect the susceptibility in terms of lesion number (Kahn and Libby, 1958; Roumen, 1992), did not affect LP. It is concluded that the LP period is not important as a component of PR to leaf blast.

References

Bonman, J.M., B.A. Estrada and J.M. Bandong, 1989. Leaf and neck blast resistance in tropical lowland rice cultivars. Plant Disease 73:388-390.

Brodni, U., J. Castaño-Zapata and R.R. Nelson, 1988. Evaluation of rate limiting resistance to blast disease in rice cultivars using sub-lethal concentrations of a systemic fungicide. J. Phytopathology 122:174-180.

Castaño, J., D.R. Mackenzie and R.R. Nelson, 1989. Components analysis of race nonspecific resistance to blast disease of rice caused by *Pyricularia oryzae*. J. Phytopathology 127:89-99.

Ezuka, A., 1979. Breeding for and genetics of blast resistance in Japan. In: IRRI, Proceedings of the rice blast workshop, pp. 27-48. IRRI, Los Baños, Laguna, Philippines.

Jeanguyot, M., 1983. Lutte contre la pyriculariose du riz. Mémoires et Travaux de l'IRAT 2. IRAT, Nogent-sur-Marne, France, 55 pp.

Kahn, R.P. and J.L. Libby, 1958. The effect of environmental factors and plant age on the infection of rice by the blast fungus, *Piricularia oryzae*. Phytopathology 48:25-30.

Kiyosawa, S., D.J. Mackill, J.M. Bonman, Y. Tanaka, Y. and Z.Z. Ling, 1986. An attempt of classification of world's rice varieties based on reaction pattern to blast fungus strains. Bull. Natl. Inst. Agrobiol. Resour. Japan 2:13-37.

Kwon, S.H., J.M. Oh and H.S. Song, 1974. Studies on the relationship between chemical contents of rice plants and resistance to rice blast disease. Korean J. Plant Prot. 13:33-39.

Matsuyama, N., 1975. The effect of ample nitrogen fertilizer on cell-wall materials and its significance to rice blast disease. Ann. Phytopath. Soc. Japan 41:56-61.

Notteghem, J.L., 1989. La création de variétés résistantes. Cas du riz et de la pyriculariose. Bull. Soc. Bot. de France 136: Actual. Bot. 227-237.

Ou, S.H., 1985. Rice diseases. 2nd Edition. Commonwealth Mycological Institute. Kew, Surrey, England, 380 pp.

Parlevliet, J.E., 1979. Components of resistance that reduce the rate of epidemic development. Ann. Rev. Phytopathol. 17:203-222.

Parlevliet, J.E., 1983. Can horizontal resistance be recognized in the presence of vertical resistance in plants exposed to a mixture of pathogen races. Phytopathology 73:379.

Rodríguez, M. and G.E. Gálvez, 1975. Indications of partial resistance of rice to the fungus *Pyricularia oryzae* Cav.. In: G.E. Gálvez (Ed), Proceedings of the seminar on horizontal resistance of the blast disease of rice, Series CE-No. 9, pp. 137-154. CIAT, Cali, Colombia.

Roumen, E.C., 1992. Effect of leaf age on components of partial resistance in rice to leaf blast. Euphytica 63:271-279.

Sah, D.N. and J.M. Bonman, 1992. Effects of seedbed management on blast development in susceptible and partially resistant rice cultivars. J. Phytopathology 136:73-81.

Sakurai, Y. and K. Toriyama, 1967. Field resistance of the rice plant to *Piricularia oryzae* and its testing method. In: T. Ogura (Ed), Rice diseases and their control by growing resistant varieties and other measures, Proc. Symp. Tropical Agric. Researches, Sept. 1967, pp. 123-135. Ministry of Agric. and Forestry, Tokyo, Japan.

Silue, D., J.L. Notteghem and J. Chaume, 1992. Étude de la résistance spécifique d'*Oryza glaberrima* Steud. à la pyriculariose du riz. Agron. Trop. 46:121-129.

Toriyama, K., 1975. Recent progress of studies on horizontal resistance in rice breeding for blast resistance in Japan. In: G.E. Gálvez (Ed), Proceedings of the seminar on horizontal resistance of the blast disease of rice, Series CE-No. 9, pp. 65-100. CIAT, Cali, Colombia.

Tokunaga, Y., T. Katsube and Y. Koshimizu, 1966. Studies on the relationship between metabolism of rice plant and its resistance to blast disease. 3. Correlation of nitrogen metabolism and blast disease in rice plant. Bull. Tohoku Nat. Agric. Exp. Sta. Japan 34:37-79.

Yeh, W.H. and J.M. Bonman, 1986. Assessment of partial resistance to *Pyricularia* oryzae in six rice cultivars. Plant Pathology 35:319-323.

Yorinori, J.T. and H.D. Thurston, 1975. Factors which may express general resistance in rice to *Pyricularia oryzae* Cav.. In: G.E. Gálvez (Ed), Proceedings of the seminar on horizontal resistance of the blast disease of rice, Series CE-No. 9, pp. 117-136. CIAT, Cali, Colombia.

Yunoki, T., A. Ezuka, Y. Sakurai, H. Shinoda and K. Toriyama, 1970. Studies on the varietal resistance to rice blast. 3. Testing method for field resistance on young seedlings grown in greenhouse. Bull. Chugoku Agr. Exp. Sta. Japan, Series E 6:1-19.

Zadoks, J.C., 1971. Systems analysis and the dynamics of epidemics. Phytopathology 61:600-610.

Zadoks, J.C. and R.D. Schein, 1979. Epidemiology and plant disease management. Oxford University Press, U.K. 427 pp.

Small differential interactions for partial resistance in rice cultivars to virulent isolates of the blast pathogen

Summary

Six rice genotypes, differing in partial resistance, were exposed to three isolates of the blast pathogen. Of the variance due to host and pathogen genotypes, 39% was due to host genotype effects, 60% was due to isolate effects, and only 1% was due to host genotype x isolate interactions. Although small, this interaction variance was highly significant and mainly due to the IR50 x W6-1 and IR37704 x JMB8401-1 host-isolate combinations. Although behaving largely as race-non-specific (large main effects only), the partial resistance cannot be classified as race-non-specific. The results suggest that minor genes for partial resistance operate in a gene-for-gene relationship with minor genes in the pathogen.

Introduction

Blast disease of rice, caused by *Magnaporthe grisea* (anamorph *Pyricularia oryzae*) is an important disease in most of the world's rice growing areas. A high degree of control against the disease can be obtained by planting resistant cultivars. However, in only a few cases breeders have been successful in breeding cultivars with resistance that is durable. The complete resistance that is usually selected for, is controlled by hypersensitivity genes. This type of resistance is highly race-specific, and the pathogen population appeared to be able to adapt to such cultivars quite easily, often causing a quick break down of the resistance (Ezuka, 1972; Jeanguyot, 1983). Partial resistance (PR), on the other hand, is reported to be associated with durability (Ezuka, 1979; Yeh and Bonman, 1986). Unfortunately, selection for PR to blast is difficult. In the field, a mixture of races is usually present and this complicates screening for PR due to epistatic effects of hypersensitivity genes on the expression of PR (Ezuka, 1979; Notteghem, 1993). As an alternative, Niizeki (1967) and Sakurai and Toriyama (1967) recommended to screen for PR in the greenhouse using a single isolate with as many virulence factors as possible. Since PR in the temperate Japonica cultivars appeared to be largely race-non-specific (Ezuka, 1972; Yunoki et al., 1970), the same approach was followed for tropical Indica cultivars in a study by Roumen (1992).

However, what may be true for temperate Japonica cultivars is not necessarily true for tropical Indica cultivars. It is by no means certain that using a single virulent race for screening of PR is representative for all virulent races. Therefore, the research described in this paper aimed at detecting interactions between virulent pathogen races and rice genotypes for PR to leaf blast.

Material and methods

The rice genotypes CO39, IR36,

IR37704-98-3-2-2, IR50, IR64 and IR66 were used, representing a range of PR to leaf blast (Roumen, 1992). In the remainder of this paper, IR37704-98-3-2-2 is abbreviated to IR37704. The pathogen isolates used were Po6-6, JMB8401-1 and W6-1. These isolates can be considered distinct races since they induce a differential reaction on certain rice genotypes (Table 4-1). However, on the rice genotypes used in the present study, these isolates produced a susceptible infection type.

Three consecutive experiments were planted, with an interval of three weeks between each experiment. Plants were grown in plastic trays (24 x 30 cm) in a greenhouse. In total, 18 trays were sown per experiment. Per tray, six rows of 10 plants were sown with the six rice genotypes randomized over the rows.

Table 4-1. Infection type[1] of seven selected rice genotypes after inoculation at the sixth leaf stage with three *Magnaporthe grisea* isolates

Rice	Isolate		
Genotype	JMB8401-1	W6-1	Po6-6
Surjamkuhi	6	0	0
Azucena	3	3	5
Lubang	2	1	5
Tres Meses	0	1	5
Malos	0	3	5
Kuroka	0	5	6
C22	0	6	6

[1] 6: large spindle shaped sporulating lesions without dark margin; 5: large spindle shaped sporulating lesions with dark margin; 4: small spindle shaped sporulating lesions with dark margin; 3: more or less round, small sporulating lesions with dark margin; 2: more or less round, brown, non-sporulating lesions; 1: tiny pinpoint size dark non-sporulating lesions; 0: no visible symptoms

Plants were grown under non-flooded conditions. When the plants reached the second leaf stage, nitrogen was applied at an equivalent of 5 g/m^2 by adding an ammoniumsulfate solution to each tray. The same amount of nitrogen was applied when the plants reached the fourth leaf stage and again one day before inoculation. The plants were inoculated when most of the plants had reached the sixth leaf stage. In the morning of the day of inoculation, the point of emergence of the youngest leaf was marked with a felt-tip pen. Six (random) trays were inoculated per isolate.

Spores of each isolate were produced by culturing the isolates on a medium in petri dishes, similar as described by Bonman et al. (1986). JMB8401-1 and W6-1 were grown on rice polish agar (20 g rice polish, 5 g saccharose and 18 g bacto-agar per litre distilled water, with a pH set to 6), but Po6-6 was grown on prune agar, since this isolate sporulates much better on this medium (3 pieces of prunes, 1 g yeast extract, 5 g bacto-agar per litre distilled water, with a pH set to 6). Inoculation was done shortly before sunset inside a plastic cage (one cage per isolate). Per batch of six trays, 400 ml of a solution with 5 x 10^4 spores/ml in 0.5 % gelatine was sprayed over the plants as a fine mist using an electric sprayer. After inoculation, all trays were placed together in another plastic cage to ensure a high humidity until the next morning. The trays were then returned to the greenhouse.

Six days after inoculation, the number of sporulating lesions in the leaves

of the main culm was counted, and the total number of sporulating lesions and the number of leaves with at least one sporulating lesion were calculated for each plant.

Statistical analysis (ANOVA) was done using the mean values of each plant row as experimental unit. The experiments were analyzed as a split plot design with trays as main plots and rows as sub plots. For the analysis, GENSTAT was used on a VAX mainframe computer. Since the number of sporulating lesions that is formed was previously shown to be the most important component of PR in these rice genotypes (Roumen, 1992), interactions for the number of lesions were assumed to be representative for interactions for PR.

Results

The capacity to induce sporulating lesions on the tested rice genotypes differed remarkably between the pathogen isolates. Isolate W6-1 caused the largest number of lesions on the genotypes in all three experiments, which was about five times more than the number of lesions resulting from inoculation with isolate JMB8401-1 (Table 4-2).

The host genotype effects on lesion number were very similar for the three isolates, despite the large difference for their aggressiveness (Table 4-3). Nearly all variation associated with host-pathogen effects was explained by main effects of isolates and genotypes (60 and 39% respectively), while only 1% was due to host genotype x isolate interactions (Table 4-4). Most of the small, but highly significant genotype x isolate interaction was traced back to the IR50 x W6-1 and IR37704 x JMB8401-1 combinations (Table 4-3).

Table 4-2. The average number of sporulating lesions on leaves of the main culm for three *Magnaporthe grisea* isolates in three experiments, relative (%) to the mean value of isolate W6-1

Isolate	Experiments			Mean
	1	2	3	
W6-1	100 a[1]	100 a	100 a	100 a
Po6-6	24 b	78 a	31 a	44 b
JMB8401-1	17 b	23 b	23 b	21 c

[1] Within experiments, each value is the mean across six genotypes. 100%=8.4, 9.3, and 11.4 lesions/plant for experiment 1, 2, and 3, respectively.

Within columns, values followed by different letter are significantly different (Bonferroni's test for inequalities; α=0.05).

Furthermore, the ANOVA indicated a very small genotype x experiment interaction. This interaction appeared to be entirely derived from a significantly ($P \leq 0.05$) lower number of sporulating lesions on IR50 in series 2 than in series 1 and 3, regardless of isolate.

The number of sporulating lesions that developed on the genotypes appeared to be closely associated to the average number of leaves on the main culm (from the top) with at least a single sporulating lesion. Regardless of isolate, the rank correlation coefficient between the two parameters was 0.92.

The average number of leaves per plant (from the top) with at least one sporulating lesion significantly differed between the isolates ($P \leq 0.05$), indicating that some isolates have a greater capacity to successfully infect relatively old leaf tissue than others (Table 4-5). Although treatment differences were

Table 4-3. Average number of sporulating lesions in leaves of the main culm of six rice genotypes, relative to the value for CO39 for three isolates of *Magnaporthe grisea* in three experiments (Exp.)

Genotype	Isolate Po6-6				Isolate W6-1				Isolate JMB8401-1				Mean
	Exp.				Exp.				Exp.				across
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	isolates
CO39	100	100	100	100	100	100	100	100	100	100	100	100	100 a[3]
IR50	40	31	44	38	55	38	52	48[1]	38	22	39	33	40 b
IR37704	38	36	41	38	30	40	40	37	22	16	25	21[2]	32 b
IR66	19	23	19	20	30	24	19	24	21	14	19	18	21 c
IR36	10	11	16	12	14	14	9	12	13	11	17	14	14 d
IR64	7	8	7	7	10	10	6	9	5	4	10	6	7 e

[1] Significantly higher ($P \leq 0.01$) than the expected value of 37 without genetic interaction.
[2] Significantly lower ($P \leq 0.01$) than the expected value of 37 without genetic interaction.
[3] Values followed by different letters are significantly different (Bonferroni's test for inequalities; $\alpha = 0.5$).

Table 4-4. Combined ANOVA over three experiments for the number of sporulating lesions per plant using the mean plant value per row as experimental unit after log(x+1) transformation of the data

Source of variation	df	SS	MS	F	
Experiments	2	9.26	4.63		
Isolates	2	71.10	35.55	95.8	$P \leq 0.001$
Main-plot residual	49	18.18	0.37		
Host genotype	5	113.71	22.74	300.3	$P \leq 0.001$
Isolates x Host genotypes	10	5.29	0.53	7.0	$P \leq 0.01$
Experiments x Host genotypes	10	1.84	0.18	2.4	$P \leq 0.05$
Sub-plot residual	242	18.33	0.08		
Grand total	320	237.72			
Estimated grand mean	1.430				
Total df	324				
Number of missing values	3				

less clear when expressed in number of leaves than in number of lesions, counting the number of leaves with lesions still detected the relatively small interaction between rice line IR37704 and isolate JMB8401-1 (Table 4-5).

Discussion

The result that most of the variation for PR between the rice genotypes was of a race-non-specific nature is in agreement with the general outcome of studies on interactions between rice genotypes and blast isolates for PR that were carried out in Japan (Sakurai and Toriyama, 1967; Kozaka, 1975; Ezuka, 1972, citing Asaga and Yoshimura, 1969; Toriyama, 1975, citing Niizeki, 1967, and Hirano and Matsumoto, 1971). Some exceptionally large genotype x isolate interactions for PR were observed by Yunoki et al. (1970), caused by the presence of a single gene, named Pi-f (Toriyama et al., 1968). Bonman et al. (1989) also observed a relatively large genotype x isolate interaction for PR, perhaps due to a gene similar to the Pi-f gene. In their study, Philippine isolates were far less aggressive on Korean rice cultivars than were Korean isolates, but all isolates were equally aggressive on the Indian cultivar CO39. In the present study, despite the considerable difference in aggressiveness between the isolates, the genotype ranking for PR did hardly change. Regardless of the race, PR was highest in IR64 and IR36, and screening for PR using just one of these three races would not have changed results of selection. The PR expressed in the rice genotypes was in good agreement with that in an earlier study (Roumen, 1992).

Table 4-5. Average number of leaves on the main culm[1] with at least one sporulating lesion for six rice genotypes after inoculation with three isolates of *Magnaporthe grisea*, relative to the value of CO39 (=100%)

Genotype	Isolate			Mean
	Po6-6	W6-1	JMB8401-1	
CO39	100	100	100	100 a[2]
IR50	74	71	72	72 b
IR37704	78	78	56[3]	71 b
IR66	71	66	51	63 b
IR36	42	37	41	40 c
IR64	42	31	26	33 c
100%=	1.6 p^2	2.0 q	1.2 r	(leaves/plant)

[1] Mean of three experiments
[2] Values followed by different letters are significantly different (Bonferroni's test for inequalities at $\alpha=0.05$).
[3] Significantly lower ($P \leq 0.05$) than the expected value of 72 in case of no genetic interaction.

Considerable difference for aggressiveness among isolates was also observed in a field experiment where six defined isolates were tested to 15 rice genotypes in isolated field plots in Japan (Central Agricultural Experiment Station, 1970; cited in Ezuka, 1972). However, part of the difference in aggressiveness between the isolates in the present study could well be the result of some effect of the media on which the isolates were cultured. Some isolates are known to perform better on some media than on others (Otsuka et al., 1965) and this might influence the ability of the harvested spores to infect rice leaves.

The result that small, but significant interactions between rice genotypes and blast isolates were detected despite a relatively small sample size,

indicates that such interactions are not uncommon. Genetic studies on other cultivars have shown PR to be oligo- or polygenically controlled with small effects for each gene (Higashi and Kushibuchi, 1978; Higashi and Saito, 1985; Notteghem, 1985), and among the cultivars used in the present study, evidence for oligo- or polygenic control of PR was found for IR36 and IR64 (Roumen, 1993). Therefore, the small interactions suggest that PR to leaf blast in these cultivars is controlled by minor genes which operate in a gene-for-gene relationship with minor genes in the isolates of the blast pathogen, similar as described by Parlevliet (1978) for PR genes to leaf rust in barley. As shown in a model study of Parlevliet and Zadoks (1977) and confirmed by an extensive study of this model using simulation (Jenns and Leonard, 1985), a gene-for-gene relationship in a polygenic system with relatively small effects of each gene would largely behave as race-nonspecific. Assuming a polygene for polygene relationship, the significant interactions for IR50 and IR37704 suggest that these cultivars each contain at least one PR gene that is not present in any of the other tested cultivars.

The high correlation between the number of sporulating lesions and the number of leaves on the main culm developing such lesions regardless of isolate and despite large differences in aggressiveness between the isolates, strongly supports earlier findings that a relatively high PR in a genotype is closely associated to a rapid increase of resistance with aging of the newly emerging leaves to high resistance levels (Roumen et al., 1992. Roumen, 1992).

References

Asaga, K. and S. Yoshimura, 1969. Relation between field resistance of rice varieties to blast and the races or strains of the causal fungus. Ann. Phytopath. Soc. Japan 35:385-386. (in Japanese)

Bonman, J.M., T.I. Vergel de Dios and M.M. Khin, 1986. Physiologic specialization of *Pyricularia oryzae* in the Philippines. Plant Disease 70:767-769.

Bonman, J.M., J.M. Bandong, Y.H. Lee, E.J. Lee and B. Valent, 1989. Race specific partial resistance to blast in temperate Japonica rice cultivars. Plant Disease 73:496-499.

Central Agricultural Experiment Station. 1970. Yearbook 1969, pp. 32-35. (in Japanese)

Ezuka, A., 1972. Field resistance of rice varieties to blast disease. Rev. Plant Protec. Res. 5:1-20.

Ezuka, A., 1979. Breeding for and genetics of blast resistance in Japan. In: IRRI, Proceedings of the rice blast workshop, pp. 27-48. Los Baños, Laguna, Philippines.

Higashi, T. and K. Kushibuchi, 1978. Genetic analysis of field resistance to leaf blast (*Pyricularia oryzae*) in rice. Jap. J. Breeding 28:277-286.

Higashi, T. and S. Saito, 1985. Linkage group of field resistance genes of upland rice variety Sensho to leaf blast caused by *Pyricularia oryzae*. Jap. J. Breeding 35:438-448.

Hirano, T. and K. Matsumoto, 1971. Resistance of the rice varieties to several C races of blast fungus. I. Studies on variation of field resistance by races and fungus strains. Jap. J. Breeding 21:110-111. (in Japanese)

Jeanguyot, M., 1983. Rice blast and its control. Mémoires et travaux de L'IRAT no. 3. IRAT, Nogent-sur-Marne, France. 53 pp.

Jenns, A.E. and K.J. Leonard, 1985. Reliability of statistical analyses for estimating relative specificity in quantitative resistance in a model host-pathogen system. Theor. Appl. Genet. 69:503-513.

Kozaka, T., 1975. Recent advances in studies on horizontal resistance to blast disease of rice in Japan. In: G.E. Gálvez (Ed), Seminar on horizontal resistance of the blast disease of rice. Series CE-9, pp. 101-116. CIAT, Cali, Colombia.

Niizeki, H., 1967. On some problems in rice breeding for blast resistance, with special reference to variation of blast fungus. Recent Adv. Breeding 8:71-78. (in Japanese)

Notteghem, J.L., 1985. Définition d'une stratégie d'utilisation de la résistance par analyse génétique des relations hôte-parasite. Cas du couple riz-*Pyricularia oryzae*. Agron. Trop. 40:129-147.

Notteghem, J.L., 1993. Durable resistance to rice blast disease. In: Th. Jacobs and J.E. Parlevliet (Eds), Durability of disease resistance, pp. 125-134. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Otsuka, H., K. Tamari and N. Ogasawara, 1965. Variability of *Piricularia oryzae* in culture. In: IRRI, Proceedings symposium on rice blast disease, pp. 69-109. John Hopkins Press, Baltimore, Maryland, U.S.A.

Parlevliet, J.E., 1978. Race-specific aspects of polygenic resistance of barley to leaf rust, *Puccinia hordei*. Neth. J. Pl. Path. 84:121-126.

Parlevliet, J.E., and J.C. Zadoks, 1977. The integrated concept of disease resistance; A new view including horizontal and vertical resistance in plants. Euphytica 26:5-21.

Roumen, E.C., 1992. Effect of leaf age on components of partial resistance in rice to leaf blast. Euphytica 63:271-279.

Roumen, E.C, 1993. Inheritance of host plant effect on the relative infection efficiency of *Magnaporthe grisea* in rice cultivars. (submitted).

Roumen, E.C., J.M. Bonman and J.E. Parlevliet, 1992. Leaf-age related partial resistance to *Pyricularia oryzae* in tropical lowland rice cultivars as measured by the number of sporulating lesions. Phytopathology 82:1414-1417

Sakurai, Y. and K. Toriyama, 1967. Field resistance of the rice plant to *Piricularia oryzae* and its testing method. In: T. Ogura (Ed), Rice diseases and their control by growing resistant varieties and other measures. Proc. Symp. Tropical Agriculture Researches. Sept. 1967, pp. 123-135. Ministry of Agriculture and Forestry, Tokyo, Japan.

Toriyama, K., 1975. Recent progress of studies on horizontal resistance in rice breeding for blast resistance in Japan. In: G.E. Gálvez (Ed), Seminar on horizontal resistance of the blast disease of rice. Series CE-9, pp. 65-100. CIAT, Cali, Colombia.

Toriyama, K., T. Yunoki, and H. Shinoda, 1968. Breeding rice varieties for resistance to blast. II. Inheritance of high field resistance of Chugoku No. 31. Jap. J. Breeding 18:145-146.

Yeh, W.H. and J.M. Bonman, 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathology 35:319-323.

Yunoki, T., A. Ezuka, T. Morinaka, Y. Sakurai, H. Shinoda and K. Toriyama, 1970. Studies on the varietal resistance to rice blast. 4. Variation of field resistance due to fungus strains. Bull. Chugoku Agr. Expt. Sta. Japan, Series E 6:21-41.

Partial resistance to neck blast influenced by stage of panicle development and rice genotype

Summary

Neck nodes of eight rice genotypes were inoculated with a virulent isolate of the blast pathogen at four slightly different, increasingly older stages of panicle development shortly after flowering. Resistance to infection as well as resistance to growth of the pathogen after infection was assessed. Significant differences between genotypes were found for both components of resistance. Resistance to growth of the pathogen sharply increased with aging of the neck node as was indicated by a large reduction of the length of the lesions on the culm, but resistance to infection did not change much. The lesion length was closely related to yield loss. A small delay in time of infection can lead to a large decrease in yield reduction. Meaningful comparison of partial resistance to neck blast between genotypes therefore requires infection at an identical stage of panicle development. In the field, where panicles are in different stages of development, selection of genotypes that do show the symptoms of neck blast together with relatively little yield loss in infected panicles is recommended.

Introduction

Blast disease of rice (*Oryza sativa* L.), caused by *Magnaporth grisea* (Hebert) M.E. Barr (anamorph *Pyricularia oryzae*), is geographically wide spread and can cause serious damage to the crop. Two phases are commonly recognized: leaf blast and panicle blast (Jeanguyot, 1983; Ou, 1985). Infection of leaves can cause damage to the rice crop by reducing the green leaf area and in severe cases an entire planting can be destroyed. However, infection of panicles usually is economically more important, as panicle infection can directly cause severe yield losses.

Blast is a typical disease of young plant tissues. Within cultivars, after inoculation with virulent isolates, partial resistance (PR) in newly formed leaves quickly increases with aging of the leaf (Kahn and Libby, 1958; Goto et al., 1961; Notteghem and Andriatompo, 1979). Across cultivars, a shorter period during which newly formed leaves remain susceptible to infection was found to be closely associated with higher levels of PR (Roumen et al., 1992, Roumen, 1992).

Increase of resistance with aging also occurs in panicles and neck nodes (Amin, 1983; Willis et al., 1968; Shindo and Asaga, 1989). Neck nodes are particularly susceptible to infection, and since the neck node is at the panicle base, a single infection can result in the complete loss of the panicle. Neck blast is therefore considered a very serious form of the disease.

Similar to what has been found for leaves, the increase of PR (and perhaps also of tolerance) with aging of the neck node might differ between rice genotypes. If resistance and/or tol-

erance to neck node infection builds up very quickly, a genotype should develop less neck blast and thus suffer less yield loss in the field compared to other genotypes grown under the same environmental conditions. The experiments reported here aimed therefore at assessing PR in neck nodes of various ages in different rice genotypes.

Materials and methods

The research was conducted at the International Rice Research Institute (IRRI). Two trials were carried out, the first in a greenhouse and the second in a screenhouse. Six rice cultivars and two breeding lines were studied (Table 5-1). The breeding lines IR35546-17-3-1-3 and IR37704-98-3-2-2 are abbreviated as IR35546 and IR37704. Pathogen isolate Po6-6 was used for inoculations as this isolate induced a typical susceptible infection type on the leaves of each of these genotypes in previous tests. Isolates that are virulent to leaves, are nearly always also virulent to necks (Shindo and Asaga, 1989)

Plants were raised in plastic pots of 20 cm diameter containing a clay soil. The soil was not puddled and water could drain easily. Seeds were germinated in petri dishes. A few days after germination, each pot was planted with four or five germinated seeds. The pots were then placed in water filled beds. In each trial, 22 pots were planted per genotype, except for the susceptible check IR50, of which two sets of 22 pots were planted, seven days apart. The planting schedule was timed in such way that all genotypes were expected to flower at about the same time.

The genotypes were randomized in four blocks with five pots per entry and one block with two pots per entry. Within the blocks, the pots of the same genotype were kept together. Nitrogen fertilizer was applied in three splits of 5 g/m^2 equivalents each as a solution of ammoniumsulfate. The first split was applied when the plants had three to four leaves, the second during the tillering phase, and the third when the flag leaves emerged.

The neck nodes were inoculated at different ages using the time of flowering of the panicle as a standard. The day at which 30 to 50% of the spikelets of a panicle flowered was marked as day 0. Neck nodes were either inoculated at day 0, 2, 4 or 6, or received a control treatment. The day of flowering and the intended inoculation treatment was marked on a tag.

Inoculation was done shortly before sunset. Tags were cut in half to avoid double inoculations. The inoculation method differed between the trials. In the greenhouse trial, a small piece of cotton-wool was placed around each neck node at day 0 with the top edge barely covering the node. The cotton-wool was then loosely covered with a piece of aluminum foil to hold it in place and to retain the moisture after inoculation. Inoculum was applied using a syringe by dropping 3 ml of a suspension adjusted to 5 x 10^4 conidia /ml against the culm on the cotton-wool. For each of the 20 pots per entry, equal numbers of panicles were randomly assigned to each of the four inoculation treatments, while the neck node of one randomly chosen panicle was inoculated with just water on day 0 (control). The aluminum foil and cotton-wool were removed about two

weeks after inoculation. For two pots per entry, the panicle neck nodes were not prepared with cotton-wool or aluminum foil, but inoculated with just water to study the effect of the methodology on grain yield.

In the screenhouse trial, the panicles were distributed over the control and inoculation treatments in the same manner as in the greenhouse trial. Inoculation occurred by placing a drop of a sticky spore solution to the neck node containing 4% carboxylmethylcellulose and 5 x 10^4 conidia/ml. The same solution without spores was applied to control panicles on day 0. The screenhouse bed was covered with a plastic sheet during the nights to prevent drying out of the inoculum. No difference in plant reaction between the two inoculation methods was detected in a separate test using IR50 (Roumen, unpublished data).

The infection rate was calculated as the percentage of inoculated neck nodes developing a sporulating lesion. The number of filled, half filled and empty seeds was counted per panicle and the seed weight was measured excluding the hulls of empty seeds. The percentage non-empty grain and its 1000 seed weight were calculated for each panicle. In the greenhouse trial, at the time of harvest, the lesion length was measured as the length of the disease symptoms from the neck node downwards (mm) and the diameter of each neck node was measured with a callipers. In the screenhouse trial, the distinction between healthy and diseased tissue was unclear. Most of the neck nodes and culms with blast symptoms, but not those without, also showed symptoms of infection by what seemed to be mostly saprophytic organisms. Thus, only the presence or absence of neck node infection was scored. The development of the saprophytes following infection by *M. grisea* was probably the result of the fact that the plants in the screenhouse often remained wet during long periods. The measurements of neck node diameter in the first trial were used to check whether the diameter influenced the disease development and/or the damage due to the disease. Neck node diameter varied considerably both between and within genotypes (data not shown). However, analysis showed that, both within and between genotypes, neck node diameter varied completely independent from the variation in infection rate and the variation in percentage filled grain and grain weight.

Preliminary analysis showed that the placement of cotton-wool and aluminum foil in the greenhouse trial caused a yield reduction in itself (7% averaged over genotypes), due to an increase of empty grains. Preliminary analysis also showed that neck nodes that were inoculated with the pathogen, but remained free of symptoms, could be regarded as necks that were inoculated with just water. Results of such panicles were therefore added to the control group in the final analysis of yield loss. The number of panicles per plant differed between genotypes, and treatment means were usually based on a varying number of observations. For measurements on infected panicles, the number of observations for a treatment mean also depended on the infection rate. As an indication of the sample size within each of the two trials, about 90% of the treatment means for infected panicles were based on more than 30 observations

while about 25% were based on more than 50 observations. In statistical analyses of treatment means, differences for the number of observations were ignored.

Results

All genotypes developed typical neck blast symptoms. In both trials, symptoms appeared between 7 and 9 days after inoculation, without apparent differences between genotypes. The initial symptoms looked very similar to the initial symptoms of sporulating blast lesions in leaves. A small grey spot developed, rarely longer than 2 mm, sometimes with a thin black margin. Unlike in leaves, the grey colour disappeared within 2 to 3 days, and the growing lesions became red-brown. In some cases, mostly when infection occurred at day 0, the panicle dried out completely within a few hours after the first symptoms were visible. This gave the panicle a typical white appearance and always resulted in 100% yield loss.

Varying with genotype and time of inoculation, the infection rate ranged from 45 to 93% in the first and from 25 to 96% in the second trial (Table 5-1). A small part (less than 3%) of the control panicles also developed neck blast. This was probably caused by secondary infection since the symptoms developed much later than 10 days after flowering and remained slight. In these panicles no yield loss was observed.

In some cases, such as for IR36,

Table 5-1. Percentage panicle neck nodes developing blast symptoms after inoculation at either 0, 2, 4, or 6 days after flowering for eight rice genotypes in two trials

Genotype	Trial 1					Trial 2					Mean of 2 trials
	0	2	4	6	Mean	0	2	4	6	Mean	
IR37704	80	80	71	79	77	84	96	94	90	91	84 a[2]
IR62	63	65	72	66	67	85	92	79	85	85	76 ab
IR35546	73	75	84	77	77	82	78	69	52	70	74 ab
IR50[1] 1	91	77	74	72	78	72	71	74	68	71	72 ab
2	93	81	69	60	76	71	69	56	52	62	
IR36	85	56	52	52	61	76	91	80	76	81	71 ab
IR60	64	70	73	67	69	70	80	72	54	69	69 ab
IR64	86	70	62	60	70	57	70	65	44	59	64 bc
IR66	52	45	59	60	54	25	33	45	46	37	46 c
Mean	74 a[2]	68 a	68 a	66 a	69	69 a	76 a	71 a	63 a	70	

[1] IR50 was planted twice, the second planting one week after the first. [2] Treatment means followed by different letters are significantly different. (Bonferroni's test for inequalities was used at $\alpha=0.05$)

Table 5-2. Analysis of variance of the infection rate (%) of the neck node after inoculation at different times shortly after flowering for eight rice genotypes in two trials

Analysis of variance					
Source	df	SS	MS	F-ratio	P
Trial	1	14.1	14.1	0.09	n.s
Genotype	7	7057.9	1008.3	6.7	≤0.005
Inoculation time	3	540.4	180.1	1.2	n.s
Genotype x Inoc. time	21	1588.9	75.7	0.5	n.s
Error	31	4689.7	151.3		

C.V. = 18%

IR50 and IR64 in the first trial and for IR35546 in the second trial, the infection rate decreased if neck nodes were inoculated later after flowering (Table 5-1). However, the results for the two trials were inconsistent and the ANOVA showed there was no significant effect of the time of inoculation on the infection rate for any of the genotypes (Table 5-2). In some genotypes, the average infection rate differed considerably between the trials:

Table 5-3. Length (mm) of blast lesions on the culm after inoculation of the neck node at different days shortly after flowering for eight rice genotypes

Genotype	Days				Mean
	0	2	4	6	
IR37704	37 a[2]	26 b	18 b	18 b	24.8 p[3]
IR35546	23 a	25 a	20 a	25 a	23.3 pq
IR64	32 a	21 b	18 b	17 b	22.0 pqr
IR36	31 a	20 ab	15 b	15 b	20.3 pqr
IR60	32 a	17 b	13 b	16 b	19.5 qr
IR50[1] 1	33 a	15 b	11 b	10 b	17.3 rs
2	26 a	19 ab	13 bc	10 c	17.0 rs
IR62	13 a	7 a	11 ab	5 b	9.0 s
IR66	8 a	8 a	5 a	6 a	6.8 s
Mean	26.1	17.6	13.8	13.6	

[1] IR50 was planted twice in each experiment, the second planting one week after the first.

[2] *Different letters within rows indicate significant difference between treatments* (Kruskal-Wallis test; comparison of multiple treatments at $\alpha=0.05$)

[3] Means followed by different letters indicate significant differences among genotypes (Friedman's test at $\alpha=0.05$)

in IR36 and IR62, the average infection rate in the second trial was much higher than in the first, while the reverse was true for IR64 and IR66. Despite the large fluctuations, the infection rate significantly differed between genotypes, mainly because the infection rate in IR66 was considerably lower than in most other genotypes (Table 5-1). Its mean infection rate across the trials was only 55% of that in IR37704.

In general, a later time of infection resulted in a clear reduction of lesion length (Table 5-3). For most genotypes, lesion length was reduced about 50% when infection occurred at day 4 instead of at day 0. Relatively small lesions developed in IR62 and IR66 regardless of the time of infection. In IR35546, lesion length was relatively small when infection occurred at the time of flowering, but longer than in any of the other genotypes when infection occurred on day four or day six.

Visual impression was that the average lesion length was much larger in the screenhouse- than in the greenhouse trial for all genotypes. The time of flowering in the screenhouse trial coincided with a dark, rainy period with relatively cool temperatures, favourable for blast development.

Infection significantly reduced the percentage filled grains. The degree of the reduction was strongly influenced by the time of inoculation (Table 5-4). Except in a few cases, the percentage filled grains was smallest when inocu-

Table 5-4. Percentage filled[1] grains in infected panicles of eight rice genotypes after inoculation of the neck node with the blast pathogen at different days shortly after flowering relative to uninfected controls

Genotype	Trial 1				Trial 2				Mean of 2 trials				Control
	0	2	4	6	0	2	4	6	0	2	4	6	
IR37704	51	84	91	97	55	81	83	94	53	82	87	95	100
IR50[2] 1	59	90	98	100	56	75	85	98	54	78	90	92	100
2	56	79	96	94	45	69	79	73					
IR35546	65	69	81	75	56	77	77	74	61	73	79	74	100
IR60	72	94	95	90	55	66	73	92	63	80	84	91	100
IR36	67	90	93	91	69	69	79	94	67	79	86	92	100
IR64	73	93	97	93	65	59	73	71	69	76	85	82	100
IR62	84	94	101	98	62	73	81	91	73	84	91	95	100
IR66	106	109	112	118	72	81	87	96	89	95	99	107	100
Mean	70	89	96	95	59	72	78	87	66 d[3]	81 c	88 bc	91 b	100 a

[1] Including seeds which were partly filled
[2] IR50 was planted twice, the second planting one week after the first.
[3] Treatment means followed by different letters are significantly different (Bonferroni's test for inequalities (α=0.05) was used after arcsin$\sqrt{x}$ transformation of the data)

lation was done at the time of flowering and increased the later infection had occurred. Sometimes, even a delay of only two days resulted in a sharp increase of the percentage filled grains, particularly when inoculation was delayed from day 0 to day 2. Meanwhile, the reduction in infected panicles was considerably larger in the second than in the first trial, especially when infection occurred at the time of flowering. This indicates that the effect of neck node infection on the percentage filled grains can vary considerably with environmental conditions.

The effect of infection also depended on genotype. In both trials, the reduction of the percentage filled grains in IR50 and IR35546 was larger than in IR66 at any time of inoculation ($P \leq 0.05$). In general, the differences between genotypes were largest when infection occurred at flowering and diminished when the interval between flowering and inoculation increased. However, due to a large variation between panicles within the genotypes, none of the differences between genotypes for a particular day of inoculation were significant, not even for inoculation at day 0. Unlike for most other genotypes, the reduction of the percentage filled grains in IR35546 diminished relatively little with longer intervals between flowering and inoculation, and the reduction remained considerable even after inoculation at day 6. This suggests that some genotypes are prone to yield loss due to a reduced percentage filled grain for a

Table 5-5. Average weight of non-empty grains in infected panicles of eight rice genotypes after inoculation of the neck node with the blast pathogen at different days shortly after flowering relative to uninfected controls

Genotype	Trial 1				Trial 2				Mean of 2 trials				Control
	0	2	4	6	0	2	4	6	0	2	4	6	
IR37704	68	89	92	97	62	71	81	96	65 m[2]	81 m	87 m	94	100
IR35546	82	89	94	93	65	82	88	95	74 m	86 m	91	94	100
IR50[1] 1	82	97	98	100	72	84	89	94	77	89	95	97	100
2	81	94	100	96	71	81	92	97					
IR36	78	91	97	96	80	81	85	91	79	86	91	94	100
IR60	95	100	101	100	79	89	94	98	81	91	95	97	100
IR64	86	95	98	98	85	84	85	88	85	90	92	93	100
IR62	95	100	101	100	79	89	94	98	87	94 n	97 n	99	100
IR66	98	98	100	99	94	91	88	94	96 n	95 n	94	96	100
Mean	85	95	98	98	77	84	88	94	81 d[3]	89 c	93 b	96 b	100 a

[1] IR50 was planted twice, the second planting one week after the first.

[2] For the same day of inoculation, among genotypes, means followed by -m- are significantly lower than means followed by -n-.

[3] Means of different inoculation days followed by different letters are significantly different. (Bonferroni's test for inequalities was used at $\alpha=0.05$ after arcsin$\sqrt{x}$ transformation of data).

Table 5-6. Average loss of grain yield (%) in infected panicles of eight rice genotypes after inoculation of the neck node with the blast pathogen at various days shortly after flowering relative to un-infected controls. Means of two trials

Genotype	Day of inoculation				Control
	0	2	4	6	
IR37704	65 m[1]	33	24	8	0
IR50	59	30	15	12	0
IR35546	56	38	28	30	0
IR60	49	26	19	11	0
IR36	47	31	21	13	0
IR64	41	31	22	23	0
IR62	36	20	11	7	0
IR66	14 n	10	5	-3	0
Mean	46 d[2]	27 c	18 bc	13 b	0 a
Mean of trial 1	38	15	6	7	
Mean of trial 2	53	40	30	18	

[1] For the same day of inoculation, among genotypes, means followed by -m- are significantly lower than means followed by -n-.

[2] Means of different inoculation days followed by different letters are significantly different. (α=0.05; Bonferroni's test for inequalities after arcsin$\sqrt{x}$ transformation of data).

longer period than others.

The average grain weight of the non-empty grains relative to the control showed a similar pattern as the percentage filled grains, but on the whole, the effect of infection on grain weight was smaller. The correlation coefficient (Pearson r) between the two yield components was 0.89 in trial 1 and 0.71 in trial 2. The degree of the reduction was again larger in the second trial than in the first (Table 5-5). In the first trial, hardly any reduction in grain weight (2% average over the genotypes) occurred if necks were infected at four or six days after flowering. In the second trial, the reduction was 12% after inoculation at day 4, and 6% after inoculation at day 6. Differences between genotypes were again largest when infection occurred at the time of flowering (Table 5-5).

Yield loss in infected panicles was estimated by multiplying the relative losses due to empty grains with those due to reduced grain weight. Since both yield components showed greater loss in the second trial than in the first, grain yield losses were considerably higher in the second trial (Table 5-6). In accordance with the pattern of its components, yield loss was highest when infection occurred at the time of flowering and was sometimes markedly reduced with a relatively small increase of the time between flowering and inoculation. Some genotypes con-

Table 5-7. Average loss of grain yield (%) over all inoculated panicles of eight rice genotypes after inoculation of the neck node with the blast pathogen at various days shortly after flowering relative to un-infected controls. Means of two trials

Genotype	Day of inoculation				Control
	0	2	4	6	
IR37704	59 m[1]	30	20	7	0
IR50	48	22	10	7	0
IR35546	44	29	21	20	0
IR36	38	25	21	9	0
IR60	32	21	14	7	0
IR64	29	22	14	11	0
IR62	28	18	9	5	0
IR66	3 n	3	2	-2	0
Mean	35 d[2]	21 c	14 b	8 b	0 a
Mean of trial 1	38	15	6	7	
Mean of trial 2	53	40	30	18	

[1] Mean for IR37704 was significantly lower than for IR66.
[2] Means for different days of inoculation followed by different letters are significantly different. (α=0.05; Bonferroni's test for inequalities after arcsin$\sqrt{x}$ transformation of data)

sistently suffered less yield loss than others regardless of the time of inoculation and especially after infection at the time of flowering, large differences among genotypes were observed. However, due to a very large variation of yield loss between individual panicles, the differences were not significantly different except that between IR66 and IR37704 at day 0 (Table 5-6).

Across genotypes and times of inoculation, the lesion length (measured in greenhouse trial only) showed a high linear correlation with yield loss in infected panicles relative to the control (r=0.91) and with its components grain weight and percentage filled grains (r= -0.89 and -0.88). Assuming that the lesion length was related to the size of the *M. grisea* colony in the culm, a smaller yield loss appeared to be closely associated with increase of resistance to the growth of the pathogen.

Yield loss in a field situation is not only determined by the yield reduction in infected panicles, but also by the proportion of panicles that develops neck blast. Estimated yield loss after correction for the infection rate is shown in Table 5-7. Because IR66 combined a low infection rate with a relatively low reduction of yield in infected panicles, total yield loss in IR66 was much less than in the other genotypes and was relatively small even when inoculum was applied at the time of flowering.

Discussion

No clear relation was found between the time of inoculation and the infection rate of neck nodes, indicating that aging of the neck node did not affect resistance to penetration and establishment of the pathogen much. This result seems to be in contrast to the results of Willis et al. (1968) and Amin (1983), who found that the infection rate was inversely related to the stage of panicle development at time of inoculation. The disparity, however, may be explained because of the wider range in stages of panicle development studied in their work, which was 20 days in the work of Amin (1983) and at least 10 days in the work of Willis et al. (1968), against six days in the present study.

The present data show that the infection rate at the time of flowering and shortly thereafter, may substantially differ between genotypes. Since the highest yield losses are observed if infection occurs at early stages of panicle development, a high PR to penetration and establishment of the pathogen at early stages of panicle development is a very desirable trait. Therefore, selection for a relatively low infection rate of the neck node after inoculation to virulent isolates at the time of flowering could be an efficient method to improve PR to neck blast.

Unlike resistance to infection, resistance to growth of the pathogen after its establishment rapidly increased with aging of the neck node. Moreover, the data suggest that resistance to growth of the pathogen increased faster with time in some genotypes than in others.

The length of the lesion on the culm was found to be closely associated to yield reduction. In the screenhouse trial, where lesion length was longer than in the greenhouse trial, larger yield losses occurred, and within the greenhouse trial, the lesion length and yield loss were highly correlated. To prevent yield loss, a high PR to growth of the pathogen at early stages of panicle development is probably more important than a relatively fast increase of resistance with aging, since the level of resistance at early stages of panicle development determines the potential damage that can be done by infection. Increase of PR with aging in IR50 was relatively fast, but the PR at the time of flowering was relatively low, and this genotype is relatively susceptible in the field (Bonman et al., 1989). Thus, besides selection for a low infection rate to virulent isolates, selection for relatively small lesions on the culm after infection at early stages of panicle development is expected to help curbing yield losses due to neck blast.

The result that yield loss was reduced when neck node infection occurred later after flowering is well documented (Katsube and Koshimizu, 1970; Prabhu and de Faria, 1982; Takasaki, 1988). More interesting is the finding that, due to the rapid increase in resistance to growth of the pathogen with aging of the neck node, even a two days difference for the stage of panicle development at the time of infection can have a large effect on yield reduction and the yield components grain weight and the percentage filled grains. For the grain weight, a similar result was found by Prabhu and de Faria (1982) in an upland field experiment in Brazil, in which yield loss due to reduction of the grain weight in the cultivar IAC 1246 fell from 38% to 14% when infection

was delayed only three days. Thus, even a slightly different flowering time among genotypes may seriously disturb objective evaluation of neck and/or panicle blast resistance.

Since the infection rate is expected to be directly related to the resistance to infection and the yield loss in infected panicles was closely associated to the resistance to growth of the pathogen, differences in yield loss in the present experiments (Table 5-7) may be considered to reflect to a fair extent differences in the combined effects of the partial resistance components between genotypes. Based on the yield loss after inoculation at the time of flowering (Table 5-7), PR of IR35546 was thus estimated to be similar to that of the susceptible check IR50, while PR of IR37704 was even lower. IR36, IR60, IR62 and IR64 were judged to show more PR to neck blast than IR50, but much less than IR66, which was considered by far the most resistant genotype.

Six of the genotypes tested in this study were also evaluated in a field study by Bonman et al. (1989). In this field study, resistance to neck blast was measured using a scale developed by Ahn and Mukelar (1986) and PR was assessed as the mean percentage severe neck blast over five trials. The agreement for ranking of PR of the genotypes between the studies was reasonably high ($r_s = 0.71$) considering the differences in disease assessment, and the large impact of environmental influences on expression of PR in general. However, a remarkable discrepancy was observed for IR37704. In sharp contrast with the present results, PR of IR37704 in the field was significantly higher than that of IR50 and was close to that of IR36 or IR66. This discrepancy might be due to escape in the field. In the present study, neck nodes and panicles in IR37704 reached a much higher position above the flag leaf than in the other genotypes. In a field situation, a rapid extrusion of the neck node to a relatively high position above the flag leaf may help avoiding infection by increasing the distance between the panicle and the other plant parts. Moreover, the micro-environmental conditions for neck nodes that stick out far above the flag leaf are less likely to be favourable for infection than for neck nodes remaining close to the canopy. Since avoidance mechanisms are considered durable (Parlevliet, 1981), it may be useful to study effects of the position of the neck node and the panicle on occurrence of neck blast in more detail.

Since proper assessment of PR to neck blast is much more laborious than assessment of PR to leaf blast, the relation between PR to neck blast and PR to leaf blast is of special interest. Field experiments indicated that PR to neck- and to leaf blast do not necessarily correspond, although for most genotypes the reactions show good agreement (Jeanguyot, 1983; Bonman et al., 1989; Shindo and Asaga, 1989). In field studies, contrasting behaviour between the two plant parts may be attributed partly to differences in flowering time between genotypes interacting with variations in the environmental conditions, and to differences in the pathogen population between the leaf stage and the panicle stage. In the present study, variation in the development stages of the panicle was greatly reduced by inoculating individual necks of known stage of development. Using the same isolate, PR to leaf blast was measured in detail

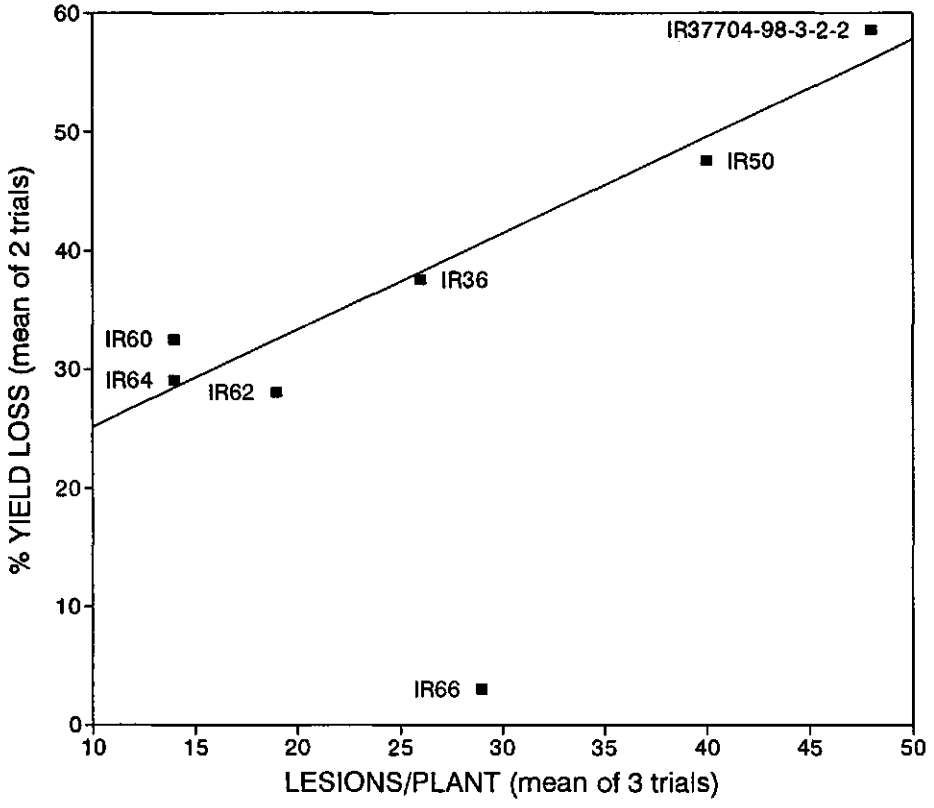

Fig. 5-1. Relation between yield loss due to neck blast after infection with isolate Po6-6 at the time of flowering and partial resistance to leaf blast to this isolate for seven rice genotypes

for all genotypes except for IR35546 (Roumen, 1992). Comparison of the PR to leaf blast with the yield loss that occurred when panicles were inoculated at the time of flowering, indicated that PR to neck blast closely corresponded with PR to leaf blast, except for IR66 (Fig. 5-1). In this genotype, PR to neck blast was much higher than expected based on its PR to leaf blast. A similar result was obtained for this genotype in field experiments (Bonman et al., 1989). The reaction of IR66 strongly supports the opinion that PR to neck blast can not always be predicted from PR to leaf blast. According to Shindo and Asaga (1989), in genotypes with relatively low PR to leaf blast in the seedling stage, but with relatively high PR to neck blast, the reaction of leaves became consistent with that of the neck node only in the upper 2-3 leaves of the plant including the flag leaf. PR to neck blast was also reported to be more closely related to PR to leaf blast in adult plants than in seedlings by Hwang et al. (1987). Unlike in the field experiments of Bon-

man et al. (1989), after artificial inoculation, PR to neck blast in IR37704 was in good agreement with its PR to leaf blast (Fig. 5-1), supporting the explanation that the relatively low neck blast in this genotype in the field may have been caused by escape.

When the pathogen population consists of a mixture of races, the effective inoculum dose may differ between genotypes if they carry different race-specific major genes. In the field, a low incidence of neck blast in a genotype may then be the result of a low frequency of the race(s) with the matching virulence gene(s) and not of a high PR to infection (Parlevliet, 1983). The disturbing effects of race-specific major genes can be illustrated with an example from the field study of Bonman et al. (1989), in which line IR35546-52-3-3-2 was among the most resistant entries out of 27 lines, while its sister line IR35546-17-3-1-3 was the most susceptible entry of all. Therefore, although a low infection rate is a very desirable trait, strong selection pressure for low incidence of neck blast under field conditions is unlikely to be efficient for improving PR as it will tend to select major genes.

Since the yield loss in panicles with neck blast strongly depends on the time of infection, monitoring the appearance of neck blast symptoms regularly after flowering may help explain why a certain genotype suffers yield loss in one experiment but not in another. Regularly monitoring the appearance of the symptoms would also enable identification of genotypes that develop relatively short lesions on the culm and that suffer relatively limited yield loss in infected panicles despite infection early after flowering.

References

Ahn, S.W. and A. Mukelar, 1986. Rice blast management under upland conditions. In: F. Shideler and C.V. Mendoza (Eds), Progress in Upland Rice Research, pp. 363-374. IRRI, Manila, Philippines.

Amin, S.M., 1983. Neck rot infection in rice: varietal resistance, panicle phenology and yield reduction. Mardi Res. Bull. 11:79-83.

Bonman, J.M., B.A. Estrada and J.M. Bandong, 1989. Leaf and neck blast resistance in tropical lowland rice cultivars. Plant Disease 73:388-390.

Goto, K., K. Hirano and K. Ohata, 1961. Susceptibility of leaf of rice to blast in relation to leaf age and position. 1. Variation of susceptibility among different leaf positions and grades of emergence of the top leaf. Spec. Bull. Okayama. Pref. Agric. Exp. Stn. Japan 58:77-88.

Hwang, B.K., Y.J. Koh and H.S. Chung, 1987. Effects of adult plant resistance on blast severity and yield of rice. Plant Disease 71:1035-1038.

Jeanguyot, M., 1983. Lutte contre la pyriculariose du riz. Mémoires et Travaux de l' IRAT no. 2. IRAT, Nogent-sur-Marne, France. 55 pp.

Kahn, R.P. and J.L. Libby, 1958. The effect of environmental factors and plant age on the infection of rice by the blast fungus, *Piricularia oryzae*. Phytopathology 48:25-30.

Katsube, T. and Y. Koshimizu, 1970. Influence of blast disease on harvests of rice plants. 1. Effect of panicle infection on yield components and quality. Bull. Tohoku Natl. Agric. Exp. Stn. Japan 39:55-96.

Notteghem J.L. and G.M. Andriatompo, 1979. Étude de quelques facteurs de la résistance horizontale du riz à la pyriculariose. Agron. Trop. 34:180-195.

Ou, S.H., 1985. Rice diseases. 2nd edition. *Commonwealth Mycological Institute*. Kew, Surrey, England. 380 pp.

Parlevliet, J.E., 1981. Race-non-specific disease resistance. In: J.F. Jenkyn and R.T. Plumb (Eds), Strategies for the control of cereal disease, pp. 47-54. Blackwell Scient. Publ.. Oxford, U.K.

Parlevliet, J.E., 1983. Can horizontal resistance be recognized in the presence of vertical resistance in plants exposed to a mixture of pathogen races ? Phytopathology 73:379.

Prabhu, A.S. and J.C. de Faria, 1982. Relacionamentos quantitativos entre brusone nas folhas e paniculas e seus efeitos sobre enchimento e peso dos graos em arroz de sequeiro. Pes. Agopec. Bras. 17:219-223.

Roumen, E.C., 1992. Effect of leaf age on components of partial resistance in rice to leaf blast. Euphytica 63:271-279.

Roumen, E.C., J.M. Bonman and J.E. Parlevliet, 1992. Leaf-age related partial resistance to *Pyricularia oryzae* in tropical lowland rice cultivars as measured by the number of sporulating lesions. Phytopathology 82:1414-1417.

Shindo, K. and K. Asaga, 1989. Studies on new method to evaluate panicle resistance of rice varieties to rice blast. Bull. Tohoku Natl. Agric. Exp. Stn. Japan 80:1-51.

Takasaki, T., 1988. Relationship between infection time of neck node blast in rice plant and symptoms or yield reduction by the disease. Bull. Fukuoka Agric. Res. Cent. Japan, Series A 8:23-28.

Willis, G.W., R.D. Allowitz and E.S. Menvielle, 1968. Differential susceptibility of rice leaves and panicles to *P. oryzae*. Phytopathology 58:1072. (abstract)

Inheritance of host plant effect on the relative infection efficiency of *Magnaporthe grisea* in rice cultivars

Summary

The inheritance of the host plant effect on the relative infection efficiency for leaf blast was studied in the crosses IR36/CO39 (partially resistant x highly susceptible) and IR36/IR64 (both partially resistant). Gene action appeared multiplicative on the natural scale. After log transformation, additive effects described most of the genetic variation in the cross IR36/CO39. Additive and dominance effects were about equal in magnitude in the cross IR36/IR64. Dominance was towards increased resistance. No transgression occurred in the cross IR36/CO39. The number of genes that reduce lesion number was estimated to be none in CO39 and five or more in IR36. The cross IR36/IR64 showed transgression in both directions, and IR36 and IR64 each contain at least one gene that is not present in the other cultivar. The heritabilities (narrow sense) in the F_2 were low (range 0.06-0.16). Heritabilities based on F_3 lines were much higher (range 0.41-0.68). Lesion numbers in F_3 lines were reasonably correlated with those in F_5 progenies derived from the same F_2 plant (r was $\pm$ 0.6 in both crosses). Partial resistance can be effectively improved by selecting the most resistant plants from the most resistant F_3 lines.

Introduction

Magnaporthe grisea, causing blast disease of rice, occurs in nearly all rice growing areas (Ou, 1985). The disease can seriously damage the crop, but effective control is possible by planting resistant cultivars. Highly resistant cultivars can be obtained with relative ease by selecting genotypes with effective hypersensitivity genes. Unfortunately, the resistance of such cultivars usually breaks down soon after the cultivars are released (Ahn and Mukelar, 1986; Ezuka, 1972). On the other hand, in some traditional and improved cultivars that develop lesions of a typical susceptible type, the progress of the disease remains limited and these cultivars are rarely damaged. The resistance in these cultivars, called partial resistance (Parlevliet and van Ommeren, 1975,) has remained effective for many years and seems to be durable *sensu* Johnson (1984). A problem is that the expression of partial resistance (PR), unlike that of the hypersensitivity resistance, is strongly influenced by environmental conditions, complicating its selection, especially in the early generations of a breeding cycle when replicated testing is often not possible.

With more knowledge of the inheritance of PR, the efficiency of selection in segregating generations could be improved. The most important component of PR to leaf blast in rice is the relative infection efficiency (RIE), measured as the number of sporulating lesions that develops (Yunoki et al., 1970; Notteghem, 1985; Roumen, 1992a), and it is this component that was studied to get more insight into

the genetics of PR in this pathosystem.

Materials and methods

Plants of the crosses IR36/CO39 and IR36/IR64 were studied. CO39 is highly susceptible (Mackill et al., 1988; Roumen, 1992a) and is assumed to carry few, if any, genes that hamper the formation of sporulating lesions (hereafter called lesions). Genetic analysis of PR using molecular techniques in a cross between this cultivar and the resistant cultivar Moroberekan did not show any PR enhancing factors in CO39 (Wang et al., 1993). The PR of IR36 is sufficiently high to prevent losses due to blast under normal growing conditions, and has lasted although IR36 has been grown on a wide scale for many years (Yeh and Bonman, 1986; Bonman and Mackill, 1988). The PR of IR64 resembles that of IR36 in field tests (Bonman et al., 1989; Sah and Bonman, 1992). In the greenhouse, IR64 develops a slightly lower number of lesions than IR36, but their size is larger (Roumen, 1992a and 1992b). This suggests that the PR in these cultivars is controlled by partly different genes.

Over 250 F_2 plants were grown per cross, all derived from the same F_1 plant. F_3 lines were grown from 50 F_2 plants taken at random. Of a randomly selected F_3 plant per line, five F_4 plants were grown. The seed of four of these F_4 plants was harvested to produce 50 groups of four F_5 lines (hereafter called F_5 groups).

For the evaluation of the inheritance, reserve seed of the 50 F_3 lines and seed of the 50 F_5 groups was sown together with seed of the parents and of the F_1. This was done in two trials per cross. Each of the 25 F_2 progenies per trial consisted of ten F_3 plants and ten plants of each of the four F_5 lines, which were grown in a completely randomized design together with 10 F_1 plants, and 20 plants of each parent. The trials of the cross IR36/IR64 included 20 CO39 plants as a check. The plants were grown in a greenhouse in plastic trays (30 x 24 x 10cm) in six rows of five plants. All generations were sown directly in the trays, except the F_1, which was sown in petri dishes and planted a few days after germination. Nitrogen fertilizer and water were applied as previously described (Roumen, 1992a). The plants were inoculated with the virulent isolate Po6-6 inside a plastic cage (2 x 2m) when most plants had six leaves on the main culm. Per m^2 cage floor area, 400 ml of a conidia suspension with 5×10^4 conidia /ml in 0.5% gelatine was evenly sprayed over the plants as a fine mist shortly before sunset. The plants were kept in the cage at 100% humidity overnight and returned to the greenhouse the next morning. The number of lesions in each leaf on the main culm was counted six days after inoculation and their total number was calculated per plant.

Preliminary analysis indicated that gene effects (number of lesions) were multiplicative on the natural scale. Therefore, genetic parameters were estimated after log transforming the data using the function $\ln(x+1)$, with x being the number of lesions per plant relative to the mean number for CO39. Genetic analysis was done assuming an additive-dominance genetic model. The midparent value (m), and the additive ([d]), and dominance ([h]) effects in each cross were estimated by

weighed least squares analysis of the generation means across the two trials, using the reciprocal of the variance of the means as weight (Mather and Jinks, 1982). For estimation of the heritability, a separate ANOVA was performed per trial for the F_3 and F_5 generations. The phenotypic variance components of the F_3 and F_5 were estimated from the equations of their expected mean squares (Table 6-1). In addition, the covariance between the F_3 lines and the corresponding F_5 groups was calculated. The expected contributions of the additive variance (D), the dominance variance (H), and the environmental variance (E) to these phenotypic variances and the covariance are listed in Table 6-2 (Mather and Jinks, 1982). The environmental variance within each trial was estimated solving the equations of the residual variances within the F_3 and F_5 lines. The estimates were substituted in the equations, and the additive variance was then estimated as the average of the solution for each equation ignoring dominance variance. Disregarding the dominance variance is unlikely to bias the estimate of the additive variance much, because the parameters for H in most of the equations were much smaller than those for D (Table 6-2). No estimates were made for H. Heritabilities were calculated as the expected additive variance divided by the phenotypic variance.

The frequency of off-spring with parental values occurring in the F_3 and F_5 was used for estimating the number of effective factors controlling the RIE in the cross IR36/CO39. Assuming that all favourable alleles are in the more resistant parent (IR36), and with -k- factors segregating, the chance of finding at least one F_3 line, F_5 group, or F_5 line with the genotype of the susceptible parent among 50 progenies is $1-(1-P)^{50}$, with P being the chance of recovering this genotype in a single F_3 line, F_5 group, or in at least one F_5 line within a single F_5 group. For an F_3 line, P is approximately $(¼)^k$, the chance that a random F_2 plant is like the susceptible parent. The additional chance that the susceptible genotype is

Table 6-1. Degrees of freedom (df) and expected mean squares (EMS) for the ANOVAs of the F_3 and F_5 generations

Generation	Source	df	EMS
F_3	Lines	24	$\sigma^2F3_2 + 10\sigma^2F3_1$
	Residual	225	σ^2F3_2
F_5	Groups	24	$\sigma^2F5_3 + 10\sigma^2F5_2 + 40\sigma^2F5_1$
	Lines within Groups	75	$\sigma^2F5_3 + 10\sigma^2F5_2$
	Residual	900	σ^2F5_3

σ^2F3_1 = variance between means of F3 lines
σ^2F3_2 = variance within F3 lines
σ^2F5_1 = variance between F5 groups
σ^2F5_2 = variance between means of F5 lines within groups
σ^2F5_3 = variance within F5 lines

Table 6-2. Expected contributions of the additive (D), dominance (H) and environmental (E) variances to phenotypic variances and a covariance realized in trials of two rice crosses assuming an additive-dominance genetic model

Variance component		Expected contributions of the additive, dominance and the environmental variances						
$10\sigma^2F3_1$	=	5.25	D	+	0.25	H	+	E
σ^2F3_2	=	0.25	D	+	0.125	H	+	E
$40\sigma^2F5_1$	=	31.3125	D	+	0.65625	H	+	E
$10\sigma^2F5_2$	=	1.3125	D	+	0.1875	H	+	E
σ^2F5_3	=	0.0625	D	+	0.03125	H	+	E
COV(F3,F5)	=	0.5	D	+	0.015625	H		

σ^2F3_1 = variance between means of F_3 lines
σ^2F3_2 = variance within F_3 lines
σ^2F5_1 = variance between means of F_5 groups
σ^2F5_2 = variance between means of F_5 lines within groups
σ^2F5_3 = variance within F_5 lines
COV(F3,F5) = covariance between means of F3 lines and the corresponding F5 groups

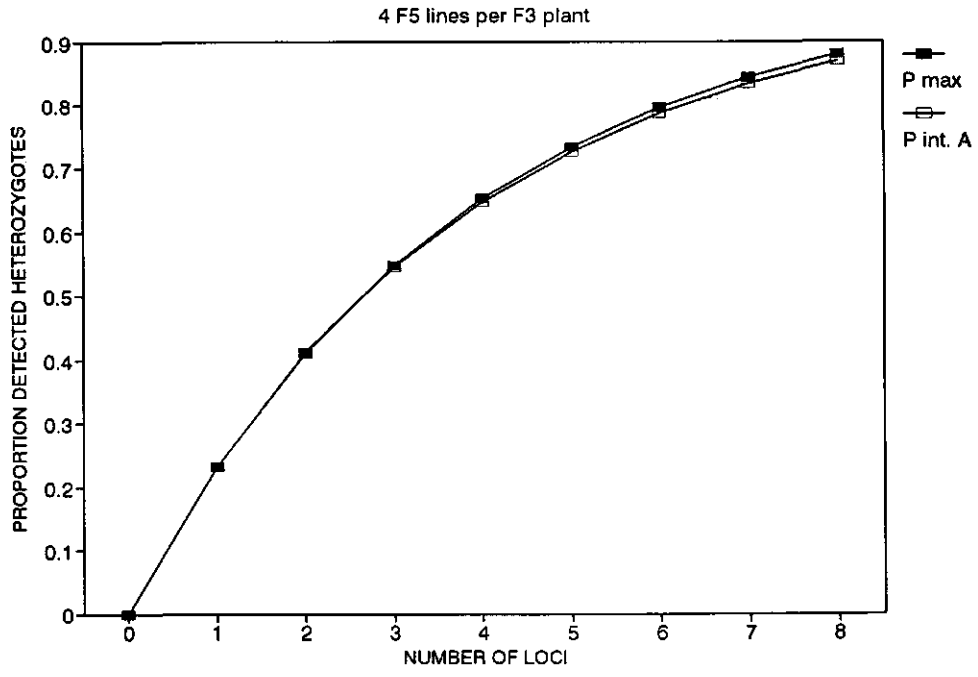

Fig. 6-1. The relation between the proportion of heterozygous F_3 plants and the number of segregating loci as estimated by P_{max} and $P_{int.A}$ (Towey and Jinks, 1977) when four F_3-derived F_5 progenies are tested.

recovered in all 10 F_3 plants when the F_2 plant is heterozygous, is negligible. Likewise, P is approximately $(3/8)^k$ for a F_5 group, i.e. the chance that the susceptible parent is recovered in a random F_3 plant. Finally, P is approximately

$$\sum_{s=0}^{k} \binom{k}{s} \cdot (3/8)^{k-s} \cdot (1/4)^s \cdot (1-(1-1/4^s)^n)$$

for the F_5 lines within a group, with s being the number of loci for which the F_3 plant was heterozygous and n=4 since four F_4 plants were grown per F_3 plant.

Estimates for the number of segregating loci in both crosses were obtained from $(\Sigma d_a)^2/D$, the squared sum of the additive gene effects divided by the additive variance (Mather and Jinks, 1982). For the cross IR36/CO39, the squared sum of the additive gene effects was estimated as $[d]^2$, and as the square of half the difference between the parents, i.e. $1/4 \cdot (P_1-P_2)^2$. For the cross IR36/IR64, it was estimated as the square of half the range that was observed among the F_5 lines, $1/4 \cdot (F_5max-F_5min)^2$. Estimates were also obtained by genotype assay (Jinks and Towey, 1976; Towey and Jinks, 1977). The differences between the line means within the 50 F_3-derived F_5 groups were evaluated by t-tests based on the residual variance of each ANOVA for making an estimate of the proportion of heterozygous F_3 plants in each cross. The estimated proportion was then related to the number of effective factors using the equations for Pmax and for Pint.A as proposed by Towey and Jinks (1977) and Mulitze and Baker (1985a). The number of effective factors as estimated by these equations when four F_3-derived F_5 lines are evaluated, is shown in Fig. 6-1.

Table 6-3. Relative number of lesions in the parents and in the F_1, F_3 and F_5 generations of the crosses IR36/CO39 and IR36/IR64 (mean of two trials per cross)

	Cross IR36/CO39	Cross IR36/IR64
IR64	...	7.7
IR36	10.6	13.4
F_1	31.1	6.6
F_3	28.3	10.4
F_5	31.6	11.0
CO39	100.0	100.0

Results

Gene action. The number of lesions increased with the advance of generations in the cross IR36/IR64, indicating dominance of resistance, but no increasing trend was observed in the cross IR36/CO39 (Table 6-3). In both crosses, the F_5 generation means were close to the (midparent) value that was expected when gene action is multiplicative without epistasis (Table 6-3). The scaling tests indicated that the inheritance in both crosses was satisfactorily explained by additive and dominance effects; Chi square values for models without epistatic interaction were low (Table 6-4). The pooled dominance effects were towards higher resistance. As expected from the generation means, gene action in the cross IR36/CO39 was mostly additive. The dominance effects in this cross were not significant. In the cross IR36/IR64, additive and dominance effects were both significant and were

Table 6-4. Estimates for the midparent value (m), and the pooled additive ([d]) and dominance ([h]) effects for two crosses. The estimates were calculated by joint scaling tests using log transformed data and assuming just additive (model 1) or additive and dominant gene effects (model 2). The goodness of fit (X^2;df) and the significance (P) of these models are included

	Cross IR36/CO39		Cross IR36/IR64	
	model 1	model 2	model 1	model 2
m	3.04	3.08	2.04	2.07
[d]	1.41	1.38	0.27	0.30
[h]		0.43		0.35
X^2	1.93	1.60	2.61	0.18
df	3	2	3	2
P	0.50-0.75	0.25-0.50	0.25-0.50	0.90-0.95

Table 6-5. ANOVA summaries of the F_3 and F_5 generations after log transformation of the data, for two trials of the crosses IR36/CO39 and IR36/IR64

Cross	Source		df	MS	F	significance
IR36/CO39 trial 1	F_3	Line	24	3.70	2.256	P≤0.01
		Residual	224	1.64		
	F_5	Group	24	12.96	8.596	P≤0.01
		Line/Group	75	2.17	1.439	P≤0.05
		Residual	900	1.51		
IR36/CO39 trial 2	F_3	Line	24	1.86	3.183	P≤0.01
		Residual	224	0.58		
	F_5	Group	24	5.14	9.584	P≤0.01
		Line/Group	75	0.66	1.241	n.s.
		Residual	894	0.54		

Table 6-5. ANOVA summaries... continued

Cross	Source		df	MS	F	significance
IR36/IR64 trial 1	F_3	Line	24	2.61	4.173	P≤0.01
		Residual	224	0.62		
	F_5	Group	24	7.27	12.840	P≤0.01
		Line/Group	75	1.25	2.203	P≤0.01
		Residual	896	0.57		
IR36/IR64 trial 2	F_3	Line	24	2.04	1.607	P≤0.05
		Residual	225	1.27		
	F_5	Group	24	6.64	5.960	P≤0.01
		Line/Group	75	1.57	1.408	P≤0.05
		Residual	897	1.11		

about equal in magnitude (Table 6-4). A subsequent scaling test assuming complete dominance in this cross, showed a very good fit of expected and realized generation means (X^2_{3df} =0.23; P≥0.995).

Heritability. The ANOVAs for each trial are shown in Table 6-5. Conform expectation, the phenotypic variance within F_5 lines was smaller than that within F_3 lines in both trials of each cross, whereas the variance between F_2-derived lines was larger in the F_5 than that in the F_3. The values for E, estimated from the residual variances of the F_3 and F_5, and the values for D, obtained after substituting the estimates for E in the equations of Table 6-2, are shown in Table 6-6. The heritabilities for individual plants in the F_2 calculated from these estimates were 0.08 and 0.12 for the trials 1 and 2 of the cross IR36/CO39, and 0.16 and 0.06 for those of the cross IR36/IR64. The corresponding heritabilities for F_3 lines were 0.47 and 0.58 in the cross IR36/CO39, and 0.68 and 0.41 in the cross IR36/IR64.

Transgression. The distribution of the F_3 and F_5 line means of the cross IR36/CO39 was centred around the expected mid-parent value of 32.5% (=number of lesions relative to CO39), and nearly all means were

Table 6-6. Estimates for the additive (D) and the environmental (E) variance in two trials of two rice crosses

Variance component	Cross	
	IR36/CO39	IR36/IR64
Trial 1		
D	0.25	0.21
E	1.46	0.55
Trial 2		
D	0.14	0.14
E	0.52	1.06

between those of the parents (Fig. 6-2). The means of the F_3 lines and those of the F_5 groups that were derived from the same F_2 plant were reasonably well correlated (r=0.62). None of the progeny lines developed as many lesions as CO39. A small percentage of the F_3 and F_5 lines developed fewer lesions than IR36 (Fig. 6-2), but except for one (0.5%) of the F_5 lines, the differences between these lines and IR36 were not significant (LSD; $P \leq 0.05$). Because no clear transgression occurred, all genes for a reduced RIE that segregated in this cross are apparently from IR36.

Within the F_3 and the F_5 of the cross IR36/IR64, many lines developed either less lesions than IR64 or more than IR36 (Fig. 6-2). A lower number of lesions in the F_3 was again correlated with a lower number of lesions in the corresponding F_5 group derived from the same F_2 plant (r=0.61). The percentage of lines with means outside the parental range was 76% for the F_3, and 71% for the F_5. Most of the differences between these lines and IR36 or IR64 were not significant. However, two (4%) of the F_3, and twelve (6%) of the F_5 line means were significantly lower than that of IR64. In addition, one (2%) of the F_3, and ten (5%) of the F_5 line means were significantly higher than that of IR36 (LSD; $P \leq 0.05$). Because a significant proportion of the F_5 lines showed significant transgression towards lower or higher resistance, IR36 and IR64 each have at least one gene controlling the RIE that is not present in the other cultivar.

Estimates for the number of effective factors. Because the phenotype of CO39 was not recovered in any progeny line of the cross IR36/CO39, five or more independently segregating genes that reduce the RIE are probably present in IR36. If four loci were segregating in this cross, the probability of retrieving at least one F_5 line with the phenotype of CO39 would be 0.97. Based on the value of 1.41 for [d] (Table 6-3) and the average value of 0.195 for D (Table 6-6), the minimum number of effective factors in IR36 is estimated at 9 or 10. Based on the difference between the parents, the minimum number is 7 or 8. The genotype assay indicated that in the F_3, 40% of the plants were heterozygous, since twenty F_5 groups contained lines with significantly different means (LSD; $P \leq 0.05$). For this proportion, the number of effective factors is between 2 and 3 (Fig 6-1).

The transgression that occurred in both directions in the cross IR36/IR64, rules out the possibility that all the favourable alleles were concentrated in either of the parents. No estimate for the number of segregating loci was thus calculated from [d] or the difference between the parents and the additive variance. Based on the phenotypic range of the F_5 lines and the average additive variance of the two trials, the number of segregating loci was estimated to be 10 or 11. The percentage F_3 plants in this cross that was declared heterozygous by genotype assay was 48%, and the estimate for the number of segregating loci in IR36/IR64 by this method was thus between 2 and 3 (Fig. 6-1).

Discussion

Because most of the gene action in the cross IR36/CO39 on the log transformed scale appeared additive,

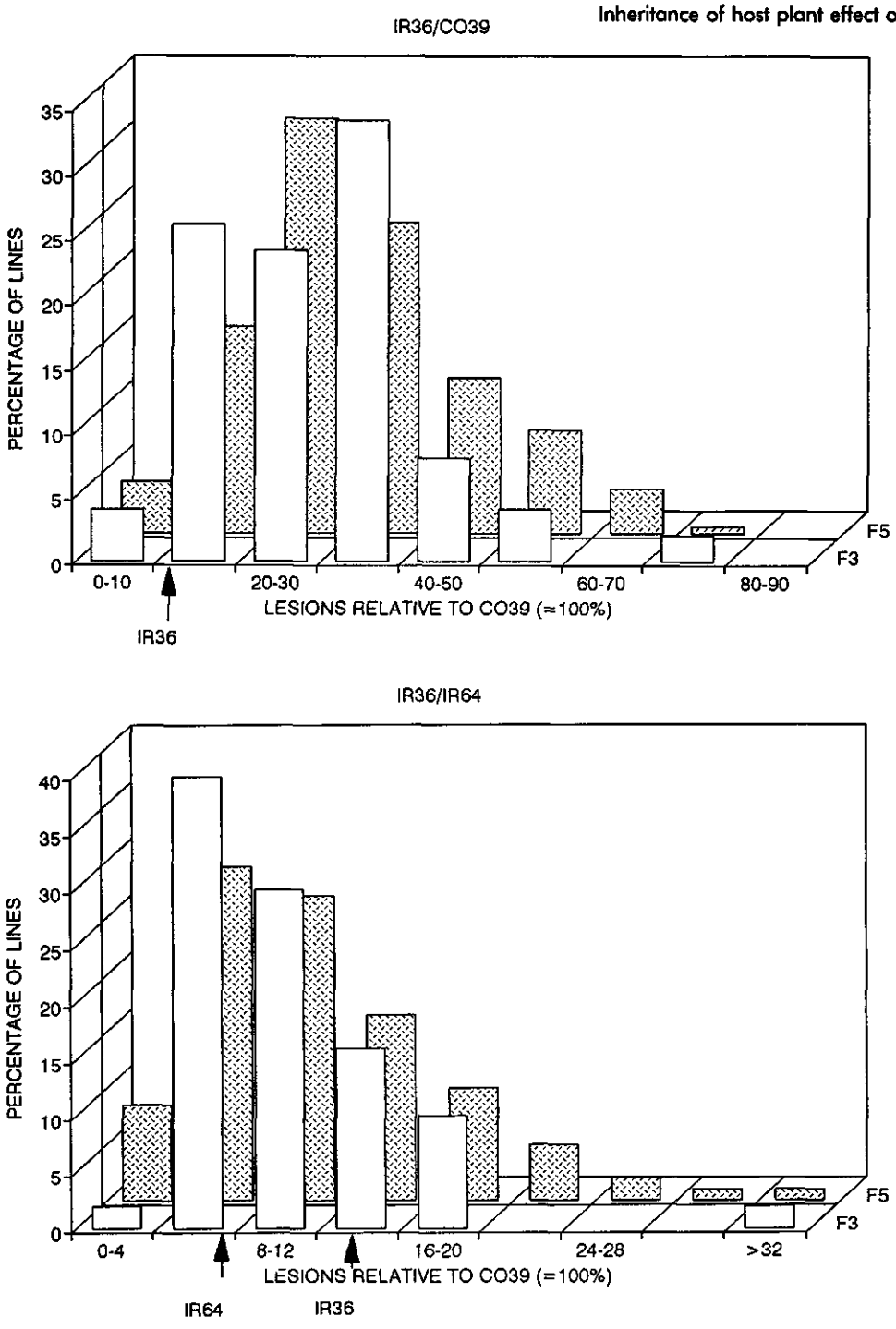

Fig. 6-2. Distribution of the average RIE of F_3 and F_5 lines derived from 50 random F_2 plants in the crosses IR36/CO39 and IR36/IR64

finding several progeny lines with a phenotype that was lower than or similar to that of IR36, but finding none that was as high as that of CO39, was somewhat unexpected (Fig. 6-2). The discrepancy can be explained by the decreasing absolute gains that occur for each additional gene in a multiplicative acting genetic system. For a RIE of IR36 that is 10% that of CO39, that is under control of five genes with equal effects, and assuming no RIE-reducing genes in CO39, the presence of each of these genes is expected to multiply the RIE with the factor 0.63. The expected RIE in a pure line from the cross IR36/CO39 with one gene is then 63% that of CO39 (37% difference to CO39), and in a line with four genes, it is 16% that of CO39 (6% difference to IR36). With an error of 6 to 10%, lines with 4 genes could develop a similar or even slightly lower number of lesions than the resistant parent, whereas lines with one gene would not develop as many lesions as CO39.

The result that the inheritance of the RIE can be described by an additive-dominance genetic model, is in agreement with that of Lin (1986), and Notteghem et al. (1981) who also investigated the inheritance of this component of PR to leaf blast. However, Wang et al. (1989) whose study included backcrosses, reported some epistatic interaction among loci was likely. As in the present study, Lin (1986) and Wang et al. (1989) found the pooled dominance effects to be directed towards increased resistance, suggesting that most non-additive genes for a reduced RIE have a dominant expression. However, Notteghem et al. (1981) found that the number of lesions in the F_1 in a diallel of five cultivars was on average 41% more than that in the parents, and concluded that dominance and recessiveness of genes affecting the RIE occurred in about the same frequency.

In agreement with results of most other inheritance studies of PR to leaf blast (Higashi and Kushibuchi, 1978; Higashi and Saito, 1985; Wang et al., 1993), the present study indicates that PR to leaf blast is controlled by several genes. In the present study, the estimate for the number of segregating loci in the cross IR36/CO39 that is based on the proportion of the offspring with a parental phenotype is considered the most reliable, because this estimate just assumes mendelian segregation of genes and, unlike the other estimates, it is not sensitive to alterations of the scale (Mather and Jinks, 1982). Since no clear transgression was observed in the cross IR36/CO39, all segregating loci were probably derived from IR36. This cultivar is concluded to contain at least five genes that reduce the RIE. Supporting the result of Wang et al. (1993), none were detected in CO39. However, the possibility that CO39 carries RIE-reducing genes cannot be entirely excluded. One of the F_5 lines from the cross IR36/CO39 developed significantly fewer lesions than IR36, and, of course, CO39 and IR36 may have one or more RIE-reducing genes in common. The conclusion that IR36 contains at least five RIE reducing genes is supported by the estimate of the sum of the squared additive gene effects divided by the additive variance, but the estimate from the genotype assay method seems to indicate the contrary. Mulitze and Baker (1985a and 1985b) showed that estimates based on genotype assay depend on the number of plants that is assessed and on the

magnitudes of the type I and type II errors in the statistical tests. High type I errors cause an upward bias of the estimate, whereas low heritabilities mean large type II errors, which cause a downward bias of the estimate. For single plant based heritabilities as low as found in the present study, a considerable downward bias of the estimate by genotype assay is expected, even though differences between line means were evaluated at a relatively high type I error rate (Mulitze and Baker, 1985b). Evaluating differences among four line means by LSD tests at $\alpha=0.05$ is equivalent to performing a F-test at $\alpha=0.30$.

The transgression in the cross IR36/IR64 showed that IR64 and IR36 each have at least one gene that is not present in the other cultivar. However, it is very unlikely that the RIE of IR64 is controlled by just one gene. If so, this gene would have a very large effect (-92% compared to CO39). Assuming that IR64 without this hypothetical gene would have a phenotype as CO39, about half of the F_5 lines (lacking this gene) should have developed more lesions than IR36 and the distribution of these lines would be expected to show a peak near 32.5% of the number of lesions of CO39 (Fig. 6-2). The biometric estimates support the presence of at least as many segregating loci in the cross IR36/IR64 as in IR36/CO39. This suggests that a considerable amount of genetic variation exists among cultivars with a good agronomic performance, and breeding for higher PR to leaf blast should thus be possible without having to step back in terms of yield or other agronomically important traits.

Based on the heritability estimates, selection of individual plants in the F_2 is unlikely to be efficient for improving PR. However, combining plant and line selection in early generations, such as selection of the best plants within the better F_3 lines, is expected to be reasonably efficient, since the heritability of F_3 lines was reasonably high in both crosses. In addition, a reasonably high correlation between the F_3 line means and the F_5 group means derived from the same F_2 plant was observed in both crosses. Because the RIE is controlled by several genes, selection in early generations will be necessary for efficiently accumulating RIE reducing genes into new cultivars in breeding programs.

References

Ahn, S.W. and Mukelar, A., 1986. Rice blast management under upland conditions. In: F.J. Shideler (Ed), Progress in upland rice research, Proceedings of the 1985 Jakarta conference, pp. 363-374. IRRI, Philippines.

Bonman J.M. and D.J. Mackill, 1988. Durable resistance to rice blast disease. Oryza 25:103-110.

Bonman, J.M., B.A. Estrada and J.M. Bandong, 1989. Leaf and neck blast resistance in tropical lowland rice cultivars. Plant Disease 73:388-390.

Ezuka, A., 1972. Field resistance of rice varieties to blast disease. Rev. Plant Prot. Res. 5:1-21.

Higashi, T. and K. Kushibuchi, 1978. Genetic analysis of field resistance to leaf blast (*Pyricularia oryzae*) in rice. Japan. J. Breeding 28:277-286.

Higashi, T. and S. Saito, 1985. Linkage groups of field resistance genes of upland rice variety Senshou to leaf blast caused by *Pyricularia oryzae* Cav. Jap. J. Breeding 35:438-448.

Jinks, J.L. and P. Towey, 1976. Estimating the number of genes in a polygenic system by genotype assay. Heredity 37:69-81.

Johnson, R., 1984. A critical analysis of durable resistance. Ann. Rev. Phytopathol. 22:309-330.

Lin, S.C., 1986. Genetic analysis of minor gene resistance to blast in Japonica rice. In: IRRI, Rice Genetics, Proceedings of the International Rice Genetics Symposium 27-31 May 1985, pp. 451-469. Island Publishing House Inc., Manila, Philippines.

Mackill, D.J., J.M. Bonman, P.D. Tenorio and T.I. Vergel de Dios, 1988. Near-isogenic Indica rice lines with blast resistance genes. Rice Genetics Newsletter 5:98-101.

Mather, K. and J.L. Jinks, 1982. Biometrical genetics, The study of continuous variation, Third edition. Chapman and Hall Ltd. New York, London. 396 pp.

Mulitze, D.K. and R.J. Baker, 1985a. Evaluation of biometrical methods for estimating the number of genes. 1. Effect of sample size. Theor. Appl. Genet. 69:553-558.

Mulitze, D.K. and R.J. Baker, 1985b. Evaluation of biometrical methods for estimating the number of genes. 2. Effect of type I and type II statistical errors. Theor. Appl. Genet. 69:559-566.

Notteghem, J.L., M. Chatel and R. Dechanet, 1981. Analyse diallele de deux caracteristiques de la résistance du riz à *Pyricularia oryzae*. In: Symposium sur la résistance du riz à la pyriculariose. pp. 313-330. IRAT-GERDAT, Montpellier, France.

Notteghem, J.L., 1985. Définition d'une stratégie d'utilisation de la résistance par analyse génétique des relations hôte-parasite. Cas du couple riz-*Pyricularia oryzae*. Agron. Trop. 40:129-147.

Ou, S.H., 1985. Rice diseases. 2nd edition. Commonwealth Mycological Institute, Surrey, England. 380 pp.

Parlevliet, J.E., and A. van Ommeren, 1975. Partial resistance of barley to barley leaf rust, *Puccinia hordei*. II. Relationship between field trials, microplot tests and latent period. Euphytica 24:293-303.

Roumen, E.C., 1992a. Effect of leaf age on components of partial resistance to leaf blast in rice. Euphytica 63:271-279.

Roumen, E.C., 1992b. Small differential interactions for partial resistance in rice cultivars to virulent isolates of the blast pathogen. Euphytica 64:143-148.

Sah, D.N and J.M. Bonman, 1992. Effects of seedbed management on blast development in susceptible and partially resistant rice cultivars. J. Phytopathology 136:73-81.

Towey, P. and J.L. Jinks, 1977. Alternative ways of estimating the number of genes in a polygenic system by genotype assay. Heredity 39:389-410.

Wang, G, D.J. Mackill, J.M. Bonman, S.R. McCouch and R.J. Nelson, 1993. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics (in press)

Wang, Z., D.J. Mackill and J.M. Bonman, 1989. Inheritance of partial resistance to blast in Indica rice cultivars. Crop Sci. 29:848-853.

Yeh, W.H. and J.M. Bonman, 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathology 35:319-323.

Yunoki, T., A. Ezuka, Y. Sakurai, H. Shinoda and K. Toriyama, 1970. Studies on the varietal resistance to rice blast. 3. Testing methods for field resistance on young seedlings grown in greenhouse. Bull. Chugoku Agr. Exp. Stn. Japan, Series E 6:1-21.

Response to selection for high and low partial resistance to leaf blast in F_2 populations of three rice crosses

Summary

Divergent selection for higher and lower partial resistance to leaf blast was applied in F_2 populations of crosses between the rice cultivars IR36, IR64 and CO39 after exposure to a virulent isolate. IR36 and IR64 are partially resistant while CO39 is highly susceptible. As selection criteria the number of sporulating lesions in leaves of the main culm or the lesion density in the topmost leaf relative to other F_2 plants with the same stage of development were used. A highly significant response to selection was obtained in most cases, but the efficiency of the selection was low. Realized heritabilities varied from 0.14 to 0.25 depending on the cross and were similar for both selection criteria. Selection for improved partial resistance to leaf blast is possible as early as the F_2. The efficiency of selection is probably much higher if replicated tests could be made, and better results are therefore expected if selection among F_3 lines is carried out. The results indicated that the relatively low infection efficiency in IR36 and IR64 is oligo- or polygenically controlled.

Introduction

Blast disease of rice (*Oryza sativa*) is caused by the fungal pathogen *Magnaporthe grisea* (anamorph *Pyricularia oryzae*). The disease can cause serious damage to the crop, but can be effectively controlled if resistant cultivars are grown. Highly resistant cultivars can be bred by incorporating genes that trigger a hypersensitive reaction to the invading fungus, but this type of resistance usually becomes ineffective soon after a cultivar is released. On the other hand, partial resistance (PR), characterized by a reduced epidemic development despite a susceptible infection type (Parlevliet and van Ommeren, 1975) does not seem to erode quickly when used (Bonman and Mackill, 1989; Notteghem et al., 1980).

When cultivars are exposed to a virulent isolate in the greenhouse, cultivar differences for the relative infection efficiency (RIE), measured as the number of sporulating lesions that develop in the leaves, correspond well to those for PR in the field for temperate and tropical, lowland and upland rice cultivars (Sakurai and Toriyama, 1967; Notteghem, 1985; Yeh and Bonman, 1986; Roumen, 1992). After exposure to an isolate that is virulent to the parents, selection for a reduced number of sporulating lesions in the early generations of crosses can perhaps be used to improve PR to blast. The present study investigates the response to such a selection in the F_2 generation.

Material and methods

F_2's of the three crosses between CO39 (highly susceptible) and IR36 and IR64 (both partially resistant) were used. At least 350 F_2 plants of each cross were grown as described

previously (Roumen, 1993), together with 60 plants of each parent. All F_2 plants of one cross were derived from a single F_1 plant. Plants were inoculated with isolate Po6-6 when most plants had six or seven leaves on the main culm. Shortly before inoculation, the degree of emergence of the youngest leaf was marked with a waterproof felt-tip pen. Inoculation was done inside a plastic cage shortly before sunset. Per m^2 cage floor area, 400 ml of a conidia suspension with 5 x 10^4 conidia/ml in 0.5% gelatine was evenly sprayed over the plants as a fine mist. The plants were kept in the cage overnight at 100% humidity and returned to the greenhouse the next morning.

The length and width of the topmost leaf at the time of the inoculation was measured. For the leaf width, the widest leaf part was measured. However, leaf width was measured at about three quarters distance from the tip in very young leaves with a triangular shape. The leaf area was calculated as 0.7 x length x width. The number of sporulating lesions in each of the leaves of the main culm was counted six days after inoculation and their total number was calculated per plant. The lesion density in the top leaf was calculated by dividing the number of lesions in this leaf by the leaf area.

For the selection among the F_2 plants two criteria were used: the number of sporulating lesions that developed in the leaves of the main culm, and the lesion density in the youngest leaf relative to the mean of other plants that were in the same stage of leaf expansion. The latter criterium was included to compensate for differences in stage of development between plants. Resistance to infection by blast is known to increase with the age of the leaves and with the age of the plants (Roumen, 1992; Kahn and Libby, 1958). Small differences in plant development may thus influence the number of lesions that develops.

For each of the two criteria the most resistant and most susceptible plants in the F_2 were selected. At least 20 plants with a low number of lesions, 20 with a high number of lesions, 20 with a low lesion density in the top leaf and 20 with a high lesion density in the top leaf were identified. The criteria were applied independently and plants could be selected by both criteria. As a result, the number of selected plants varied per cross. The selected plants were transferred to trays filled with puddled soil and were grown under flooded conditions with ample nitrogen to obtain a fair amount of F_3 seed. Some of the transplanted plants died, especially in the cross IR36/IR64 where there was a stemborer problem.

The F_3 was grown and inoculated in the same manner as the F_2. Fifteen F_3 plants were grown from each selected F_2 plant in a completely randomized design, together with 30 plants of each parent. After inoculation, the number of sporulating lesions in the leaves of the main culm was counted per plant. The response to selection was evaluated comparing the number of lesions that developed in the selected F_2 plants with those that developed in the F_3 lines. The efficiency of the selection (realized heritability) was calculated as the difference between the selected F_2 groups, divided by the corresponding difference between the F_3 groups (Guthrie et al., 1984; Wang et al., 1989).

Results

The average number of sporulating lesions per plant in the F_2 of the crosses IR36/CO39 and IR64/CO39 was much closer to the mean of the more resistant parent than to that of CO39 (Fig. 7-1). No indication of transgressive segregation was observed in the F_2 of either cross. The mean of the F_2 in these crosses was close to the geometric mean of the parents suggesting that favourable alleles that reduce the RIE operate in a multiplicative manner. The distribution of the F_2 of the cross IR36/IR64 was similar to that of its parents, but in this cross some transgression towards higher susceptibility seemed to occur (Fig. 7-1).

The average number of lesions per plant of the selected F_2 fractions and their F_3 progenies relative to the mean value of the more susceptible parent are shown in Table 7-1. The number of lesions were expressed as a percentage of the mean value of the more susceptible parent to standardize for the different number of lesions that developed in the F_2 and F_3 trials. The realized heritabilities h_n^2 varied between 0.14 and 0.25 depending on the cross. The efficiency of the two criteria was about the same. Despite the low heritabilities, a significant response to selection was obtained in all cases except in the cross IR36/CO39 after selection based on relative lesion density (Table 7-1).

The distribution of the F_3 lines is

Table 7-1. Number of sporulating lesions per plant in selected F_2 plants and their F_3 progenies relative to the mean value of the more susceptible parent (100%), and realized heritabilities h_n^2 for two selection criteria applied in three crosses between rice cultivars. CO39 was the more susceptible parent in the crosses IR36/CO39 and IR64/CO39 and IR36 was the more susceptible parent in the cross IR36/IR64

Cross	F_2			F_3				Number of lines	
	selection group		difference	selection group		difference			
	high	low		high	low		h_n^2	high	low
criterium: lesions									
IR36/CO39	63.1	3.8	59.3	48.2	36.5	11.7 **	0.20	26	24
IR64/CO39	96.3	1.9	77.3	33.1	16.0	17.1 **	0.18	21	20
IR36/IR64	314.7	8.1	306.6	110.0	68.1	41.9 **	0.14	15	18
criterium: relative lesion density									
IR36/CO39	42.7	13.1	29.6	46.1	40.8	5.3 n.s.	0.18	22	22
IR64/CO39	83.8	9.5	74.3	31.2	12.6	18.6 **	0.25	20	20
IR36/IR64	269.3	19.2	250.1	110.7	76.3	34.4 *	0.14	15	16

*, ** Significant at the 0.025 and 0.005 levels of probability, respectively, based on a F-test of the line means.

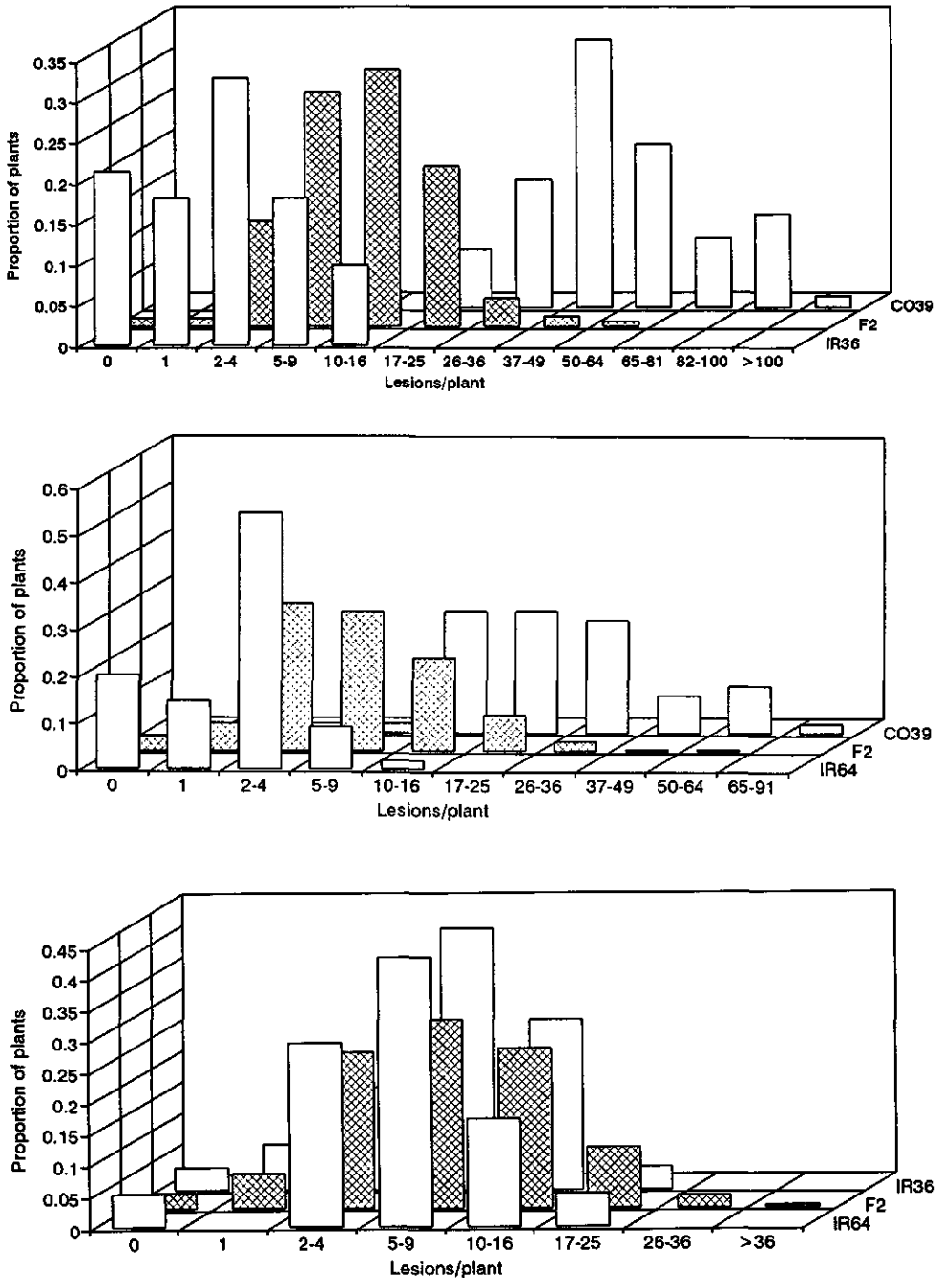

Fig. 7-1. Frequency distribution of the number of sporulating lesions in leaves of the main culm of the F_2 and parental genotypes for the rice crosses IR36/CO39, IR64/CO39, and IR36/IR64.

shown in Fig. 7-2. Within the F_3 of the crosses IR36/CO39 and IR64/CO39 no transgression was observed. All lines developed significantly less lesions than CO39 in the cross IR36/CO39, and all but one developed significantly less lesions than CO39 in IR64/CO39 (t-test; $\alpha=0.05$). Summed over both selection criteria, two F_3 lines developed slightly less lesions than the more resistant parent in the cross IR36/CO39 and one F_3 line developed slightly less lesions than the more resistant parent in the cross IR64/CO39. These differences were not significant. In the cross IR36/IR64, 19 out of the 49 F_3 lines developed more lesions than the more susceptible parent IR36 and eight lines developed less lesions than the more resistant parent IR64. The number of lesions in one of these F_3 lines was significantly higher than in IR36 and in another it was significantly lower than in IR64 (t-test; $\alpha=0.05$) indicating transgressive segregation in this cross.

Discussion

The present study shows that the number of lesions that develops after exposure to a virulent isolate of the blast pathogen can be significantly reduced by single plant selection in early generations. However, the efficiency of the selection was too low to merit practical use of the applied selection criteria by breeders, mainly because they are too time consuming. The realized heritabilities for the number of lesions in the crosses IR36/CO39 and IR36/IR64 are slightly higher than those calculated for these crosses in an earlier study (Roumen, 1993). The realized heritabilities in the present study are similar to those obtained by Wang et al. (1989) who also applied divergent selection in the F_2 using lesion number as one of their criteria.

Compensation for differences in stage of plant development by selecting those plants that had a low or high lesion density in the youngest leaf compared to other plants that were in a similar stage did not improve the efficiency of selection. In fact, the number of lesions in the leaves of the main culm seemed to be the more reliable selection criterium. In the cross IR36/CO39, where the selection based on relative lesion density gave no significant response, the realized selection differential in terms of lesion number between the high and low F_2 groups was relatively small.

The highly significant response to selection indicated that the low heritability was due to a very large environmental variation between plants and not so much due to lack of genetic variation in the crosses. The efficiency of selection is probably much improved if repeated measurements could be made. Better results are therefore expected if selection among F_3 lines is carried out.

Because transgression occurred both towards higher resistance and towards higher susceptibility in the cross IR36/IR64, IR36 and IR64 each contain at least one RIE reducing gene that is not present in the other cultivar. The lack of transgressive segregation in the F_3 of the crosses IR36/CO39 and IR64/CO39 despite selection towards extreme genotypes indicates that CO39 has no genes that reduce the RIE that are not also present in IR36 or IR64. The distribution of the F_3 lines of these crosses indicates oligo- or polygenic control of the low number of

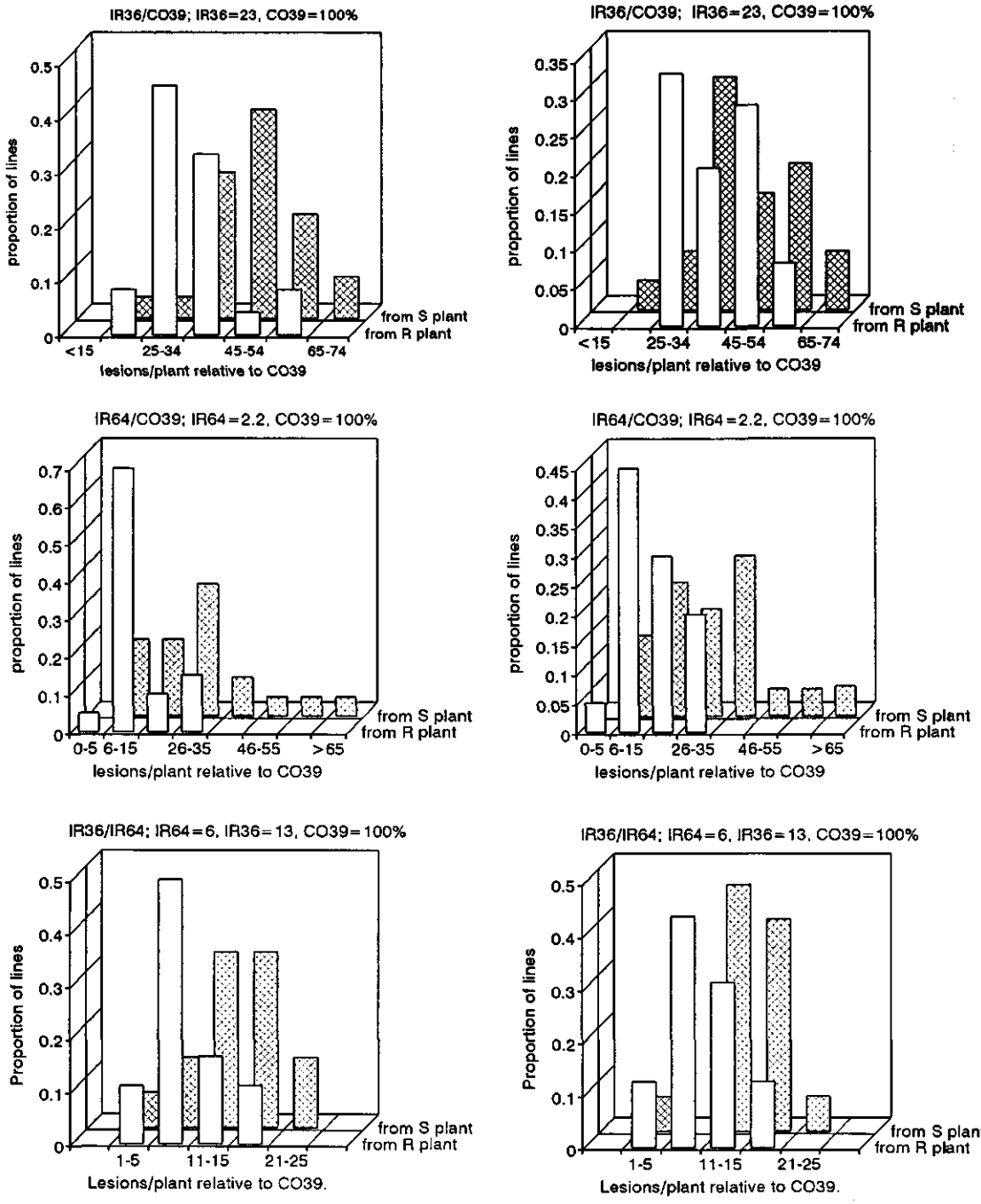

Fig. 7-2. Distribution for the mean number of lesions per plant for F_3 lines after divergent selection in the F_2in three rice crosses for two selection criteria. The F_2 plants were selected based on the number of sporulating lesions in leaves of the main culm (left side) or the density of sporulating lesions in the top leaf among plants with a similar stage of plant development (right side).

lesions in IR36 and IR64 (Fig. 7-2), which is in agreement with the result of an earlier study of these cultivars (Roumen, 1993), and which supports the broadly held view that PR to leaf blast usually is under polygenic control (Ezuka, 1979). The fact that the proportion of the F_3 lines with a phenotype as the more resistant parent in the crosses IR36/CO39 and IR64/CO39 was higher than the proportion with a phenotype as the more susceptible parent, may be explained by a progressively smaller (absolute) effect of each additional gene when more genes are already present (Roumen, 1993). Because a low RIE is an important component of PR to leaf blast and this trait is controlled by several genes, selection in early generations is recommended for improving PR to blast disease.

References

Bonman J.M. and D.J. Mackill, 1988. Durable resistance to rice blast disease. Oryza 25:103-110.

Bonman, J.M., B.A. Estrada and J.M. Bandong, 1989. Leaf and neck blast resistance in tropical lowland rice cultivars. Plant Disease 73:388-390.

Ezuka, A., 1979. Breeding for and genetics of blast resistance in Japan. In: IRRI, Proceedings of the rice blast workshop, pp. 27-48. IRRI, Los Baños, Philippines.

Guthrie, D.A., E.L. Smith and R.W. McNew, 1984. Selection for high and low grain protein in six winter wheat crosses. Crop Sci. 24:1097-1099.

Kahn, R.P. and J.L. Libby, 1958. The effect of environmental factors and plant age on the infection of rice by the blast fungus, *Piricularia oryzae*. Phytopathology 48:25-30.

Notteghem, J.L., G.M. Andriatompo, M. Chatel and R. Dechanet, 1980. Techniques utilisées pour la sélection de variétés de riz possédant la résistance horizontale à la pyriculariose. Ann. Phytopahol. 12:199-226

Notteghem, J.L., 1985. Définition d'une stratégie d'utilisation de la résistance par analyse génétique des relations hôte-parasite. Cas du couple riz-*Pyricularia oryzae*. Agron. Trop. 40:129-147.

Parlevliet, J.E., and A. van Ommeren, 1975. Partial resistance of barley to leaf rust *Puccinia hordei*. II. Relationship between field trials, microplot tests and latent period. Euphytica 24:293-303.

Roumen, E.C., 1992. Effect of leaf age on components of partial resistance to leaf blast in rice. Euphytica 63:271-279.

Roumen, E.C. 1993. Inheritance of host plant effect on the relative infection efficiency of *Magnaporthe grisea* in rice cultivars. submitted.

Sakurai, Y. and K. Toriyama, 1967. Field resistance of the rice plant to *Piricularia oryzae* and its testing method. In: T. Ogura (Ed), Rice diseases and their control by growing resistant varieties and other measures, Proc. Symp. Tropical Agriculture Researches, pp. 123-135. Ministry of Agriculture and Forestry, Tokyo, Japan.

Wang, Z., D.J. Mackill and J.M. Bonman, 1989. Inheritance of partial resistance to blast in Indica rice cultivars. Crop Sci. 29:848-853.

Yeh, W.H. and J.M. Bonman, 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathology 35:319-323.

How to select for a reduced infection efficiency of *Magnaporthe grisea* into rice leaves, an important component of partial resistance to leaf blast

Summary

The number of sporulating lesions that develop in leaves of rice genotypes after exposure to virulent isolates of the blast pathogen is closely associated with partial resistance to the disease in the field. However, counting the lesions is cumbersome and the practical use of this trait in breeding more resistant cultivars is limited. Samples of rice genotypes, exposed to virulent isolates in the 6th leaf stage, showed a high correlation between the number of sporulating lesions that developed in leaves on the main culm and the number of these leaves with at least one such lesion. In experiments with pure lines, the rank correlation ranged from 0.86 to 0.98 and in F_3 lines of three crosses the rank correlation ranged from 0.78 to 0.90. Unlike the number of sporulating lesions, the presence of sporulating lesions in leaves can be assessed easily and relatively fast. The number of leaves with lesions may therefore serve as a first, fairly rapid selection criterium for improving partial resistance to leaf blast.

Introduction

The number of sporulating lesions that develop in leaves of rice (*Oryza sativa*) after exposure to virulent races is an important component of partial resistance (PR) to the blast pathogen (*Magnaporthe grisea*, anamorph *Pyricularia oryzae*) in both temperate and tropical rice genotypes (Yunoki et al., 1970; Yeh and Bonman, 1986; Roumen, 1992a). Improving PR by selection for a reduced number of sporulating lesions after artificial inoculation may be a more efficient alternative than selection for a low amount of disease in the field. In greenhouse studies, good control of races is possible, avoiding the disturbing influence by effective major genes that usually occurs in the field (Sakurai and Toriyama, 1967, Ezuka, 1979; Notteghem, 1993). Greenhouse studies also enable a better control of environmental conditions, reducing the large variation in disease development due to unpredictable environmental effects on the expression of PR. Unfortunately, assessment of the number of sporulating lesions is very laborious, limiting the practical use of this characteristic for improving PR in breeding programs.

Only young leaf tissue is susceptible to the blast pathogen (Kahn and Libby, 1958; Notteghem and Andriatompo, 1979). The PR of newly emerged rice leaves rapidly increases with age and mature leaf tissue is completely resistant. The period after which a leaf becomes completely resistant depends on the host genotype. In partially resistant genotypes, new leaves become completely resistant faster than in highly susceptible genotypes (Roumen et al., 1992). So the total number of sporulating lesions and the number of

leaves from the top with at least one such lesion is reduced in cultivars with higher PR. High correlations were indeed found between the number of sporulating lesions in leaves of the main culm and the number of those leaves with at least one sporulating lesion regardless of isolate (Roumen, 1992b). Unlike the total number of sporulating lesions, the presence or absence of sporulating lesions in leaves can be assessed with relative ease. The research in this paper studies the potential of the number of leaves with lesions as a practical selection criterium for improving PR to leaf blast.

Material and methods

Two experiments were carried out using pure lines. Most of the genotypes were previously studied in the field (Bonman et al., 1989) and/or in the greenhouse (Roumen, 1992a and 1992b). In a third experiment, segregating progenies were studied.

Experiment 1. Twenty genotypes (those shown in Table 8-1 and IR22103-26-6-2, IR68, IR70 and IR74) were exposed to the isolates V850145, Po6-6, Po3-81, and NBGA84-01. Plants were grown in plastic pots (10 cm diameter) similar as described by Roumen (1992a). Twenty pots were planted per genotype with seven plants per pot. Four blocks were created, each with five pots per genotype. Genotypes were randomized as rows within blocks. The plants of one block were inoculated with one of the four isolates when most plants had developed six leaves on the main culm. Inoculation was done shortly before nightfall inside plastic cages as described previously (Roumen, 1992a). Per block, 400 ml of a conidia suspension containing circa 5×10^4 conidia/ml was sprayed over the plants as a fine mist. The plants were kept in the plastic cage at 100% R.H. overnight and then transferred to a humid room kept at 25 C until the time of scoring, six days later.

For the genotypes with a susceptible infection type (IT), the number of sporulating lesions in each leaf on the main culm was counted and the total number of lesions and the number of leaves with at least one lesion were calculated.

Experiment 2. Fifteen genotypes (those shown in Table 8-2, IR68, IR31802-48-2-2-2, and IR52) were planted in three plastic trays (24 x 30 cm) with five genotypes per tray in single rows of 20 plants each. Conditions for plant growth were similar as described previously (Roumen, 1992b). The plants were inoculated with isolate Po6-6 when most plants had developed six leaves on the main culm. The plants were inoculated shortly before nightfall in a plastic cage with 200 ml of a conidia suspension with 5×10^4 conidia/ml. The plants were kept at 100% R.H. in the cage overnight and then transferred to a humid room kept at 25 C until the time of scoring. Collection of data was the same as for experiment 1.

Experiment 3. The data of a study that investigated the response to selection for PR in the F_2 (Roumen, 1993) were utilized to assess the relation between the total number of sporulating lesions and the number of leaves with such lesions in segregating progenies. 80 F_3 lines of the cross IR36/CO39, 64 F_3 lines of the cross IR64/CO39, and 49 F_3 lines of the cross IR36/IR64

were tested. The F_3 lines consisted each of 15 plants which were grown in a completely randomized design. The plants were raised in the greenhouse in small trays (30 x 24 cm), with six rows of five plants per tray. The plants were inoculated with isolate Po6-6 when most plants had six or seven leaves on the main culm. Similar as in experiments 1 and 2, inoculation was done shortly before sunset inside a plastic cage. Per m^2 cage floor area, 400 ml of a conidia suspension with 5 x 10^4 conidia/ml in 0.5% gelatine was evenly sprayed over the plants as a fine mist. The plants were kept in the

Table 8-1. Number of sporulating lesions/plant in leaves on the main culm, and the number of those leaves with at least one lesion in 16 rice genotypes after exposure to four isolates of the blast pathogen, relative to the values of CO39

Genotype	Lesions					Leaves				
	Isolate					Isolate				
	V85-0145	Po6-6	Po3-81	NBGA 84-01	Mean	V85-0145	Po6-6	Po3-81	NBGA 84-01	Mean
CO39	100	100	100	100	100	100	100	100	100	100
IR29723-88-2-3-3	..[1]	74	83	54	70	..	74	82	74	77
IR37704-98-3-2-2	..	49	53	29	44	..	67	76	57	67
IR29725-22-3-3-3	43	26	34	..	34	79	55	57	..	64
IR50	59	17	14	..	30	76	56	44	..	58
IR28150-84-3-3-2	..	21	36	18	25	..	63	75	49	62
IR35546-17-3-1-3	..	24	34	14	24	..	57	54	40	50
IR66	29	27	15	2	18	75	58	49	9	48
IR52	24	18	12	..	18	82	52	46	..	60
IR29692-99-3-2-1	..	23	9	13	15	..	55	42	52	50
IR36	23	11	10	..	15	49	39	39	..	43
IR35361-59-3-3-2	..	13	11	5	10	..	56	46	27	43
IR62	17	5	6	..	9	54	34	33	..	40
IR35293-125-3-2-3	9	11	6	..	9	38	51	30	..	39
IR60	9	4	4	..	6	46	26	24	..	32
IR64	..	7	6	2	5	..	34	31	18	27

[1] resistant infection type, no sporulating lesions were formed.

Two values for the number of lesions relative to CO39 are considered significantly different if the ratio of larger value divided by the smaller value is 5.9 or higher (α=0.05; Based on Scheffé's test after log transformation of the relative data).

cage overnight at 100% R.H. and then returned to the greenhouse. The number of sporulating lesions in leaves on the main culm and the number of leaves with such lesions were counted six days after inoculation.

Results

Experiment 1. Isolates Po6-6 and Po3-81 induced a susceptible IT on the same 16 of the 20 host genotypes. The isolates V850145 and NBGA-84-01 showed a more restricted, and sharply contrasting, virulence pattern and each induced a susceptible IT on nine host genotypes (Table 8-1). None of the isolates induced a susceptible IT on IR68, IR70, IR74 and IR22103-26-6-2 (therefore not shown in Table 8-1). Within genotypes, the variation for the number of sporulating lesions that developed per plant in the leaves of the main culm was very high. The average coefficient of variation for the number of sporulating lesions between plants of the same genotype was 102%. Between genotypes, the average number of sporulating lesions relative to the value of CO39 varied sometimes considerably from one isolate to another (Table 8-1). These differences were not significant, except for the much lower relative value for IR66 induced by isolate NBGA84-01 compared to that of the other isolates. In spite of the large error, the rank correlation between the number of sporulating lesions in the leaves of the main culm and the number of those leaves with at least one lesions was very high for each of the isolates (range 0.86-0.98).

Table 8-2. Number of sporulating lesions/plant in leaves of the main culm and the number of these leaves with at least one sporulating lesion for 12 rice genotypes after exposure to isolate P06-6 of the blast pathogen

Genotype	Lesions	Leaves
IR37704-98-3-2-2	21.9	2.6
IR29723-88-2-3-3	13.7	2.5
IR35546-17-3-1-3	8.9	1.6
IR29692-99-3-2-1	7.6	1.6
IR28150-84-3-3-2	6.4	1.7
IR35293-125-3-2-3	4.0	1.3
IR35361-59-3-3-2	2.8	1.3
IR29725-22-3-3-3	2.6	1.2
IR62	1.6	0.8
IR64	0.9	0.6
IR58	0.4	0.3
IR60	0.3	0.3

Experiment 2. IR68 and IR31802-48-2-2-2 developed a resistant IT and the plants of IR52 were chlorotic. In the remaining 12 genotypes, a lower number of sporulating lesions per plant was again closely associated with a lower number of leaves with such lesions (rank correlation = 0.97) in spite of the small number of plants that was grown (Table 8-2).

Experiment 3. Across the F_3 lines, the average number of sporulating lesions in the leaves of the main culm and the average number of those leaves with lesions were also highly correlated. The rank correlation between the number of sporulating lesions per plant and the number of leaves with such lesions was 0.78 for the F_3 lines of the crosses IR36/CO39 and IR34/IR64 and 0.90 for the cross IR64/CO39. Compared with the value of the unselected population, the average number of sporulating lesions per plant in the best 12.5% or best 25% of the F_3 lines using the number of leaves with lesions as selection criterium was almost as strongly reduced as that when the number of lesions itself would have been used (Table 8-3).

Discussion

Although a reduced number of sporulating lesions is the most important component of PR to leaf blast, it is difficult to use in a breeding program. Counting the number of lesions is too time consuming. Because the number of lesions within a given genotype often varies markedly from plant to plant, only large differences between genotypes can be assessed reliably by visual observation. However, when observed in detail, even small cultivar differences in number of sporulating lesions appear reproducible. The present study shows that such small differences can be more easily assessed by scoring the number of leaves with sporulating lesions. The average number of leaves with sporulating lesions in pure lines was highly correlated with the number of sporulating lesions that developed, even when relatively few plants per entry were grown in a

Table 8-3. Mean number of sporulating lesions/plant in the best 12.5% and the best 25% of the F_3 lines of three crosses using the number of leaves with sporulating lesions (leaves) or the number of leaves with sporulating lesions itself (lesions) as criteria. The mean of all F_3 lines is also shown (unselected control)

	Cross					
	IR36/CO39		IR64/CO39		IR36/IR64	
Selection criterium	12.5%	25%	12.5%	25%	12.5%	25%
Lesions	40.2	44.1	0.6	0.9	1.5	1.7
Leaves	43.0	50.1	0.7	1.0	1.9	1.9
Average of all F_3 lines	66.9	66.9	2.6	2.6	3.8	3.8

simple experimental lay-out. High correlations were also obtained in segregating progenies. Selection for a reduced number of sporulating lesions can thus effectively be achieved by selection of entries that have relatively few leaves with sporulating lesions, a much faster and easier approach.

Cultivar differences for PR in the field have been found to be associated to the differences for the number of sporulating lesions that develop on these cultivars in the greenhouse after exposure to a virulent isolate in tropical and temperate, and upland and lowland rice cultivars (Sakurai and Toriyama, 1967; Notteghem et al., 1980; Yeh and Bonman, 1986; Roumen, 1992a). Because of the close association between the two traits, selection for a reduced number of leaves with sporulating lesions is expected to improve PR in the field almost as good as selection for the number of sporulating lesions itself would do.

Assessment of the number of leaves with sporulating lesions can perhaps also be used if a mixture of isolate races is used for inoculation. A genotype that contains major genes effective to just a small proportion of the races, but in which new leaves rapidly build up resistance to all races in a mixture, is expected to have a low number of infected leaves and a high level of PR, although a large number of lesions may develop. On the other hand, a genotype that builds up PR with increase of leaf age slowly, but that contains an effective major gene to nearly all the races in a mixture, will still develop some lesions on older leaves. Such a genotype could thus have a high number of leaves with sporulating lesions although relatively few lesions may develop in total. Since PR and its components can only be assessed when a genotype is exposed to a virulent isolate, the possibility to use mixtures of isolates would be very useful; single isolates which can attack all or even a large proportion of the genotypes are hard to find, especially in the case of tropical rice genotypes (Kiyosawa et al., 1986). When mixtures of isolates are used for inoculation, counting the number of sporulating lesions cannot be used as a reliable estimator of PR (Parlevliet, 1983), unless the investigator knows which major genes are present in the genotypes and which avirulence genes are present in the pathogen races. For tropical rice genotypes this is hardly ever the case (Kiyosawa et al., 1986; Yu et al., 1987).

References

Bonman, J.M., B.A. Estrada and J.M. Bandong, 1989. Leaf and neck blast resistance in tropical lowland rice cultivars. Plant Disease 73:388-390.

Ezuka, A., 1979. Breeding for and genetics of blast resistance in Japan. In: IRRI. Proceedings of the rice blast workshop, pp. 27-48. IRRI, Los Baños, Philippines.

Kahn, R.P. and J.L. Libby, 1958. The effect of environmental factors and plant age on the infection of rice by the blast fungus, *Piricularia oryzae*. Phytopathology 48:25-30.

Kiyosawa, S., D.J. Mackill, J.M. Bonman, Y. Tanaka, Y. and Z.Z. Ling, 1986. An attempt of classification of world's rice varieties based on reaction pattern to blast fungus strains. Bull. Natl. Inst. Agrobiol. Resour. Japan 2:13-37.

Notteghem, J.L., 1993. Durable resistance to rice blast disease. In: Th. Jacobs and J.E. Parlevliet (Eds), Durability of disease resis-

tance, pp. 125-134. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Notteghem, J.L. and G.M. Andriatompo, 1979. Étude de quelques facteurs de la *résistance horizontale du riz à la pyriculariose*. Agron. Trop. 34:180-195.

Notteghem, J.L., G.M. Andriatompo, M. Chatel and R. Dechanet, 1980. Techniques utilisées pour la sélection de variétés de riz possédant la résistance horizontale à la pyriculariose. Ann. Phytopathol. 12:199-226.

Parlevliet, J.E., 1983. Can horizontal resistance be recognized in the presence of vertical resistance in plants exposed to a mixture of pathogen races ? Phytopathology 73:379.

Roumen, E.C., 1992a. Effect of leaf age on components of partial resistance to leaf blast in rice. Euphytica 63:271-279.

Roumen, E.C., 1992b. Small differential interactions for partial resistance in rice cultivars to virulent isolates of the blast pathogen. Euphytica 64:143-148.

Roumen, E.C., 1993. Response to selection for high and low partial resistance to leaf blast in F_2 populations of three rice crosses. (submitted)

Roumen, E.C., J.M. Bonman and J.E. Parlevliet, 1992. Leaf age related partial resistance to *Pyricularia oryzae* in tropical lowland rice cultivars as measured by the number of sporulating lesions. Phytopathology 82:1414-1417.

Sakurai, Y. and K. Toriyama, 1967. Field resistance of the rice plant to *Piricularia oryzae* and its testing method. In: T. Ogura (Ed), Proceedings of the symposium; Rice diseases and their control by growing resistant varieties and other measures, pp. 123-135. Ministry of Agriculture and Forestry, Tokyo, Japan.

Yeh, W.H. and J.M. Bonman, 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathology 35:319-323.

Yu, Z.H., D.J. Mackill and J.M. Bonman, 1987. Inheritance of resistance to blast in some traditional and improved rice cultivars. Phytopathology 77:323-326.

Yunoki, T., A. Ezuka, Y. Sakurai, H. Shinoda and K. Toriyama, 1970. Studies on the varietal resistance to rice blast. 3. Testing methods for field resistance on young seedlings grown in greenhouse. Bull. Chugoku Agr. Exp. Stn. Japan, Series E 6:1-21.

General discussion

Importance of components of partial resistance to blast

Leaf blast. The outcome of the present study that the relative infection efficiency (RIE) is the most important component of PR to leaf blast in rice cultivars, agrees with results from other studies of components of partial resistance (PR) to blast. Similar as for the presently studied tropical lowland cultivars, significant differences between cultivars for RIE have been observed for temperate rice cultivars and for cultivars adapted to tropical upland conditions (Sakurai and Toriyama, 1967; Yunoki et al., 1970a; Notteghem et al., 1980). The RIE appears to be important regardless of the varietal or evolutionary groups that are discerned in rice (Japonica/Indica, tropical/temperate, lowland/upland).

The cultivar differences for the RIE are largely due to cultivar differences for the increase of resistance with aging of the leaves. A certain time after appearance, newly emerged leaves become completely resistant to infection, even by virulent isolates. In cultivars with a high PR, the RIE in young leaves is relatively low and the resistance in leaves of these cultivars increases quickly with age (chapters 1 and 2).

The sporulation capacity of lesions is a function of the size of the grey centre of lesions (Dhua, 1989; Toyoda and Suzuki, 1952; Yamaguchi and Ito, 1979), and a restricted size of these centres is thus associated to PR to leaf blast. In the present study, the average size of this grey centre was found to vary between cultivars, but the differences were considerably smaller than for the RIE (chapter 2).

The latent period (LP) of leaf blast infection depends on the temperature (Yoshino, 1971). Under the tropical conditions of the present study, the LP was just four days (chapters 2 and 3). In the field, a blast epidemic results from a polycyclic infection process, and due to its short LP, leaf blast has the typical characteristic of a compound interest disease (Zadoks and Schein, 1979). Epidemiological simulation studies have shown that prolongation of the LP in such compound interest diseases is a very effective way to reduce the epidemic (Zadoks, 1971). Indeed, for other compound interest diseases, such as the leaf rusts of barley and wheat, a prolonged LP is closely associated to higher PR (Parlevliet et al., 1980; Broers, 1989). Therefore, the result of the present study that LP is not important at all as a component of PR to leaf blast was surprising. Among cultivars that developed a susceptible infection type, differences for LP were either absent or very small and these differences were not associated to the level of PR expressed by these cultivars in the field. Neither was the LP affected by the amount of nitrogen that was applied, or the age of leaves, although these factors are known to affect PR to blast considerably (Ou, 1985).

Neck blast. In principle, the RIE (measured as the proportion of neck nodes that develop a susceptible type lesion after exposure to a virulent isolate) is also an important component of PR to neck blast. The present study showed that the incidence of neck in-

fection significantly differed between cultivars (chapter 5). However, unlike in leaves, the potential damage due to neck infection depends on whether a lesion is formed or not, and not on how many lesions are formed. Relative differences between cultivars for the incidence of neck infection are thus more likely to fluctuate between experiments than those for the number of lesions in the leaves. Under high inoculum pressure, the number of neck nodes that develops blast may be uniformly high in spite of significant cultivar differences for resistance to penetration and establishment of the pathogen. The incidence of neck infection may thus fail to reflect cultivar differences for PR, depending on the inoculum pressure that was applied.

On the other hand, the total degree of hyphal growth (lesion size) in neck nodes is not likely to be much influenced by the number of successful penetrations that occurred, because the colonies are competing for the same space. The present study showed that increased resistance to the growth of the pathogen was closely related to a more restricted yield loss. The lesion size is probably a more important component of PR to neck blast than it is to leaf blast.

The LP in neck nodes was about four days longer than that in leaves. But, as was the case for leaves, no cultivar differences were observed. There were no indications that LP is important as an component to neck blast.

Inheritance of partial resistance

In the tropical lowland rice cultivars studied a present, the most important component of PR to leaf blast, the RIE, appeared to be controlled by several genetic factors with relatively small effects (chapters 6,7). Oligo- or polygenic inheritance of PR in the durably resistant cultivar IR36, as suggested by Wang et al. (1989), was confirmed. Non-monogenic control of PR has earlier been demonstrated to occur in temperate cultivars (Ezuka, 1979), and tropical cultivars that are adapted to upland cultivation (Notteghem, 1986). In agreement with present results (chapter 6), other studies found that PR to blast is governed by a fair number of loci. Higahsi and Saito (1985) conducted a very elaborate study making crosses between the Japanese upland cultivar Sensho and a set of testers with marker genes. Evaluating the linkage between PR and the markers, they concluded that the PR of Sensho is controlled by more than five genetic factors. Recently, Wang et al. (1993) studied the inheritance of PR in a cross of Moroberekan with the highly susceptible cultivar CO39 by means of molecular genetic techniques. The PR in Moroberekan, an upland cultivar with durable resistance to leaf blast, was found to be under polygenic control involving at least eight chromosomal regions in the genome. Supporting the results of the present study (chapters 6,7), no PR enhancing genetic factors were found in CO39.

Genetic variation of PR to blast is usually satisfactorily explained by models with additive and dominant genetic effects (chapter 6; Higashi and Kushibuchi, 1978; Lin, 1986; Notteghem et al., 1981). Dominant expression of PR enhancing genetic factors prevails over recessiveness (chapters 6; Higashi and Saito, 1985; Wang et al., 1989). Small interactions between host

genotypes and different virulent pathogen isolates were found (chapter 4), suggesting that genetic factors enhancing PR operate in a gene-for-gene system with genetic factors in the pathogen isolates.

The present study suggests that there's no lack of genetic variation for RIE-reducing genes within cultivated rice. Finding genetic variation within the IRRI breeding materials that show PR was not too difficult although these are genetically closely related, as was shown by e.g. Lin (1992).

Assessment of PR to blast

As already mentioned in the introduction and in several other chapters of this thesis, accurate evaluation of PR in the field is hindered if cultivars contain hypersensitivity genes that are effective to all or a part of the genotypes within the pathogen population (Ezuka, 1972; Parlevliet, 1983; Notteghem, 1986). In Japan, where this problem was first recognized, two methods were developed to reduce or eliminate the disturbing effects of hypersensitivity (major) genes and improve the assessment of PR. In the first method, comparison of PR among cultivars in the field is restricted to those cultivars that are likely to have the same major gene(s) (Narita and Iwata, 1964 in: Ezuka, 1972; Ezuka et al., 1969a and 1969b). A lot of information concerning which major genes are present in Japanese cultivars is available because of extensive and systematic inoculation studies combined with genetic analysis of the hypersensitivity resistance in these cultivars (Kiyosawa, 1972; Goto and Yamanaka, 1968). In the second method, PR is assessed after artificial inoculation in greenhouse tests using a single isolate that is virulent to the cultivars of interest (Sakurai and Toriyama, 1967; Yunoki et al., 1970a).

Unlike in Japan, systematic inoculation studies combined with genetic analysis of hypersensitivity resistance in tropical rice cultivars are relatively rare. The work that has been done indicates that nearly all tropical cultivars contain major genes (Notteghem, 1989). In many cases, these are different from any of those identified in Japan (Kozaka et al., 1970; Kiyosawa et al., 1986). Moreover, cultivars with more than one hypersensitivity gene appear to be the rule rather than the exception (Tanaka, 1986; Yu et al., 1987, Mackill and Bonman, 1992). Notteghem (1989; 1993) concludes that the situation in tropical rice cultivars is much more complicated than in temperate rice cultivars. He considers the disturbing effects of effective major genes in tropical rice a major problem for the improvement of PR. Indeed, in a field test conducted at IRRI, Ahn and Ou (1982), found the amount of disease on cultivars in small field plots to be highly correlated ($r=0.89$) to the proportion of virulent races in the pathogen population.

As long as knowledge of the major genes in tropical rice cultivars remains limited, field comparison of PR among groups of tropical rice cultivars with the same major gene(s) can not be made. However, the greenhouse alternative appears to work equally well for the tropical cultivars of the present study as it did for the temperate cultivars studied in Japan. In field tests at IRRI in which the major gene(s) of these cultivars were not effective, the rank order of the cultivars corresponded well with that of the greenhouse

test (chapter 2; Bonman et al., 1989a). It is not unlikely that isolates similar in virulence pattern as isolate Po6-6 were present in the field trials of Bonman et al. (1989a). The inoculum came from the IRRI blast nursery, where isolates with a virulence pattern similar to that of Po6-6 are easily obtained.

Assessment of PR in the greenhouse has the advantage that it requires less seed than field assessment, making it more suitable for assessing PR in early generations of a breeding cycle. The monocyclic greenhouse test also eliminates any interplot interference, which can be a problem, especially if the amount of seed is limited and only small field plots can be grown (Notteghem, 1985). A disadvantage of greenhouse assessment as done in Japan and in the present study is that counting the number of lesions is too time consuming, limiting its use for practical breeding purposes. To speed up assessment, large differences can, of course, be observed visually. The present study suggests that, for identification of smaller cultivar differences, counting the number of (successively older) leaves from the top with one or more sporulating lesions may be used as a much faster alternative to counting of lesions (chapter 2, 4, 8).

In field tests, epistatic effects of major genes may be largely eliminated if an isolate with virulence to the test cultivars of particular interest is established as the dominant pathogen genotype. A possibility is inoculation of that isolate to the spreader row in upland miniplot nurseries designed after Notteghem and Andriatompo (1977) or Marchetti (1983). Marchetti and Xinghua (1986) followed this approach in the Southern USA, but found that naturally occurring pathogen genotypes had become prevalent in certain cultivars. Especially in tropical areas where rice and rice blast are present year round, establishing a single isolate as the dominant pathogen genotype may not always be easy to achieve.

Race-specificity of partial resistance

The small, but significant interactions occurring between the limited number of rice genotypes and virulent pathogen isolates investigated in the present study (chapter 4), indicate that PR to leaf blast is race-specific and that race-specific interactions are not uncommon. However, the magnitude of the interactions were relatively small and the ranking order of cultivars was not significantly altered due to isolates. This outcome agrees with that of most other studies that investigated the expression of PR to different virulent isolates of the blast pathogen (Yunoki et al, 1970b; Hirano and Matsumoto, 1971; Asaga and Yoshimura, 1969). However, some large genotype x isolate interactions have been observed. The best documented case is probably the PR of the Japanese cultivar St-1, that has been found to be controlled by a single gene, named Pi-f (Toriyama et al., 1968; Yunoki et al., 1970b). In Japan, the general believe is that large host-pathogen interactions for PR to blast are rare (Ezuka, 1979). Bonman et al. (1989b) observed that Korean cultivars expressed a much lower RIE to Philippine isolates than to Korean isolates, whereas the RIE for the check cultivar CO39 was the same. No genetic study of this phenomenon was made.

Mechanism and durability of PR

Although a reduced RIE is the most important component of PR, failure of infection seems to be the rule even in compatible combinations of host and pathogen genotypes. Notteghem and Andriatampo (1979) reported that under highly favourable conditions for infection, about one out of 200 deposited conidia on expanding fourth leaves of the highly susceptible cultivar Rojofotsy 1285 was able to induce a sporulating lesion. In the present study, the inoculum density that was applied in the trials described in chapter 2 was equivalent to 1481 conidia (on average) per cm^2 floor area. This resulted in a highest lesion density of 12.7 sporulating lesions/cm^2, occurring in the youngest leaves of the most susceptible cultivar present (CO39; October series). The large difference between these numbers supports a very low infection frequency, although no approximate estimates could be calculated because the inoculum was not directed to the rice leaves at a fixed angle, and conidia deposition was by turbulent impact.

Resistance during penetration through the epidermal cell wall. Histologic studies also indicate that failure of infection into the epidermal cells of the leaf blade is common, even in highly compatible host-pathogen genotype combinations (Peng and Shishiyama, 1988; Koga, 1989; Heath et al., 1990). This failure is partly due to resistance that is expressed during the penetration phase through the epidermal cell wall. Cell wall appositions are sometimes formed, but their importance seems to be limited (Hashioka et al., 1968, Peng and Shishiyama, 1989; Heath et al., 1990). At many sites where penetration fails, no visible host cell reaction is observed, not even in cultivars with highly effective hypersensitivity genes (Koga, 1989).

Among the different cells in the rice leaf epidermis, successful penetration (identified by hyphal growth in the host cell underneath the appressorium) is highest into bulliform cells (Hashioka et al., 1967; Yoshii, 1936, Yoshino, 1971). These are large, thin walled cells found only in the upper surface of the rice leaf that are involved in leaf rolling during drought stress. The relatively low resistance to penetration into the thin walled bulliform cells fits with observations that the upper surface of rice leaves is more susceptible than the lower surface (Kahn and Libby, 1958; Notteghem and Andriatompo, 1979) and suggests that mechanical resistance during penetration could be an important aspect of PR.

The importance of mechanical resistance to penetration is supported by recent studies that show that the ability to build up hydrostatic pressure in appressoria is essential for penetration of the pathogen through the host cell walls. Appressoria can build up a hydrostatic pressure as high as 8 MPa (Howard et al., 1991). The ability to generate this hydrostatic pressure is linked to the melanization of the appressorial cell wall. Mutants that are unable to synthesize melanin cannot infect rice (Howard and Ferrari, 1989; Chumley and Valent, 1990). Infection is also prevented if the melanin synthesis is blocked by applying a fungicide as tricyclazole (Woloshuk et al., 1983). The magnitude of the hydrostatic pressure that is established in appressoria was related to the percentage successful penetration into rice

leaves as well as into artificial nonbiodegradable membranes of different surface hardness (Howard et al., 1991).

The extent of cultivar differences for resistance to penetration into the leaf blade and its effect on PR is unclear. Most histologic studies in which the penetration was assessed were aimed at evaluating differences between compatible and incompatible host-pathogen genotype combinations. In a study of leaf sheaths, not leaf blades, Kaur et al. (1974) found the successrate of penetration into four cultivars, some of which contained effective major genes, to range from 41% to 78%. In a later study (Kaur et al., 1984), the successrate of penetration into the leaf sheath was concluded to be under polygenic control and inheriting independently from hypersensitivity resistance. In leaf blades, Peng and Shishiyama (1988) found the percentage successful penetration at 48 hrs. after inoculation in a group of twelve cultivars to range from 24% to 46%, depending on the cultivar. Koga et al. (1986), Koga (1989), and Peng and Shishiyama (1988 and 1989) concluded that penetration into the leaves was not affected by the presence of effective hypersensitivity resistance. However, other studies, such as those by Yoshino (1972), and Koga and Kobayashi (1982) reported that the percentage successful penetration into rice leaves of incompatible combinations was reduced. Kiyosawa and Lee (1975) found a similar result for incompatible combinations in a study of leaf sheaths. So far, no systematic histological study has been made on the penetration process among cultivars without effective hypersensitivity genes but with known differences for PR.

Although the relation between cultivar differences for resistance to penetration and PR remains unclear, resistance to penetration is similarly affected by increase of leaf age as PR. Yoshino (1971) and Heath et al. (1990) found that successful penetration is sharply reduced in older leaves. Because mechanical pressure by the pathogen appears essential for penetration, the additional increase of failure with increase of leaf age could be explained by a rapid cell wall hardening, cell wall thickening, or a combination of both as the leaf matures. Ito and Sakamoto (1939, in: Suzuki, 1965), investigating the relation between resistance to mechanical puncture of rice leaves and resistance to blast, indeed found that the puncture resistance increases with aging of leaves and of plants. The mechanical resistance was measured using a torsion balance equipped with an iron needle that was 30 μm diameter at the tip. Interestingly, these authors reported that the puncture resistance was also reduced when higher amounts of nitrogen were applied or when soil moisture was low. The puncture resistance thus varies in a similar pattern with ambient and plant conditions as PR (Ou, 1985). Part of the increased resistance to penetration in old leaves probably results from deposition of silicon-compounds in the epidermal cell walls (Yoshida et al., 1962). Volk et al. (1958) reported that a higher silicon availability markedly increased PR to leaf blast in young leaves. However, after having conducted more studies, Ito and Sakamoto (1940, In: Suzuki, 1965), concluded that puncture resistance not only depends on mechanical resistance of the cell wall, but on cytoplasmatic turgor

as well. More insight in the importance of resistance to mechanical penetration as a component of PR might be obtained by conducting a comparative study of cultivar differences for the increase of PR as displayed between IR36 and e.g. CO39 with aging of the leaf (chapters 1, 2) and increase of puncture resistance. Should a close relationship exist, selection for higher resistance to mechanical puncture may provide a possibility to select for higher PR in the absence of the pathogen.

Increased mechanical resistance to penetration is likely to be effective regardless of the pathogen genotype. Pathogen genotypes could compensate for increased mechanical resistance by generating an even higher hydrostatic pressure in the appressoria, but this will require more energy. Developing the sources to provide this extra energy probably involves a slow process of genetic adaptation. Nevertheless, genetic variation among pathogen genotypes for the ability to generate a certain hydrostatic pressure may exist, possibly explaining why some isolates induce more lesions than others. For example, the Japanese isolate 'Ken 54-20' induces more and larger sporulating lesions than isolate 'Ken 54-04' (Kiyosawa, 1966, In: Toriyama, 1975). Isolate 'Race 3' in a study of Kahn and Libby (1958) induced invariably more sporulating lesions than the other isolates used, and was better adapted to infect older plants. In the present study, isolate W6-1 induced more lesions than isolate Po6-6 (chapter 4). The high aggressiveness of isolate W6-1 was associated with an increased ability to infect older leaves. Measuring the hydrostatic pressure in appressoria of isolates of different aggressiveness may be another manner to assess the importance of mechanical pressure towards PR to blast.

Resistance after penetration. Resistance after penetration is probably even more important as a component of PR to blast than resistance during the penetration phase. The present study (chapter 3) indicates that small host-pathogen interactions are common, and these are more likely to originate from resistance expressed during the growth of colonies than during penetration. The hypothesis that a major part of PR is expressed after penetration is also supported by the large effect of an application of nitrogen fertilizer shortly before inoculation. In the course of the present work, it was discovered that adding 5 g/m^2 nitrogen shortly (24 hrs or less) before inoculation almost guaranteed a successful inoculation and promoted development of a fair amount of typical susceptible lesions. Compared to a control, adding some nitrogen shortly before inoculation always resulted in a considerable increase of the number of sporulating lesions that developed (e.g. in experiment 2 of chapter 4, the number of lesions increased more than two fold). The remarkable increase of susceptibility occurring within such a short time is more likely to result from a facilitated growth after penetration, e.g. due to an increase of soluble nitrogen in the cytoplasm, than from a sudden reduction of resistance to penetration of the cell wall.

PR that occurs after penetration and that reduces or arrests the colony growth, is macroscopically expressed as a smaller size of sporulating lesions, a reduction of the number of sporulating lesions that is formed, and/or changes in the ratio between

sporulating and non-sporulating lesions (chapter 2, Goto et al., 1961, Goto and Yamanaka, 1968; Yorinori and Thurston, 1975). Even within leaf parts of the same age, PR is not uniformly expressed and a mixture of lesion types is often observed. Kozaka (1979) attributes the development of a mixture of lesion types to a varying degree of susceptibility of the different epidermal cells. Bulliform cells, besides being relatively easily penetrated, also sustain a more pronounced hyphal growth than other infected epidermal cells (Yoshino, 1971; Koga, 1989). PR to growth of colonies rapidly increases with leaf age. In older leaves, the average size of sporulating lesions is reduced and relatively more non-sporulating lesions are formed (chapter 2; Goto et al., 1961). Heath et al. (1990) reported that the initial growth rate of colonies at infection sites in older leaves of the highly susceptible cultivar M-201 was already less than that of colonies in young leaf tissue. This suggests that the higher resistance to colony growth in older leaves is expressed immediately after penetration of the fungus.

The time after penetration that browning of host cells occurs near an infection site is probably closely associated with the degree of PR that is expressed. Colonies that emerged as dark brown spots (in which browning of host cells thus occurred relatively early), invariably developed into non-sporulating lesions, and these appeared clearly ahead of the whitish spots that developed into sporulating lesions. Takahashi (1956) found that, in a cultivar with a susceptible infection type, browning of host cells occurred sooner after inoculation in older leaf sheaths than in younger leaf sheaths. In the present study, dark margins formed earlier around sporulating lesions in older leaves than in younger leaves (chapter 1, 2), and observations indicated that the growth of sporulating lesions was reduced or stopped as soon as these margins developed. Comparative studies of the host cell reactions of incompatible and compatible host-pathogen combinations show that browning of host cells occurs at part of the infection sites whether effective hypersensitivity resistance is expressed or not. However, unlike in incompatible combinations, browning of host cells in compatible combinations is not preceded by rapid granulation of the cytoplasm of infected cells. In addition, the onset of browning of host cells is markedly delayed and this factor was found to be associated with continued hyphal growth (Koga, 1989; Peng and Shishiyama, 1989). Perhaps a susceptible type lesion develops in compatible combinations whenever the fungal colony is able to outgrow the area where browning of host cells occurs during the first days after penetration. Comparative histopathological studies of cultivars with a susceptible infection type, but with different degrees of PR could check this hypothesis.

Whatever may be the exact mechanism(s), after rapidly increasing with tissue age, the PR becomes complete. The increase of PR with aging of host tissue has been reported for rice cultivars from different geographic origin, belonging to different morphological groups and is expressed under widely different environmental conditions. Moreover, it is expressed regardless of the pathogen genotype used. Although some genetic variation seems to exist within the pathogen for the ability to

infect old rice tissues (chapter 4, Kahn and Libby, 1958), apparently the pathogen is unable to rapidly develop races that are able to infect much older leaves. This indicates that the resistance in cultivars that rapidly acquire the mature tissue resistance has a good chance of being durable. Depending on the importance of resistance to mechanical penetration in older leaves, adaptation of the pathogen by evolving genotypes that establish a higher hydrostatic pressure may be limited by the energy that can be stored in the conidia. Any increased energy cost needed for penetration may have the disadvantage that less energy will be available to rapidly develop a nutrient absorbing hyphae after penetration has succeeded, limiting the initial growth of the pathogen and enhancing the chance that the infection is contained by host cell reactions.

Recommendations for accumulating genes for PR

Since the present and other studies demonstrate that PR is usually based on several genes, the chance of getting all the favourable alleles together in advanced progeny lines will be relatively small without selection in early phases of a breeding cycle. Selection in early generations of the breeding cycle is therefore recommended.

Because nearly all tropical rice cultivars contain one or more hypersensitivity genes, in the early generations of a breeding cycle, these will segregate along with genes for PR. In most cases, the hypersensitivity resistance shows a simple dominant inheritance (Kiyosawa, 1972, Yu et al., 1987), so any disturbing effect by hypersensitivity resistance is usually avoided if PR of the progenies is assessed using isolates that are virulent to both parents. Here the problem arises that the distinction into virulent and avirulent isolates is not always so easy. For example, intermediate infection types characterized by the presence of small sporulating lesions may be the result of a hypersensitivity gene with an incomplete effect, but also of high PR when the environmental conditions are unfavourable for disease development. Repeated testing, and including susceptible and PR check cultivars in inoculation tests can help deciding if intermediate infection type reactions are caused by incomplete hypersensitivity resistance or adverse environmental conditions. Sometimes, major genes with an incomplete effect are detected by testing more isolates at the same time. In inoculation tests conducted in the course of the present study, isolate V86025 repeatably induced a more susceptible infection type on the cultivar Iguape Cateto than isolate Po6-6. But isolate Po6-6 did not systematically induce a more resistant infection type on the other tested cultivars (Table 9-1). In studies done elsewhere, Iguape Cateto is reported to have a fair amount of PR (Notteghem, 1981). Until the reaction of Iguape Cateto to isolate V86025 was observed, one could not be certain if the small sporulating lesions that developed in this cultivar after exposure to isolate Po6-6 were the result of a high PR or of a single gene causing incomplete hypersensitivity resistance.

Once a suitable virulent isolate is identified, selection could be done in the greenhouse following the methodology of plant cultivation and inocula-

Table 9-1. Infection type[1] of seven selected rice genotypes after inoculation at the sixth leaf stage with two *Magnaporthe grisea* isolates (the same result was obtained in two separate tests)

Rice genotype	Isolate	
	Po6-6	V86025
Maratelli	6	6
IRAT 216	0	0
OS 6	3	6
Iguape Cateto	3	6
Malos	5	5
Yamada Bake	6	6
C 22	6	0

[1] 6: large spindle shaped sporulating lesions without dark margin 5: large spindle shaped sporulating lesions with dark margin 4: small spindle shaped sporulating lesions with dark margin 3: more or less round, small sporulating lesions with dark margin 2: more or less round, brown, non-sporulating lesions 1: tiny pinpoint size dark non-sporulating lesions 0: no visible symptoms

tion that has been used throughout this volume. After assessment of PR, the best lines and plants can be treated with a fungicide and transplanted to the field to allow observation of other important agronomic traits and to obtain a good grain production. The present study shows that selection for higher PR in early generations of a breeding program is feasible. The RIE, the most important component of PR, can be significantly reduced by selection as early as the F_2 (chapter 7). However, due to the high error of individual plant assessment for the RIE, selection among F_3 lines is much more effective (chapter 6). Selection of the most resistant plants from lines that have relatively few leaves with sporulating lesions and in which sporulating lesions only form in the youngest leaf tissue is recommended (chapter 8).

The same procedure can also be carried out in the field at locations where natural infection does not occur often. E.g., at the IRRI farm, the dew period in the nights is often too short for natural infection to occur and natural disease pressure is low. Prolonging the leaf wetness period is possible by covering the plots with plastic during night time. In such situation, segregating lines planted in upland miniplots can be assessed after artificial inoculation in the same manner as in the greenhouse. The inoculum can be sprayed directly on the test plants, or be used to infect spreader plants first. Field inoculation will be particularly useful for assessing progenies of upland- and other cultivars that are adapted to cultivations in which no transplanting is practised.

References

Ahn, S.W. and S.H. Ou, 1982. Quantitative resistance of rice blast disease. Phytopathology 72:279-282.

Asaga, K. and S. Yoshimura, 1969. Relation between field resistance of rice varieties to blast and the races or strains of the causal fungus. Ann. Phytopath. Soc. Jpn. 35:385-386. (in Japanese)

Bonman J.M., B.A. Estrada and J.M. Bandong, 1989a. Leaf and neck blast resistance in tropical lowland rice cultivars. Plant Disease 73:388-390.

Bonman, J.M., J.M. Bandong, Y.H. Lee, E.J. Lee and B. Valent, 1989b Race-specific partial resistance to blast in temperate japonica rice cultivars. Plant Disease 73:496-499.

Broers, L.H.M., 1989. Partial resistance to wheat leaf rust in 18 spring wheat cultivars. Euphytica 44:247-258.

Chumley, F.G. and B. Valent, 1990. Genetic analysis of melanin-deficient, nonpathogenic mutants of *Magnaporthe grisea*. Mol. Plant-Micr. Interact. 3:135-143.

Dhua, U., 1989. Relation between size, colour and spore liberation from rice blast lesions. J. Phytopathology 124:27-30.

Ezuka, A., 1972. Field resistance of rice varieties to blast disease. Rev. Plant Protec. Res. 5:1-21.

Ezuka, A., 1979. Breeding for and genetics of blast resistance in Japan. In: IRRI, The rice blast workshop, pp. 27-48. IRRI, Los Baños, Laguna, Philippines.

Ezuka, A., T. Yunoki, Y. Sakurai, H. Shinoda, and K. Toriyama, 1969a. Studies on the varietal resistance to rice blast. 1. Tests for genotype of "true resistance". Bull. Chugoku Agr. Exp. Stn. Japan, Series E 4:1-31. (English abstract)

Ezuka, A., T. Yunoki, Y. Sakurai, H. Shinoda, and K. Toriyama, 1969b. Studies on the varietal resistance to rice blast. 2. Tests for field resistance in paddy fields and upland nursery beds. Bull. Chugoku Agr. Exp. Stn. Japan, Series E 4:33-53. (English abstract)

Goto, K. and S. Yamanaka, 1968. Studies on the race of rice blast fungus. Bull. Coll. Agric. Utsunomiya Univ. Japan 7:21-71.

Goto, K., K. Hirano and K. Ohata, 1961. Susceptibility of leaf of rice to blast in reference to leaf age and position. I. Variation of susceptibility among different leaf positions and grades of emergence of top leaf. Spec. Bull. Okayama Pref. Agric. Exp. Stn. Japan 58:77-88.

Heath, M.C., B. Valent, R.J. Howard and F.G. Chumley, 1990. Interactions of two strains of *Magnaporthe grisea* with rice, goosegrass and weeping lovegrass. Can. J. Bot. 68:1627-1637.

Hashioka, Y., H. Ikegami, O. Horino and T. Kamei, 1967. Fine structure of the rice blast. I. Electronmicrographs of *Pyricularia oryzae* and the epidermal cell walls of rice plants. Res. Bull. Fac. Agric. Gifu Univ. Japan 24:66-77.

Hashioka, Y., H. Ikegami and T. Murase, 1968. Fine structure of the rice blast. III. The mode of invasion of *Pyricularia oryzae* into rice epidermal cells. Res. Bull. Fac. Agric. Gifu Univ. Japan 26:23-30.

Higashi, T. and K. Kushibuchi, 1978. Genetic analysis of field resistance to leaf blast (*Pyricularia oryzae*) in rice. Japan. J. Breeding 28:277-286.

Higashi, T. and S. Saito, 1985. Linkage groups of field resistance genes of upland rice variety Sensho to leaf blast caused by *Pyricularia oryzae*. Jap. J. Breeding 35:438-448.

Hirano, T. and K. Matsumoto, 1971. Resistance of the rice varieties to several C races of blast fungus. I. Studies on variation of field resistance by races and fungus strains. Jap. J. Breeding 21:110-111. (in Japanese)

Howard, R.J. and M.A. Ferrari, 1989. Role of melanin in appressorium function. Exp. Mycology 13:403-418.

Howard, R.J., M.A. Ferrari, D.H. Roach and N.P. Money, 1991. Penetration of hard substrates by a fungus employing enormous turgor pressures. Proc. Natl. Acad. Sci. U.S.A. 88:11281-11284.

Ito, S. and M. Sakamoto, 1939 and 1940. Studies requested by he Ministry of Agriculture and Forestry, Japan. Annual Report on rice blast. Hokkaido Univ. Botan. Lab. Fac. Agr.

Kahn, R.P. and J.L. Libby, 1958. The effect of environmental factors and plant age on the infection of rice by the blast fungus, *Piricularia oryzae*. Phytopathology 48:25-30.

Kaur, S., P. Kaur and S.Y. Padmanabhan, 1974. A method of quantification of the infection phenomenon in blast disease of rice for the use in general studies. Indian Phytopathology 27:624-627.

Kaur, S., P. Kaur and S.Y. Padmanabhan, 1984. Inheritance of resistance to *Pyricularia oryzae* Cav. at penetration and establishment phase. Indian Phytopathology 37:100-106.

Kiyosawa, S., 1966. Resistance of some rice varieties to a blast fungus strain, Ken 54-04: An analysis of field resistance. Agr. Hort. 41:1229-1230.

Kiyosawa, S., 1972. Genetics of blast resistance. In: Rice Breeding, pp. 203-225. IRRI, Los Baños, Philippines.

Kiyosawa, S. and E.J. Lee, 1975. Expression of genes for true and field resistance to blast disease in cell of leaf sheath of rice plant. Jap. J. Breeding 25:145-154.

Kiyosawa, S., D.J. Mackill, J.M. Bonman, Y. Tanaka and Z.Z. Ling, 1986. An attempt of classification of world's rice varieties based on reaction pattern to blast fungus strains. Bull. Natl. Inst. Agrobiol. Resour. Japan 2:13-37.

Koga, H., 1989. Histopathology of resistant and susceptible rice plants inoculated with *Pyricularia oryzae* Cavara. J. Agr. Res. Quarterly 23:8-16.

Koga, H. and T. Kobayashi, 1982. Comparison of the leaf infection process of *Pyricularia oryzae* Cav. in rice leaves of compatible and incompatible combinations. Ann. Phytopathol. Soc. Japan 48:506-513.

Koga, H., O. Horino and T. Kobayashi, 1986. Ultrastructural studies on the resistance mechanisms of rice plants infected with *Pyricularia oryzae* Cav.. Bull. Hokuriku Natl. Agric. Exp. Stn. Japan 28:1-56.

Kozaka, T., 1979. Breeding for and genetics of blast resistance in Japan. In: IRRI, The rice blast workshop, pp. 3-25. IRRI, Los Baños, Laguna, Phillipines.

Kozaka, T., S. Matsumoto and M. Yamada, 1970. Reactions of foreign rice varieties to major races of *Pyricularia oryzae* in Asia. Bull. Natl. Inst. Agric. Sci. Japan, Series C 24:113-152.

Lin, S.C., 1986. Genetic analysis of minor gene resistance to blast in Japonica rice. In: IRRI, Rice Genetics, Proceedings of the International Rice Genetics Symposium 27-31 May 1985, pp. 451-469. Island Publishing House Inc., Manila, Philippines.

Lin, M., 1992. Ancestral contribution to IRRI rice varieties (IR5-IR62). Jap. J. Breeding 42:437-442.

Mackill, D.J. and J.M. Bonman, 1992. Inheritance of blast resistance in near-isogenic lines of rice. Phytopathology 82:746-749.

Marchetti, M.A., 1983. Dilatory resistance to rice blast in USA rice. Phytopathology 73:645-649.

Marchetti, M.A. and L. Xinghua, 1986. Screening techniques to identify slow-blasting rice lines. In: F.J. Shideler and C.V. Mendoza (Eds), Progress in upland rice research, Proceedings of the 1985 Jakarta conference, pp.317-326. IRRI, Los Baños, the Philippines.

Narita, T. and T. Iwata, 1964. Varietal difference in severity of blast occurrence in late-sown upland nursery beds. Special report on diseases and insect pest outbreak forecast work, Ministry of Agriculture and Forestry, Tokyo, Japan 18:59-60. (in Japanese)

Notteghem., J.L.. 1981. Analyse des résultats d'inoculation de 67 varietes de riz par 15 souches de *Pyricularia oryzae*. In: Symposium sur la résistance du riz a la Pyriculariose, Montpellier, France, 18-21 mars 1981. IRAT-GERDAT, Montpellier, France.

Notteghem, J.L., 1985. Définition d'une stratégie d'utilisation de la résistance par ana-

lyse génétique des relations hôte-parasite. Cas du couple riz-*Pyricularia oryzae*. Agron. Trop. 40:129-147.

Notteghem, J.L., 1986. Blast resistance methodologies in the Ivory Coast, 1972-1985. In: F.J. Shideler and C.V. Mendoza (Eds), Progress in upland rice research, Proceedings of the 1985 Jakarta conference, pp.305-316. IRRI, Los Baños, the Philippines.

Notteghem, J.L., 1989. La création de variétés résistantes. Cas du riz et de la pyriculariose. Bull. de la Société Bot. de France 136:227-237.

Notteghem, J.L., 1993. Durable resistance to rice blast disease. In: Th. Jacobs and J.E. Parlevliet (Eds), Durability of disease resistance, pp. 125-137. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Notteghem, J.L. et G.M. Andriatompo, 1977. Mesure au champ de la résistance horizontale du riz à *Pyricularia oryzae*. Agron. Trop. 32:400-412.

Notteghem, J.L. et G.M. Andriatompo, 1979. Étude de quelques facteurs de la résistance horizontale du riz à la pyriculariose. Agron. Trop. 34:180-195.

Notteghem, J.L., G.M. Andriatompo, M. Chatel and R. Dechanet, 1980. Techniques utilisées pour la sélection de variétés de riz possédant la résistance horizontale à la pyriculariose. Ann. Phytopathol. 12:199-226.

Notteghem, J.L., M. Chatel and R. Dechanet, 1981. Analyse diallele de deux caracteristiques de la résistance du riz à *Pyricularia oryzae*. In: Symposium sur la résistance du riz à la pyriculariose, pp. 313-330. IRAT-GERDAT, Montpellier, France.

Ou, S.H., 1985. Rice diseases, 2nd edition. Kew Surrey, England. 385 pp.

Parlevliet, J.E., W.H. Lindhout, A. van Ommeren and H.J. Kuiper, 1980. Level of partial resistance to leaf rust, *Puccinia hordei*, in West-European barley and how to select for it. Euphytica 29:1-8.

Parlevliet, J.E., 1983. Can horizontal resistance be recognized in the presence of vertical resistance in plants exposed to a mixture of pathogen races ? Phytopathology 73:379.

Peng, Y.L. and J. Shishiyama, 1988. Temporal sequence of cytological events in rice leaves infected with *Pyricularia oryzae*. Can. J. Bot. 66:730-735.

Peng, Y.L. and J. Shishiyama, 1989. Timing of a cellular reaction in rice cultivars associated with differing degrees of resistance to *Pyricularia* oryzae. Can. J. Bot. 67:2704-2710.

Sakurai, Y. and K. Toriyama, 1967. Field resistance of the rice plant to *Piricularia oryzae* and its testing method. In: T. Ogura (Ed), Rice diseases and their control by growing resistant varieties and other measures, Proceedings symposium on tropical agricultural researches, pp. 123-135. Ministry of Agriculture and Forestry, Tokyo, Japan.

Suzuki, N., 1965. Mechanism and genetics of resistance. In: The rice blast disease, Proceedings of a symposium at the International Rice Research Institute, pp. 277-301. John Hopkins Press, Baltimore, Maryland, U.S.A.

Takahashi, Y., 1956. Studies on the mechanism of resistance of rice plants to *Piricularia oryzae*. II. Pathological changes microscopically observed in host cells in which fungus hyphae do not grow well. Yamagata Univ. Agr. Sci. Bull. Japan 2:37-51.

Tanaka, Y., 1986. Gene analysis for major resistance genes to rice blast in some Brazilian upland varieties. In: F.J. Shideler and C.V. Mendoza (Eds), Progress in upland rice research, Proceedings of the 1985 Jakarta conference, p.295-304. IRRI, Los Baños, the Philippines.

Toriyama, K., 1975. Recent progress of studies on horizontal resistance in rice breeding for blast resistance in Japan. In: G.E. Gálvez (Ed), Proc. seminar on horizontal resistance to the blast disease of rice, CIAT series CE-9, pp. 65-100. Cali, Colombia.

Toriyama, K., T. Yunoki and H.Shinoda, 1968. Breeding rice varieties for resistance to blast. II. Inheritance of high field resistance of Chugoku 31. Jap. J. Breed. 18 (suppl. 1):145-146.

Toyoda, S. and N. Suzuki, 1952. Histochemical studies on the lesions of rice blast caused by *Piricularia oryzae* Cav. I. Some observations on the sporulation on lesions of different types occurring on leaves of the same variety. Ann. Phytopathol. Soc. Japan 17:1-4.

Volk, R.J., R.P Kahn and R.L. Weintraub, 1958. Silicon content of the rice plant as a factor influencing is resistance to infection by the blast fungus, *Piricularia oryzae*. Phytopathology 48:179-184.

Wang, G., D.J. Mackill, J.M. Bonman, S.R. McCouch and R.J. Nelson, 1993. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Submitted.

Wang, Z., D.J. Mackill and J.M. Bonman, 1989. Inheritance of partial resistance to blast in Indica rice cultivars. Crop Science 29:848-853.

Woloshuk, C.P., H.D. Sisler and E.L. Vigil, 1983. Action of the antipenetrant, tricyclazole, on appressoria of *Pyricularia oryzae*. Physiol. Plant Pathol. 22:245-259.

Yamaguchi, T. and I. Ito, 1979. Sporulation potentials of rice blast lesions on rice cultivars differing in field resistance to blast. Ann. Phytopath. Soc. Japan 45:695-698.

Yorinori, J.T. and D.H. Thurston, 1975. Factors which may express general resistance in rice to *Pyricularia oryzae* Cav. In: G.E. Gálvez (Ed), Proc. seminar on horizontal resistance to the blast disease of rice, CIAT series CE-9, pp. 117-135. Cali, Colombia.

Yoshida, S., Y. Ohnishi and K. Kitagishi, 1962. Histochemistry of silicon in rice plants. III. The presence of cuticle-silica double layer in the epidermal tissue. Soil Science and Plant Nutrition 8(2):1-5.

Yoshii, H., 1936. Pathological studies on rice blast, caused by *Piricularia oryzae*. II. On the mode of infection of the pathogen. Ann. Phytopathol. Soc. Japan 6:205-219.

Yoshino. R., 1971. A method for detecting hyphae of rice blast in leaf blade epidermis. Proc. Assoc. Plant Prot. Hokuriku Japan 19:14-17.

Yoshino, R., 1972. Influence of temperature on the incubation period of *Pyricularia oryzae* and early detection of lesions by staining with iodine potassium iodide. Rev. Plant Prot. Res. 5:105-107.

Yu, Z.H., D.J. Mackill and J.M. Bonman, 1987. Inheritance of resistance to blast in some traditional and improved rice cultivars. Phytopathology 77:323-326.

Yunoki, T., A. Ezuka, Y. Sakurai, H. Shinoda, and K. Toriyama, 1970a. Studies on the varietal resistance to blast. 3. Testing method for field resistance on young seedlings grown in greenhouse. Bull. Chugoku Agr. Exp. Stn. Japan, Series E 6:1-19. (English abstract)

Yunoki, T., A. Ezuka, T. Morinaka, Y. Sakurai, H. Shinoda, and K. Toriyama, 1970b. Studies on the varietal resistance to rice blast. 4. Variation of field resistance due to fungus strains. Bull. Chugoku Agr. Expt. Stn. Japan, Series E 6:21-41.

Zadoks, J.C., 1971. System analysis and the dynamics of epidemics. Phytopathology 61:600-610.

Zadoks, J.C. and R.D. Schein, 1979. Epidemiology and plant disease management. Oxford University Press, New York., 427 pp.

Summary

A detailed study of three components of partial resistance (PR) to leaf blast in tropical lowland rice genotypes was made. Among the components relative infection efficiency (RIE), measured as the number of sporulating lesions that developed, lesion size, and latent period, the RIE appeared to be the most important component. Genotypes with a higher level of PR developed less lesions which usually had smaller sporulating areas compared to the susceptible check. No difference of any importance between genotypes was found for latent period. Genetic analysis showed that the RIE is controlled by several minor genes. Small differential interactions between host-cultivars and virulent pathogen genotypes were detected, indicating that RIE-reducing genes in the host operate on a gene-for-gene basis with genes in the pathogen. Only young leaf tissue is susceptible to the blast pathogen. PR rapidly increases with aging of the leaf, and the higher the level of PR of a genotype, the faster the leaf tissue became resistant. A higher level of resistance was mainly expressed as a more rapid reduction of the number of sporulating lesions.

Genotypes developing fewer lesions per plant in the greenhouse demonstrated a higher level of PR in field studies. The total number of sporulating lesions per plant and the number of leaves (from the top) in which these lesions developed were closely associated in pure, as well as in segregating lines. Therefore, counting the number of leaves with sporulating lesions instead of counting all sporulating lesions per plant will suffice when selection for improved PR to leaf blast is applied. Since PR to blast is controlled by several genes, selection for improved PR should preferably occur in early generations of a breeding program. The present study shows that such selection is feasible. The RIE was significantly reduced by selection in F_2 populations. However, due to the high error of individual plant assessment, selection among lines was much more effective. Selection of the plants with the fewest lesions from the most resistant F_3 lines of crosses is recommended to accumulate genes for PR to blast.

A higher PR to leaf blast was usually, but not always, linked to a higher PR to neck blast. As in leaves, PR to neck blast rapidly increased with aging. This was mainly expressed as a rapid increase of resistance to growth of the pathogen after infection. The lesion length was closely correlated to yield loss that occurred. Because PR to neck blast can be affected considerably even by small differences in development stage of the panicle, meaningful comparison of PR to neck blast between genotypes requires infection at an identical stage of panicle development. Inoculation of neck nodes at the time of flowering is recommended for screening PR to neck blast. Promising entries are those in which typical neck blast symptoms are observed after exposure to a virulent isolate, but in which relatively few necks become infected, and in which the yield loss in panicles that develop neck blast remains relatively small.

Samenvatting

Drie componenten van partiële resistentie (PR) tegen "leaf blast" werden bestudeerd in rijstrassen voor de teelt in het tropische laagland. Metingen aan de relatieve infectie frequentie (RIE), de grootte van het sporulerend oppervlak in lesies en de latentie periode, toonden aan dat de RIE de meest belangrijke component was (de RIE werd bepaald door het aantal gevormde sporulerende lesies te tellen na blootstelling aan een virulent isolaat). Een genetische analyse liet zien dat een lage RIE op meerdere genen berust, elk met een relatief klein effect. Er werden kleine, significante en herhaalbare, interacties voor de RIE tussen de waard- en schimmelgenotypen aangetoond, wat erop duidt dat er een gen-om-gen relatie bestaat tussen rijst en "blast" voor genen die de RIE beïnvloeden. Jong bladweefsel is het meest vatbaar voor "blast". Met het ouder worden van de bladeren nam de PR snel toe en na een aantal dagen was de resistentie kompleet. De toename van de PR kwam voornamelijk tot uiting als een snelle afname van het aantal sporulerende lesies per bladoppervlakte-hoeveelheid. Daarnaast was ook het sporulerende oppervlak per lesie kleiner. In rijstrassen met hogere PR duurde het korter voordat bladeren kompleet resistent werden.

Rijstrassen met minder sporulerende lesies per plant in de kas waren ook meer resistent in een veldsituatie. Het aantal lesies per plant en het aantal bladeren (vanaf de top) waarin sporulerende lesies konden ontwikkelen, hingen nauw samen zowel in zuivere als in splitsende lijnen. Daarom is het voor het beselecteren van PR niet nodig alle lesies te tellen, maar kan men volstaan met het tellen van het aantal bladeren met lesies. Omdat een hoge PR op meerdere genen berust, is selectie op deze eigenschap in vroege generaties wenselijk. Selectie voor PR in vroege generaties bleek zeer goed mogelijk. De RIE kon significant verlaagd worden door in F_2 populaties te selecteren. Maar omdat het aantal lesies dat zich vormt op een individuele plant, sterk beïnvloed wordt door kleine milieu-verschillen, was lijnselectie veel effectiever. Selectie van de planten met de minste lesies in de meest resistente F_3 lijnen wordt aanbevolen om lijnen te krijgen waarin de verschillende PR genen tegen "blast" uit beide ouderrassen samengevoegd zijn.

Een hogere PR tegen infectie van bladeren ging meestal, maar niet altijd, vergezeld met een hogere PR tegen infectie van de knoop aan de halmbasis ("neck blast"). Net als in bladeren nam de PR in de knoop aan de halmbasis snel toe met het ouder worden van het weefsel, wat zich vooral uitte als het kleiner blijven van de lesies na infectie van oudere knopen. De lesie-lengte hing nauw samen met het opbrengstverlies. Bij evaluatie van PR tegen infectie van de halmbasis is het dus belangrijk om zelfs kleine verschillen in ontwikkelingsstadium tussen rijstlijnen zoveel mogelijk te vermijden. Om PR tegen "neck blast" te verbeteren wordt aanbevolen om tijdens de bloei te inoculeren. Vervolgens kan men binnen de groep rijstlijnen die een vatbaar infectie type ontwikkelen, selecteren voor lijnen waarin relatief weinig halmen aangetast zijn en die een relatief klein opbrengstverlies hebben in die halmen die wel aangetast zijn.

Curriculum Vitae

The author was born November 25, 1960 in Roosendaal, The Netherlands. He obtained his Gymnasium-B diploma in June 1979 at the Norbertuscollege in Roosendaal. In September 1979, he started a study at the Wageningen Agricultural University, initially as a student tropical agriculture, but soon switching to plant breeding. In June 1986, he completed this study and was awarded the 'ingenieur' diploma (cum laude) with main focus on plant breeding (tropical orientation), weed sciences, and plant nematology. A large part of his study was spent abroad. Between 1983 and 1985, he participated in the alfalfa breeding program of the Ottawa Research Station, Agriculture Canada, in the bean breeding program of the Centro Internacional de Agricultura Tropical, Cali, Colombia, and in the malting barley breeding program of the University of Saskatchewan, Saskatoon, Canada.
A literature review that he wrote, called 'The role of plant breeding in the yield potential improvement of maize (*Zea mays* L.) since the introduction of hybrid varieties.', was given the 'Broekema' award in June 1988.
After his studies, from February 1987 to November 1991, he was employed as an associate expert by the section Research and Technology Transfer of the Netherlands' Ministry of International Development Cooperation, and worked at the International Rice Research Institute at Los Baños, the Philippines. The work done there resulted in the present thesis.