Margery F. Koch

Aspects of quantitative resistance to *Xanthomonas campestris* pv. *oryzae* in rice

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PROPOSITIONS

1. Resistance to bacterial blight in rice is less desirable than resistance to *Xanthomonas campestris* pv. *oryzae*.

2. Demonstrated close linkage of resistance genes in many plant species, such as rice, barley and flax, suggests that these genes can be expected to have gene products with similar primary functions.

   Ellis, J.G., G.J. Lawrence, W.J. Peacock, A.J. Pryor, 1988. Approaches to cloning plant genes conferring resistance to fungal pathogens. Annual Review Phytopathology 26:245-263.

3. All resistance in rice to *Xanthomonas campestris* pv. *oryzae* can be observed to be quantitative, according to the definition of Yamada (1984), if the susceptible parent used in the test cross is not much more susceptible than the resistant rice cultivar.

   Yamada, T., 1984. Studies on genetics and breeding of resistance to bacterial leaf blight in rice. VI. Inheritance of quantitative resistance of the variety IR28 to bacterial groups II, II, IV of *Xanthomonas campestris* pv. *oryzae* of Japan. Japan. J. Breeding 34:181-190.

4. Ogawa's genetic analysis of resistance to *Xanthomonas campestris* pv. *oryzae* based on slowing of lesion development 18 days after inoculation is disputable.

   Ogawa, T., T. Yamamato, H. Kaku, S. Taura, G.S. Khush, T.W. Mew, 1988. Resistance to Rice Bacterial Leaf Blight. IRRI/Government of Japan Collaborative Research Project. 299pp.

5. Selection for quantitative resistance to *Xanthomonas campestris* pv. *oryzae* has always been carried out when the most resistant individuals of a screening for qualitative major-gene resistance are chosen from a segregating population.

6. International multilocational site testing is appropriate for assessing resistance to biological stresses such as pathogens and insects, while multiyear testing is more appropriate for assessment of tolerance to abiotic stresses such as drought.

7. Breeding for yield stability is more difficult than breeding for yield increases, and breeding programs for yield stability will therefore take longer to give results.

8. The promising developments in direct seeding of irrigated and rainfed rice brings with it the disadvantage of fewer jobs for women workers.

9. The relevance of IARC's research can better be measured by the interest and cooperation among the national programs than in the number of hectares high yielding varieties planted.

10. The limits to racial classification of a pathogen appear to depend as much upon the diligence of the workers as upon the biology of the system.

    Caten, C.E., 1987. Concept of race in plant pathology. In: Populations of Plant Pathogens, Eds. Wolfe, M.S., C.E. Caten. Blackwell Scientific, Oxford. 280pp.

11. The racial classification system in *Xanthomonas campestris* pv. *oryzae* should be replaced by the designation of alleles for virulence and avirulence found present in an isolate, based on tests with the recently developed near-isogenic lines.

12. The potential long term negative impact of substitution of traditional tropical products with biotechnologically altered temperate-grown products is greater than the potential shorter term positive impact of biotechnology to improve the staple food crops of developing countries.

13. Techniques such as protein electrophoresis and genotypic homology studies will lead to a taxonomic grouping of *Xanthomonas* which is more related to the evolution of the genus than the present classification based on chemotaxonomic characteristics.

14. The uncertainty concerning causes for the increase in atmospheric methane in the past decades, and the contribution of paddy rice culture to this increase, should rather lead to an increased willingness to accept the interrelationship of all environmental changes than to a call to limit rice production.

15. The *kroket* is to the Netherlands what the *loempia shanghai* is to the Philippines.

Margery F. Koch
Aspects of quantitative resistance to *Xanthomonas campestris* pv. *oryzae* in rice
Wageningen, 12 January 1990

AUTHOR'S ABSTRACT

Quantitative resistance (QR) to *Xanthomonas campestris* pv. *oryzae* (Xco), the causal organism of bacterial blight in rice, is evident as reduced lesion length and slowed lesion development relative to lesions in susceptible cultivars. QR was evident at all growth stages and leaf ages as a reduction in lesion length following clip inoculation with virulent isolates of the pathogen. QR affects the ability of bacterial cells to establish and multiply within a leaf. No confirmed evidence of cultivar x isolate interactions of QR factors was found. Varying levels of incomplete race-specific major gene resistance were found and were difficult to distinguish from differences in QR.

Differences between cultivars for lesion number following spray inoculation varied from the differences in lesion length following clip inoculation, indicating the presence of an extra component of resistance limiting bacterial entry into intact leaves.

Multiple QR resistance factors were shown to be present in four moderately resistant cultivars. A minimum of five factors was found in varying combinations in six rice cultivars. The residual effect of the Xa-4 gene for resistance, or a closely linked gene, also imparted some level of QR. Selection using one isolate was effective in improving the level of QR to another isolate.

a childhood dream come true -
four years playing in the mud

FOREWORD

This thesis would not have been possible without the help of all those at the IRRI involved in the "Partial resistance in rice" project. Some gave their help in the organization and guidance of the research. Others gave themselves, in the long hours and much sweat needed to carry out the work. All were wonderful in their support during the four years I spent at IRRI and I will not forget their friendship and hospitality.

Foremost I would like to thank Dr. T.W. Mew, head of the Department of Plant Pathology of IRRI and specialist on bacterial diseases of rice, for his patience and support of the work. It was his broad vision of research on all aspects of rice diseases which lead to the "Partial Resistance" project. His foresight has resulted in an active department where new ideas and techniques have been incorporated into the research by an enthusiastic group of researchers. The activity in the labs and greenhouses was incredible! I am also grateful to Dr. Mew for giving me the chance to participate in the Bacterial Blight Workshop in 1988. That was an important week for me.

Valuable technical support and welcome advice for the genetic studies were given by Dr. Khush and the members of the Plant Breeding Department. Dr. HilleRisLambers and Dr. Dave Mackill were always willing to consider my questions over a cup of coffee. Dr. Ogawa was very kind to suggest cultivars for study and to supply me with seed of IR9101-46.

My thanks to the others in the Bacterial Blight group of the Plant Pathology Department for their continued help. Nollie Vera Cruz was always able to answer my questions concerning lab or field work, having herself done it all before. Thanks for the friendly discussions and for having patience with me when I needed too many workers and too much screenhouse space. Sonny Reyes was always a capable guide to the IRRI system. Mayette Baraoidan was an excellent teacher of the phage technique to measure bacteria. Edward Medalla managed to keep me happy whenever I asked for helpers. Fanny Garcia was the person who was always there when you needed her, also when I needed to hear a friendly word.

The men in the greenhouse were the ones who did the work which is reported here, especially measuring the kilometers of lesions. Floring Balenson was the best field aide one could wish for. My heartfelt thanks for the long hours and expert care of the screenhouse and field plots. Planting day was never a worry if he was there. Eddie Hernandez and Vic Banasihan, I was lucky to have had such good helpers. Fayette Ebron, Rey Mani, James Ortegas, and the others of the bacterial blight group all helped to make my stay in the Philippines worthwhile.

The Pathology lab wouldn't have run smoothly without the work of Sonia Ebron. Lab experiments wouldn't have been possible without the work of Mando, who made the media, and Epoy, who made sure that there were enough test tubes. Gloria worked hard to get everything typed the same day it was handed in. If she was too busy I could always turn to Yollie

and Bennie for help. Boyet Lazaro was the cheerful artist who made the many graphs I needed, some of which are found in this thesis.

The students from Wageningen, Hanneke Buiel, Peter van Duin, Carel Jaspers, Aart Osman and Annemieke de Vos, helped in the research with their work, their enthusiasm and their questions. Each gave me a chance to see my research in a different light, and all discovered the Philippines in their own very individual way. They will recognize some of their work in this thesis, while some of their work most important to me is left for future chapters of the BB story.

My thanks to Huub for the detailed statistical analysis of part of the work, and for teaching me Lotus and Statistix, so that I could continue on my own.

My thanks to others at IRRI for their friendship during the four years I lived there. Dr. S.W. Ahn asked me every day "what's new", which led to hours of talk about the topic at hand. His good advice will be important to me for years to come. Dr. Paul Teng brought his great activity (and wonderful wife) to Los Banos for a few years. Fausto Nuque, Mike Bonman, Anke, Joe, Tita, Velet helped whenever they could. Veerle, Kit, Imke, Rob, Hans, Nagamani, Hei, Debbie, Ekkehart, Tika, Zhang Qi, Kevin, Myungi,Amara, Seri, Eddie, Rebecca, John, Cisca, Lina, Wong and Gisela all shared in part of the life. The members of the Dutch community at Los Banos, Dirk and Katy HilleRisLambers, Michael and Maureen Rombach, Frits and Cobie Penning de Vries, Don Jansen, Eddie Roumen, were able to create "gezelligheid" in true style. Annie M. made us all welcome.

My promotor, Jan Parlevliet, was the visitor most eagerly awaited each year, in the beginning as the "prof", and at the end of the four years as a friend. I slowly learned what it means to really look at data with patience and an open mind. The science of translating data into tables and words which simplify rather than confuse the reader was not the least of his valuable lessons. Outside the office and in the Philippine sun the prof turned into a cheerful traveller with good stories and a sympathetic ear. His enthusiasm in the results and interest for all topics of life in our little community did wonders to raise the spirits at critical moments.

As I write this, back in Wageningen, the memories of the good and not so good times return. Gon van Laar has helped to bridge the gap between "there" and the next stop, where ever that may be. Her expertise and efficiency in thesis production helped speed the appearance of this book. You are right, Gon, it will never be a best-seller but it is what I like to do. Thanks for helping put things in perspective.

Theo Jacobs gave valuable comments on the last chapters of this book. He, Fien, Charlotte and Rients made me feel welcome at the Dept. of Plant Breeding in Wageningen.

Last but not least I'd like to thank my parents for giving me the benefit of the doubt and the chance to do what I like, however far away that may take me.

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CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION

Bacterial blight (BB), a major bacterial disease of rice (*Oryza sativa* L.), is found in most irrigated, rainfed and deep water temperate and tropical rice growing areas, including all Asian countries, areas of West Africa, Australia, South America and the Caribbean (Ou, 1985; Mew, 1987). The disease was recently identified in United States (Xu and Gonzalez, 1988). BB is seldom found in dryland rice culture. BB is a very destructive disease of rice and can cause up to 30% loss in temperate regions and as much as 50-80% in tropical Asia (Ou, 1985). Effective chemical control is not available, and the disease is mainly controlled by cultivar resistance and clean cultural practices.

Symptoms were first shown to be caused by a bacterial organism in 1908 (Ou, 1985). The pathogen causing the disease has been classified as *Xanthomonas campestris* pv. *oryzae* (Ishiyama 1922) Dye (Ou, 1985), and is abbreviated here as Xco.

SYMPTOMS

Infection on rice leaves is first noticeable as small water-soaked or greyish-white areas at the upper leaf margins or at wounds in the leaf blade. On susceptible cultivars the infection develops rapidly into long, thin lesions with a slightly wavy margin along the edge of the leaf blade or above and below the wound. Lesions gradually widen to cover the entire leaf blade and may extend down the leaf sheath. Lesions are largely necrotic, with a thin grey-white or water-soaked margin, especially at the lower edge. Saprophytic fungi may grow on the necrotic tissue, giving this a greyish appearance. A yellow area sometimes develops along the margin of infected tissue, especially in moderately resistant cultivars; this area later turns necrotic.

When conditions are favourable bacterial masses will be extruded from active lesions onto the abaxial leaf surface. Heavy dew promotes such ooze production. The milky ooze drops dry into small yellow beads

which fall off when touched.

Glumes of developing grains may also develop lesions. These appear as brownish or yellow spots surrounded by a grey-white or water-soaked margin. Such grains are usually incompletely filled and break easily.

Early infections in the humid tropics may cause "kresek" and pale yellow symptoms (Mew, 1987). Kresek symptoms are caused by systemic infections of young seedlings, usually through root or leaf wounds caused during transplanting. One to two weeks after infection leaves turn a dull greyish-white and roll along the midrib. The leaf soon withers and the entire seedling or young tiller may die within a few weeks. Plants which survive may remain stunted with few tillers. New growth of systemically infected plants often appears pale yellow when the leaf unfolds, although the leaf later develops a light green color. Fields which are early and heavily infected with Xco show empty patches and plants with chlorotic, uneven growth.

LIFE CYCLE

In the humid tropics bacteria can survive in rice stubble and weeds (*Leersia*, *Leptochloa* spp.) growing in or near harvested rice fields (Ou, 1985). Stubble is probably the major source of infection of a new crop (Ou, 1985). Infected glumes of seeds may also contain bacteria, although survival on infected seeds has been shown to decrease rapidly with time under moist warm conditions, and transmission from glumes to germinating seedlings has not been proven (Anon., 1987).

The bacteria are deposited on the leaf surface by splashing water such as rain or by heavy dew (Mizukami and Wakimoto, 1969). It is not reported how long the bacteria can remain viable on the leaf surface, or whether Xco can multiply epiphytically. Wounds caused by transplanting, wind or weeding are favourable points of entry. Strong winds during storms and tropical typhoons damage the leaf margins and result in heavy leaf blight infections in fields planted with susceptible cultivars when the bacteria are present. The bacteria may also enter through water pores on hydathodes near the tip of the leaf (Mew et al., 1984).

The bacteria enter the xylem, either directly through wounds, or following multiplication in the epitheme below the hydathodes (Tabei, 1977). Bacteria multiply and spread in the xylem vessels of the vascular system. In susceptible cultivars symptoms develop three to seven days after infection of the vascular system (Parry and Callow, 1986).

Droplets of ooze from infected leaves produce inoculum for secondary infections. Rain can spread bacteria from leaf to leaf or plant to plant within a field. The infection can be spread through irrigation water to neighbouring fields (Mizukami and Wakimoto, 1969).

RESISTANCE

Highly resistant reactions

The first report of resistance to BB dates from 1923, when a native Japanese cultivar resistant to BB was used in a breeding program aimed at developing new cultivars resistant to this disease (Ezuka and Sakaguchi, 1978). Since then thousands of cultivars and lines have been screened for resistance to BB in various countries using varying isolates of Xco.

Hypersensitive resistance to Xco is not known (Parry and Callow, 1986) although browning has been reported in reaction to infection of lines carrying the Xa-3 gene for resistance (Kaku and Kimura, 1978). Resistant reactions are typified by greatly reduced lesion size. Reduced lesion size has been shown related to reduced bacterial numbers in leaves (Kaku and Kimura, 1983). Both virulent and avirulent bacteria can multiply to some extent in infected leaves and highly resistant reactions resulted in up to 100-fold less bacteria per leaf than a susceptible reaction (Leach et al., 1989).

Genetic analyses has shown the existence of at least 12 genes for resistance (Xa genes), each resulting in a large decrease in lesion size following inoculation with avirulent isolates of BB (Ogawa et al., 1988). All are race-specific. Some differences between the resistance reactions related to the different major genes have been recorded. In Japan a near-immune reaction to infection following prick inoculation with avirulent isolates has been reported for cultivars carrying the Xa-1 and Xa-2 genes (Ezuka and Sakaguchi, 1978). Small (<1 cm) but still active lesions develop in the Philippines on cultivars carrying the Xa-4 and xa-5 genes (Mew et al., 1981). Kaku and Kimura (1978) reported that the Xa-3 gene produces browning on the lesion edge. In the Philippines the resistance of cultivars carrying the Xa-3 gene significantly limits lesion size only on adult rice plants (Zhang and Mew, 1985). Cessation of lesion extension 18-22 days after inoculation has also been suggested as a specific effect of the Xa-3 gene in the Philippines (Ogawa et al., 1988).

A decline in the resistance of improved cultivars, often only short-

ly after their release, was noticed in Japan in the 1950's (Ezuka and Sakaguchi, 1978). This lead to a division of isolates into groups according to their virulences to a differential series of cultivars (Ezuka and Horino, 1974a). Virulence groups I, II, and III showed significant quantitative differences in symptom development with clear cultivar x isolate interactions. Isolates of virulence group I were only virulent on cultivars thought to carry no genes for resistance, isolates of virulence group II were virulent on the Kogyoku group of cultivars and isolates of group III were virulent on both the Kogyoku and the Rantai Emas group of cultivars. Resistant cultivars had lesions of less than 16 mm^2 while susceptible cultivars had lesions of at least 25 and usually more than 400 mm^2. A similar rapid decline in the resistance of the improved cultivar IR20 was noticed shortly after its large-scale introduction in the Philippines (Tin Win, 1973). The increased lesion size of the new isolates was shown to be specific for the resistance gene of IR20, Xa-4 (Mew and Vera Cruz, 1979).

In large screenings of collections, isolates from tropical countries were felt to be more aggressive than temperate (Japanese) isolates (Buddenhagen and Reddy, 1972). Australian isolates were also shown to differ greatly from Asian isolates for reactions on a small number of cultivars. However, because a continuous range of symptoms from highly resistant to highly susceptible was found, specific interactions were not considered proof of physiological specialization.

The above-mentioned interactions were all found by identifying cultivars with increasing levels of resistance to specific isolates. These interactions could have been due to an increased aggressiveness of isolates in reaction to the introduction of increasingly resistant cultivars. Distinct differential interactions conclusively proving a physiological specialization were reported by Ezuka and Horino (1974a) using 14 Japanese isolates and three cultivars. Mew et al. (1982) also demonstrated large specific cultivar x isolate interactions using seven representative Philippine isolates and five differential cultivars. The cultivar Cas 209 showed a reversal in reactions to existing Philippine races in relation to the cultivar IR20.

Five virulence groups are now defined in Japan and six groups are defined in the Philippines, using two differential series distinct for each country. Working with a large number of isolates collected from seven S.E. Asian countries, Yamamoto and Ogawa (1988) defined 26 distinct races of Xco. This great variation in virulence is reflected in the fact that none of the major genes are effective in all rice-growing areas.

Few, if any genes, are effective in certain areas of Bangladesh and India (Yamamoto and Ogawa, 1988).

Quantitative resistance

Significant quantitative differences in disease reaction can be shown among cultivars considered susceptible to isolates of BB. Many cultivars are considered to be moderately resistant or moderately susceptible. Yamada (1984) defined the resistance which results in a continuous distribution of F_2 plants for lesion size following a cross between a moderately resistant and a highly susceptible rice cultivar as quantitative resistance. He called highly resistant reactions which result in discrete groups in the F_2 qualitative resistance.

Watanabe (1976) studied crosses of ten cultivars showing moderate resistance and reported quantitative inheritance of the resistance, based on F_1 and F_2 data. Yamada (1984) also showed the moderate resistance of IR28 to race II, III and IV isolates to be quantitative, and the highly resistant reaction of this cultivar to race I and V isolates to be qualitative. Yamada assumed that the quantitative resistance is based on multiple resistance factors, while the qualitative resistance is known to be based on major genes. If quantitative resistance is indeed polygenic and based on other factors than the known major genes, then it could be important for areas where major gene resistance is not effective. A combination of major gene resistance with such minor resistance factors would protect against fluctuations in resistance if a major gene should be matched by increased virulence in the pathogen population.

Yamamoto et al. (1977) studied cultivars showing moderate resistance to Indonesian isolates of Xco. The highly significant correlations between the reactions of the isolates on a range of cultivars lead the authors to consider quantitative resistance to be horizontal, following the definition of van der Plank (1968). The possibility that this resistance would then also be durable increased interest in quantitative resistance.

Moderate resistance may appear quantitative in the F_2 distribution but may be due to the incomplete action of a major gene, in which case race-specificity would be expected. Moderate resistance may also be due to race-specific oligogenes or polygenes. Race-specificity of small resistance factors is difficult to demonstrate, especially when the variation in the resistance reaction is relatively large. Parlevliet (1981a) noticed small but significant interactions evident in the above-mentioned

data of Yamamoto et al. (1977) and suggested that the moderate resistance described might not be horizontal. He felt that the system might be similar to the partial resistance in barley to barley leaf rust (Parlevliet, 1983).

This partial resistance in barley to leaf rust is clearly distinguishable from the hypersensitive resistance caused by major genes for resistance. Partial resistance is typified by a susceptible reaction to infection, with a reduced number of infections, a prolonged latency period and a reduced sporulation. Partial resistance in barley has been shown to be polygenically inherited and the resistance of certain partially resistant cultivars has proven durable over the past decades (Parlevliet, 1981b).

THIS THESIS

The purpose of this research was to further investigate the nature of quantitative resistance to Xco and to develop guidelines for its assessment and use in cultivar improvement.

Quantitative resistance is here used according to the definition of Yamada (1984), as described in the previous section on quantitative resistance reactions. The quantitative nature of a resistance is only apparent when the F_2 population of a cross has been tested. The smaller the difference between the reactions of the parent cultivars, the greater the likelihood that a resistance will appear quantitative. It is therefore possible that a cultivar which was found in one study to have quantitative resistance can be found in another study, when crossed with a more susceptible cultivar, to have a qualitative resistance.

Rice cultivars show a continuous range of variation in resistance to Xco from highly susceptible to highly resistant. For the purposes of this research the cultivars can be grouped according to their level of resistance relative to a highly susceptible cultivar as follows:

Highly susceptible (HS)	85-100%	of the maximum disease reaction
Susceptible (S)	67-85	" "
Moderately susceptible (MS)	33-66	" "
Moderately resistant (MR)	16-33	" "
Resistant (R)	6-15	" "
Highly resistant (HR)	0-5	" "

This study was mainly carried out with cultivars which can be considered to be moderately resistant, moderately susceptible, and susceptible to Philippine race 2 and race 6 isolates of Xco. Highly resistant and resistant cultivars were not used, as it was expected that they would prove to have one or two major genes for resistance, and would therefore be found to have a qualitative resistance, i.e., a resistance which results in clearly distinguishable groups in the F_2 generation. Because moderately resistant and moderately susceptible cultivars were used the chance increases that a resistance will appear quantitative due to the smaller difference between parents of a cross. The choice of the susceptible cultivar is therefore critical. At the time the study was begun it was believed that the cultivar TN1 was highly susceptible. However, based on the results of the cultivar screening and on the genetic study, it is apparent that lines and cultivars exist which are more susceptible than TN1, and this cultivar is therefore classified as susceptible.

Cultivar choice was based on data of the multilocational trials of the International Rice Bacterial Blight Nursery of the International Rice Testing Program (Anon., 1985) of the International Rice Research Institute at Los Baños, Philippines, and on discussions with researchers at this institute. Preliminary screenings of more than 100 entries with a race 2 and a race 6 isolate were then carried out. In these screenings absence of large differences in the reaction to the two isolates was considered to be of major importance. A final set of six cultivars was chosen for intensive study and for genetic analysis. To facilitate comparisons these cultivars were all of a short stature. Additional cultivars were used in more extensive tests.

Chapter 2 of this thesis is a review of parameters which have been used for quantitative assessment of resistance, especially those related to symptom development. Chapter 3 demonstrates that lesion length is the most appropriate parameter for comparing moderately resistant and moderately susceptible cultivars. Chapter 4 shows that quantitative resistance is indeed related to a reduction in the number of bacteria in inoculated rice leaves, and that lesion size is highly correlated with differences in the size of the bacterial population. Chapter 5 addresses the effect of plant age and leaf age on quantitative resistance. Chapters 6 and 7 investigate the use of two alternative parameters for screening for quantitative resistance, and show that these parameters distinguish cultivars in much the same way as when lesion length measurements are used. Possible components of resistance which may affect symptom development, and the ability of two methods of inoculation to identify these components,

are discussed in Chapter 8. Chapter 9 addresses the question of the race-specificity or non-specificity of quantitative resistance, using a number of Philippine Xco isolates and moderately resistant and moderately susceptible cultivars. The genetic basis of the quantitative resistance of four moderately resistant cultivars, and the response to selection for larger and smaller lesions is presented in Chapter 10. Chapters 11 and 12 offer some conclusions and practical advice concerning the nature and use of quantitative resistance for cultivar improvement and crop protection.

CHAPTER 2

METHODS OF ASSESMENT OF RESISTANCE TO *XANTHOMONAS CAMPESTRIS* pv. *ORYZAE* IN RICE

SUMMARY

A literature review of methods of assessment of resistance to *Xanthomonas campestris* pv. *oryzae* is given. Clip or prick inoculation allows assessment of resistance to bacterial blight (BB) by estimating the percentage diseased leaf area (%DLA), by measuring the lesion length or by using a score based on one of these parameters. The %DLA can be rapidly estimated, but rice cultivars with longer leaves may appear more resistant than cultivars with short leaves. The %DLA of large lesions is less accurately estimated than that of small lesions, and %DLA is therefore less suited for assessment of moderate resistance. Lesion lengths require more time to measure but can more accurately distinguish between minor differences in resistance. Scales based on %DLA or lesion length have the same qualities as the original parameters, but may not facilitate fine distinctions between plants. Attention to lesion type in highly resistant lines may help to distinguish between plants.

Factors affecting primary and secondary establishment of BB can be measured following spray inoculation and secondary infection. The relationship between these factors and those affecting lesion size following clip inoculation has not been well studied. Interplot interference may severely affect incidence values when the inoculum is spread through a single water system to all cultivars.

Alternative methods for assessing resistance are needed to characterize the resistance mechanisms found and to aid in distinguishing between otherwise similar levels of resistance. Alternative methods have not shown a qualitative difference between compatible and incompatible interactions, with the possible exception of histological evidence of the presence of fibrillar material in incompatible reactions.

INTRODUCTION

Resistance in rice to *Xanthomonas campestris* pv. *oryzae* (Xco), the causal organism of bacterial blight (BB), can be identified by a lowered disease level apparent at one or more stages of development following contact between the host and the parasite (Parlevliet, 1981b). Since clear differences in lesion type are not generally found in BB differences in resistance are measured by the presence and size of the lesions (Kaku and Kimura, 1978).

Assessment of resistance is needed for:

1. screening of a large collection of cultivars,
2. characterization and genetic study of the resistance, and
3. utilization of the resistance in a breeding program.

The choice of methods of assessment for each stage can determine the correctness and accuracy of the conclusions reached, but it can also determine the size limits imposed on a program.

Most assessment procedures depend on artificial inoculation of the leaf, whole plant or field. While spray inoculations most resemble field infection, inoculation by clipping or pricking gives a higher chance of infection and allows better quantification of the reaction. Clip and prick inoculation deposit the inoculum directly in the vascular system of the leaf, bypassing the natural points of entrance at the hydathodes (Preece, 1982). Plant age, plant part inoculated, inoculation procedure, inoculum concentration, days after inoculation for evaluation can all play a role in the ease and power of resolution of assessment.

Many of the tests discussed in this chapter can be carried out on a single plant, or even a part of one plant. Such tests are suitable for use in selection among segregating populations, where each plant must be assessed individually. Field tests usually require a stable genetic composition of a population, and as such are mainly suitable for resistance assessment in the beginning of a program, when selection must be made among prospective parent cultivars, or in the most advanced stages, when more uniform lines exist.

TYPES OF RESISTANCE

Mew (1987) refers to seedling, adult plant, and moderate resistance to BB. Seedling resistance is gained by any one of a number of known

major genes, and is characterized by a high level of resistance throughout the crop growth period (Ezuka and Horino, 1976). Adult plant resistance is characterized by a high level of resistance in adult plants but a highly susceptible reaction at the seedling stage (Zhang and Mew, 1985). Moderate resistance is characterized by reduced symptom expression in relation to a highly susceptible check cultivar (Yamada, 1986a; Wasano and Dhanapala, 1982). While the kresek wilt of rice seedlings caused by Xco has not been studied as thoroughly, resistance has been identified (Watanabe, 1976). The relation between kresek wilt and leaf blight is not clearly documented (Horino and Yamada, 1982).

ASSESSMENT BASED ON LEAF SYMPTOMS

Assessment of resistance will depend to a large extent on measurements of visual symptoms of the disease because of the speed and ease with which large numbers of plants can be scored. Other tests are generally slower and more tedious, or cost more to perform. These tests must clearly add to our knowledge of the material and improve our ability to distinguish between plants before being adopted (Large, 1966).

Assessment following clip or prick inoculation

Assessment of resistance is most often carried out on plants which have been inoculated by pricking (Ezuka and Horino, 1974b) or clipping (Kauffman et al., 1973). The relation in symptom development between these two methods has been demonstrated (Mew et al., 1981). Both methods are sensitive to the concentration of the inoculum used, and a suspension with at least $1x10^8$ cfu/ml is needed to avoid escapes and for full development of the lesion (Cho, 1975). At booting stage prick inoculated plants are usually scored 21 days after inoculation; clip inoculated plants can be scored between 14 and 21 days after inoculation. Younger plants may be scored earlier (Mew et al., 1981). A less frequently used alternative is to score plants when the lesions on the susceptible check reach a predetermined length (Ogawa and Yamamoto, 1987).

Percentage diseased leaf area and assessment scales

Estimations of the percentage diseased leaf area (%DLA) allow rapid scoring of large numbers of plants, although estimates can vary signifi-

cantly between scorers when more than one person does this work (Baw, 1985). In seedlings lesions can vary from nearly 100% to less than 1 %DLA while on plants of maximum tillering stage or older the maximum expected is about 50 %DLA. Highly resistant reactions will result in a %DLA of not more than 5% and often less than 1% (Baw, 1985).

Estimations of %DLA, even when aided by field keys, are more precise at the extremes than in the midrange as visual accuracy is proportional to the logarithm of the intensity of the impulse (Horsfall and Barrett, 1945). This will result in large differences in the error variance between highly resistant and moderately susceptible cultivars, so that when this type of screening is done using %DLA a transformation may be required before an analysis of variance can be carried out (Finney, 1973).

For speed of scoring many prefer to use a disease scale based on %DLA (Horsfall and Cowling, 1978). Such scales divide the reactions into a number of defined classes. The well-known Horsfall-Barrett scale (HB) was designed on a logarithmic scale for estimations of %DLA up to 50% and for estimations of the remaining green leaf area from 50-100 %DLA (Horsefall and Cowling, 1978). This division of classes largely replaces the need for a logarithmic transformation of the data, which is often used to account for differences in the variance of cultivars. When %DLA estimates are averaged the arithmetic mean is found. When the scores of the equally spaced codes of the HB scale are averaged the logarithim of the geometric mean of the %DLA is calculated. This is less distorted by extreme individual scores (Large, 1966), again reducing error variance. The HB scale itself has not often been used to assess bacterial blight; two other scales have been developed for this purpose.

Ezuka and Horino (1974b) developed a scale to score prick-inoculated leaves (Table 2-1). This scale has eight classes (coded 0-7) ranging from symptomless to 100% coverage of the leaf area above the inoculation point. Tests are most often done on flag leaves in the assumption that this reaction is the most stable (Mew, 1987). The scale deviates from the HB scale in the great emphasis it puts on resistant reactions. Distinctions are made between symptomless plants, plants showing a slight discoloration around the point of inoculation, and plants with lesions of less than 15 mm length. These extra classes allow greater discrimination when selecting for high resistance.

The Standard Evaluation System (SES) of the International Rice Research Institute (Anon., 1980) was developed to score clip-inoculated leaves on booting or flowering plants. The scale has six classes (coded 0,1,3,5,7,9) ranging from symptomless to 100 %DLA (Table 2-2). The last

Table 2-1: A scale developed by Ezuka and Horino (1974b) for assessing bacterial blight lesions resulting from inoculation of rice leaves at booting stage by the double pin prick method. The ratio of the lesion length to the leaf length from the inoculation point to the leaf tip is assessed.

Code	Description
0	No symptoms
1	Slight discoloration at the inoculation point
2	Lesions less than 15 mm in length
3	Lesions 1/4 of the length from the inoculation point to the leaf tip
4	Lesions between 1/4 and 1/2 of the length
5	Lesions between 1/2 and the whole length
6	Lesions covering the whole length but some green area remaining
7	Lesions covering the whole area above the inoculation point

Table 2-2: A scale developed for the standard evaluation system (SES-scale) of the International Rice Research Institute (Anon., 1980). Rice plants at booting stage are clip inoculated and the mean percentage diseased leaf area (%DLA) of the upper three leaves is assessed.

Code	Description
0	No lesion
1	< 1 %DLA
3	1-5 %DLA
5	6-25 %DLA
7	25-50 %DLA
9	50-100 %DLA

class contains all severities greater than 50 %DLA, an area which is not usually found in plants after maximum tillering stage, but which is often found in seedlings. Highly resistant cultivars score a 1 or a 3, while highly susceptible cultivars will receive a 7 or a 9. Horino et al. (1981) found a significant correlation between the scale of Ezuka and Horino (1974b) and the SES scale (r=0.753) and between the former scale and the absolute lesion length (r=0.728).

Baw (1985) compared the %DLA, SES, HB and a modified HB scale for assessment of 39 cultivars ranging from highly resistant to highly susceptible at seedling and maximum tillering stage. He concluded that there was good agreement between all scales except when a great range of disease reactions fall into one class, as can happen when young plants are scored with the SES scale. The differences between the cultivars were clearer using the %DLA estimations than with a scale; the %DLA score also distinguished more statistically different classes than parameters based on multiple scorings of %DLA on single leaves. Further discussion of the use of multiple scores for deriving parameters to describe the infection process in a single leaf are discussed in a later section of this chapter.

Lesion length

Several workers have preferred to use the absolute lesion length (Yoshimura et al., 1984) or lesion area (Yamamoto et al., 1977) for studies on BB. Unlike the scattered symptoms caused by spraying or natural infection, the single lesion resulting from clip or prick inoculation is well-defined and large enough to measure, although agreement must be reached on the inclusion or exclusion of chlorotic tissue in the measured length. Measuring requires more time than estimations of %DLA, although electronic equipment may help to make rapid measurements possible.

While %DLA measures the relative damage to the leaf caused by Xco, lesion length is a direct measure of the extent of the rice-Xco interaction. When screening cultivars for resistant lines to use in a breeding program one can expect great variation in the leaf size. It is possible that a longer lesion may receive a lower score than a shorter lesion, if scored on a relative basis, when the leaf area of the former is much greater than that of the latter. While the damage level is greatest to the smaller leaf a breeder must expect that if the resistance is transferred to a new plant type the damage level may change. (Lesion length and leaf length have not been found to be correlated (Chapter 3, this thesis)). With varying plant types it is probably better to score for

absolute lesion size than to depend on a relative value. Baw (1985) showed a high correlation ($r>0.95$) between lesion length and %DLA for four cultivars at maximum tillering, but these correlations were made for each cultivar separately. The slopes of the four regression lines varied from 0.60 to 1.05, indicating differences which could affect the assessment during screening of varied material.

Because lesion length measurements are continuous and not subject to the discrete grouping of %DLA or scales, the power of discrimination of small differences is expected to be greater. This may be important in distinguishing among highly resistant individuals, which would all fall into a single class of a scale, but it is especially important when selecting for moderate resistance, as it is in this range that the eye can less accurately detect small differences. This can also mean that fewer leaves need be scored to achieve a desired level of variance.

To speed and simplify scoring while retaining lesion length as the parameter of selection, a scale based on absolute lengths can be used. Goto (1978) developed such a scale to classify the reactions of rice cultivars to bacterial foot rot, *Erwinia chrysanthemi*. This scale has 11 classes, ranging from symptomless to complete destruction of the tillers. Lesions are classified into groups by 2.5 cm lesion length intervals, up to 20 cm. Saha (1984), working with maximum tillering and booting plants, used four classes based on lesion length, corresponding to resistant (0-4 cm), moderately resistant (5-9 cm), moderately susceptible (10-15 cm) and susceptible (greater than 15 cm) for scoring BB. It is better if the class limits of such a scale are only determined when the susceptible reaction of a standard cultivar is known, as this may vary considerably between tests.

Genetic studies for characterization of a resistance require information on the distribution of the disease reaction in the population. Confirmation of monogenic resistance will require division of the population into resistant and susceptible types. Because there are no qualitative differences between resistant and susceptible lesion types, the limits of the two groups need not necessarily be determined beforehand (Saha, 1984). A study of the histogram of the F_2 generation should show whether a natural division of the population into groups exists. In this way shifts in the level of the entire population due to variation in inoculum concentration, environment, or the genetic background are not confounded with the resistance in question.

When a scale is used for genetic analysis instead of %DLA or lesion length measurements, the nature of the scale can affect the apparent dis-

tribution of a population. If the classes have unequal ranges of disease reactions this can result in skewness of a normally distributed population when the histogram is drawn with equally spaced classes. This is especially important when studying polygenic effects where the range and shape of the histogram are indicative of the number of genes involved (Mathers and Jinks, 1971). The scale of Ezuka and Horino (1974b) has been used for genetic studies of quantitative resistance (Wasano and Dhanapala, 1982; Yamada, 1986a) although the class sizes are not equal. The above-described scale of Saha (1984) has uniformly spaced classes but divide the population into only four classes.

Assessment following spray inoculation

Spray inoculation has been used both for one time assessment of plants of varying ages and to begin an epidemic in small field plots in which repeated scores will be taken. Spraying one day before transplanting (Anon., 1987) and spreader rows have been used to start an epidemic (Choi et al., 1985). Spray inoculation and root dipping have been used for assessment of kresek wilt (Watanabe, 1976).

Primary infections can be assessed using incidence (number of plants showing symptoms, expressed as a percentage of the total (Large, 1966)) or severity. Incidence is very suited to scoring for kresek wilt and the SES scale (Table 2-2) has been used for assessing incidence of kresek. The number of leaves per plant showing symptoms, expressed as a percentage of the total, has also been used. However, this parameter is more variable as plants differ greatly in leaf number with age and cultivar.

When the epidemic is followed throughout the crop growth period the changes in incidence and severity can be used to express its course over time. At IRRI (Anon., 1986) differences between rice cultivars were found for the linear rate of increase of a BB infection. Other methods of assessing the course of the epidemic, such as the area under the disease process curve (Waggoner, 1986), should be tried.

Because the inoculum is spread through the water interplot interference may affect the assessment of disease incidence. For this reason also comparisons of epidemics by measuring Xco concentrations in paddy water are not expected to give good results.

The relationship between incidence, which measures the number of successful infections, and severity, which measures the size of an infection, is not well documented. Resistance factors relating to entry may be independent of factors relating to spread within a leaf following es-

tablishment (Buddenhagen, 1983). Mew et al. (1984) showed that the exclusion of bacteria from the water pores in an incompatible reaction was an active process of resistance by the plant. Until further evidence has been gained the two parameters should be viewed separately.

ALTERNATIVE ASSESSMENT PARAMETERS

While visual symptom assessment will remain the major parameter used in research on BB, other parameters have been used to characterize resistance. Such measurements generally are more tedious but the added information may offer potential insights into the host-parasite interaction, and may help distinguish between otherwise similar reactions when selecting.

Rate of lesion development

The large differences in the reported time of appearance of symptoms of BB (Kaku and Kimura, 1983; Horino and Hifni, 1978; Yoshimura et al., 1984; Rao et al., 1979) may be due to differences in the plant age, the inoculation method and the concentration of the isolates used. Major differences between cultivars following inoculation with a single isolate have not been reported. Differences in time of appearance of bacterial ooze have not been reported and would be very difficult to measure.

Differences between cultivars for the rate of lesion extension have been reported (Yoshimura et al., 1984; Baw, 1985) using either lesion length or percentage leaf area infected as a measure of daily lesion development. Logit and Gompertz transformations of the original data have been used (Yoshimura et al., 1984; Baw, 1985) for comparison. The integral of the lesion extension curve (ADPC) was also used as a measure of comparison by Baw (1985). While it could be expected that information from repeated scorings would improve the ability to distinguish between cultivars, Baw (1985) concluded that a single assessment better distinguished 39 cultivars ranging from highly resistant to highly susceptible than the parameters based on repeated scores. For general screening purposes the rate of lesion extension seems to be excessively time consuming for the added information gained. However, greater attention to the form of the curves may show small but important differences in the rates at which lesions begin and end extension.

Bacterial counts from infected leaves

Sap extracted from leaves infected with an incompatible isolate has been shown to reach a maximum bacterial count 10^2-10^3 fold lower than sap from the same cultivar infected with a compatible isolate (Kaku and Kimura, 1983; Barton-Willis et al., 1986). There is some evidence (Kaku and Kimura, 1983) that a relationship between lesion lengths and bacterial concentrations can be found for susceptible reactions where the cultivars vary in quantitative resistance. A 10^1-10^2 fold difference in bacterial counts between highly susceptible cultivars and a more moderately susceptible cultivar has been noticed by Kaku and Kimura (1983) 12 days after inoculation.

An indirect method of quantifying the bacteria was tried by Horino and Yamada (1975), who measured the exudation from leaf segments directly after cutting. Their exudation index combined observations on the size of the bacterial cloud from each vascular bundle with a count of the number of bundles exuding bacteria. The index was used to rate 20 cultivars one day before symptom appearance and was found to better distinguish between cultivars than a 0-5 rating of lesion size (Horino and Hifni, 1978). It is unclear whether the advantage of the exudation index would remain when lesion length or %DLA were used instead of a disease index.

It is known that bacterial presence does not always lead to symptom development (Goto, 1965). The relation between lesion size and bacterial concentration can be confounded with factors related to the sensitivity of the tissue to destruction in the presence of bacteria. For this reason two tests have been used to follow latent bacterial presence in apparently healthy tissue. Parry (1985) followed the spread of virulent and avirulent bacteria during the first 172 hours of infections in IR20 and Cas 209. He cut inoculated leaves into 2 cm segments and scored for the presence of Xco colonies after four days incubation on peptone sucrose agar. Large differences in the movement were found between the two cultivars but little difference could be found between the two bacterial isolates for each cultivar. Goto (1965) developed a technique for coloured dye uptake in detached leaves. Using a basic fuschine solution he showed that the area of vascular blockage was considerably larger than the area of visible lesions. Reddy and Kauffman (1973) used this technique to show differences between a resistant and a susceptible cultivar.

Electrolyte conductivity

The conductivity of the leaked electrolytes has been used to compare cultivars for damage caused by the bacteria to the leaf tissue. Kohno et al. (1981) report that three days after infection fluid from a compatible reaction showed greater conductivity than that from an incompatible reaction. These differences remained detectable for up to 18 days after infection (Rao et al., 1979).

Infectivity titration

Infectivity titrations can be used to derive a number of parameters useful in varietal screening (Ercolani, 1984). The dose-response curve can be used to compare the test cultivars for the dosage needed to elicit a certain response, usually the effective dose at which 50% of the inoculated units show symptoms (ED_{50}) or to compare the response at one or more dosages. The slope of the line at the ED_{50} point can also be used to compare reactions (Ercolani, 1984). Two cultivars with equal ED_{50} but different dose response curves will show differences at higher and lower dosages. The researcher must therefore realize that when using a single value for comparison caution should be taken that this value be related to concentrations expected in field infections (Ercolani, 1984).

IRRI (Anon., 1981) reported differences in the ED_{50} of Cas 209 for compatible and incompatible reactions at different growth stages. Cho (1975) found differences in the dose-response curves of seven cultivars using four concentrations varying from 10^5-$2x10^9$ cfu/ml. The lesion size in compatible reactions increased with the dosage. This quantitative relationship between dose and reaction should be further studied.

Epiphytic growth, surface reactions and chemotactic responses

Phytopathogenic bacteria have been found to survive and multiply on leaf surfaces, and this can provide inoculum for disease. Studies with *Pseudomonas* species and with *Xanthomonas campestris* pv. *phaseoli* have shown that epiphytic populations of pathogenic bacteria "are generally lower on resistant compared to susceptible cultivars of a given host species" (Hirano and Upper, 1983). Because methods to quantify epiphytic populations are tedious and the results very variable, direct measurement of this value does not appear a useful parameter for cultivar screening, although it might explain unexpectedly low or high field resistance of a

cultivar as compared to levels following clip or prick inoculation.

One aspect of this epiphytic bacterial interaction is the attractive quality of host exudates. The ability of rice water-pore exudate to attract Xco was studied by Feng and Kuo (1975). The number of bacterial cells attracted from a central source into a capillary tube filled with exudate was compared. Exudates from highly susceptible cultivars and synthetic medium attracted up to eight times as many bacteria as exudates from non-hosts, several resistant cultivars and distilled water. Different isolates were not tried.

Histological evidence

Histological studies of compatible and incompatible interactions have shown differences in the spread of the bacteria (Kaku and Kimura, 1983). In addition there was evidence of cellular disintegration, both in the vascular bundle or in the adjacent parenchymatous tissue, as the bacteria translocate from the point of inoculation, except in symptomless types of reactions (Kaku and Kimura, 1978).

Electron micrograph studies showed dramatic change in the integrity of the host vessel wall three days after inoculation. The inner vessel wall was loosened and released into the vessel. Bacteria within the vessel were misshapened and immobilized in a mass of fibrillar material (FM). When the cytoplasm of vascular parenchyma cells was disrupted the bacteria appeared to be dead (Horino, 1978). Some FM was found in compatible interactions 20 days after inoculation but the bacteria were not as misshapened as in the incompatible reaction (Horino and Yamada, 1982). The time taken until FM appears may be a parameter for varietal comparisons.

Mew et al. (1984) reported differences in the reaction of water pores following contact with compatible or incompatible bacteria. This reaction should be further studied on more cultivars.

ASSESSMENT OF VARYING TYPES OF RESISTANCE

Seedling resistance

Seedling resistance is usually identified following clip or prick inoculation in both seedling and maximum tillering or booting stage. A single isolate of known virulence is usually sufficient to indicate the presence or absence of the resistance. As the most highly resistant

plants are usually sought, great attention should be paid to detecting fine distinctions between plants showing a resistant reaction. Attention to lesion type and speed of appearance may aid in developing the resistance to its most full expression.

Adult plant resistance

Identification and characterization of adult plant resistance will necessarily involve tests at various stages to determine when the resistance begins to develop, when it reaches its maximum expression, as well as the maximum level of the resistance. These tests should be done as staggered plantings to avoid confounding influence of environment or inoculum concentration with maturity dates. Prior tests should be done to determine when protection against bacterial blight is first needed, and inoculation for selection should be done no later than at this stage, if the resistance is to be satisfactory. Care must be taken to consider flowering dates during selection to avoid selecting for earliness (Zhang and Mew, 1985).

Moderate resistance

This can be identified by clip or prick inoculation with a single isolate. Yamada (1986a) recommends using a highly aggressive isolate for this purpose. Interest in moderate resistance centers on its quantitative nature, as durable resistance tends to be quantitative (Parlevliet, 1979). The quantitative nature of moderate resistance can be tested with a cross with a susceptible check cultivar. Where bimodality in the F_2 generation is found the presence of a weak major gene is likely. It can also be helpful to test the cultivar with varied isolates. Large interactions can indicate a mono- or oligogenic basis of the resistance.

Resistance to kresek wilt

Dipping the roots of seedlings immediately before transplanting was shown to be efficient in inducing severe kresek infection (Zaragoza and Mew, 1979). A kresek wilt strain of Xco should be chosen for this purpose (Mew, 1978). The incidence of symptoms can be assessed at varying days after infection.

CHAPTER 3

APPROPRIATE PARAMETERS FOR ASSESSMENT OF BACTERIAL BLIGHT IN RICE

SUMMARY

Lesion lengths, leaf lengths and leaf widths were measured on infected leaves two weeks following clip inoculation of 64 rice cultivars with two virulent isolates of *Xanthomonas campestris* pv. *oryzae*. No significant correlation was found between the lesion length and the leaf dimensions, indicating that physical leaf size does not affect the spread of the bacteria once these have entered the leaf. Lesion length is therefore an acceptable parameter for assessing disease reactions, and is to be preferred above the estimated percentage diseased leaf area for assessing moderate resistance, where fine distinctions between cultivar reactions are hard to estimate. Xylem vessel diameter was not found to be significantly correlated with lesion length of 16 cultivars inoculated with a virulent isolate, indicating that physical size of the vascular system was not a restrictive factor in determining the level of susceptibility.

INTRODUCTION

Bacterial blight (BB) of rice has been controlled by cultivars resistant to the causal organism, *Xanthomonas campestris* pv. *oryzae* (Xco). These cultivars have a high level of resistance and lesion size following clip inoculation is limited to less than 5% and sometimes less than 1% of the leaf area at all growth stages (Mew et al., 1981). However, the resistance has been found to decrease within a few years following introduction of such a resistant cultivar due to changes in the genetic make-up of the bacterial population (Vera Cruz and Mew, 1989). For this reason interest in the quantitative resistance (QR) of moderately resistant (MR) cultivars has increased as a possible source of more durable resistance.

In breeding programs large numbers of lines are screened for their

resistance to BB by estimating the percentage diseased leaf area (%DLA) following clip or prick inoculation. However when the purpose is to select among MR lines or individuals %DLA estimates are less appropriate. An MR reaction typically results in lesions covering 10-30 %DLA, or more when the plants are young. The human eye can fairly accurately distinguish between 1, 3 and 5 %DLA but becomes less precise as the lesion area increases. Instead of %DLA therefore, actual lesion length has been used to distinguish between MR cultivars or individuals following clip or prick inoculation. This allows for more accurate measurement, especially in the midrange.

Another problem with assessing MR using %DLA is the variation in leaf sizes between cultivars and development stages. Since the lesion is estimated in relation to leaf size, differences in lesion size may be overshadowed by larger differences in leaf size. A considerable error may be introduced by using %DLA when cultivars or different plant stages are compared. Use of lesion length measurements would eliminate this problem.

Before adopting lesion length as a parameter for assessment of MR, we must be certain there is no relationship between lesion length and leaf size (leaf length and width). If an association is found, lesion length must be considered as unsuitable for making comparisons of resistance between plants. A significant correlation of lesion length with leaf characteristics would also imply a physiological relationship between leaf size and the bacterial movement in the vascular system.

A test for such a correlation between lesion length and leaf length both within and between cultivars was carried out using the data from a cultivar screening for moderate resistance to BB. To further investigate the possible relationship between leaf dimensions and lesion length the diameter of the central xylem vessel, a major pathway for bacterial spread following clip inoculation, was measured and correlated with the lesion length.

MATERIALS AND METHODS

Lesion length and leaf size

Seed of 64 rice cultivars of varying origins were obtained from the International Rice Germplasm Collection at the International Rice Research Institute. Seeds were sown in lowland soil in 33x26x10 cm plastic trays

and the 21 day old seedlings were transplanted to 20x2.5 m beds in a protected screenhouse. Per cultivar 12 hills in a single plot were grown. Plants were fertilized with ammonium sulfate (21-0-0, 175 kg/ha total) applied in two applications, one before planting and one before maximum tillering.

Xco isolates PX0126 (race 2) and PX099 (race 6) were stored at -10 °C in skim milk suspensions until needed. The isolates were revived on peptone sucrose agar (PSA) slants incubated at 30 °C and transferred once to fresh slants for further increase of inoculum. Inoculum was prepared by suspending the bacteria in distilled water and adjusting the optical density to 1.00 (590 nm), to give a concentration of approximately $5x10^9$ cfu/ml.

Plants were inoculated at 65 days after seeding using the clipping method described by Kauffman et al. (1973). Tillers of each hill were divided into two groups before inoculation so that each hill could be inoculated with two isolates. Lesion length and leaf length of 20 leaves per cultivar were scored 14 days after inoculation. The width of the midleaf section was also measured for leaves inoculated with PX099.

Xylem diameter study

Sixteen rice cultivars varying from MR to highly susceptible were selected from the above 64 cultivars. Seeds were germinated and transplanted as described above. Plots of six hills per cultivar were planted in a randomized block design in three replications.

Isolate PX099 was maintained as described above. Plants were clip inoculated 64 days after seeding. Seven days after inoculation 10 leaves were harvested from each plot and measured for lesion length, leaf length, and leaf width. A small piece from the front of the active lesion was removed for microscopic analysis.

To measure the natural change in the xylem diameter within a leaf uninoculated leaves of the highly susceptible cultivar TN1 and the MR cultivar Cisadane were also harvested and pieces were cut from the leaf at 0, 1, 5 and 15 cm from where the inoculation point would have been.

Leaf pieces were fixed for a minimum of 48 hours in formaline acetic acid (FAA) and then dehydrated in increasing concentrations of tertiary butyl alcohol. The pieces were embedded in paraffine, mounted on wooden blocks and sectioned to 12-15 µm thickness with a rotary microtome. Sections were mounted on slides using Haupt's adhesive (Johansen, 1940) and stained with thionine and orange G (Stoughton, 1930). The sections

Table 3-1: Mean lesion lengths, leaf lengths, and leaf widths (in cm) measured on 64 rice cultivars clip inoculated with two isolates of *Xanthomonas campestris* pv. *oryzae*.

Cultivar	Isolate PXO99			PXO126	
	Lesion length	Leaf length	Leaf width	Lesion length	Leaf length
Zhu-xi 26	20.5	25.4	1.3	10.9	29.2
BR161-25-25	6.7	37.5	1.3	6.0	35.0
Milyang 42	12.7	39.7	1.6	7.9	38.9
TNAU 658	27.0	39.7	1.3	11.0	36.3
Peng chiu moung	20.9	43.1	1.1	7.5	40.0
IR40	12.3	44.3	1.3	7.7	43.1
Hashikalmi	4.1	45.6	1.6	1.1	41.7
Nigeria 5	5.4	45.7	1.1	6.3	50.2
BR51-282-8	12.0	46.0	1.5	7.4	43.2
Shaitan dumra	29.4	46.6	1.7	11.6	47.2
BR171-2B-8	7.5	46.8	1.6	5.9	42.9
ARC 7325	8.0	47.4	1.5	3.8	48.5
TKM 6(B)	10.1	47.8	1.0	6.0	41.6
Cisadane	4.1	48.6	1.2	5.4	42.1
Pelita 1/1	15.0	49.1	1.5	8.8	57.1
IR54	5.6	49.7	1.2	5.0	51.7
DV-2	28.9	50.2	1.7	10.1	48.6
Nam-sa-gui 19	12.2	50.9	1.6	12.1	42.3
TN1	25.3	51.1	1.4	10.9	47.2
BR319-1-HR38	10.0	51.4	1.7	5.3	42.6
GH 105	6.9	51.5	2.0	6.5	47.5
AUS 230	2.2	51.9	1.4	1.5	47.3
Kwang-cer-ai 5	21.8	51.9	1.4	6.9	48.0
Ta-poo-cho-z	12.0	52.4	1.0	5.1	50.0
Ayung	7.5	52.5	1.6	5.8	53.2
Kalimekri	1.5	53.2	1.2	1.2	51.5
RP 633-76-1	3.6	53.5	1.2	0.8	47.9
ADT 18	27.4	54.8	1.4	11.2	49.6
IR26	14.3	55.3	1.3	8.8	49.3
Hsinchu 56	11.3	55.5	1.1	2.3	53.5
IR5	14.1	55.8	1.3	8.8	52.9
Dourado agulla	9.4	56.2	1.5	7.3	44.2

Table 3-1 (continued)

Cultivar	Isolate PXO99			PXO126	
	Lesion length	Leaf length	Leaf width	Lesion length	Leaf length
IR9101-46	22.9	56.5	1.4	11.8	54.7
B441B-126-3-2-1	13.8	56.8	1.3	6.1	55.3
Pankaj	11.6	58.6	1.4	7.5	57.6
CO 39	14.1	58.6	1.4	7.2	55.2
Warrangal 1263	20.2	60.2	1.2	11.6	55.5
IR4442-46-3-3-3	7.2	60.9	1.2	7.4	57.5
MI-48	12.5	61.5	1.2	6.5	57.9
Century patna 23	17.9	64.4	1.1	8.6	62.7
IR48	10.2	65.2	1.3	4.4	64.5
Mahsuri	10.8	66.4	1.1	6.9	66.0
Pae moku	9.8	66.5	1.9	1.0	59.1
BJ 1	3.3	67.6	1.1	1.9	62.4
Tahun gembrong	10.8	68.0	1.5	4.9	67.8
BPI 76	23.1	68.1	1.4	7.1	66.1
Gokaung	11.1	68.5	1.3	9.4	80.7
Sigadis	14.8	71.2	1.3	11.1	73.2
Early prolific	14.3	71.3	1.3	1.7	72.3
Remadja	11.9	71.4	1.4	7.1	66.8
Sigadia	5.9	72.4	1.5	11.7	68.8
JC 70	29.2	74.2	2.2	15.0	72.5
Elwee	15.3	75.1	1.3	9.0	72.8
Lead rice	19.5	75.4	1.3	7.3	70.6
RD 6	13.0	77.0	1.4	8.0	75.8
GEB 24	14.3	77.1	1.0	10.0	71.2
Ngome Tia	15.7	77.4	1.6	9.9	73.1
Ranbirga	10.0	79.2	1.0	3.4	77.4
Ramadja	11.5	79.4	1.4	8.3	75.7
Niaw sanpan tawn	18.1	79.8	1.6	9.5	73.6
ARC 6018	12.7	79.9	1.1	8.3	70.1
Akundi	15.2	81.5	1.3	0.6	76.2
Meegauk	7.6	82.0	1.1	6.4	74.1
PTB 33	15.6	82.7	1.0	7.5	77.1

were viewed at 1000x magnification under a light microscope. The length and width of the center of the two central xylem vessels was measured using a microfilameter. The diameter (d) of a vessel was calculated by converting the surface of the measured ellipse into that of a circle of equal surface using the formula:

$d = \sqrt{L_1 \times L_2}$ L_1=ellipse length, L_2=ellipse width

The error in the diameter as a result of deviations from the perpendicular orientation of the leaf piece with the direction of movement of the microtome was found to be at most 5% and was therefore disregarded.

RESULTS

Lesion length and leaf size

Average lesion lengths of the 64 cultivars varied from 1.5 to 29.4 cm for PX099 and from 0.8 to 15.0 cm for PX0126 while leaf lengths varied from some 25 cm to 83 cm (Table 3-1). There was no significant rank correlation between lesion length and leaf length for all cultivars combined (r_s=0.156 for PX099 and r_s=0.068 for PX0126). Omission of the most resistant cultivars, which may have had some effect of a major gene, changed the r_s value for PX099 to -0.27, which is significant (P=0.05) but still very low, and changed the correlation for PX0126 to 0.109, which is not significant. Within cultivars correlations between lesion length and leaf length were occasionally found to be significant for either PX099 or PX0126, as is to be expected considering the number of leaves per cultivar measured (20) and the large number of cultivars tested. The majority of the cases showed no significant correlation between lesion length and leaf length.

The percentage diseased leaf length infected (lesion length/leaf length x100, %DLL) was compared with the lesion length to estimate the importance of variations in leaf length on assessment when a relative assessment parameter is used. Rank correlation (r_s) between lesion length and %DLL was 0.64 for plants inoculated with isolate PX099 and 0.40 for plants inoculated with isolate PX0126.

Average leaf widths varied from 1.0 to 2.2 cm (Table 3-1). The correlation between leaf width and lesion length for PX099 was not significant (r_s=0.165). Omission of the most resistant cultivars did not

improve this relationship. Leaf length was not correlated with leaf width (r_s=-0.165).

Xylem diameter study

Within the 16 cultivars xylem vessel diameter varied from 32.5 to 55.9 µm, with an average of 41.8 µm (Table 3-2). The lesion length appeared to be correlated with the vessel diameter (r=0.72**). However, this correlation was found to be largely caused by the correlation between two extreme values, that of JC70, a very highly susceptible cultivar with extremely long, broad leaves and large vessels, and IR40, a MR cultivar

Table 3-2: Lesion lengths, leaf lengths and widths, and xylem vessel diameters measured on 16 cultivars following clip inoculation with race 6 isolate PXO99 of *Xanthomonas campestris* pv. *oryzae*. Values in a column followed by a common letter are not signifcantly different (P=0.05, Bonferroni's test for inequalities).

Cultivar	Lesion length		Leaf length		Leaf width		Vessel diameter	
	mean (cm)	var. (-)	mean (cm)	var. (-)	mean (cm)	var. ($x10^{-3}$)	mean (µm)	var. (-)
Cisadane	4.1f	3.4	32.7d	0.0	1.2cdefg	1.2	41.0cde	0.3
IR54	5.3ef	3.1	34.8d	0.5	1.2defg	0.7	39.1cde	0.2
IR40	5.5ef	1.2	32.5d	5.0	0.9h	0.6	32.5f	0.4
IR4442-46	5.9ef	1.5	34.8d	4.1	1.1g	1.2	36.6ef	0.8
BR319-1-HR38	5.9ef	2.1	41.4bcd	13.0	1.4bc	3.0	43.9c	8.0
BR161-25-25	6.0def	0.4	35.2d	0.8	1.1fg	2.0	40.1cde	2.0
BR171-2B-8	6.2cdef	1.2	39.7bcd	8.3	1.4b	3.9	42.0cd	0.8
IR58	6.2cdef	3.7	32.7d	1.6	1.2cdefdg	1.3	37.2def	0.3
Biplab	6.7cdef	2.4	40.2bcd	0.5	1.3bcdef	2.4	42.0cd	0.8
BR51-282-8	7.5cde	0.9	38.7cd	10.7	1.3bcde	6.3	40.5cde	6.2
IR9101-124	7.7cde	1.6	35.3d	0.2	1.2cdefg	4.4	42.0c	6.1
Gaja baru	8.2bcde	7.7	49.6ab	4.0	1.3bcd	3.2	43.4c	1.7
IR28	9.4bcd	0.5	38.2cd	1.7	1.2defg	0.1	39.5cde	0.8
Tiri 253	9.5bc	7.2	46.2bc	50.4	1.4b	0.9	49.4b	1.7
TN1	11.5ab	1.2	41.9bcd	11.0	1.2efg	4.9	42.1c	0.6
JC70	14.4a	5.8	57.0a	13.5	1.8a	7.2	55.9a	9.3
mean	7.5		39.2		1.3		41.8	

which has very narrow vessels. When one of these values was eliminated from the analysis no significant correlation remained (r=0.44 and 0.50, respectively).

Leaf length and leaf width were both found to be significantly correlated with xylem vessel diameter (r=0.88** and r=0.92**, respectively). The vessels in the midrib were shown to widen in the direction of the leaf base. The average increase in diameter was 0.43 µm/cm between 0 and 15 cm below the point where the leaf begins to narrow towards the leaf tip.

DISCUSSION

Lesion length is not correlated with leaf length or leaf width. If there is an association between lesion length and xylem vessel diameter it does not appear to be a strong one. The lack of correlation between lesion length and leaf length and width permits the use of lesion length for assessing resistance to Xco without fear of confounding influences of leaf size. Surprisingly, leaf length was not strongly correlated with leaf width.

A significant correlation between lesion length and the diameter of the xylem vessel of the leaf midrib was not clearly shown. Xylem vessel diameter appeared to be correlated with both leaf length and width, although this conclusion is based on evidence from 16 cultivars only. Because the tissue for measuring xylem vessel diameter was taken from the lesion front the xylem was measured more towards the base of leaves of more susceptible cultivars than of more resistant cultivars. The general tendency for xylem vessels to widen toward the leaf base could have resulted in a seeming correlation. The fact that no correlation was found supports the lack of a strong relationship between these two factors.

Because of the speed with which %DLA can be assessed it will remain the preferred parameter for mass screening or for screening for highly resistant cultivars. However, lesion length is a more accurate measure than %DLA for comparisons of reactions of cultivars or individual plants with moderate or low levels of resistance. Lesion length is a continuous scale which can be measured at all lesion lengths with equal accuracy. Estimates of %DLA fall into discrete groups limited by our ability to distinguish differences; the law of Weber-Fetcher, as interpreted by Horsfall and Barrett (1945) shows that the range of actual areas we group together increases logarithmically as the lesion area increases to 50%,

and then decreases again as the lesion area approaches 100% as we estimate the remaining green area with increasing accuracy. In the range expected for MR and moderately susceptible reactions (5-50 %DLA) the range of values which will be grouped into one %DLA class can be quite large. This will limit the ability to distinguish differences or will require that more individual measurements be made.

Lesion length is even more to be preferred above %DLA when making detailed comparisons of cultivars with varying leaf lengths, such as when both traditional and improved short statured rice cultivars are compared. With a given lesion length cultivars with long leaves will be scored by %DLA as more resistant than cultivars with short leaves while when lesion length is used as a parameter they would be scored as equally resistant. For example a lesion of about 20 cm length in Warrangal 1263 (PX099) will give a totally different %DLA than an equally long lesion in Zhu-xi 26, as the respective leaf lengths were 60.2 and 25.4 cm (Table 3-1). Since the aim will be to transfer the resistance to a new cultivar the actual host-parasite interaction, as measured by lesion length, is a better parameter for selection than %DLA, which in fact measured leaf damage caused by the disease.

Both wide and long leaves appear to have larger diameter xylem vessels. The lack of a clear correlation between lesion length and xylem vessel diameter is thus in agreement with the above results. A relationship between bacterial blight development and xylem vessel diameter was suggested by the fact that the lesion following clip inoculation is usually longest at the midrib (containing the largest xylem vessel) and is shorter at the leaf margins. Park and Cho (1972) also investigated the possibility that the physical dimensions of the xylem vessel of a leaf may affect bacterial movement. They reported a lack of correlation between resistance of six Japonica cultivars and xylem vessel diameter but found a relationship between the xylem vessel length and the level of resistance.

Race-specific resistance in rice to Xco has been demonstrated, and the lesions which develop on rice leaves following infection by virulent isolates of Xco are probably a result of active chemical processes involving both host and parasite and affecting bacterial movement, bacterial multiplication and plant cell damage, rather than purely a result of passive physical processes. Any physical effect of xylem dimensions would imply that another mode of quantitative resistance was also present; to date no evidence for such an alternative source of variation in resistance has been demonstrated in rice against Xco.

CHAPTER 4

POPULATION TRENDS OF *XANTHOMONAS CAMPESTRIS* pv. *ORYZAE* IN SUSCEPTIBLE AND MODERATELY RESISTANT RICE CULTIVARS

SUMMARY

Lesion length and bacterial counts were assessed from leaves of moderately resistant to highly susceptible rice cultivars inoculated with a virulent isolate of *Xanthomonas campestris* pv. *oryzae*. Three separate tests were made on clip-inoculated plants 55-63 days after sowing. The bacterial counts per leaf increased rapidly from the first day after inoculation and continued to increase until 14 days after inoculation. Highly susceptible cultivars were found to have more bacteria per leaf than moderately susceptible cultivars from three days onward. Significant and high correlations between lesion length and bacterial counts of the lesion border area were found for 10-14 days after inoculation, the r-values ranging from 0.60** to 0.85**.

INTRODUCTION

Resistance in rice to bacterial blight, caused by *Xanthomonas campestris* pv. *oryzae* (Xco), is assessed on the basis of disease severity (% diseased leaf area, %DLA) or absolute lesion length following inoculation with a concentrated suspension of the pathogen. A hypersensitive response is not known in this host-pathogen system (Parry and Callow, 1986) and other qualitative signs of resistance are only sometimes seen (Kaku and Hori, 1977). Cultivars highly resistant to Xco have been developed (Horino et al., 1981) and their resistance has been shown to result from the action of one or more of 14 race-specific major genes (Mew et al., 1982). Symptoms of such resistant cultivars remain restricted to very small or small lesions. Moderately resistant (MR) and moderately suscep-

tible (MS) cultivars show reduced symptom development, as compared to highly susceptible cultivars (S), following infection with virulent isolates of Xco. Moderate resistance has in some cases been shown to be an incomplete reaction of known major genes to certain isolates (Yoshimura et al., 1984). In other cases it has been shown to be quantitatively inherited (Yamada, 1986a).

Kaku and Kimura (1983, 1987) have shown that highly resistant reactions are related to lower levels of bacteria in the infected leaf. They found that bacterial populations of a compatible interaction stabilized at a level of about 10^8 cfu/leaf. Populations of an avirulent isolate inoculated to the same cultivar began to increase at the same rate as the virulent isolate but stabilized at levels 10-100 times lower.

Moderately resistant and susceptible cultivars differ quantitatively for lesion size following inoculation with a virulent isolate. Such differences could be due to reduced bacterial growth within the leaf, but could also be due to reduced tissue damage with equally large bacterial populations in the MR cultivars. Population trends of MR and MS cultivars were therefore measured and the results compared with the lesion size of each cultivar to determine if reduced lesion size is related to a reduction in the bacterial population.

MATERIALS AND METHODS

Experiment 1

Seeds of ten cultivars ranging from S to MR were sown in a seedbed and transplanted 17 days later to a lowland bed in screenhouse. Cultivars were planted in rows of six plants per cultivar in three replications in a randomized block design. A split application of 21-0-0 ammonium sulfate nitrogen fertilizer was applied (175 kg/ha in total) and insect control (monocrotophos) was carried out when needed.

Plants were inoculated 63 days after sowing with the virulent race 2 isolate IRN812, which grows well on a semispecific medium composed of peptone sucrose agar (PSA) to which 100 mg/l cyclohexamide, 10 mg/l trichloroacetic acid and 5 mg/l trimethoprim had been added after sterilization (T.W. Mew, pers. comm). The isolate was stored at -10 °C in skim milk until needed. Inoculum was prepared from a three day old culture grown on PSA at 30 °C. The bacteria were suspended in distilled water and the optical density (590 nm) of the suspension was adjusted to 1.00 (approxim-

ately $1x10^9$ cfu/ml). The top two fully extended leaves of each tiller were inoculated by clipping 2-3 cm off the leaf tip using scissors dipped in the inoculum suspension.

Leaves were harvested 1, 2, 4, 7, 11 and 14 days after inoculation (dai). Leaf width at the clip point or leading edge of the lesion and, where applicable, lesion length were measured. Leaves were trimmed to 10 cm from the clip point (1, 2, 4, 7 dai) or to 2 cm lesion plus 8 cm green leaf directly below the lesion edge (11 and 14 dai). Such a section was taken to avoid using necrotic tissue which was felt to be the source of contamination during isolation. Three leaves per cultivar per replicate were ground with a leaf and bud press (Erich Pollahne, Wenningsen, West Germany) washed with 10 ml sterile water and were serially diluted. From each chosen concentration 1 ml of suspension was mixed with approximately 5 ml of warm (40 °C) semispecific medium and spread in petri plates. Colonies were counted 4 days after plating.

Experiment 2

Bacterial populations were measured on six of the ten cultivars used in Experiment 1. Four of these, Cisadane, BR51-282-8, IR28 and IR40, have been found to be moderately resistant to moderately susceptible and two, TN1 and IR9101-46, have been found to be highly susceptible to virulent isolates (Chapter 9, this thesis). Seeds were germinated on moist filter paper and seedlings were transferred to six 33x26x11 cm plastic trays filled with lowland soil when the coleoptile was 1-2 cm long. Each tray contained six plants of each of the six cultivars, planted in rows following a randomized block design within the tray. Trays were placed on tables in the greenhouse. Nitrogen fertilizer and insect control were applied as needed. Before inoculation the best four trays (four replicates) were selected for use and the plants were trimmed so that only the main tillers remained. Plant were inoculated 55 days after sowing.

To facilitate isolation of the bacteria, a spectinomycin resistant mutant 306, derived from the race 2 isolate IRN793 (E. Ardales and H. Leung, IRRI, pers. comm.), was used in Experiments 2 and 3. Virulence of the mutant was verified by inoculation on the above-mentioned test cultivars. Lesion lengths were found comparable with the original isolate. A single colony subisolate was selected for further use and was stored in skim milk at -10 °C until needed. Inoculum preparation and inoculation was carried out as described for Experiment 1.

One leaf per cultivar per replication was harvested for extraction

at 1 hr, 1, 3, 5, 7 and 11 dai. The uppermost inoculated leaf was harvested from each test plant. Clip width and, where applicable, lesion length were measured on each leaf immediately after harvest. Bacteria were extracted by grinding each leaf separately in a leaf and bud press. The entire leaf was used on 7 and 11 dai, in contrast to Experiment 1, where only a section from the lesion border area was used. Serial dilutions were made in distilled water and plated on PSA to which spectinomycin (100 mg/l) had been added after sterilization. Plates were incubated at 30 °C and colonies were counted four days after plating.

Experiment 3

Seeds of six cultivars used in Experiment 2 plus four other cultivars including the highly resistant cultivar IR1545-339 (xa-5 gene for resistance) were germinated on moist filter paper and transferred to 15 cm diameter clay pots filled with lowland soil. Each pot contained three plants and one pot was equal to one replication. Pots were arranged in four replications in a randomized block design on tables in the greenhouse. Nitrogen fertilizer and insect control (monocrotophos) were applied as needed. Plants were inoculated 55 days after sowing.

Storage, multiplication and inoculation of isolate 306 was carried out as described in Experiment 1. One leaf per cultivar per replication was harvested on 8, 10, 12 and 14 dai. Lesion length and leaf width at the lesion front were measured and the leaf was trimmed to the lower 5 cm lesion plus the adjacent 5 cm green tissue. These 10 cm sections were macerated as described above, serially diluted and plated on PSA containing spectinomycin (100 mg/l).

RESULTS

Experiment 1

Mean bacterial counts increased from 8.0×10^2 cfu/leaf one dai to 1.1×10^7 cfu/leaf section 14 dai. The greatest logarithmic increase was between one and four dai (Table 4-1). Levels increased from 1 to 4 dai, remained about equal 4-7 dai, increased again by 11 dai and remained constant between 11 and 14 dai. A significant difference between cultivars for bacterial counts was found on 11 and 14 dai.

Lesions were first seen four dai. Significant cultivar differences

Table 4-1: Lesion lengths and bacterial counts[1] measured on clip-inoculated leaves of ten rice cultivars at varying days after inoculation (Experiment 1).

Cultivar	Days after inoculation											
	1		2		4		7		11		14	
	lesion (cm)	count ($x10^2$)	lesion (cm)	count ($x10^4$)	lesion (cm)	count ($x10^5$)	lesion (cm)	count ($x10^5$)	lesion (cm)	count ($x10^6$)	lesion (cm)	count ($x10^6$)
IR4442-46	0	8.9 ± 1.7a[2]	0	2.1 ± 0.9a	0a	8.6 ± 1.8a	2.5a	2.0 ± 2.5a	5.4 a	5.8 ± 1.7ab	6.7 a	3.6 ± 1.0a
BR51-282-8	0	7.6 ± 1.6a	0	3.3 ± 1.4a	0.1a	6.4 ± 5.7a	4.3abc	6.5 ± 0.9a	7.5 a	15.6 ± 4.3bcd	8.6 ab	4.9 ± 0.6ab
IR40	0	9.2 ± 3.0a	0	2.2 ± 0.3a	0a	11.5 ± 2.5a	3.3a	2.5 ± 0.4a	6.6 a	17.8 ± 3.0cd	9.4 ab	8.5 ± 0.5ab
Cisadane	0	5.2 ± 1.3a	0	6.0 ± 4.9a	0a	22.1 ±10.5a	3.4a	5.8 ± 2.1a	7.7 a	7.0 ± 4.3abc	12.5 ab	4.1 ± 6.7a
BR171-2B-8	0	7.9 ± 0.2a	0	3.5 ± 1.2a	0a	4.1 ± 1.0a	4.0ab	4.4 ± 2.0a	7.2 a	4.4 ± 1.1a	13.6 abc	3.1 ± 1.6a
Biplab	0	8.9 ± 2.5a	0	2.7 ± 1.5a	0.1a	12.8 ± 4.4a	3.6a	4.2 ± 3.5a	8.4 a	11.8 ± 0.3abcd	14.3 bc	7.4 ± 2.4ab
IR28	0	7.5 ± 0.8a	0	2.7 ± 2.5a	0.1a	7.0 ± 5.3a	6.3bcd	1.9 ± 2.1a	11.2 ab	20.8 ± 4.6d	15.1 bcd	7.5 ± 2.4ab
IR9101-46	0	8.9 ± 1.4a	0	6.6 ± 5.0a	0a	23.1 ± 5.7a	4.8abcd	9.4 ± 3.2a	15.5 b	49.7 ± 5.8f	20.4 cde	18.7 ± 5.3b
Warrangal	0	10.0 ± 4.4a	0	4.0 ± 1.9a	0a	15.6 ± 5.3a	6.3cd	13.4 ± 2.0a	16.2 b	36.7 ± 0.5e	21.7 de	18.4 ± 7.7b
TN1	0	5.8 ± 0.9a	0	4.6 ± 3.6a	1.0a	14.1 ± 1.9a	7.1d	9.1 ± 0.5a	15.4 b	42.7 ± 8.8ef	22.3 e	36.6 ± 16.8c
mean	0	8.0	0	3.8	0.1	12.5	4.6	5.9	10.1	21.2	12.7	11.2

1) cfu / leaf (1-7 dai), cfu/10 cm section of leaf (11 and 14 dai).

2) ^{10}log values of numbers within a column followed by the same letter are not significantly different (P=0.05, Bonferroni's test for inequalities).

Table 4-2: Lesion lengths and bacterial counts[1] measured on clip-inoculated leaves of six rice cultivars at varying days after inoculation (Experiment 2).

Cultivar	Days after inoculation											
	0		1		3		5		7		11	
	lesion (cm)	count ($x10^4$)	lesion (cm)	count ($x10^4$)	lesion (cm)	count ($x10^7$)	lesion (cm)	count ($x10^7$)	lesion (cm)	count ($x10^8$)	lesion (cm)	count ($x10^8$)
Cisadane	0	1.1±0.3a[2]	0	1.3±1.1a	0	1.3±0.5abc	0.5ab	7.6 ±2.7ab	0.9a	1.2±0.2ab	7.0a	2.2 ±0.8a
IR40	0	1.0±1.0a	0	0.9±0.5a	0	1.0±0.3ab	0.1a	5.6 ±1.2a	1.0a	1.3±0.2abc	8.3a	4.6 ±1.1ab
BR51-282-8	0	1.7±1.0a	0	4.8±1.5a	0	1.4±0.2bc	1.0b	6.6 ±1.8ab	1.5a	2.5±1.0cd	8.7a	3.3 ±2.0a
IR9101-46	0	1.7±0.7a	0	3.2±2.5a	0	1.8±0.4bc	0.4a	13.0 ±1.2bc	1.7a	2.1±0.1cd	9.2a	12.0 ±1.9c
IR28	0	1.0±0.2a	0	2.2±0.4a	0	0.6±0.2a	0.2a	5.3 ±0.6a	1.2a	0.8±0.2a	9.7a	4.3 ±1.5ab
TN1	0	1.2±0.3a	0	4.2±1.1a	0	2.7±0.3c	1.1b	17.0 ±1.2c	3.3b	2.8±0.3d	15.3b	9.3 ±2.6bc
mean	0	1.3	0	2.8	0	1.5	0.6	9.2	1.6	1.8	9.7	6.0

[1]) cfu/leaf.

[2]) ^{10}log values of numbers within a column followed by the same letter are not significantly different (P=0.05, Bonferroni's test for inequalities).

for lesion lengths were found on 7, 11 and 14 dai (Table 4-1). A significant correlation between lesion length and ^{10}log bacterial count was found for 11 and 14 dai (r=0.85** and 0.74**, resp., calculated over all individual leaves of all cultivars). Adjustment for clip or leaf width differences decreased the correlation slightly.

Experiment 2

Mean bacterial counts per leaf increased from 1.3×10^4 cfu/leaf one hour after inoculation to 6.0×10^8 cfu/leaf 11 dai (Table 4-2). An increase in the bacterial populations was first seen three dai, at which time significant cultivar differences for ^{10}log bacterial counts were found. By five dai the two S cultivars TN1 and IR9101-46 had significantly higher levels than the four MR cultivars. This difference remained until 11 dai. Although the lesion length of the susceptible cultivar IR9101-46 was unusually low 11 dai the susceptible nature of this cultivar was apparent in the higher bacterial counts found. Adjustment of values for the clip or leaf width differences did not greatly affect the results.

Lesions were first seen five dai and all inoculated leaves showed lesions seven dai (Table 4-2). Significant differences between cultivars were found for lesion lengths both 7 and 11 dai. The correlation between lesion length and ^{10}log bacterial counts was significant on both days (r=0.54** and 0.60**, respectively, calculated over all individual leaves).

Experiment 3

Mean bacterial counts ranged from 7.7×10^7 cfu/leaf 8 dai to 18.1×10^7 14 dai (Table 4-3). Bacterial counts appeared to increase from 8 to 14 dai, although all extractions were from leaf pieces with 5 cm lesion and 5 cm adjacent green tissue. Bacterial counts in the highly resistant cultivar IR1545-339 were much lower than in MR, MS and S cultivars on all days. Significant differences between other cultivars were also found (Table 4-3). Lesion length was significantly correlated with ^{10}log bacterial counts of the 10 cm leaf sample on all days (r=0.66**, 0.72**, 0.79**, and 0.82** on dai 8, 10, 12 and 14, respectively). The correlation remained highly significant when the resistant cultivar IR1545-339 was excluded from the calculations.

Table 4-3: Lesion lengths and bacterial counts[1] measured on clip-inoculated leaves of ten rice cultivars at varying days after inoculation (Experiment 3).

Cultivar	Days after inoculation							
	8		10		12		14	
	lesion (cm)	count ($x10^7$)	lesion (cm)	count ($x10^7$)	lesion (cm)	count ($x10^7$)	lesion (cm)	count ($x10^7$)
IR1545-339	0.7a[2]	0.2 ±0.1a	1.1 a	0.5 ±0.2a	1.0 a	0.3 ±0.2a	1.7a	0.6 ± 0.3 a
IR40	4.8bcd	4.8 ±2.1b	7.0 bc	10.2 ±4.1b	7.7 b	8.4 ±0.3bcd	8.6b	10.6 ± 2.4 bcd
BR171-2B-8	5.7bcd	8.7 ±1.8b	7.4 bcd	9.5 ±2.0b	9.5 b	7.5 ±0.7bcd	9.0b	3.3 ± 7.7 ab
Biplab	2.5ab	4.8 ±1.8b	7.1 bc	5.2 ±3.8b	8.4 b	7.5 ±1.6bcd	9.1b	5.7 ± 3.9 bc
IR28	5.8bcd	3.8 ±3.5b	9.5 cd	4.9 ±1.6b	10.4 bc	3.4 ±1.8b	11.2b	7.6 ± 4.3 bc
IR54	3.8abc	4.7 ±1.9b	4.9 ab	7.4 ±2.5b	8.9 b	4.9 ±3.6bc	11.3b	20.7 ± 9.1 cd
BR51-282-8	6.3cd	11.4 ±3.3b	9.7 cd	12.7 ±1.8b	15.3 c	32.8 ±5.4d	14.9c	22.4 ±12.9 cd
TN1	7.0cd	13.3 ±3.4b	10.1 cd	14.2 ±1.1b	12.6 bc	18.5 ±6.2cd	15.2c	22.6 ±10.2 cd
IR9101-46	8.0d	15.0 ±3.9b	11.3 d	13.3 ±3.4b	12.3 bc	16.9 ±7.0cd	15.5c	40.6 ±13.5 d
Cisadane	8.2d	10.0 ±1.2b	10.8 cd	20.8 ±7.1b	12.2 bc	16.3 ±1.9cd	15.9c	51.4 ± 7.7 d
mean	5.3	7.7	7.9	9.9	9.8	11.6	7.7	18.1

1) cfu/10 cm leaf section.

2) ^{10}log values of numbers within a column followed by the same letter are not significantly different (P=0.05, Bonferroni's test for inequalities).

DISCUSSION

Bacterial populations began to increase between one and three days after inoculation (dai) and continued increasing until 11 or 14 dai. The most rapid increase occurred between one and three dai. Large bacterial populations were present before symptoms appeared.

Cultivars first differed in bacterial populations three dai in Experiment 2 but only 11 dai in Experiment 1. This difference may have been due to the improved extraction technique used in Experiments 2 and 3. The fungal growth inhibitors added to the semispecific medium used in Experiment 1 were found to have some effect on Xco growth as well. Small differences between cultivars may have therefore gone undetected in the earlier harvests of this experiment.

The significant correlation between bacterial population and lesion length observed indicates that bacterial presence within the rice leaves plays an important role in determining the length of lesions which appear following inoculation with Xco. The linear correlation between the logarithm of the bacterial counts and lesion lengths was much better than between the absolute counts and the lesion lengths. Since logarithmic transformation of unlimited bacterial growth results in a straight line when graphed against time, symptoms are probably related more to the bacterial growth rate than to simple bacterial presence.

Among the susceptible and moderately resistant cultivars tested in all three experiments bacterial population levels ranged from 4-14 fold difference between the lowest and the highest counts 7-14 dai, depending on the experiment. In comparison, the highly resistant cultivar IR1545-3-39 used in Experiment 3 ranged from a 5-10 fold lower level than the most resistant of the MR cultivars, and up to 67 fold lower than the susceptible cultivar TN1.

Populations of virulent and avirulent bacteria prick inoculated on cultivars with the resistance gene Xa-3 have been reported to differ by 10^2-10^3 fold (Kaku and Kimura, 1987). Leach et al. (1989) reported differences of up to 10^5 fold 12 dai, working with seedlings of the cultivars IR20 and Cas 209 inoculated with virulent and avirulent isolates using a Hagborg inoculator. Since seedlings are known to be more susceptible than adult plants (Chapter 5, this thesis; Mew et al., 1982) the high bacterial counts reported by Leach et al. (1989) further support the evidence for a general relationship between symptom development and bacterial populations. In contrast to these results, Parry and Callow (1986) found little difference between bacterial counts of compatible and incompatible reac-

tions. They attributed the difference in symptoms to a difference in the sensitivity of the host tissue to the pathogen.

Differences in susceptibility of cultivars, as assessed by lesion size, can result from:

1. differences in the ability of the bacteria to spread within a leaf,
2. differences in the ability of the leaf to support bacterial growth,
3. differences in the sensitivity of the leaf tissue to the presence of the bacteria.

Leach et al. (1989) tested factor 1 by following bacterial spread within a leaf by laying out 2 cm pieces of infected tissue and scoring for the presence or absence of bacteria in these pieces. They found that virulent bacteria spread further and faster than avirulent bacteria.

A measure of factor 2 would be the number of bacteria per cm lesion area. However, when the ^{10}log counts were divided by the lesion length, the correlation between lesion length and this value was somewhat lower than the correlation between the lesion length and the original counts from the whole leaf. This was probably due to the large proportion of bacteria in the tissue not yet showing symptoms and the possible variation in the sensitivity of the leaf tissue. Variations in the absolute size of the water-soaked part of the lesion may also affect the final count. The exact lesion area must be more accurately measured if small differences in the bacterial counts per unit area or in the sensitivity of the tissue to the presence of the bacteria are to be shown. The relatively large lesion size for the bacterial counts measured on IR28 (Table 4-3) may indicate that the third factor also plays a role, although probably not as large as that stated by Parry and Callow (1986).

Another factor, the ability of bacteria to survive in necrotic tissue, will affect the total amount of inoculum in a field, and is therefore important for determining the rate of spread of bacteria in a field. Bacterial populations can only stabilize when bacterial death is equal to bacterial growth. Differences in the rate of death in necrotic tissue (or failure of extraction) will therefore result in differences in the time at which the populations per leaf stabilize. Parry and Callow (1986) reported bacterial counts to have stabilized by the time the first symptoms appeared, while results here showed the total bacterial population to still increase 14 dai. This factor is expected to vary with isolate, cultivar or plant age, but also with the extraction procedure used. It is important that all bacteria per leaf be extracted when such comparisons are to be made.

CHAPTER 5

EFFECT OF PLANT AGE AND LEAF AGE ON THE QUANTITATIVE RESISTANCE OF RICE CULTIVARS TO *XANTHOMONAS CAMPESTRIS* pv. *ORYZAE*

SUMMARY

Lesion lengths were measured following clip inoculation of six moderately resistant and susceptible rice cultivars of seven plant ages ranging from 40-77 days after sowing with two isolates of *Xanthomonas campestris* pv. *oryzae*. Resistance in booting and post-flowering stages were further tested in a second experiment. The resistance, assessed by lesion length, increased considerably with age, the fastest increase occurring between 30 and 50 days after sowing. Between maximum tillering and flowering the increase in resistance was very small. Quantitative resistance was evident at all growth stages as a reduction in lesion length relative to susceptible cultivars. Lesions of all cultivars decreased in absolute length about equally, but the relative decrease in lesion length was greater with moderately resistant cultivars than with highly susceptible cultivars. Immature extending leaves were compared with the fully extended leaves directly below the extending leaves for susceptibility to bacterial blight. Immature leaves were more susceptible than extended leaves, but immature leaves of moderately resistant cultivars were less susceptible than those of highly susceptible cultivars. In order to compare entries of different earliness it is advised to assess lesion length when all entries are between maximum tillering and flowering.

INTRODUCTION

Moderately resistant (MR) and moderately susceptible (MS) cultivars are often found when screening rice cultivars for resistance to *Xanthomonas campestris* pv. *oryzae* (Xco), the causal organism of bacterial blight (BB). While a resistant reaction results in lesions of 0-15% of the length of the lesions of the most susceptible cultivar, MR and MS reac-

tions can be defined as having lesions ranging from 16-33% and 34-66%, respectively, of the length of the lesions on a highly susceptible cultivar (Chapter 1, this thesis; Mew and Vera Cruz, 1979). Lesions following clip inoculation of MR cultivars are usually between 5 and 10 cm in length, as compared to 25 cm or longer in the susceptible cultivar, and as much as 30 cm in a highly susceptible cultivar.

Ezuka and Horino (1976), and Mew et al. (1981) observed that young plants of susceptible cultivars tend to have longer lesions than older plants although the disease score usually does not drop by more than one or two points of the Standard Evaluation Scale (Chapter 2, this thesis). Cultivars with adult plant resistance based on the major gene Xa-3 have been shown to drop by as much as the full rating scale, starting as highly susceptible in the seedling stage and ending as highly resistant before booting (Zhang and Mew, 1985). MR and MS cultivars have also been shown to vary in reaction, although less consistently (Mew and Vera Cruz, 1979). Highly resistant reactions are usually stable throughout the growth cycle (Ezuka et al., 1974; Mew, 1987).

Changes in the expression of the resistance reaction of several MR and MS cultivars were studied to determine the extent with which the expression of the resistance varies with leaf position and leaf age. This can help determine whether a moderate level of resistance is likely to be acceptable to protect a crop during the whole cropping season. It can also help in the proper evaluation of resistance when screening entries with different maturity dates.

MATERIALS AND METHODS

The rice cultivars Cisadane, BR51-282-8, IR28, IR40, TN1, and IR9101-46, were used. The first four cultivars show MR or MS reactions and the last two cultivars were included as susceptible checks (Chapter 3, this thesis). To facilitate comparisons the chosen entries were all of a short stature.

In the first experiment sowing was done on day 1, 8, 15, 22, 29, 36, and 47 and plants were transferred to 18 cm diameter clay pots on greenhouse tables one week after germination, six plants per pot. Pots were filled with lowland rice paddy soil. Ammonium sulfate (175 kg/ha) was given in two applications and normal insect control (monocrotophos) was applied when needed. All side tillers were regularly trimmed away so that only the main tiller could develop. Leaf position was regularly noted

during growth.

Pots were arranged in three replications of a split plot design with sowing dates as main plots and cultivars as subplots (three pots per subplot). During inoculation three plants in each of the three pots per cultivar were inoculated with each of two isolates, thus forming subsubplot units of nine individual plants.

Virulent single colony subisolates of race 2 isolate PX086 and race 6 isolate PX099 were stored at -20 °C until needed. Fresh inoculum was prepared by culturing the bacteria on peptone sucrose agar (PSA) slants at 30 °C for 72 hours. The culture was suspended in 20 ml distilled water to give a density of ca. $1x10^9$ cfu/ml. The top two fully developed leaves of all plants were inoculated by dipping scissors into the bacterial suspension and then clipping 2 to 3 cm from the leaf tip (Kauffman et al., 1973). Inoculation was done 77, 70, 63, 56, 49, 42, and 30 days after sowing, respectively. Lesion lengths were measured 14 and 21 days after inoculation.

In the second experiment sowing was done at intervals in order to synchronize maximum tillering, late booting and post-flowering of all six cultivars. Experimental design and culture were as described above, except that eight plants in four pots formed the subsubplot experimental unit, and that the side tillers were allowed to develop. Plants were inoculated as described above; in the last two growth stages only flag leaves were inoculated. Lesion lengths were measured 14 days after inoculation.

The difference in reaction of immature leaves and fully developed leaves was tested on plants of the cultivars Cisadane, BR51-282-8, IR40 and TN1. Plants were grown in the greenhouse in 18 cm diameter clay pots, three plants per pot. At 60 days after sowing tillers were selected which had a topmost immature leaf not longer than half of the size of the leaf directly below; tightly rolled leaves were also included in the test. On each plant both of these leaves were clip inoculated as described above with isolate PX086 and lesion lengths were measured 14 days after inoculation. The experiment was carried out twice, using three pots per cultivar in a completely randomized design.

RESULTS

Experiment 1

Only IR28 and TN1 had reached flowering at the time of inoculation, although all cultivars except Cisadane had flowered by the time scoring was finished, 21 days after inoculation. Lesion lengths of the top two leaves were recorded separately but the data from these were later averaged because there was only a very slight difference between the reactions of the two leaves, and this difference was the same for all cultivars. The scores of the individual leaf positions will be further discussed below, in relation to leaf age.

At 14 days after inoculation lesions on plants inoculated 30 days after seeding were considerably longer than those of all other sowing dates. Lesions of plants 56 days or older appeared to be somewhat shorter than those of 42-49 day old plants, although the effect was not significant at the 5% level (Table 5-1). The leaf positions of each cultivar inoculated at each plant age are given in Table 5-2.

Significant differences between the cultivars were observed at all plant ages. Among the susceptible cultivars, TN1 was more susceptible than IR9101-46. Among the MR-MS group, IR28 was less resistant than the others and Cisadane was the most resistant. No difference was observed (P=0.05) between IR40 and BR51-282-8. No significant age x cultivar interaction was found; all cultivars decreased an average of 9.9 cm between the youngest and the oldest planting group. However, when one considers the relative decrease in lesion length of each cultivar it is clear that lesions on MR-MS cultivars decreased relatively more with increasing plant age than susceptible cultivars (Table 5-4).

Isolate PX099 produced longer lesions than PX086 14 days after inoculation. A highly significant interaction between cultivar and isolate could be traced to the level of resistance of the group of MR-MS cultivars relative to the two susceptible cultivars. The difference between these two groups was much larger when plants were infected with PX099 than with PX086 (Table 5-1).

When lesions were measured 21 days after inoculation plants infected with PX086 had longer lesions than those infected with PX099, a reversal in relation to the measurements at 14 days after inoculation (Table 5-3). PX086 therefore showed a greater increase in lesion length than PX099 in this time interval, despite the fact that it had shown slower growth in the first 14 days of infection. However, the difference in cm between the

Table 5-1: Lesion lengths (cm) of the upper two leaves 14 days after clip inoculation of six rice cultivars of seven ages using two isolates of *Xanthomonas campestris* pv. *oryzae*.

Isolate	PXO86 (mean 9.7 cm)						PXO99 (mean 12.6 cm)						mean
Days from sowing	Cultivar Cisadane	BR51-282-8	IR40	IR28	IR9101-46	TN1	Cisadane	BR51-282-8	IR40	IR28	IR9101-46	TN1	
30	15.9	14.8	13.6	15.4	20.4	20.7	13.6	18.2	15.8	17.3	22.6	26.1	17.9a[2]
42	6.8	8.3	7.0	12.5	14.0	15.8	8.1	12.2	10.3	13.1	21.0	22.6	12.7b
49	5.9	6.4	9.4	9.6	14.4	14.2	7.2	7.0	12.5	10.9	19.5	20.8	11.5b
56	4.0	6.7	7.0	10.9	9.6	10.8	7.6	7.1	9.0	9.7	15.6	16.3	9.5b
63	4.0	4.8	7.4	7.1	9.9	11.1	6.3	6.0	7.8	8.0	15.5	16.9	8.7b
70	3.9	6.0	6.5	9.0	10.7	16.2	4.8	7.1	7.1	9.8	18.4	20.4	10.0b
77	3.1	5.6	4.1	4.6	9.0	11.2	3.8	6.3	4.9	7.5	16.9	18.8	8.0b
mean	6.2c[1]	7.5c	7.8c	9.9b	12.6a	14.3a	7.3d	9.1cd	9.7c	10.9c	18.5b	20.3a	

[1]) Standard error of an average 0.38; cultivar means within each isolate group followed by the same letter are not significantly different (P=0.05), as determined by Bonferroni's test for differences.

[2]) Standard error of an average 0.775.

MR-MS and susceptible cultivars remained larger with PX099 than with PX086, despite the nearly equal lesion lengths of the two isolates on the susceptible check cultivar TN1. The relative decrease in lesion length between younger and older plants when scored 21 days after inoculation was about the same as that when scored 14 days after inoculation (Table 5-4).

Experiment 2

Plants inoculated were in the maximum tillering, late booting/flowering, and post-flowering stages. Post-flowering plants were two weeks older than those in the late booting/flowering stage, and the grains were already developing. Only flag leaves were inoculated on booting/flowering and post-flowering plants. Plants of the last two stages were thus older than the plants measured in Experiment 1, with the exception of IR28 and TN1, which had already flowered at the time of inoculation of Experiment 1.

Table 5-2: Position of the upper two inoculated leaves of six rice cultivars of seven sowing dates.

Days from sowing	Cultivar Cisadane	BR51-282-8	IR40	IR28	IR9101-46	TN1
30	8/7	8/7	8/7	9/8	8/7	8/7
42	10/9	10/9	10/9	11/10	10/9	10/9
49	12/11	11/10	11/10	12/11	11/10	11/10
56	13/12	12/11	12/11	13/12	12/11	12/11
63	14/13	13/12	13/12	13/12	13/12	13/12
70	15/14	14/13	13/12	14/13	14/13	14/13
77	16/15	15/14	14/13	14/13	15/14	14/13
flag leaf position	18	16	15	14	16	14

Table 5-3: Lesion lengths (cm) of the upper two leaves 21 days after clip inoculation of six rice cultivars of seven ages using two isolates of *Xanthomonas campestris* pv. *oryzae*.

Isolate	PXO86 (mean 18.9 cm)						PXO99 (mean 17.8 cm)						mean
Days from sowing	Cultivar Cisadane	BR51-282-8	IR40	IR28	IR9101-46	TN1	Cultivar Cisadane	BR51-282-8	IR40	IR28	IR9101-46	TN1	
42	16.2	19.8	17.7	26.4	31.2	39.9	12.2	17.7	18.0	20.9	30.2	33.1	23.6a[2]
49	11.0	15.9	23.9	24.5	30.1	31.2	10.6	11.0	19.5	18.7	29.8	31.3	21.5ab
56	6.7	12.8	13.3	24.2	19.8	24.6	10.5	9.9	12.2	17.8	24.7	24.3	16.7bc
63	7.3	12.4	16.8	15.2	21.4	26.0	8.6	9.3	16.2	12.3	25.2	24.7	16.3bc
70	7.1	12.7	13.3	18.4	23.3	31.2	6.9	11.4	10.3	15.1	28.7	30.0	17.4bc
77	5.9	13.4	11.4	10.9	19.1	27.0	6.2	8.7	9.3	13.2	23.1	28.5	14.7c
mean	9.0e[1]	14.5d	16.1d	19.9c	24.2b	30.0a	9.1c	11.3bc	14.2b	16.3b	27.0a	28.7a	

[1]) Standard error of an average 0.70; cultivar means within each isolate group followed by the same letter are not significantly different (P=0.05), as determined by Bonferroni's test for differences.

[2]) Standard error of an average 0.92.

Table 5-4: Lesion lengths of six rice cultivars inoculated 77 days after sowing with isolates PXO86 and PXO99 of *Xanthomonas campestris* pv. *oryzae*, expressed as a percentage of the lesion lengths of plants inoculated 40 days after sowing. Lesions were measured 14 and 21 days after inoculation.

Cultivar	Days after inoculation 14		21	
	PXO86	PXO99	PXO86	PXO99
TN1	71	83	68	86
IR9101-46	64	80	61	76
IR28	33	57	41	63
IR40	58	48	64	52
BR51-282-8	67	52	68	49
Cisadane	46	47	36	51

Significantly shorter lesions were measured on the flag leaves of booting and post-flowering plants than on those at maximum tillering, for all cultivars tested (Table 5-5). There was no difference between lesion lengths of the booting and the post-flowering stages. The two highly susceptible cultivars were again clearly distinguishable from the other four cultivars in all stages, but the distinction was clearest at the maximum tillering stage because of the smaller experimental error found here. The relative resistance of the four MR-MS cultivars was more difficult to ascertain. Cisadane was the most resistant cultivar at all three growth stages; there was no significant difference between the other three cultivars. No significant difference between cultivars was found for the decrease in lesion length with increasing age when the decrease was expressed in cm lesion length. However, lesions of MR-MS cultivars decreased relatively more than of susceptible cultivars (Table 5-5).

Differences between the two isolates were found at maximum tillering and at post-flowering stages, but not at the booting stage. PXO99 was more aggressive than PXO86 at maximum tillering, while PXO86 was more aggressive than PXO99 on post-flowering plants, based on the lesion length on TN1.

Table 5-5: Lesion lengths (in cm, average of the upper two leaves) measured 14 days after clip inoculation of six rice cultivars of three growth stages with two isolates of *Xanthomonas campestris* pv. *oryzae*.

Isolate	PXO86							PXO99						
Growth stage	Cultivar Cisadane	BR51	IR40	IR28 -46	IR9101	TN1	mean	Cultivar Cisadane	BR51-282-8	IR40	IR28	IR9101 -46	TN1	mean
MT[1]	9.2	11.3	10.4	11.4	16.9	17.6	12.8 b	10.9	13.6	12.1	12.9	17.3	19.4	14.3a
B/F[2]	4.2	5.4	8.1	6.8	12.6	15.4	8.8 cd	2.9	6.1	7.6	6.8	10.2	13.9	7.9d
PF	4.7	8.4	7.4	6.3	13.1	15.0	9.2 c	4.2	7.2	7.2	7.3	9.7	11.8	7.9d
mean	6.0d[3]	8.4c	8.2c	8.6c	14.2b	16.0a	10.2	5.8d	8.9c	9.0c	9.0c	12.4b	15.0a	10.0
PF+B/F as % MT	48	61	74	57	76	86	70	33	49	61	55	58	66	55

[1]) MT=maximum tillering, B/F = booting/flowering, PF = two weeks post-flowering.

[2]) Flowering is for each cultivar, respectively, 103, 85, 88, 68, 82 and 75 days after sowing.

[3]) Standard error of the mean for cultivars is 0.351, for growth stages is 0.248.

Leaf age tests

Lesions on immature leaves were found to be 2.2 cm longer than on the mature leaf directly below, averaged over both tests of the four cultivars studied (Table 5-6). This difference is significant (P=0.05), indicating that immature leaves are slightly more susceptible than mature leaves. Immature leaves of TN1 were more susceptible than immature leaves of the three MR cultivars, and no significant cultivar effect was found for the difference between the lesion lengths on the immature and the mature leaves. This indicates that the resistance factor(s) is already expressed in immature leaves of MR cultivars.

Data from Experiment 1 were used to compare leaves of the same leaf position but of varying leaf age. For each leaf position the mean lesion length was compared when the leaf was inoculated when it was the youngest fully extended leaf and when it was the second leaf from the top (Table 5-7). No significant difference was found between the mean of the two leaf ages.

Table 5-6: Lesion lengths (in cm) of unfolding and fully extended leaves of four rice cultivars inoculated 65 days after sowing with race 2 isolate PXO86 of *Xanthomonas campestris* pv. *oryzae*. Lesions were measured 14 days after clip inoculation.

Leaf age	Cultivar Cisadane	BR51-282-8	IR40	TN1	mean
unfolding	11.8	11.1	11.0	19.9	13.5
fully extended	8.6	8.7	9.8	18.1	11.3
difference	3.2	2.4	1.2	1.8	2.2

Table 5-7: Lesion lengths of leaves of varying ages measured on two rice cultivars, Cisadane and TN1, clip inoculated with two isolates of *Xanthomonas campestris* pv. *oryzae* at four leaf positions. Lesions measured 14 days after inoculation.

Cisadane

Leaf positon	Isolate PXO86			PXO99		
	A[1]	B[2]	A-B	A	B	A-B
12	6.0	3.8	2.2	7.7	7.0	0.7
13	4.1	4.3	-0.2	7.5	6.3	1.2
14	3.9	4.0	-0.1	6.4	4.5	1.9
15	3.8	3.1	0.7	5.2	3.5	1.7

TN1

Leaf positon	Isolate PXO86			PXO99		
	A[1]	B[2]	A-B	A	B	A-B
10	16.2	14.3	1.9	22.9	20.9	2.0
11	14.4	10.5	3.9	20.6	17.7	2.9
12	11.3	10.7	0.6	15.0	16.9	-1.9
13	11.6	12.6	-1.0	16.9	18.8	-1.9

[1]) Leaves just unfolded.

[2]) Leaves one or two weeks after unfolding.

DISCUSSION

All cultivars showed the same general trend towards reduced susceptibility to bacterial blight (BB) with increasing age. Even on TN1, a susceptible cultivar, lesions on flag leaves were considerably shorter than lesions on leaves of an earlier growth stage. A similar trend was found by Kauffman et al. (1973) and Ezuka et al. (1974) for susceptible and MS cultivars. Mew et al. (1981) also found that lesions on susceptible cultivars at booting stage were shorter than those on the same cultivars at 30 days after seeding. Devadath and Padmanabhan (1969), however, tested 20 cultivars against 9 Indian isolates of Xco and found

that the seedlings in all cases showed greater resistance than the flag leaves. These inoculations were not done simultaneously for both growth stages so that differences in inoculum concentration and environment may have affected the results.

Differences in the levels of resistance of the six cultivars were detectable at all plant ages tested, from 30 days to postflowering. Although the cultivars were always clearly divided into two groups, those which were highly susceptible and those with a moderate level of resistance, the difference between these two groups was more apparent with isolate PX099, the more aggressive of the two isolates, as measured on the susceptible cultivar TN1.

Differences between cultivars of the moderately resistant group were difficult to ascertain at all plant ages, based on lesions measured 14 days after inoculation. In Experiment 1 there was no distinction found among the four MR-MS cultivars except at 30 and at 70 days after sowing, where the cultivars with the highest and the lowest disease levels were significantly different. In Experiment 2 some distinction between the MR-MS cultivars was found at maximum tillering, but not at flowering or post-flowering. The two cultivars which had flowered at the time of inoculation in Experiment 1 did not change their level of resistance relative to the other cultivars at that time. TN1, in fact, appeared to increase slightly in susceptibility just before flowering. It is therefore clear that plant age does not greatly affect the ability to distinguish among MR cultivars. This would indicate that screening for moderate resistance can be done at all stages of growth, provided that there are no large differences in maturity. In case of such differences it is better to test somewhere between 50 and 70 days after sowing since changes in susceptibility are small in this period. The isolate chosen should be both virulent to all cultivars tested and highly aggressive.

The gradual decrease in lesion length with increasing plant age and the final moderate level of resistance suggests that the decrease is probably not the same as the decrease due to the adult plant resistance reported by Mew et al. (1981) and by Zhang and Mew (1985). This adult plant resistance is typified by a change from a clearly susceptible reaction in the seedling stage to a highly resistant reaction at booting. In cultivars known to possess monogenic adult plant resistance the major decrease has been shown to occur between 60 and 80 days from sowing (Mew et al., 1981), although in other cultivars with adult plant resistance of unknown genetic background the change has been shown to occur earlier and to be more gradual (Zhang and Mew, 1985). All cultivars tested in this

study decreased equally in lesion length with increasing plant age, although relative decreases were greater with MR-MS cultivars than with susceptible cultivars. The largest decrease in lesion length occurred fairly early, between 30 and 42 days after seeding.

The somewhat longer lesions on immature leaves is probably due to other factors than the resistance investigated here. The resistance in MR cultivars was already expressed in the leaves before they reached their fully extended size.

Longer lesions on leaves of young plants result in considerably more leaf damage than the shorter lesions on the considerably longer leaves of more mature plants. Flag leaves of rice plants are generally again shorter, but the resistance is at its greatest expression at this time. The limitation of lesion size in MR cultivars may mean that damage from BB is significantly reduced after about 42 days from sowing. Studies should be carried out to test if a moderate level of resistance is sufficient to effectively protect a crop from BB damage.

CHAPTER 6

THE USE OF THE RATE OF LESION EXTENSION AS PARAMETER FOR MEASURING RESISTANCE TO *XANTHOMONAS CAMPESTRIS* pv. *ORYZAE* IN RICE

SUMMARY

Two susceptible and four moderately susceptible rice cultivars were clip inoculated with three *Xanthomonas campestris* pv. *oryzae* (Xco) isolates at two plant ages. Development of bacterial blight lesions on clip inoculated leaves was followed from lesion appearance until shortly before leaf senescence. All lesions appeared four to five days after inoculation. A quadratic model was found to explain 99% of the variance in the measured values. Mean lesion length and mean daily rate of lesion length increase were highly correlated. Younger plants and susceptible cultivars had higher mean rates of increase than older plants and moderately susceptible cultivars, respectively. The rate of extension decreased somewhat with time for Xco isolates PX071 and PX099 and increased or remained the same for isolate PX086. There were no major differences between cultivars for the change in the rate of extension over time. Because of this there were no major changes in the relative levels of resistance of the six cultivars over time. Multiple scorings are therefore not expected to give extra information about this factor. The interactions between isolates observed may indicate a characteristic of Xco isolates worth further study.

INTRODUCTION

Resistance in rice to bacterial blight (BB), caused by *Xanthomonas campestris* pv. *oryzae* (Xco), is usually assessed 14 or 21 days after artificial inoculation with a concentrated bacterial suspension. A hypersensitive response is not known in this host-pathogen system (Parry and Callow, 1986), and a continuous range of lesion sizes is usually found

when screening cultivars (Chapter 3, this thesis). Cultivars can be designated as resistant (R), moderately resistant (MR), moderately susceptible (MS), or susceptible (S) to each specific isolate of the pathogen (Horino et al., 1981; Mew et al., 1981). Although this division is arbitrary and without clear-cut distinctions, high levels of resistance have been shown to be the result of the action of race-specific major genes (Ezuka and Horino, 1974b; Mew and Vera Cruz, 1979). Moderate reactions have in some cases been shown to be an incomplete reaction of known major genes to certain isolates (Yoshimura et al., 1984) and in other cases to be quantitatively inherited (Yamada, 1986a).

Following clip inoculation with a virulent isolate the bacteria move via the vascular system downwards towards the leaf base and lesions appear four to five days after inoculation. A lesion is distinct and large and its length can easily and accurately be measured. If repeated measurements are made on a single leaf the daily rate of increase in length can be calculated. This daily rate may be constant but may also change during the infectious period. When the daily rate of lesion extension varies during the infectious period, assessment of symptom development at different days after inoculation could then lead to different conclusions regarding the relative resistance of various cultivars.

Evidence for such interactions was sought by closely following the progress of lesions on various cultivars, using three Xco isolates and at varying plant ages. Such a comparison can offer insight into the way in which lesions develop, and possibly show differences in the host-pathogen interactions between seemingly similar cultivars.

MATERIALS AND METHODS

The rate of lesion extension was measured on the six cultivars Cisadane, IR40, IR28, BR51-282-8, IR9101-46 and TN1. The first four cultivar possess the Xa-4 gene for resistance and are moderately resistant or moderately susceptible to isolates virulent to the Xa-4 gene, while the latter two are susceptible and without the Xa-4 gene. Seeds were pregerminated on moist filter paper and transferred to 33x26x11 cm plastic trays filled with lowland soil when the first leaf was 1-2 cm long. Each tray contained six plants of each of the six cultivars. The cultivars were planted in rows following a randomized block design within the tray. Trays were randomized on the greenhouse or phytotron tables. Nitrogen fertilizer (21-0-0 ammonium sulfate) was applied regularly during growth.

Bacterial isolates were stored in skim milk suspensions at -20 °C until needed. Isolates were revived on peptone sucrose agar (PSA) slants incubated at 30 °C and transferred once before inoculation. Inoculum was prepared by suspending 72 hour old cultures of Xco in 20 ml distilled water per slant, and adjusting the optical density to 1.00 (590 nm), giving a suspension of about $1x10^9$ cfu/ml. The uppermost fully extended leaf of the main tiller of each plant was inoculated by dipping a scissor in freshly prepared inoculum and clipping off the top 1-2 cm.

Leaves were numbered four days after inoculation and the lesion lengths of each leaf was recorded on alternate days, beginning on day 5 and continuing until the leaf began to senesce. Five leaves per cultivar per isolate per replicate were measured.

In Experiment 1 (Apr. - June 1986) the extension of lesions caused by three isolates at two plant ages was tested under greenhouse conditions. Seeds were sown on day 0 and on day 20, and plants were inoculated when 60 and 40 days old, respectively. Isolates of race 2 (PX086), race 4 (PX071) and race 6 (PX099) were used. Races 2 and 6 are virulent to the Xa-4 gene. Race 4 is incompletely virulent to this gene (Yoshimura et al., 1984) and cultivars with an Xa-4 gene show an MR reaction when inoculated with PX071. Three trays (replicates) of each isolate x growth stage combination were measured.

In Experiment 2 (Apr. - June 1987) the extension of lesions caused by infection with race 2 isolate PX086 and race 6 isolate PX099 was tested under phytotron conditions (29/21 °C, 70-90% RH, natural light). Plants were inoculated 55 days after seeding. Three trays (replicates) were tested per isolate.

The relationship between lesion length and time was fit with both a linear and a quadratic model for each plot. The predicted values for each day were compared with the observed values and the percentage variance explained by each model was calculated per plot.

RESULTS

Experiment 1

Lesions appeared in all cultivars four to five days after inoculation and were measured until leaves began to senesce at 16 days after inoculation for the younger plants and 24 days after inoculation of the older plants. Because of poor germination a different seed source of the

line IR9101 was used in the second sowing. Unfortunately these latter plants did not show the typical susceptible reaction of this line and the data for this cultivar were not used in the comparison of plant ages.

A linear model described at least 95% of the variance in the measured values in most cases, and a quadratic model described more than 99% of the variance in all cases. The quadratic model was chosen to compare the treatments. This model allows for gradual change in the daily rate of lesion extension over time.

Lesions extended significantly faster on younger plants than on older plants (Table 6-1). Lesions on younger plants increased at a mean rate of 1.55 cm per day between the fourth and the 16th day after inoculation. Lesions on older plants increased at a mean rate of 0.89 cm per day between the fourth and the 24th day after inoculation. Lesion extended

Table 6-1: Daily rate of lesion extension (in cm day^{-1}) measured on six rice cultivars of two plant ages following clip inoculation of leaves with three isolates of *Xanthomonas campestris* pv. *oryzae*.

	Days after sowing							
	40[1]				60[1]			
Isolate	PXO99	PXO86	PXO71	mean[2]	PXO99	PXO86	PXO71	mean[2]
Cultivar								
IR28	1.35	1.63	0.98	1.32c	0.56	0.90	0.44	0.61e
IR40	1.53	1.59	0.88	1.33c	0.62	0.91	0.39	0.63e
Cisadane	1.39	1.85	0.66	1.30c	0.81	0.83	0.51	0.72e
BR51-282-8	1.61	1.51	0.73	1.28c	0.81	1.20	0.48	0.85d
TN1	2.46	2.54	2.60	2.53a	1.20	1.86	1.78	1.61b
IR9101-46	-	-	-	-	1.54	1.78	1.52	1.61b
mean[2]	1.67b	1.82a	1.17c	1.55	0.92d	1.25c	0.85e	0.89

[1]) Rate determined for days 4-16 or days 4-24 after inoculation, respectively, for 40 and 60 day old plants.

[2]) Standard error of the mean for isolates is 0.041, for cultivars is 0.068. Values in a row (isolate) or both columns (cultivars) which are followed by different letters differ as determined by Bonferroni's test for inequalities (P = 0.05).

faster on the two susceptible cultivars TN1 and IR9101-46 than on the MS cultivars.

The difference between the slowest and fastest extending lesion was greater in older plants than younger plants, and this was reflected in the greater ability to differentiate between levels of resistance among cultivars inoculated 60 days after sowing than 40 days after sowing. The rate of lesion extension of the most resistant cultivar was 1.25 cm day^{-1} (51%) slower, than the most susceptible cultivar among young plants, and 1.00 cm day^{-1} slower (38%) than the most susceptible cultivar among older plants (Table 6-1). Lesion lengths on representative days after inoculation also were proportionately shorter on older moderately resistant cultivars than on younger plants (Table 6-2).

Table 6-2: Lesion lengths (cm) measured on six rice cultivars of two plant ages following clip inoculation of leaves with three isolates of *Xanthomonas campestris* pv. *oryzae*.

	Days after sowing							
	40[1]				60[1]			
Isolate	PXO99	PXO86	PXO71	mean[2]	PXO99	PXO86	PXO71	mean[2]
Cultivar								
IR28	15.9	15.9	11.1	14.3b	11.5	16.2	9.0	12.3c
IR40	17.3	15.9	9.4	14.2b	13.2	15.5	7.3	12.0c
Cisadane	16.4	20.0	7.7	14.7b	16.9	15.0	9.9	13.9c
BR51-282-8	18.8	16.2	8.6	14.6b	16.8	21.2	10.0	16.0b
TN1	27.9	23.5	25.7	25.7a	25.2	32.6	33.0	30.3a
IR9101-46	-	-	-	-	29.0	31.5	26.8	29.1a
mean[2]	18.8a	17.8a	12.6b	16.4	18.8b	22.0a	16.0b	18.9

[1]) Lesion lengths recorded 14 or 22 days after inoculation for 40 and 60 days after sowing, respectively.

[2]) Standard error of the mean for isolates is 0.673 and 0.868, for cultivars is 0.850 and 1.002, for 40 and 60 days after sowing, respectively. Values in a row (isolate) or column (cultivar) followed by different letters differ significantly (P=0.05).

On the younger plants all three isolates showed similar rates of lesion extension on TN1, indicating equal aggressiveness. However, PX071 is incompletely virulent to the Xa-4 gene and the mean rate of this isolate over all six cultivars was therefore only 1.17 cm per day, as compared to a rate of 1.67 and 1.82 for PX099 and PX086, respectively (Table 6-1). On older plants PX099 appeared to be slightly less aggressive than the other two isolates, as indicated by this parameter and by the mean lesion length of this isolate on the susceptible checks TN1 and IR9101-46 (Table 6-2). Isolate PX099 showed the largest decrease in rate of extension between the two ages (55%), while isolate PX071, which had shorter lesions in both ages, did not decrease as much with increasing plant age (73%).

The daily rate of lesion extension was highly correlated with the mean lesion length, averaged over all days (r=0.93** for both younger and older plants) and for the mean lesion length on representative days after inoculation (r=0.96** and 0.98** for younger and older plants, respectively).

Significant differences between all three isolates at both plant

Table 6-3: Rate of change in the daily rate of lesion extension (cm day^{-2}) of three isolates of *Xanthomonas campestris* pv. *oryzae* following clip inoculation of rice plants[1] of two ages.

Days after sowing	Isolate PXO99	PXO86	PXO71
40	-0.075d[2]	+0.017a	-0.031c
60	-0.031c	-0.005b	-0.021c

[1]) Average of six rice cultivars in three replications.

[2]) Standard error of an average 0.0064. Values followed by different letters differ as determined by Bonferroni's test for inequalities (P=0.05).

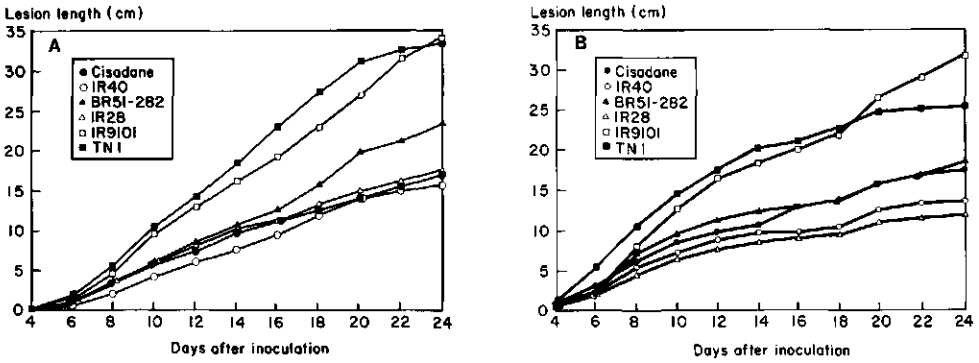

Figure 6-1: Lesion extension curves of six rice cultivars clip inoculated at 60 days after sowing with race 2 isolate PXO86 (A) and race 6 isolate PXO99 (B) of *Xanthomonas campestris* pv. *oryzae*.

ages were found for the quadratic term of the fit line, which expresses the change in the rate of extension of the lesions over time. However, no significant differences between cultivars for this parameter were found. On younger plants PX099 slowed, but did not stop extension while PX086 increased its rate of extension over time (Table 6-3). The decrease in lesion extension of PX099 was not due to a lack of fresh leaf tissue; less than 10% of the lesions on TN1, and even fewer on the MR cultivars, had reached the leaf sheath by the time measurements were stopped. On plants inoculated 60 days after sowing all isolates slowed growth somewhat but PX099 and PX071 slowed significantly faster than PX086 (Table 6-3). Growth of lesions of PX086 remained constant on IR9101-46 and BR51-282-8 but slowed slightly in TN1, Cisadane, IR28 and IR40 (Figure 6-1A). Cultivar differences for this parameter were seen most clearly in the more rapid decrease in lesion extension of PX099 in TN1 than on the other cultivars, so that by 24 days after inoculation IR9101-46 showed longer lesions than TN1 (Figure 6-1B). These interactions were not large enough to affect the classification of a cultivar as MR, MS or S with time, but changed the ranking order of the cultivars within each group.

Experiment 2

Lesions were followed from 5 to 20 days after inoculation. The lesions extended at an average rate of 1.68 cm per day (Table 6-4). The greater rate of increase in this experiment, as compared to the above results, could have been due to the cooler conditions in the phytotron, as compared to the greenhouse, or to the slightly younger age and different nutritional status of the plants.

A linear model described 94-99% of the observed variation, while a quadratic model described 99% or more in all but four of the 36 plots. Mean lesion length was highly correlated (r=0.93**) with the mean daily rate of lesion extension.

Significant differences were found between cultivars for the daily rate of extension but not for changes in this rate. Significant differences between isolates were not found for the daily rate of lesion extension but were found for the change in daily rate (Table 6-4). Isolate PX099 slowed growth over the measurement period while PX086 increased the rate of extension, as was found in Experiment 1.

Table 6-4: Mean lesion length (cm), daily rate of increase in lesion length (cm day^{-1}), and daily change in this rate (cm day^{-2}) measured on six rice cultivars following clip inoculation of leaves with isolates PXO99 and PXO86 of *Xanthomonas campestris* pv. *oryzae*.

Cultivar	Lesion length[1]			Daily rate of increase			Change in daily rate ($x10^{-2}$)		
	PXO99	PXO86	mean[2]	PXO99	PXO86	mean[2]	PXO99	PXO86	mean[2]
Cisadane	9.7	6.6	8.1d	1.3	1.0	1.2d	-0.5	1.4	0.4a
IR40	10.2	7.2	8.7cd	1.4	1.3	1.4cd	1.3	6.9	4.1a
IR28	8.0	9.7	8.9cd	1.1	1.5	1.3cd	-0.7	4.5	1.9a
BR51-282-8	11.5	10.1	10.8cd	1.5	1.6	1.6c	0.9	4.4	0.4a
IR9101-46	16.6	12.5	14.6b	2.1	2.1	2.1b	-5.3	5.5	0.1a
TN1	19.3	18.3	18.8a	2.3	2.8	2.6a	-5.1	5.7	0.3a
mean	12.6a	10.7a	-	1.6a	1.7a	-	-1.6b	4.7a	-

[1]) Lesions measured from 5-20 days after inoculation.

[2]) Standard error of the mean for lesion length is 0.817, for the daily rate of increase is 0.104, for the change in the daily rate is 0.025. Values in a row (isolate) or in a column followed by different letters differ as determined by Bonferroni's test for inequalities (P=0.05).

DISCUSSION

A quadratic equation was found to better describe the observed lesion extension than a simple linear equation. Baw (1985) also found a quadratic model appropriate to describe infection in 40-50 day old TN1 plants but that a linear relationship sufficiently described the infection in older (booting) plants. Large differences between cultivars for the daily rate of lesion extension were found, but there were few differences for the quadratic term for changes in the daily rate of lesion extension. This indicates that while the lesions extended on the different cultivars at different rates, few significant interactions over time are to be expected. The good correlations between mean lesion length and the daily rate of increase also shows this fact. Comparisons between cultivars varying from moderately resistant to highly susceptible can therefore be made any time after symptom appearance, although measurements between cultivars will probably be most meaningful in the period of rapid, more or less linear growth, before leaf senescence affects measurements. Little extra information is to be gained by measuring lesions on two or more separate occasions and using a parameter based on the lesion growth in this interval.

Baw (1985) and Yoshimura et al. (1984) used a logistic and a Gompertz model to describe the extension of bacterial blight lesions. Lesions were expressed as a percentage of the leaf area or of the leaf length, respectively. Logistic and Gompertz models are two sigmoid curves often used to describe the development of a disease in a field. This is assumed to first increase its rate of spread as inoculum builds up and then later to slow development as healthy plant tissue becomes scarce. These two conditions cannot be transferred to the monocyclic infection process studied here. There is no indication that lesions increase logarithmically in length shortly after symptoms appear. While lesions on older plants slowed growth slightly, lesions on young plants infected with PX086 appeared to increase in growth rate.

Younger plants were more susceptible than older plants, as indicated by the greater rate of lesion extension in the former age group. Since younger plants also tend to have shorter leaves the damage to younger plants is considerably more than to older plants.

The incompletely virulent race 4 isolate was distinguishable from isolates PX086 and PX099, both virulent to Xa-4, by the mean lesion length and the mean rate of extension. The latter two isolates were distinguished from each other by the difference in the change in the rate of exten-

sion over time. PX099 slowed growth while PX086 kept extending at a constant rate, or even speeded up, depending on the plant age. This seems to be a repeatable characteristic of these isolates and should be further studied.

As stated above, there is some indication that lesions, especially on older plants, slow but do not stop growth. The lesion is not usually at the leaf base when the rate of extension decreases. In the case where the leaf is fully affected, a situation found in young leaves of highly susceptible cultivars, a lesion can proceed to extend further down the leaf sheath, although this is not usually measured. Morinaka et al. (1978) found no difference in susceptibility between the tip and the midleaf section of leaves following clip inoculation. Similar results were found on TN1 plants (unpublished results, M. Koch). However Yamanaka et al. (1952) found that the leaf base was slightly less susceptible than the upper section based on the number of successful prick inoculations. Because lesions are found to slow shortly before leaf senescence it is possible that the changes in the internal environment related to senescence adversely affect the bacterial growth and multiplication. Some isolates may be more sensitive to these changes than others.

Lesions on more resistant cultivars did not begin to slow growth earlier than lesions on highly susceptible cultivars. This is in agreement with Baw (1985) who found that lesions do not stop extension for up to three weeks, even lesions of a highly resistant reaction on plants with the Xa-4 gene. Ogawa et al. (1988) also report that on plants with the Xa-4 gene for resistance lesions continued extending for up to 22 days after inoculation without a noticeable change in rate. Yoshimura et al. (1984), however, reported that lesions of avirulent race 2 isolates on cultivars with the xa-5 gene stopped growth by 18 days after inoculation. Ogawa et al. (1988) reported that lesions of isolate-cultivar combinations involving the Xa-3 gene for resistance slowed extension 18-20 days after inoculation. In some cases browning was seen to develop in these lesions. They used these two characteristics, which they attributed to the presence or absence of one of several known major genes for resistance, to separate segregating plants into resistant and susceptible groups. Lesion length was not used as a selection characteristic in these cases. Conclusions concerning slowing of lesion extension may therefore be related to the specific resistance and virulence genes being studied.

CHAPTER 7

EFFECT OF INOCULUM CONCENTRATION ON THE DEVELOPMENT OF BACTERIAL BLIGHT SYMPTOMS IN RICE

SUMMARY

The responses to eight different inoculum concentrations of *Xanthomonas campestris* pv. *oryzae* were compared using ten rice cultivars varying in susceptibility (Experiment 1) and using three rice cultivars at three development stages (Experiment 2). Significant differences between cultivars for effective dose needed to establish infections in 50% of the inoculated leaves (ED_{50}) were found in both experiments. The ED_{50} was negatively correlated (r=-0.76*) with the susceptibility, as measured by lesion length at the highest dosage. No effect of growth stage on the ED_{50} was found.

The percentage infected leaves increased with increased inoculum concentrations. The course of this increase was very rapid around the ED_{50} point, indicating a dose-dependent chance of infection of the bacteria. Lesion length increased gradually with increasing dosage.

INTRODUCTION

The chance of successful infection per unit inoculum received is an important component of resistance of plants to pathogens (Parlevliet, 1979). Natural infection of rice by *Xanthomonas campestris* pv. *oryzae* (Xco), the causal organism of bacterial blight (BB) occurs when bacteria, deposited on the leaf by splashing water, enter through wounds or via the hydathodes (Ou, 1985). Resistant cultivars show less extensive symptoms in field tests than susceptible cultivars (Chapter 8, this thesis). There could be resistance to entrance (infection) and resistance to colonization.

Resistance of rice to Xco following clip inoculation is characterized by the absence of a qualitative difference between resistant and susceptible reactions, with the possible exception of the Xa-3 gene for

resistance (Kaku and Kimura, 1978). Only one lesion type can be consistently identified and a resistant reaction can produce an active lesion, i.e. one which shows typical water-soaking (Mew, 1987). Lesion size is considered to be decisive in classifying a cultivar as resistant or susceptible to a given Xco isolate.

Based on large differences in lesion size following clip inoculation races of Xco have been demonstrated and race-specific resistance genes have been identified (Mew, 1987). In a number of cases the resistance genes give a near-complete resistance; the lesions are absent or are very small. In other cases the resistance genes have an incomplete expression showing moderate resistance (Yoshimura et al., 1984). Leach et al. (1989) have shown that even in incompatible reactions bacteria multiply and spread considerably following inoculation, but not to the extent of compatible reactions. Truly nonpathogenic bacteria, such as *Xanthomonas campestris* pv. *campestris* decline in numbers shortly after inoculation.

The likelihood of infection by bacteria can be tested with infectivity titrations (Ercolani, 1984). The dosage at which 50% of inoculations result in active infections (ED_{50}) has been used to characterize resistance of cultivars (Boelema, 1977) or pathogenicity of isolates (Vera Cruz, 1984). The slope of the curve of the linearized relationship between dose and percentage infection indicates whether the chance of infection is equal for all bacteria, regardless of dosage or whether this chance is dependent on the dose applied.

Mew et al. (1982) have shown that an avirulent isolate required a 10^{19} fold higher inoculum concentration than a virulent isolate to induce 50% infection following clip inoculation of the cultivar Cas 209. The extent of the symptoms which developed following inoculation with virulent isolates was also shown to be positively correlated with the dosage.

Infection titrations were carried out on a number of rice cultivars to further determine the relationship between inoculum dosage and susceptibility, as defined by lesion length following clip inoculation. Both a quantal response (infection or not) and a quantitative response (lesion length of successful infections) were assessed to gain information concerning the mode of establishment of the bacteria in the plant.

MATERIALS AND METHODS

Experiment 1

Ten cultivars varying in susceptibility to BB were tested with eight dilutions of the race 2 isolate PX086 of Xco. Seeds were obtained from the Plant Breeding Department and the International Rice Testing Program of the International Rice Research Institute, Los Baños, Philippines. Seeds were germinated in plastic trays (35x20x10 cm) filled with lowland soil and seedlings were transplanted (20x20 cm) to a lowland bed in a screenhouse 18 days after seeding. Plants were fertilized one week after transplanting and one week before inoculation (175 kg/ha 21-0-0 ammonium sulfate total). Insect control (monocrotophos) was applied as needed against brown plant hoppers.

Plants were arranged in a split-plot design in three replications with cultivars in the main plots and concentrations of inoculum in the subplots. A subplot consisted of six hills.

The Xco isolate PX086 was kept in skim milk culture at -10 °C. When needed the bacteria were transferred to peptone sucrose agar (PSA) incubated at 30 °C and subcultured once before use. A single three day old slant culture was mixed with 10 ml sterile water and diluted to the eight desired concentrations. A sterile dilution series was also made to determine the concentration of bacteria in the original suspension.

Plants were clip inoculated (Kauffman et al., 1973) 64 days after seeding, when all plants were just past maximum tillering. Five inoculated leaves per hill of five hills per subplot were assessed for infection and lengths of active lesions were measured 14 days after inoculation.

ED_{50} points were determined using probit analysis (Finney, 1971). The slope of the linearized probit transformed data at this ED_{50} point was calculated.

Experiment 2

The effect of plant age and the differences in resistance were tested in a second experiment. Three cultivars were tested at three development stages with eight dilutions of race 2 isolate PX086 of Xco.

Seeds of the susceptible cultivar TN1, the moderately resistant cultivar IR40 and the highly resistant cultivar IR1545-339 (xa-5 gene for resistance) were sown on days 0 (IR1545-339 and IR40), 7 (TN1), 21 (all)

and 42 (all) in order to synchronize the seedling, maximum tillering and booting stages of these three cultivars. Seeds were sown as described above and seedlings were transplanted to the screenhouse bed 18-21 days after sowing. Maintenance was carried out as described above, except that nitrogen fertilizer was given one week after the first transplanting and one week before inoculation.

Plants were arranged in a split-split plot design in three replicates with cultivars in the main plots, plant ages in the subplots and concentrations of inoculum in the subsubplots. A row of six plants formed one subsubplot unit. Extra space was allotted between certain plots to avoid heavy shading.

Inoculum was prepared as described above. Plants were clip inoculated as described above and lesions were assessed 14 days after inoculation. All clipped leaves were collected and assessed for typical blight symptoms. The numbers of leaves with and without lesion development were recorded. Lesion lengths were measured on up to 20 leaves showing symptoms.

The ED_{50} points were calculated by interpolation, as the change in percentage infections was too rapid for application of the probit analysis.

RESULTS

Experiment 1

At lower inoculum dosages symptoms were absent or were limited to small atypical lesions. These lesions were sometimes surrounded by a dark brown line, which was found to be caused by the wounding of the leaf. A similar dark brown line was also seen in leaves clip inoculated with distilled water. Typical BB lesions were sometimes found to extend from this area and these inoculations were scored as active. However, these lesions remained very limited in size. At higher dosages typical grey-green BB symptoms developed, usually without browning.

ED_{50}, probit slope and lesion length all showed significant cultivar effects, which were clearly associated (Table 7-1). ED_{50} values for inoculum concentration ranged from 5.1×10^4 to 6.9×10^5 cfu/ml. More susceptible cultivars had lower ED_{50} points and sharper slopes than more resistant cultivars. Lesion lengths resulting from inoculation with the most concentrated inoculum was correlated with the ED_{50} value ($r=-0.76^*$) (Figure

7-1). The cultivars IR28 and BR171-2B-8 had low ED_{50} values in relation to their relative resistances based on lesion lengths. Lesions tended to lengthen with increasing dosage, especially with the most susceptible cultivars Warrangal and TN1. The largest increase was found at concentrations near the ED_{50} point but some increase was even seen between more concentrated inoculum dosages.

Table 7-1: Effective dosages (ED_{50}), slope of the probit-transformed line at the ED_{50} point, and lesion lengths determined on ten rice cultivars clip inoculated with race 2 isolate PXO86 of *Xanthomonas campestris* pv. *oryzae*.

Cultivar	ED_{50} (^{10}log cfu/ml)	Probit slope	Lesion length[2] (cm)
Cisadane	5.84±0.178 a[1]	1.67 ab	5.2 d
Biplab	5.70±0.244 ab	2.00 ab	7.7 d
IR4442-46	5.44±0.409 ab	1.09 b	6.5 d
BR51-282-8	5.23±0.103 ab	1.75 ab	8.6 cd
IR40	5.10±0.170 ab	1.75 ab	8.1 cd
BR171-2b-8	4.92±0.353 ab	0.95 b	7.5 d
IR9101-46	4.87±0.101 ab	1.72 ab	15.2 ab
IR28	4.83±0.217 ab	1.28 ab	12.2 bc
TN1	4.75±0.062 b	3.35 a	19.2 a
Warrangal	4.71±0.059 b	3.63 a	17.2 a

[1]) Standard error of the mean for ED_{50} = 0.21, for slope of the probit-transformed line at the ED_{50} point = 0.42, for mean lesion length = 0.87. Values in a column followed by the same letter are not significantly different (P=0.05).

[2]) Lesion length was measured on the highest inoculum concentration treatment.

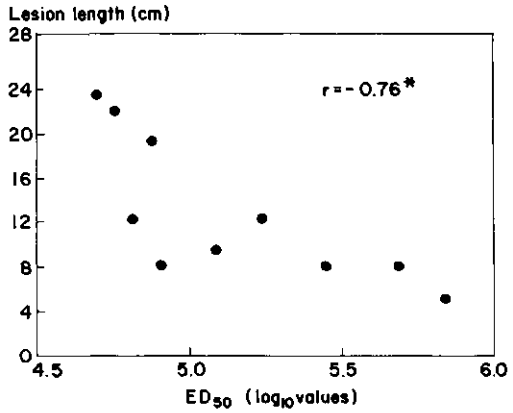

Figure 7-1: The relationship between the ED_{50} values and lesion lengths measured 14 days after clip inoculation of ten rice cultivars at maximum tillering stage with race 2 isolate PXO86 of *Xanthomonas campestris* pv. *oryzae*.

Experiment 2

All clipped leaves were collected and examined for the presence or absence of active (grey-green) lesions. More leaves per plot could be collected and assessed on the maximum tillering and adult plants than on the seedling plants.

A slight but significant difference for ED_{50} values was found among the three cultivars, with TN1 being the most susceptible (lowest ED_{50}) and IR1545-339 the most resistant (Table 7-2). The ED_{50} values were much higher, and differences between cultivars much smaller, than those found in Experiment 1. The slope of the curve at the ED_{50} point was very steep, changing from less than 10% infection to greater than 90% infection between 1.7×10^6 and 4.9×10^6. No differences between growth stages were found for the ED_{50} value (Table 7-2).

Cultivars differed strongly for mean lesion length over all dosages (Table 7-3). The large difference in lesion length between IR1545-339 and TN1 was not reflected in the very slight difference in ED_{50} (Table 7-2). Seedlings had significantly longer lesions than the plants at maximum tillering and booting stages (Table 7-2). Lesion lengths increased with concentration over all dosages (Table 7-3).

Table 7-2: ED_{50} values and mean lesion lengths determined on three rice cultivars at three growth stages following clip inoculation with race 2 isolate PXO86 of *Xanthomonas campestris* pv. *oryzae*.

Cultivar	ED_{50} (^{10}log cfu/ml)	Lesion length (cm)
IR1545-339	6.52±0.01 a[1]	1.6 c
IR40	6.46±0.07 b	8.9 b
TN1	6.45±0.03 b	16.6 a

Growth stage	ED_{50} (^{10}log cfu/ml)	Lesion length (cm)
seedling	6.47±0.05 a	10.6 a
maximum tillering	6.49±0.05 a	8.3 b
booting	6.48±0.07 a	8.3 b

[1]) Standard error of the mean for ED_{50} for cultivar = 0.01, for age = 0.01. Standard error of the mean for lesion length for cultivars = 0.44, for age = 0.42. Numbers in a column followed by the same letter are not significantly different (Bonferroni's test for inequalities, P=0.05).

DISCUSSION

The ED_{50} value was found to be correlated with lesion size, although it is a parameter with a larger variation and a smaller distinction than lesion length. No differences between growth stages were found for this value, although it is well documented that seedling are more susceptible than plants at maximum tillering and booting stages, when assessed by lesion size (Ezuka and Horino, 1976; Mew et al., 1981).

The relationship between ED_{50} and resistance to BB is similar to that reported for the cultivar Cas 209 challenged with virulent and avirulent isolates of Xco (Mew et al., 1982) although the difference between resistant and susceptible reactions reported by them was much larger than the differences reported here. Mew et al. (1982) also found no clear difference between seedling and maximum tillering plants for the ED_{50} value

Table 7-3: Lesion lengths (in cm) of three rice cultivars at three growth stages 14 days after clip inoculation with eight concentrations of *Xanthomonas campestris* pv. *oryzae* isolate PXO86.

Cultivar	Inoculum concentration	Lesion length Seedling	Tillering	Booting
TN1	$3x10^4$	0[1]	0	0
	$3x10^5$	2.2	0	2.3
	$7x10^5$	0	5.9	8.1
	$2x10^6$	12.6	9.1	14.6
	$5x10^6$	35.0	24.4	22.9
	$8x10^6$	33.1	25.9	21.8
	$4x10^7$	37.6	26.4	24.1
	$2x10^8$	36.6	30.9	25.6
IR40	$3x10^4$	0	0	0
	$3x10^5$	0	1.2	1.4
	$7x10^5$	0.8	1.0	1.1
	$2x10^6$	7.4	9.8	3.5
	$5x10^6$	15.3	13.3	13.6
	$8x10^6$	17.8	13.0	14.3
	$4x10^7$	18.0	13.5	14.4
	$2x10^8$	21.3	14.6	18.9
IR1545-339	$3x10^4$	0	0	0
	$3x10^5$	0	0	0
	$7x10^5$	0	0	0
	$2x10^6$	2.3	0.4	1.6
	$5x10^6$	2.9	2.5	2.1
	$8x10^6$	3.2	2.9	2.4
	$4x10^7$	3.5	3.1	2.6
	$2x10^8$	4.2	2.3	3.2

[1]) Standard error of the mean for TN1 = 3.31, for IR40 = 1.48, for IR1545-339 = 0.39.

of Cas 209. The resistance of Cas 209, based on the Xa-10 gene, may have a different effect from that of the resistance of IR1545-339 (Experiment 2), based on the major gene xa-5. The effect may also be different than that of moderate resistance (Experiment 1) not known to be related to any single gene.

Vera Cruz (1984) reported ED_{50} values of 10^5-10^6 cfu/ml inoculum on susceptible cultivars using pin-prick inoculation. These values are slightly higher than those reported here for Experiment 1 (susceptible cultivars) and somewhat lower than those reported for Experiment 2.

Plants in Experiment 1 appeared to be more susceptible to infection than plants in Experiment 2, as the ED_{50} values were considerably lower in the first experiment than the second. However, lesion lengths at higher concentrations were longer in Experiment 2 than in Experiment 1. While both experiments were carried out in the screenhouse, differences in temperature and humidity during inoculation and lesion development could have influenced the outcome of the inoculation. Experiment 1 was inoculated in November, during the rainy season when temperatures are relatively low and humidity is usually above 90%. Experiment 2 was inoculated in May when temperatures are higher and the humidity is relatively low. Environmental conditions conducive to the establishment of infection may be different from those affecting lesion development (Zimmerman and McDonough, 1978). Experiments under controlled conditions are needed to establish this relationship.

The ED_{50} values calculated were based on the concentration of inoculum used, not on the actual number of bacteria deposited in the leaf. Measurement of the actual numbers of cells at the inoculum site is needed to determine the chance of infection per bacteria present. The number of leaves assessed per subplot per replication (25 in Experiment 1, 20-100, depending on plant age in Experiment 2) was in some cases perhaps too low to accurately determine the percentage infection (Meynell and Meynell, 1970). Replications are not usual with ED_{50} tests. However, it was felt better to determine this value per replication, rather than combining all data from the three replicates, as the effect of the environment on this parameter is not known.

A very sharp increase in the percentage infection at the critical concentration was found for a number of cultivars in Experiment 1 and for all three cultivars in Experiment 2. A probit slope greater than 2.0003 indicates dose-dependent probability of infection, where a minimum inoculum level must be present for infection to proceed (Ercolani, 1984). Above this minimum level infection is highly likely to occur. Such proba-

bilities are expected with reactions which have to be triggered, such as a hypersensitive resistance reaction or a toxin effect, but are not normally expected for susceptible reactions, as were present here. In fact, it were the two most susceptible cultivars in Experiment 1 which had the sharpest slopes. Further tests are needed to confirm the level of the probit slope for rice-Xco interactions.

The shorter lesions seen at a low inoculum dosage appears to be due to a slight delay in appearance of the lesions and greatly reduced development after appearance, although daily measurements were not taken. Leach et al. (1989) showed that rice leaves infiltrated with a Hagborg apparatus with two concentrations of Xco differed in bacterial populations per leaf for the first five days after inoculation. After five days bacterial populations were equal. This ability to simulate a moderate resistance response in otherwise susceptible cultivars should be further studied for possible insight into the basis of quantitative resistance of rice to BB.

As a whole there seems to be a good association between ED_{50} values and the level of resistance measured by lesion length. Further research will be needed to test for cultivars which may deviate from this relationship, especially those which have a higher than expected ED_{50}, based on the lesion length measured 14 days after inoculation.

CHAPTER 8

COMPARISON OF INOCULATION METHODS ON THE ASSESSMENT OF RESISTANCE OF RICE CULTIVARS TO *XANTHOMONAS CAMPESTRIS* pv. *ORYZAE*

SUMMARY

Lesion size and lesion number were measured on cultivars of rice inoculated by clipping or by spraying with virulent isolates of *Xanthomonas campestris* pv. *oryzae* (Xco). Mean percentage diseased leaf area (%DLA) gave a similar ranking for the two inoculation methods but quantitative differences in lesion size between cultivars were much more evident after clip than after spray inoculation. Correlation between the methods was high (r=0.82**), but there were cultivars with consistently higher or lower scores after spray inoculation than expected from the clip inoculation. These cultivars also showed relatively high or low numbers of lesions following spray inoculation. Cultivars which had low scores following spray inoculation showed low disease progress during the first nine weeks after transplanting in a screenhouse field experiment. Clip inoculation assesses resistance to spread of bacteria within the leaf xylem system, probably the most important component of moderate resistance. Spray inoculation also assesses resistance to entrance of the bacteria into the leaf. In order to select rice entries with improved moderate resistance to Xco based on both components, resistance to entrance and resistance to spread, a screening based on lesion length after clip inoculation, followed by a test for lesion number after spray inoculation, is advised.

INTRODUCTION

When screening for resistance to disease one looks for a discriminative test i.e. a test in which the resistance is expressed consistently and with a low coefficient of variation. The resistance measured must be representative for the resistance in the field. In relation to this is the question whether artificial inoculation tests all components of resis-

tance. When this is not the case the level of resistance may be high but the resistance may be heavily based on one component while other useful components of resistance are lost.

In bacterial blight (BB) of rice, caused by *Xanthomonas campestris* pv. *oryzae* (Xco), screening is usually done by prick or clip inoculation of the leaves at maximum tillering or booting stage (Ou, 1985). Cultivars are assessed by the size (Chapter 2, this thesis) or occasionally the type (Kaku and Kimura, 1978) of lesions 14-21 days after inoculation (dai). Both these inoculation methods wound the leaves, depositing the inoculum directly into the vascular system. In the field natural infection may be through wounds caused by storms or made during weeding. Clipping or pricking is probably a good reflection of the reaction of cultivars to such an infection. In intact leaves, however, the bacteria can enter into a leaf through the hydathodes. An additional resistance component could be effective in limiting entrance at this point.

Among susceptible cultivars differences in lesion size have been shown following clip or prick inoculation (Yamamoto et al., 1977). When cultivars are tested by spray inoculation a similar trend is found. Morinaka et al. (1978) reported significant correlations (r=0.74-0.83) between spray and clip inoculation on 40 cultivars for each of three virulent isolates. Mew et al. (1981) also found a good relationship between clip and prick inoculation. Reddy (1989) showed a good relationship between scores from clip inoculated and from naturally infected cultivars. However, Reddy states in his paper that "existing screening techniques such as clip inoculation are inadequate to identify and exploit field resistance because they preclude exploitation of other possible forms of resistance".

Experiments were done to test the relationship between the reactions of cultivars inoculated by the clip and by the spray inoculation methods. Spray inoculated plants were assessed both for disease severity and lesion number in order to separate factors relating to bacterial entrance and establishment in a leaf from factors associated with bacterial spread and multiplication within a leaf. Disease progress over a longer period of crop growth was also followed, as it was felt that this would compound any slight difference between cultivars for factors of "field resistance", especially those not tested by clip inoculation.

MATERIALS AND METHODS

Experiment 1

Fifteen rice cultivars varying from highly resistant to highly susceptible were sown in wet seedbeds in a covered screenhouse and transplanted 21 days after seeding to an irrigated field. Plants were fertilized with 175 kg 21-0-0 ammonium sulfate per ha applied in a split application one week after transplanting and again at maximum tillering. A split-split plot design in four replications was used with isolates in the main plots, inoculation techniques in the subplots and cultivars in the subsubplots. Per subsubplot 20 hills per cultivar were planted for inoculation by clipping and 40 hills were planted for inoculation by spraying, to allow for the greater coefficient of variation of the latter method. Each subplot was surrounded by five border rows of IR40, which were planted two weeks before the test plants.

Two race 2 Xco isolates IRN812 and IRN825 and the race 6 isolate PX099 were used. All isolates were kept at -10 °C in skim milk until needed. The isolates were revived on peptone sucrose agar (PSA), transferred to fresh PSA for multiplication and then transferred to 27x11x8 cm flat-sided bottles with a thin layer of PSA for mass inoculum preparation three days before inoculation.

Inoculum was prepared by suspending the mass culture in distilled water and adjusting the suspension to an optical density of 1.00 (590 nm), roughly equivalent to $1x10^9$ cfu/ml. This was used directly for clip inoculation or was diluted to 1/10 concentration in the field immediately before spraying.

Plants were inoculated at 64 days after seeding. Clipping was done in the early afternoon using clippers with an inoculum bottle attached (Kauffman et al., 1973). Plants were sprayed to run-off just before sundown using an ordinary backpack sprayer.

The percentage diseased leaf area of 20 clipped leaves per subsubplot was assessed 14 days after inoculation (dai). Spray inoculated plants were assessed 21 dai by counting the number of leaves showing symptoms and by estimating the percentage diseased leaf area of those leaves, for up to 40 leaves per subsubplot, depending on the number of infected leaves present. Lesion area (severity) and lesion number for the spray inoculated treatments were treated separately in the analysis and were then combined for a disease index value. An arc-sine transformation was found necessary before the %DLA data could be analyzed.

Experiment 2

Seven rice cultivars were sown in plastic trays and transplanted to pots in the greenhouse seven days later, two plants per pot. Pots were arranged on greenhouse benches in a split-plot design with inoculum techniques in main plots and cultivars in subplots, in three replications. Per cultivar six and 12 pots were used for the clip and spray inoculations, respectively, per cultivar. Plants were fertilized twice with ammonium sulfate and insect control (monocrotophos) was applied as needed.

Isolate IRN812 was maintained and multiplied as described above except that both treatments received concentrated inoculum ($1x10^9$ cfu/ml). Plants were clip inoculated using scissors dipped in the inoculum. Spray inoculations were carried out in the late afternoon with 25 ml fresh inoculum per pot. The spray inoculated plants were placed under a plastic tent for 16 hours immediately after inoculation to aid infection.

All plants were scored for lesion length 21 dai. Sprayed plants were assessed on the third and fourth leaves from the top of each tiller, as these were most often the leaf positions which had been scored in the clip treatment. The lesion number was recorded from 150 leaves of spray inoculated plants per cultivar. Lesion number was scored as either 0 (no lesions), 1 (lesion on one side of the midrib) or 2 (both sides of the midrib infected) because multiple lesions on each side, if present, would have coalesced by the time of scoring.

Experiment 3

A third test was done to better investigate the relationship between lesion length following clipping and lesion number following spraying. Eighteen rice cultivars were sown and transplanted as described above to 15 cm pots, two plants per pot. Pots were arranged on greenhouse benches in a similar design as that described in Experiment 2, except that there were three pots per cultivar per replication for the clipped and six pots per replication for the sprayed plants.

Plants were inoculated 50 days after sowing by clipping or by spraying 20 ml per pot using race 2 isolate PX086, which was maintained and multiplied as described. Inoculation was done in the late afternoon and the sprayed plants were kept shaded until the following morning.

Lesion number per leaf on the top four fully unfolded leaves was scored nine days after inoculation, which was two days after the lesions first became visible as small water-soaked areas on the leaf margin.

Lesion length of the clipped leaves was scored 14 days after inoculation.

Disease progress curve

Seeds of eight cultivars were sown in wet seedbeds in the screenhouse. Xco isolates IRN812 and PX086 were maintained and multiplied as described above. Three day old cultures were suspended in 4.5 l water to give a final concentration of about $1x10^8$ cfu/ml. Per cultivar 200 ml inoculum was sprayed to run-off on approximately 1500 20 day old seedlings.

Seedlings were transplanted one day after inoculation to rectangular 1.4x3.2 (replications 1 and 2) or 1.8x2.4 m (replication 3) plots containing 136 or 130 hills (90 or 88 test hills) respectively. A split plot design with isolates as main plots and cultivars as subplots was used. A bund was built between the main plots. This limited, but did not completely eliminate, water flow between the plots with different isolates.

All test hills were assessed every 14 days for leaf blight or kresek symptoms. Kresek killed plants were considered to be missing hills once the plant was completely dead. The number of missing hills per plot was recorded weekly and subtracted from the original number of hills planted for calculation of the disease incidence. From ten hills showing symptoms the number of diseased leaves and the total number of leaves was counted. Each cultivar was scored until two weeks after flowering. Due to differences in the flowering dates some cultivars were therefore scored one or two times more than other cultivars.

The weekly incidence scores were calculated as the percentage hills showing symptoms. The area under the disease progress curve (AUDPC) was calculated using the formula (Fry, 1978):

$$AUDPC = \sum_{i=0}^{n=4} [(x_{i+1} + x_i)/2]*[t_{i+1} - t_i],$$

where x_i = the incidence at scoring i, and t_i = days after transplanting at scoring i, n = total number of observations. Only incidence data from those weeks where all eight cultivars were scored were used for calculations.

RESULTS

Experiment 1

Clip inoculation resulted in good infection of all inoculated leaves while spray inoculation gave only a limited number of lesions. The low number of successful spray inoculations indicates the difficulty in achieving good disease levels following spray inoculation.

The percentage diseased leaf area (%DLA), averaged over the cultivars and isolates, was the same for the two inoculation methods (Table 8-1). However, susceptible cultivars had longer lesions and moderately resistant cultivars had shorter lesions with clip inoculation than with spray inoculation. This gave a much greater range for the clip inoculation than for the spray inoculation (Table 8-1) and allowed better separation of the cultivars into resistant and susceptible groups. A good correlation was found between the two inoculation techniques (r=0.82**). This agreement between inoculation techniques appeared valid for all three isolates, the individual r-values being 0.84**, 0.77** and 0.88** for PX099, IRN825 and IRN812, respectively. Although the agreement was good, some cultivars such as IR40 and JC70 showed a lower %DLA than expected after spray inoculation in relation to the %DLA after clip inoculation.

IR1545-339 showed a significant race-specific effect due to the xa-5 gene for resistance to race 2 isolates (IRN825 and IRN812) (Table 8-1). While this resistance is not complete, and lesions can reach a considerable size, the difference between the race 2 and race 6 isolates was clear, especially with the clip-inoculated plants. The unexpectedly high %DLA value of Biplab for IRN825 cannot be explained. It might mean the presence of an unknown resistance gene.

The mean number of infected leaves per 40 hills ranged from 22.3 to 35.9 (Table 8-1), averaged over all three isolates. Data from the highly susceptible cultivar JC70 were not used for this parameter as a number of hills were lost following flooding early in the season and the remaining hills separated by open spaces were not felt to be representative. The total number of leaves per hill was not recorded and differences between cultivars may have existed. Cultivars, but not isolates, differed significantly for the number of infected leaves. The line BR51-282-8 showed a high number of infections in relation to its good level of moderate resistance following clipping. A significant correlation between this parameter and the %DLA of clip inoculated plants was found (r=0.73**).

A selection index was made by multiplying the score for %DLA follow-

Table 8-1: Percentage diseased leaf area (arc-sine transformed) and mean number of infected leaves of 15 rice cultivars inoculated with three bacterial blight isolates using both clip and spray inoculation procedures.

	Percentage diseased leaf area								Number of	
	Isolate									
	PXO99		IRN825		IRN812		mean		infected	Disease
Cultivar	clip	spray	clip	spray	clip	spray	clip	spray	leaves	index[1]
JC70	44.4	24.1	55.8	27.2	64.0	23.6	54.7[2]	25.0[3,4]	m[5,6]	m
IR9101-46	30.3	25.5	38.0	31.7	45.2	34.4	37.8	30.5	35.9	1095.0
Biplab	21.4	18.7	46.6	23.2	31.2	23.6	33.1	21.8	29.8	649.6
Pelita 1/1	17.6	23.2	21.6	21.9	23.2	24.6	20.8	23.2	29.1	675.1
BR171-2B-8	12.4	19.4	20.8	21.9	19.7	22.8	17.6	21.4	25.2	539.3
IR40	13.3	15.9	14.2	14.5	22.5	19.2	16.7	16.5	25.3	417.5
IR28	14.6	20.1	16.4	20.3	18.8	23.6	16.6	21.3	29.0	617.7
IR1545-339	27.1	25.9	9.5	16.6	11.7	18.3	16.1	20.3	24.8	503.4
B441B-126	14.1	20.1	14.9	20.4	18.8	20.8	15.9	20.5	26.5	543.3
BR51-282-8	13.6	19.3	14.7	20.0	16.5	23.3	14.9	20.9	30.2	631.2
Cisadane	13.0	17.1	14.3	19.7	17.4	22.0	14.9	19.6	27.5	539.0
BR319-1-HR38	13.8	19.3	12.3	18.0	16.5	19.3	14.2	18.9	29.4	555.7
IR4442-46	13.2	16.7	14.0	16.3	15.2	17.6	14.1	16.9	22.3	376.9
IR54	12.4	17.8	11.7	15.6	16.6	19.4	13.6	17.6	23.5	413.6
BR161-2B-25	11.0	16.8	12.9	15.6	14.1	19.1	12.7	17.2	25.0	430.0
Mean	18.2	20.0	21.2	20.2	23.4	22.1	20.9	20.8	27.4	569.2

[1]) Disease index = mean diseased leaf area x number of infected leaves after spray inoculation.

[2]) Standard error of the mean for cultivar = 1.46, for isolate = 1.07, for cultivar x isolate = 2.53.

[3]) Standard error of the mean for cultivar = 0.845, for isolate = 0.315, for cultivar x isolate = 1.46.

[4]) Standard error of the mean for cultivar x inoculation method = 1.193.

[5]) Standard error of the mean for cultivar = 1.700.

[6]) m = missing.

ing spray inoculation with the score for lesion number, using the mean values from all isolates. While the correlation of this index with the %DLA of clip inoculated plants was similar to that between the spray and the clip inoculated plants (r=0.83**) cultivars with smaller and/or fewer lesions than expected on the basis of clip inoculation results are more easily identified. IR40 and IR4442-46 appear to be more resistant while BR51-282-8 seems less resistant than estimated from the clip inoculation.

Experiment 2

In this greenhouse experiment lesion length was measured instead of estimating %DLA. With spray inoculation the lesions tended to extend in a narrow band along the leaf edge. The greater range between cultivars using clip inoculation allowed for a finer distinction between cultivars (Table 8-2). The high correlation (r=0.95**) found between the two inoculation methods indicates the good relationship between reactions of

Table 8-2: Lesion length, lesion number and disease index of seven rice cultivars inoculated by two procedures with race 2 isolate IRN812 of *Xanthomonas campestris* pv. *oryzae*.

Cultivar	Lesion length (cm) clip	spray	Lesion number (per leaf) spray	Disease index[3]
IR9101-46	60.8a[1,2]	58.5a	1.1a	64.4
Biplab	48.4b	48.7b	1.0a	48.7
TN1	46.7b	50.6b	1.2a	60.4
IR28	37.2c	45.0b	1.1a	49.5
IR40	34.8c	35.2c	0.7b	24.6
BR51-282-8	28.8d	38.4c	1.0a	38.4
Cisadane	20.7e	33.6c	0.9ab	30.2

[1]) Coefficient of variation 7.2% and 8.5% for clip and spray inoculations, respectively.

[2]) Numbers within a column followed by the same letter do not differ significantly (P=0.05, Bonferroni's test for inequalities).

[3]) Lesion length x lesion number.

the two tests. The cultivar IR40 showed shorter lesions after spray inoculation than expected given the length of the lesions following clipping.

The mean number of lesions per leaf showed significant differences between cultivars (Table 8-2). IR40 had significantly fewer lesions than all other cultivars except Cisadane. The disease index emphasizes this difference.

Table 8-3: Mean lesion length (in cm) following clip inoculation and mean lesion number per leaf following spray inoculation with *Xanthomonas campestris* pv. *oryzae* of 18 rice cultivars.

	Inoculation method	
Cultivar	clip	spray
TN1	18.9[1]	1.6
IR9101-46	17.9	1.0
Biplab	16.1	3.2
IR36	14.7	0.9
IR56	14.7	1.3
IR28	13.1	1.2
BR51-282-8	13.0	1.6
IR5	12.9	2.5
IR24	12.8	1.3
IR8	12.2	1.0
IR40	12.0	0.7
IR52	11.9	1.4
IR32	11.7	1.5
IR64	11.6	1.6
IR20	11.1	1.2
Cisadane	10.5	2.0
IR54	9.1	0.9
IR48	7.3	2.3
Coefficient of variation	11.1%	34.0%

[1]) Standard error of means for clip inoculation 0.83, for spray inoculation 0.3.

Experiment 3

Another, more extensive test of the relationship between lesion length following clip inoculation and lesion number following spray inoculation was made. Lesion size and lesion number were scored on the third and fourth leaf below the top unfolding leaf. Significant differences between cultivars for both lesion length and lesion number were found (Table 8-3) although the coefficient of variation for lesion number was much higher than that for lesion length. No relationship between the two parameters was found (r=0.01). As in Experiment 2, IR40 had the lowest number of lesions but an average lesion length following clipping. IR48 was the most resistant cultivar following clip inoculation but had a relatively high number of lesions. The highest values for lesion number were found on Biplab and IR5, the two cultivars with a semi-tall stature.

Experiment 4

Disease progress was followed in plots which were inoculated one day before transplanting. Disease incidence (percentage hills infected) increased during the first nine weeks after transplanting but then decreased sharply after this point (Figure 8-1), possibly in relation to a period of dry weather then. The percentage leaves per hill infected (infected hills only) decreased steadily in the plots inoculated with PX086, as the hills grew in size, but remained high in the plots inoculated with IRN812 for the first nine weeks after transplanting (Figure 8-2). This was due to the greater number of infected leaves but also to the fact that heavily infected plants tend to have fewer total number of leaves than healthy plants due to reduced tillering and faster senescence. Growth of infected plants may also have been reduced.

The AUPDC calculated for the incidence during the first nine weeks after transplanting showed significant cultivar effects but the difference between the two isolates was not significant (Table 8-4). No significant cultivar x isolate interactions were found although Biplab showed an extremely large increase in disease level, changing in fact from a relatively resistant cultivar to the most heavily infected cultivar. A significant correlation between the AUDPC results and the results of the spray inoculation test in Experiment one was found (r=0.77*) for both DLA following spray inoculation and the disease index but not between the AUDPC and the clip inoculation test (r=0.60).

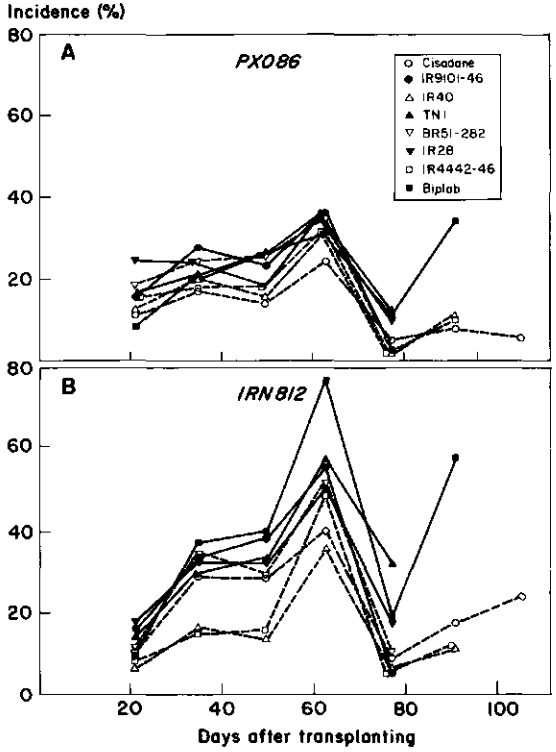

Figure 8-1: Incidence of bacterial blight (kresek and leaf blight symptoms) in rice plants spray inoculated one day before transplanting with race 2 isolates PXO86 (A) and IRN812 (B) of *Xanthomonas campestris* pv. *oryzae*.

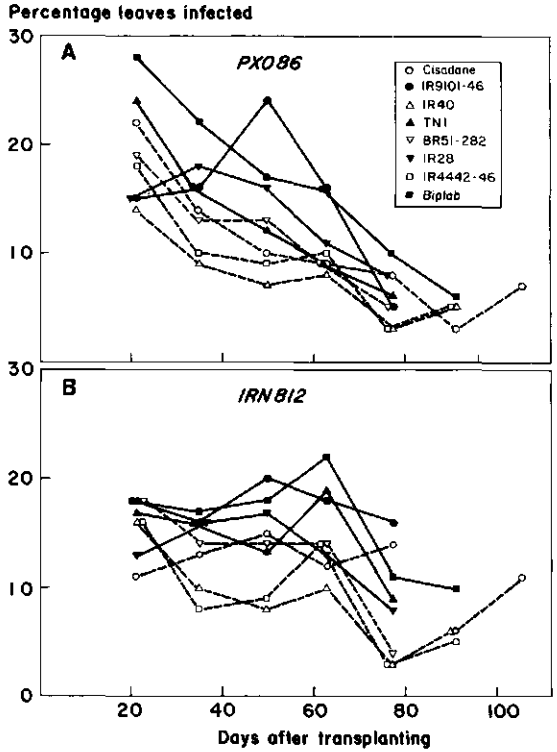

Figure 8-2: Percentage leaves per hill showing leaf blight symptoms (infected hills only) in rice plants spray inoculated one day before transplanting with race 2 isolates PXO86 (A) and IRN812 (B) of *Xanthomonas campestris* pv. *oryzae*.

Table 8-4: Area under the disease progress curve for eight rice cultivars inoculated with two isolates of *Xanthomonas campestris* pv. *oryzae*.

Cultivar	Isolate PXO86	IRN812	mean	Disease index[1]
IR9101-46	1244 cde[2,3]	1647 fg	1446	1095.0
IR28	1253 de	1565 fg	1409	617.7
Biplab	977 abcd	1776 g	1377	649.6
TN1	1211 bcde	1498 efg	1355	-
BR51-282	1241 cde	1437 ef	1339	631.2
Cisadane	808 a	1212 bcde	1010	539.0
IR4442-46	1012 abcd	915 ab	946	376.9
IR40	946 abc	789 a	868	417.5
Mean	1087	1355		

[1]) See Table 8-1.

[2]) Hill-days, average of three replications; maximum = 6300.

[3]) Numbers followed by the same letter are not significantly different (P=0.05, Bonferroni's test for inequalities). Coefficient of variation = 20%.

DISCUSSION

Lesion size (area or length) at a given time after inoculation is affected by:

1. factors related to the entrance and establishment of the host by the bacteria, and
2. factors affecting lesion extension such as bacterial movement and multiplication and sensitivity of tissue to damage.

Final symptoms following spray inoculation are a combination of factors affecting the number of lesions and factors affecting the expansion of individual lesions. Clip inoculation, because it places the bacteria directly into the vascular system, allows the measurement of factors affecting lesion expansion only.

Clip inoculation better distinguished between cultivars for small difference in resistance to BB than spray inoculation due to the smaller

coefficient of variance and the larger variation among cultivars. Clip inoculation will remain the main method of screening for resistance to BB. Because infection in the field is often through wounds caused during storms, transplanting or weeding, clip inoculation can be considered a relevant measure of field resistance.

The good correlation between spray and clip inoculation reported here indicates that a large to very large part of the resistance assessed by spraying is similar to that more accurately assessed by clipping. This is in agreement with that found by Morinaka et al. (1978). Selection for resistance can best be done by measuring lesion length following clip inoculation with a virulent isolate. There are, however, indications for a small contribution of other resistance components. This is seen by the lack of association between lesion number after spray inoculation and lesion size following clipping. Especially certain cultivars, such as IR40, seem to carry some additional resistance resulting in a reduced number of lesions.

This factor can be identified either by a monocyclic test, by counting lesion number on spray-inoculated plants, or by a polycyclic test to follow the disease progress over time. The former is a very simple greenhouse test but with a relatively high coefficient of variance. The latter test involves more space and requires repeated measurements. Under conditions at the International Rice Research Institute (IRRI) a field test relying on natural infection or using a spreader row is not likely to result in high enough levels of infection to distinguish differences between cultivars, although in other areas high levels of infection are regularly recorded (T.W. Mew, IRRI, pers. comm.). Experiment 4 reported here comes closest to following a natural epidemic in the field, although all plants were inoculated one day before transplanting. When more than one isolate is being used to test the disease progress there is a large danger of interplot interference through a common water system. Bunds or other dividers can help to limit water circulation but may not be able to prevent mixing totally.

The performance of a cultivar with moderate resistance, as assessed by lesion size following clip inoculation with a virulent isolate, could be improved if it were coupled with a resistance reducing the chance of bacterial entrance. In order to select rice entries with improved moderate resistance to Xco based on more than one component, a screening based on lesion length after clip inoculation followed by a test based on lesion number after spray inoculation seems most optimal. Only a limited number of entries with relatively short lesions, as measured in the clip inocula-

tion test, are entered in the spray inoculation test. Based on this test the cultivars with relatively high numbers of lesions are removed. If Table 8-3 is seen as such a combined screening, only the entries with a lesion size below 12.2 cm are further screened for lesion number. Especially IR54 and IR40 appear to be interesting then.

CHAPTER 9

INTERACTIONS BETWEEN SELECTED RICE CULTIVARS AND PHILIPPINE ISOLATES OF *XANTHOMONAS CAMPESTRIS* pv. *ORYZAE*

SUMMARY

A wide range of different rice cultivars were tested against more than 20 *Xanthomonas campestris* pv. *oryzae* isolates from the Philippines. Small interactions among what are considered compatible interactions were sought as evidence of minor genes for resistance. Interactions with the Xa-4 gene for resistance were included to help define the size of effects attributable to "major" and "minor" resistance genes.

The lesion length of the cultivars, averaged over all isolates, varied greatly, indicating differences in quantitative resistance. The lesion lengths of the isolates averaged over all cultivars varied considerably indicating differences in aggressiveness and/or virulence.

The reproducible cultivar x isolate interactions varied in size from large to small, but were all associated with major resistance genes Xa-4, Xa-14 and possibly Xa-10. No small cultivar x isolate interactions not associated with known major resistance genes were observed with certainty.

The isolates showed a variable reaction to the cultivars carrying Xa-4, from fully virulent to highly avirulent. The data can be explained if it is assumed that various isolates neutralize the effect of Xa-4 to a different degree. To some isolates Xa-4 shows a residual effect. This residual effect appeared race-specific and is difficult to distinguish from small race-specific effects caused by minor genes.

INTRODUCTION

Bacterial blight (BB) in rice, caused by *Xanthomonas campestris* pv. *oryzae* (Xco), has been shown to vary in reaction between cultivars and between isolates (Ezuka and Sakaguchi, 1978; Mew, 1987). No hypersensitivity has been found (Parry and Callow, 1986) although vascular brown-

ing has been identified as a resistance characteristic of some cultivars (Kaku and Kimura, 1978). Buddenhagen and Reddy (1972) and Ou (1972) tested large numbers of Xco isolates from South East Asia and found reactions varying from avirulent to highly virulent on representative cultivars. Only few significant cultivar x isolate interactions were found, and both groups of authors felt that the variation could be attributed to differences in race-non-specific resistance in cultivars and in aggressiveness in the isolates. Others also reported only minor differences between isolates for their reactions to a set of cultivars in India (Kauffman and Rao, 1972) and Sri Lanka (Watanabe, 1976). In some cases however the differences were considerable, indicating isolate specific resistance (Kauffman and Rao, 1972).

Pathogenic specialization was conclusively demonstrated in Japan (Ezuka and Horino, 1974b) and the Philippines (Mew and Vera Cruz, 1979) following introduction and extensive planting of resistant cultivars. Large interactions between identifiable races and cultivars have been shown; extensive screening has now identified 14 major resistance genes in rice cultivars (Ogawa et al., 1988) and 26 races of Xco in tropical Asian countries (Yamamoto and Ogawa, 1988).

Interest in moderately resistant (MR) and moderately susceptible (MS) cultivars has increased as resistance genes lose effectiveness to local Xco populations. The above-mentioned lack of race-specificity among susceptible cultivars led Watanabe (1976) to the hypothesis that the quantitative resistance of MR and MS cultivars is race-non-specific (horizontal) and therefore probably durable. Yamamoto et al. (1977) also concluded that quantitative resistance is horizontal after finding high correlations between isolates of various race groups for symptoms caused on 11 susceptible and MS cultivars. Yamada (1984, 1986a) found a quantitative distribution of progeny of crosses between susceptible cultivars and cultivars showing moderate resistance to three Japanese races of Xco. He felt this resistance to be race-non-specific.

Yoshimura et al. (1984) have shown that the major genes Xa-4 and xa-5 can result in either high or moderate levels of resistance to Philippine isolates of Xco, depending on the isolate used. This indicates that cultivar x isolate interactions among cultivars showing intermediate reactions could be expected, especially when these cultivars carry the Xa-4 gene. Such cultivar x isolate interactions would indicate that quantitative resistance is based at least partly on specific resistance genes of measurable effects, which may also lose effectiveness if isolates change.

A study was carried out to test MR and MS rice cultivars for the presence or absence of isolate-specific interactions to Philippine Xco isolates. Both incomplete effects of the Xa-4 gene for resistance and other possible interactions were sought.

MATERIALS AND METHODS

Experiments 1 and 2

Six cultivars were inoculated with 19 isolates and sterile water as check for cross-contamination (Experiment 1) and nine cultivars were inoculated with nine isolates and sterile water (Experiment 2). The experiments were done simultaneously in one screenhouse plot. TN1, from Experiment 1, was included as a susceptible check in analysis of Experiment 2.

Seeds of the 15 rice cultivars were obtained from the International Rice Testing Program and from the Department of Plant Breeding of the International Rice Research Institute (IRRI), Los Baños, Philippines. Seeds were sown in 33x25x10 cm plastic trays filled with upland soil fertilized with 10 kg/ha additional nitrogen. After 21 days seedlings were transplanted to two flooded 3x27 m beds in a screenhouse. A split application of ammonium sulfate nitrogen fertilizer (175 kg/ha, total) was given one week after transplanting and at maximum tillering. Cypermethrin (0.01% v/v) was sprayed against brown plant hoppers when needed.

Cultivars were arranged in rows, six hills per row, in a split plot design in two replications with isolates in the main plots and cultivars in the subplot rows. Per replication, ten main plots contained all fifteen cultivars of Experiments 1 and 2 combined and ten main plots contained only the six cultivars of Experiment 1. One bed was equal to one replication. Both experiments were carried out twice. Series 1 was seeded on July 2, 1986; Series 2, which had minor changes in isolate and cultivar choice, was seeded on September 22, 1986.

Nineteen Xco isolates from diverse origins within the Philippines were chosen from the collection at the Department of Plant Pathology of IRRI. Isolates were stored in sterile skim milk at -20 °C until needed. Cultures were revived on peptone sucrose agar (PSA) incubated for four days at 30 °C. Inoculum was prepared by transferring the bacteria to fresh PSA slants, incubating for three days at 30 °C, suspending the bacteria in 20 ml distilled water per slant and standardizing the final density to 1.0

on a Beckman spectrophotometer (590 nm). This is approximately equal to $1x10^9$ cfu/ml.

Plants were inoculated at 63 days after sowing (Series 1) or 64 days (Series 2), after all cultivars had passed maximum tillering but before heading. Fully expanded leaves were inoculated by dipping scissors in the inoculum suspension and clipping the top 1-2 cm of the leaf tip (Kauffman et al., 1973). Lesion lengths were scored on four leaves per hill from five hills 14 days after inoculation.

For each cultivar x isolate combination the deviation from the predicted lesion length was calculated with the model:

$$(cv_{ij} - TN1_j) - (\overline{cv_{i.}} - \overline{TN1_.}) \quad (cm)$$

cv_{ij} = lesion length of a particular cultivar (i) x isolate (j) combination,

$TN1_j$ = lesion length of TN1 with isolate (j),

$\overline{cv_{i.}}$ = mean lesion length of cultivar (i) over all isolates,

$\overline{TN1_.}$ = mean lesion length of TN1 over all isolates.

This model uses the lesion length of an isolate on the susceptible cultivar TN1 as a measure of the general aggressiveness of the isolate. The difference between the lesion length of TN1, averaged over all isolates, and the lesion lengths of the test cultivar, averaged over all isolates, is used as an estimate of the quantitative resistance of a cultivar. For each individual isolate, the quantitative resistance of a cultivar, relative to TN1, was compared with the mean quantitative resistance of the cultivar relative to TN1, averaged over all isolates. This value is expected to be zero, when there are no specific interactions. The deviation from zero was tested for significance using Bonferroni's test for differences (P=0.05). Significant deviations indicate specific interactions in relation to the susceptible check. Differences between other cultivars were also tested for significance using this range value, to identify differences in reactions between two cultivars for different isolates.

Experiment 3

Seeds of 20 rice cultivars from a wide range of rice growing countries of the world were obtained from the International Rice Germplasm

Collection (IRGC). Seeds were sown and plants transplanted and maintained as described above. Experimental layout was similar to Experiment 1. Seeds were sown on March 12, 1987.

Ten bacterial isolates were stored and multiplied as described in Experiments 1 and 2. Plants were inoculated 63 days after sowing by the clipping method described above. Lesion lengths were measured on four leaves per plant of five hills 14 days after inoculation.

Cultivar x isolate interactions were tested using the model described for Experiment 1 and 2, except that the mean of all cultivars was used as a measure of the general aggressiveness of an isolate, instead of a single susceptible cultivar.

RESULTS

Experiment 1

All 19 isolates produced typical BB symptoms on the rice cultivars but no lesions were found on the clipped leaves of the check plot inoculated with sterile water. The results of this plot were not included in the analysis.

Due to poor germination two sister lines of IR9101 were used for the two plantings. In Series 1, IR9101-124 was found to carry the Xa-4 gene for resistance while the line used in Series 2, IR9101-46 did not. Two isolates were replaced in the second series as they were found to be avirulent to TN1, the susceptible check. Series 1 had a higher average lesion length than Series 2, possibly due to conditions in the screenhouse after inoculation.

Large and significant differences between cultivars and between isolates were found (Table 9-1). A significant correlation was found between the aggressiveness of isolates, as defined by the length of lesions on TN1, and the range between the susceptible and MR cultivars, as measured by the difference in lesion length between TN1 and Cisadane (r_s=0.73** for Series 1 and 0.68** for Series 2). This indicates that more aggressive isolates better distinguished between cultivars than less aggressive isolates.

Significant cultivar x isolate interactions were also found. All four cultivars (five in Series 1) with the Xa-4 gene had significantly shorter lesions than TN1 with the two isolates BB1287 (race 1) and PX071 (race 4) known to be respectively, avirulent or incompletely virulent to

Table 9-1: Lesion lengths (cm) of rice cultivars averaged over isolates and of bacterial blight isolates averaged over cultivars in two series of plantings.

Cultivar	Resistance factor[1]	Series 1	2	mean
TN1	(Xa-14)	30.9[2]	26.5	28.7
IR9101-124	(Xa-4)	19.1	-	-
IR9101-46	-	-	22.6	-
IR28	(Xa-4)	19.4	14.8	17.1
BR51-282-8	(Xa-4)	18.4	13.5	16.0
IR40	(Xa-4)	15.1	11.3	13.2
Cisadane	(Xa-4)	14.2	7.9	11.1

Isolate	Race	Area of origin (Philippines)			
PXO143	3	Bohol	25.6[3]	19.7	22.7
IRN812	2	Nueva Ecija	25.3	19.9	22.6
IRN764	2	Tarlac	24.0	20.8	22.4
IRN793	2	Laguna	24.5	19.9	22.2
IRN907	2	S. Cotabota	25.1	18.5	21.8
IRN922	3	Iloilo	23.6	18.5	21.1
IRN825	2	Camarinas Sur	21.3	18.3	19.8
IRN863	2	Albay	21.8	16.7	19.3
BB1194	2	IRRI	21.6	15.9	18.8
PXO86	2	IRRI	22.2	15.1	18.7
IRN332	3	Bicol	19.5	16.9	18.2
BB1325	2	IRRI	20.7	15.3	18.0
BB1317	2	IRRI	19.7	16.0	17.9
IRN705	2	Davao	17.5	-	-
PXO99	6	IRRI	16.1	-	-
PXO122	6	IRRI	15.5	15.2	15.4
IRN718	2	Leyte	-	13.5	-
PXO71	4	IRRI	11.7	9.5	10.6
IRN738	2	Davao	-	10.5	-
BB1287	1	IRRI	7.6	9.3	8.5
IRN711	2	Leyte	7.9	-	-

[1]) Known major resistance gene present and effective in the Philippines.

[2]) Standard error of the mean for cultivars 0.34 (Series 1) 0.32 (Series 2).

[3]) Standard error of the mean for isolates 0.61 (Series 1) 0.81 (Series 2).

this gene (Table 9-2). The greatly reduced lesion lengths shows the effect of this gene on lesion development. A third isolate, BB1194, also showed shorter lesions than were predicted for all cultivars with Xa-4, although only some cultivars were significantly shorter in both plantings. This isolate appears to have a small degree of residual avirulence in relation to the Xa-4 gene for resistance, although it is highly aggressive on TN1.

Table 9-2: Lesion lengths (cm) of rice cultivars and selected bacterial blight isolates from Experiment 1 (see text).

Series 1

Isolate	Race	Cultivar TN1	IR9101-124	IR28	BR51-282-8	IR40	Cisadane
BB1287	1	35.6[1]	3.2	1.9	1.8	1.9	1.3
PXO71	4	30.5	13.4	8.1	6.2	6.7	5.2
BB1194	2	38.6	25.8	21.7	19.5	17.8	14.4
mean other isolates		30.2	20.1	21.0	20.2	16.8	15.6
IRN705	2	1.1	9.8	11.4	10.2	9.3	5.3
IRN711	2	13.4	18.2	20.3	20.9	15.4	16.8
mean other isolates		33.7	19.7	19.8	18.8	15.5	14.6

Series 2

Isolate	Race	Cultivar TN1	IR9101-46	IR28	BR51-282-8	IR40	Cisadane
BB1287	1	28.6	23.6	1.4	1.4	0.6	0.4
PXO71	4	24.6	20.5	3.9	3.6	2.1	2.3
BB1194	2	29.1	23.9	10.1	8.0	8.1	6.0
mean other isolates		25.2	21.9	14.5	15.5	12.2	8.5

[1]) Values differing by 7.0 (Series 1) and 6.4 (Series 2) are significantly different from each other, Bonferroni's test for differences (P=0.05).

Lesions resulting from two isolates, IRN705 and IRN711, were found to be significantly shorter than expected on TN1 (Table 9-2). The largest difference was found with IRN705, while IRN711 showed a smaller but still highly significant reduction of resistance on TN1. These interactions are attributed to the effect of the Xa-14 gene for resistance in TN1. TN1 has not been used as a differential cultivar to define Philippines races and therefore the differences found among race 2 isolates for virulence to this cultivar are to be expected.

Significant interactions between MS cultivars would indicate the effect of other minor genes for resistance. No such interactions were identified. In each of the two series a few interactions were significant but not reproducible as they did not occur in the other series.

Experiment 2

All 10 cultivar x isolate combinations produced typical BB symptoms while no lesions developed on the clipped leaves of the check plot.

Significant differences between cultivars and between isolates were found (Table 9-3). These differences were not clearly related to the major gene Xa-4, present in seven of the cultivars. The cultivars JC70 and TN1 were used as susceptible checks to test for residual avirulence to the Xa-4 gene, following the model described in the materials and methods. No isolate was found with such residual avirulence to the Xa-4 gene. A few significant cultivar x isolate interactions were found in each group but none of these was consistent between the two planting series, as in Experiment 1.

Experiment 3

Reactions of 20 cultivars of diverse origin were tested against ten Philippine isolates of races 2, 3 and 6. Seed of some cultivars obtained from the IRGC was found to be rather heterogeneous and differences between cultivars and isolates were therefore less clear than in Experiments 1 and 2. Significant differences between cultivars and isolates were found (Table 9-4). A significant correlation was found between the aggressiveness of isolates, as defined by the lesion length on the susceptible cultivar Wagwag, and the range between susceptible and MR cultivars, as defined by the differences in lesion length between Wagwag and the MR cultivar Pankaj (r_s=0.63*). More aggressive isolates therefore better distinguished between cultivars than less aggressive isolates, as was

Table 9-3: Mean lesion length (cm) of cultivars and isolates tested in two planting groups by clip inoculation at maximum tillering stage.

Cultivar	Resistance factor[1]	Series 1	2	mean
JC70		38.0[2]	27.7	32.9
TN1	(Xa-14)	31.6	26.3	29.0
IR8	(Xa-11)	25.1	20.1	22.6
Gaja baru	(Xa-4)	25.5	18.9	22.2
Biplab	(Xa-4)	22.1	15.7	18.9
BR171-1B-8	(Xa-4)	19.8	15.7	17.8
IR4442-46-3-3-3	(Xa-4)	20.1	14.0	17.1
IR54	(Xa-4)	19.7	14.0	16.9
BR319-1-HR	(Xa-4)	17.6	14.1	15.9
BR161-25-25	(Xa-4)	17.9	11.9	14.9

Isolate	Race	Series 1	2	mean
IRN812	2	26.2[3]	20.4	23.3
IRN793	2	25.0	19.5	22.3
IRN764	2	23.6	20.2	21.9
IRN907	2	23.8	18.3	21.1
IRN922	3	23.4	18.8	21.1
IRN825	2	20.6	18.0	19.3
IRN705	2	19.1	-	-
PXO86	2	21.3	15.0	18.2
PXO99	6	17.2	m	-
IRN738	2	-	10.0	-

[1]) Major gene suspected to be present, based on reaction to race 1 isolates and literature reports.

[2]) Standard error of the mean for cultivars 0.73 (Series 1); 0.50 (Series 2).

[3]) Standard error of the mean for isolates 0.46 (Series 1); 0.71 (Series 2).

Table 9-4: Lesion lengths (cm) of rice cultivars averaged over isolates and bacterial blight isolates averaged over cultivars.

Cultivar	IRGC number	Origin	mean
Wagwag	44803	Philippines	39.6[2]
Makalioka 34	6087	Madagascar	34.5
Khao Dawk Mali	6886	Thailand	33.9
Tadukan	9804	Philippines	29.0
Tongsan 115	19146	Indonesia	28.8
Jing gang 30[1]		China	28.7
Tetep	463	Vietnam	27.1
I141	64	India	27.0
Nahng Mon S4	708	Thailand	27.0
GEB 24	6343	India	26.5
Mineiro	9	Brazil	25.4
Engkatek	14481	Malaysia	24.6
Seraup kechil 4	13899	Malaysia	24.4
Gaukkyi	33074	Burma	23.9
Bandang putih 6	6133	Indonesia	23.4
Pankaj	3103	Bangladesh	22.8
Zhai Ye Qing 8[1]		China	22.6
Lageado	50489	Brazil	22.4
Latisail	5471	Bangladesh	11.3
Mas	3605	Indonesia	10.8

Isolate	Race	Origin (Philippines)	mean
IRN526	3	Bohol	29.4[3]
IRN922	3	Iloilo	29.0
PXO99	6	IRRI	27.9
IRN812	2	Nueva Ecija	27.1
IRN907	2	S.Cotabato	27.0
IRN863	2	Albay	25.2
IRN825	2	Camarinas Sur	24.9
IRN764	2	Tarlac	24.5
IRN332	3	Bicol	22.9
PXO86	2	IRRI	18.9

[1]) Seeds received from Mrs. Zhang Qi, CAAS, Beijing.

[2]) Standard error of the mean for cultivar 0.70, LSD = 1.63 (P=0.05).

[3]) Standard error of the mean for isolates 1.06, LSD = 2.33 (P=0.05).

found in Experiment 1.

Two cultivars, Latisail (Bangladesh) and Mas (Indonesia), were highly resistant to the race 2 isolates used, but susceptible to the race 3 and 6 isolates (see examples in Table 9-5). These two cultivars may have the Xa-10 gene for resistance, but this should be confirmed by test crosses with Cas 209, the known carrier of this gene.

Table 9-5: Lesion lengths (cm) of selected rice cultivars and selected bacterial blight isolates tested in Experiment 3 (see text).

Major interactions

	Race 3		2				
Cultivar	Isolate IRN 332	IRN 526	IRN 764	IRN 812	IRN 825	IRN 863	IRN 907
Mas	22.8[1]	26.2	0.4	0.6	0.4	0.7	0.7
Latisail	25.0	30.2	1.4	0.8	0.7	0.5	1.1

Minor interactions

Cultivar	Isolate IRN 332	IRN 526	IRN 764	IRN 812	IRN 825	IRN 863	IRN 907
Nahng Mon S4	20.4	28.6	[*20.9*	*34.1*]	[*26.0*	*35.2*]	32.7
Pankaj	20.9	26.3	[*22.9*	*22.8*]	[*21.8*	*22.5*]	27.1
Makalioka 34	31.4	37.2	31.8	32.6	37.6	[*29.8*	*39.8*]
Bandang poetih 680	15.8	22.8	21.9	27.8	26.8	[*28.8*	*24.5*]
Tongsan 115	[*30.8*]	27.7	28.0	31.0	[*27.6*]	30.1	31.7
Lageado	[*13.3*]	22.8	25.3	24.4	[*24.7*]	23.2	23.9

[1]) Bonferroni's test for differences = 6.9 cm (P=0.05).

Small but significant interactions between cultivars and isolates were found in several cases. These interactions were all of the type where two (or more) cultivars differ significantly from each other with one isolate but have a similar lesion size with another isolate (see examples in Table 9-5). These interactions suggest the effects of minor resistance genes influencing development of some isolates but not others. However, this experiment should be repeated to confirm such interactions.

DISCUSSION

Differences in resistance between cultivars tested were found to be due to two types of resistance, these being:

1. large to small race-specific effects associated with the major resistance genes Xa-4, Xa-14, and possibly Xa-10,
2. smaller effects not clearly related to a known major gene.

Yoshimura et al. (1984) showed via F_2 analysis that the size of the effect of Xa-4 was influenced by the isolates used. Results here confirmed this for Xa-4 and showed similar gradations in virulence to the gene Xa-14 in TN1. The variable degree of virulence to Xa-4 appeared independent of the aggressiveness of the isolate, as measured by the size of the lesions produced on the susceptible cultivar TN1. The two isolates which showed a strongly reduced lesion length on TN1, indicating avirulence to Xa-14, showed a different behavior. IRN711 had an aggressiveness equal to the mean of the other isolates, as measured on the cultivars other than TN1 (Table 9-2). On TN1 it was incompletely avirulent. IRN705 showed a reduced lesion length on all cultivars, and small lesions on TN1, suggesting a combination of reduced aggressiveness and incomplete avirulence to Xa-14. Aggressiveness can be affected by storage conditions of the isolate and has been shown to be variable without changing the specific virulence of an isolate (Dath and Devadath, 1982).

Apparently virulent isolates reveal large quantitative differences between cultivars for lesion length. No small significant cultivar x isolate interactions could be identified with certainty. However, the large error in lesion length measurements limited detection to differences of greater than about 7 cm, which may be quite large for minor gene effects. When the level of resistance is determined by the sum of several small factors it is difficult to separate a change in one of these factors from the experimental error (Parlevliet, 1981a). The necessary restric-

tion of the test to only Philippine isolates of Xco has been shown to limit the variation in known virulence factors (Yamamoto and Ogawa, 1988), and possibly also limits the variation in other factors in the pathogen which would lead to small interactions with minor resistance genes.

Yamada (1984, 1986a) reported lack of small race-specific interactions in segregating populations. However, the scale used by Yamada is less sensitive for variations in the moderately susceptible and susceptible range of lesion area, and it also confounds differences in lesion length with differences in leaf length. It is doubtful if he could have clearly pinpointed smaller interactions, if these were present. Yamamoto et al. (1977) reported correlations between 0.72 and 0.92 between isolates prick-inoculated onto a varying number of cultivars showing lesions greater than 25 mm^2, indicating that no large race-specific effects were present. However, minor race-specific effects among a few cultivars would not greatly lower the correlation and high correlations are therefore not to be taken as proof that such minor effects were not present. In both cases a serious study of the data for small interactions does not seem to have been made, although they appear to be present, as was already pointed out by Parlevliet (1981a). In experiments designed to test the race-specificity or non-specificity of a host-parasite interaction one seeks the exceptional interaction which will disprove the hypothesis of non-specificity; the general trend is not sufficient evidence to prove lack of specificity.

The test used here to separate residual avirulence to a major gene from differences in quantitative resistance resulting from unknown minor genes is effective. However, care must be taken that sufficient numbers of cultivars both with and without the major gene which may have residual effects are tested simultaneously. If not, large but random variability in a single check cultivar will be attributed to the effects of the major gene in the test cultivars. This was possibly the case in Experiment 1, Series 1, with BB1194, where the second susceptible check IR9101 was lost due to a change of seed source. However because similar results were found in Series 2, where IR9101-46 was highly susceptible to all isolates, the conclusion is made that BB1194 has some residual avirulence to this gene. Separation of very small residual effects of major genes from differences due to minor genes will remain difficult.

Mew and Vera Cruz (1988) refer to the quantitative differences between cultivars for resistance to bacterial blight when all cultivars are challenged by isolates presumed virulent to the major gene for resistance present in these cultivars as "background resistance". They suggest

that this might be more durable than major gene resistance. Results found here suggest that the quantitative resistance will be stable between locations in the Philippines, but that disease symptoms can be quite severe with certain isolates and under environmental conditions conducive to lesion development. Since results presented in this chapter show that the level of virulence to a major gene can vary quantitatively, a gradual increase in virulence may occur when cultivars with the major gene are grown continuously for several years over large areas. It is not known whether the same will be true for minor genes. Historical data provides better information over the expected durability of quantitative resistance than testing with a large number of isolates, however varied their origin.

If one is screening for higher levels of quantitative resistance for cultivar improvement a single isolate of proven virulence to known major genes can be used once one has ascertained that there are no important cultivar x isolate interactions. Highly aggressive isolates, as defined by the length of the lesion on the most susceptible cultivar, gave a greater range of reactions than less aggressive isolates. While the variance of lesion lengths may also increase somewhat with increasing mean lesion length, a screening is expected to be most effective in distinguishing differences in levels of quantitative resistance when a highly aggressive isolate is used.

CHAPTER 10

GENETIC ANALYSIS AND SELECTION OF FACTORS AFFECTING QUANTITATIVE RESISTANCE TO *XANTHOMONAS CAMPESTRIS* pv. *ORYZAE* IN RICE

SUMMARY

Four cultivars moderately resistant to Philippine *Xanthomonas campestris* pv. *oryzae* (Xco) races 2, 3, 5 and 6 and highly resistant to race 1 were crossed with two highly susceptible cultivars and with each other. The F_1 populations were as or more susceptible than the least resistant parent cultivar when assessed for lesion length (cm) by clip-inoculating booting plants with two race 2 and one race 6 isolates of Xco. Quantitative resistance is therefore largely recessive. The F_2 populations showed continuous distributions when assessed with the race 6 isolate PX099, although populations from crosses between two moderately resistant cultivars were positively skewed. Broad-sense heritabilities in the F_2 ranged from 0.20-0.69. Selection for varying levels of resistance was carried out in the F_2 generation. F_3 lines selected from the F_2 population modes had variances and ranges equal to those from the population extremes and larger than the variances of the parent cultivars. Quantitative resistance is therefore not due to a single additive factor.

Plant and line selection for high and low levels of resistance was carried out in the F_3 generation and assessed in the F_4 generation. Line selection was more effective than plant selection. Response to selection in the F_3 was greater than in the F_2. Lines more susceptible than both parents were recovered in the crosses between two moderately resistant cultivars, but few of these lines were as susceptible as the susceptible parent cultivars. This indicates that the moderately resistant cultivars had some resistance factors in common. A number of lines more resistant than both parents were also recovered in the crosses between two moderately resistant cultivars. All cultivars, including the susceptible cultivars, appear to have one or more factors for quantitative resistance. Moderately resistant cultivars appear to have a minimum of two factors. At least five different factors, but probably more, are thought to be present in the six cultivars.

One of these factors is probably the allele Xa-4 determining high levels of resistance to Philippine Xco race 1, or a closely linked factor. This allele was shown to have residual resistance to PX099, and reduced lesion lengths by 4-7 cm in F_2 plants. Among plants found resistant to the Xco isolate PX061, lesion length following inoculation with PX099 was also somewhat correlated (r=0.36-0.57**) to the lesion lengths when inoculated with the Xco isolate PX061. This indicates that factors for quantitative resistance affect the expression of the major gene resistance.

INTRODUCTION

Reactions of rice cultivars to *Xanthomonas campestris* pv. *oryzae*, the causal organism of bacterial blight (BB), have been shown to vary quantitatively among cultivars, by lesion area or length, but only in the case of the Xa-3 gene does resistance vary by lesion type (Mew, 1987, Kaku and Kimura, 1978). Analysis of crosses between highly resistant and highly susceptible cultivars have identified at least 12 major genes which are effective in Japan or the Philippines or both countries (Ogawa et al., 1988). Differences between isolates in these countries has demonstrated the race-specific nature of the resistance factors, and the resistance of improved cultivars has been found to lose effect after several years.

Yamada (1984) defined the terms qualitative and quantitative resistance to Xco in rice. He termed the high level of race-specific resistance of IR28 to Japanese Xco races I and V qualitative resistance, as this resulted in two clearly distinguishable groups of plants in the F_2 generation when IR28 was crossed with the susceptible cultivar Toyonishiki. When Yamada (1986a) tested the same F_2 population with races II, III and IV, to which IR28 is only moderately resistant, he found a continuous, although positively skewed distribution. Yamada termed a resistance which results in a continuous distribution for lesion size in the F_2 generation quantitative resistance. He demonstrated high correlations between the lesion size of F_3 lines without major genes when these were inoculated with representative isolates of all five japanese races. Factors for quantitative resistance were therefore shown to be different from the qualitative major gene resistance to races I and V. Selection with one isolate was effective in increasing quantitative resistance to all five races (Yamada, 1986a). Yamada therefore felt the resistance to be race-nonspecific.

However, Yamada (1986b) also found a relationship between reactions

of the qualitative resistance of F_3 lines of the above-mentioned cross to I and V with the reaction of these same lines to race II-IV. Lines which were highly resistant to races I and V were also relatively more resistant to races II, III and IV. This indicated that the gene determining qualitative resistance, or a closely linked gene, was at least partly affecting the level of quantitative resistance to races II, III and IV.

Yamada (1984, 1986a) felt the quantitative resistance defined by him to be polygenic, but did not calculate the number of factors involved. Wasano and Dhanapala (1982) analyzed a cross between Nipponbare, which they described as having durable "field resistance" with a moderate reaction to all five japanese races of bacterial blight, and the more susceptible cultivar Norin 22. The resistance was shown to have a continuous distribution in the F_2 population. Selection using one isolate improved the level of resistance in F_5 and F_6 lines to isolates of all five Japanese races (Wasano and Imoto, 1987). However, Wasano and Dhanapala (1982) concluded that only one genetic factor was determining the resistance. This is surprising, considering the proven durability of the resistance in Nipponbare, which is reported to have been grown on a large scale for many years in Japan.

In the Philippines rice cultivars resistant to Xco have been grown on a large scale since 1968 (Mew, 1987). Isolates virulent to the Xa-4 allele for resistance present in these cultivars have been found and have increased in area and prevalence over the years (Vera Cruz and Mew, 1989). Levels of bacterial blight infection have been seen to increase in cultivars which were previously highly resistant (Mew, 1987).

Cultivars with moderate resistance to virulent isolates of Xco in the Philippines have also been found. Other cultivars which were previously highly resistant were found to be moderately resistant or moderately susceptible to virulent isolates. It was hoped that the factors determining the resistance of these cultivars may be more durable than the high levels of resistance of the Xa-4 gene.

The durability of a resistance can only be determined in retrospect. However, the number of effective genes determining the resistance has been associated with the durability of a resistance. It is therefore necessary to ascertain if the moderate resistance to be used is polygenic and if it can be accumulated.

A study was initiated to analyze the genetic background of moderate resistance, and to test the ability to select for improved levels of resistance. Four moderately resistant cultivars were identified and crossed with two susceptible cultivars and with each other, to determine

the genetic basis of the moderate resistance and the differences between the four cultivars for this resistance. Selection for longer and shorter lesion length was carried out in a number of cross populations. The relationship between the presence or absence of the Xa-4 allele for resistance and quantitative differences for resistance to a virulent isolate was tested as well.

MATERIALS AND METHODS

Production of F_1 seed

Seeds from single plants of four moderately resistant rice cultivars IR40, IR28, BR51-282-8 (BR51), and Cisadane and two susceptible cultivars TN1 and IR9101-46 (IR9101) were sown at two week intervals for 6-8 weeks, depending on the cultivar. Seedlings were transplanted to a lowland screenhouse bed (20x30 cm spacing) 14 days after sowing. Plants were transferred to plastic buckets in the greenhouse one day before expected anthesis. All flowers from three or four panicles per plant were vacuum emasculated, bagged and pollinated the following morning with the desired male parent. Seeds were harvested, dried and incubated at 50 °C for three nights to break dormancy. All F_1 seeds were soaked in 0.5g/l benzamidazol for two minutes and rinsed three times immediately before sowing.

F_1 generation

F_1 seeds were sown on March 2, 1987 and transplanted 18 days later to a screenhouse bed (20x20 cm spacing). Plants were arranged in a split-split plot design in three replicates with isolate in the main plot and the F_1 crosses plus the six parent lines in the subplot. The cross IR28 x IR40 was not included in the F_1 test due to insufficient F_1 seed. This cross was multiplied separately to obtain enough seed for the F_2 test.

Six plants per subsubplot were clip inoculated with $1x10^9$ cfu/ml suspensions of Xco race 2 isolates PX086 and IRN812 and race 6 isolate PX099. Plants were scored for lesion length 14 days after inoculation. All F_1 plants of each cross were examined daily during flowering for uniformity of appearance and flowering date. Plants which began flowering more than two days earlier or later than the majority were eliminated. Rice is a strict self-pollinator and the variation in flowering dates between crosses was felt to be sufficient to prevent any seed from setting

from the rare cross-pollination. F_2 seeds were harvested when ripe and bulked per cross.

F_2 generation

The F_2 trial was grown in a flooded field on the International Rice Research Institute (IRRI) farm in the period August - December 1987. To reduce the risk of loss through typhoons and virus diseases all crosses were seeded three times (three replications) on days 1, 8 and 28. Seeds were pregerminated on moist filter paper and sown in 33x25x10 cm plastic trays. Seedlings of each cross were transplanted 21 days later to randomized plots (20x20 cm spacing, single plant per hill). Each 3.8x4.2 m plot contained 260 F_2 plants of a single cross randomized with 52 plants of each parent. Two border rows of a resistant parent were planted at the edges and plots were separated by 1 m open space. Twelve small blocks of the six parent lines, 13 plants per line, were planted at regular intervals to assess field variability. Plants were sprayed regularly with cypermethrin (0.01% v/v) to protect against green leaf hopper, the vector of tungro virus. Ammonium sulphate was applied one week after transplanting and at maximum tillering.

When the first plants of a replication flowered, 79, 79 and 77 days after sowing, respectively for the three replications, all plants were clip inoculated using the clipper apparatus described by Kauffman et al. (1973). A $1x10^9$ cfu/ml suspension of race 6 isolate PX099 was used. Lesion lengths were measured on three upper leaves per plant of up to 200 F_2 plants per cross per replication, and the three values were averaged to determine a plant's reaction. Plants which were heavily stunted by tungro virus were not scored. Especially crosses of the second replication containing TN1 and Cisadane were affected by this disease.

Per cross seed of 40 F_2 plants was harvested. These were from the 10 most resistant and the 10 most susceptible plants and from 20 plants with lesion lengths at or close to the F_2 mode. Where necessary corrections were made for differences between replication means when the selections were made.

F_3 generation

The 15 crosses were randomly divided into three planting series of five crosses each. Seeds of each of 40 F_3 lines per cross were sown in 15 cm diameter plots filled with lowland soil. Seeds of parent cultivars

were sown in 33x25x10 cm plastic trays. The three series were sown on Feb. 12, 19 and 26, 1988 and transplanted 21 days after sowing to the same IRRI experimental farm field where the F_2 had been grown (20x20 cm spacing, single plants per hill).

Each cross was arranged in a replicated block design in a 19x2.6m plot divided into two blocks (replications), each block consisting of 40 F_3 lines plus three rows each of the two parent cultivars. Two border rows of a resistant parent cultivar were placed at each edge of the plots. Rows contained 14 hills; the first and last two hills were considered borders. Plants were sprayed weekly with cypermethrin for hopper control.

Plants were clip inoculated as described above 72, 72 and 76 days after sowing for the three series, respectively, using race 6 isolate PX099. Up to eight plants per F_3 line per replication were scored for lesion length 14 days after inoculation. Three lesions per plant were measured. Plants heavily infected by tungro virus were not scored but lightly infected plants were used.

Per cross five plants of each of the two most resistant (shortest lesions) and the two most susceptible (longest lesions) lines were individually harvested. The five most resistant and the five most susceptible plants from each cross were also harvested. Plants from four crosses could not be harvested due to tungro virus damage. Tests of the F_3 populations of three of these crosses were repeated in the screenhouse following the same procedure and layout described above. Lesion lengths measured in the screenhouse test were used for analyzing the response to selection in the F_2 generation, but not for harvesting of F_4 seed.

F_4 generation

The F_4 populations of the crosses were tested in an irrigated field in Masapang, Laguna, the Philippines to escape the infection by tungro virus endemic in the IRRI farm. The populations were divided into two planting groups, germinated on July 23 and 30, 1988. Seedlings were sown as described for the F_3 populations and transplanted 21 and 14 days after sowing, respectively, to the field to single plant hills. Plots were arranged as described for the F_3 generation with 30 F_4 lines plus three rows of each parent planted in two blocks (replications). Two border rows were included at each end of the plot. Each row contained 14 plants, of which the first and last two were considered borders.

Plants were clip inoculated as described for the F_2 plots using Xco race 2 isolate IRN812. The race 6 isolate used in the previous genera-

tions was not permitted for use outside the IRRI farm. Plants were inoculated 76 days after sowing and 8 plants per line per replication were scored for lesion length 12 days after inoculation.

Analysis

Broad-sense heritabilities (h^2) of the F_2 populations per cross per planting series were calculated using the formula (Mathers and Jinks, 1971):

$$h^2 = (varF_2 - varE)/varF_2 \qquad varE = (varP_1 + varP_2)/2$$

The response to selection (h^2 = response/selection) in the F_2 and F_3 generations was calculated as the ratio of the difference between lesion lengths of groups of progeny lines of susceptible (S) and resistant (R) plants, F_3(S-R), and the difference between the originally selected group of plants, F_2(S-R) (Wasano and Dhanapala, 1982). The response to selection in the F_3 was calculated separately for plants selected on the basis of line means and plants selected on the basis of individual plant reactions.

A scaling test to determine the influence of factors other than additivity and dominance was felt to be necessary. Since the F_1 was grown in a separate trial than the F_2, the F_1 population means were adjusted using the ratio of the midparent values of the F_2 and the F_1 generations, per cross, per F_2 planting series. This value, plus the associated adjusted variance, was used in the formula:

$$4F_2 - 2F_1 - P1 - P2/\sqrt{(16varF_2 + 4varF_1 + varP1 + varP2)}$$

A t-test for significant deviation of this value from zero was carried out. While the use of F_1 values from a different test carried out under different conditions means that conclusions must be viewed with great caution, it was felt that consistently large deviations from zero would be an indication that additive and dominance effects were not the only factors of importance in determining the level of resistance to Xco.

Test for residual effects of the Xa-4 allele

The F_2 seed of five crosses and the associated parent cultivars were sown as described for the F_2 generation and transplanted 21 days later to

lowland screenhouse beds. Crosses IR28 x TN1 and BR51 x TN1 were sown January 15, 1988; crosses IR28 x IR9101, BR51 x IR9101 and Cisadane x TN1 were sown June 1, 1988. Per cross plants were arranged in randomized rows with 24 F_2 rows and three rows each of the two parent cultivars. Each row contained 13 single plants hills of which the first and the last were considered border hills. Plants were sprayed as needed with cypermethrin and fertilized twice with ammonium sulphate.

At booting stage tillers of each hill were divided into two groups, to be inoculated with two isolates, using coloured plastic twine. Each hill was clip inoculated with both Xco isolate PX061, avirulent to the Xa-4 allele for resistance, and with PX099, virulent to this allele. Isolates were stored and inoculum was prepared as described in Chapter 9. Lesion length of three to five leaves per plant per isolate were scored 14 days after inoculation and averaged to determine the plant's reaction.

From each cross seed of 40 randomly chosen F_2 plants resistant to PX061 and 10 randomly chosen plants susceptible to this isolate was individually harvested. In some crosses extra F_2 plants which could not be clearly designated as resistant or susceptible to PX061 were also harvested. After sun drying and incubation for three nights at 50°C seeds of each F_3 line, plus the associated parent cultivars, were sown (June 1 and November 9, 1988, respectively for the two groups of crosses) in 15 cm diameter pots filled with lowland soil and transplanted 21 days later to screenhouse beds. Per cross three rows of each parent and two rows of each F_3 line were transplanted in a completely randomized plot design, with 13 plants per row. Lines where the resistance to PX061 was in doubt were planted in separate plots, with one row of 17 plants per line. Plants were maintained as described for the F_2 generation.

Tillers of all plants at booting stage were divided into two groups using coloured plastic twine. Three leaves per plant were clip-inoculated with each isolate, PX061 and PX099, and lesion lengths were scored 14 days after inoculation. Lines which were to be tested for resistance to PX061 were only inoculated with this isolate.

Analysis of residual effects

Only paired lesion length measurements were included in the calculations. When one inoculation did not succeed the value from the other inoculation was eliminated. Plants were divided into resistant and susceptible groups according to the F_2 population distributions with PX061. Mean lesion lengths of these groups, when inoculated with PX099, were

compared with a t-test for differences to determine the effect of the major gene on the reaction following inoculation with PX099. The correlations between the paired lesion length measurements for all F_2 plants were also calculated. The correlations between the paired lesion length measurements for only F_2 plants resistant to PX061 were calculated to determine the effect of quantitative factors on the expression of the resistance to PX061.

RESULTS

Results of the tests will first be discussed in relation to the individual generations, to give an indication of the efficiency of the selection carried out and of the mean reactions of the different groups of crosses. Results of individual crosses, over all four test generations, will be handled after this. Conclusions concerning the number and action of resistance factors will be made in relation to specific crosses and to groups of crosses with common parents.

Response to selection

F_1 generation

The F_1 was tested in the screenhouse with three isolates of Xco. PX0812 was more aggressive than PX099 and PX086 on TN1. The range (in cm) between the most resistant and the most susceptible parent cultivar was also largest with IRN812 and somewhat smaller with PX099 and PX086.

Mean lesion lengths for the F_1 populations were in general longer than the mid-parent values, indicating dominance for susceptibility. This was most pronounced with isolate PX099, where F_1 mean values often exceeded the lesion lengths of both parent cultivars (Table 10-1). Among the group of crosses between MR parents only the lesion length of the F_1 of the cross BR51 x IR40 fell within the range of the two parents with all three isolates, suggesting that the resistance factors in these two parents were either the same or inheriting in an additive way.

F_2 generation

All F_2 populations were tested with PX099 in an irrigated field in three separate planting series. Mean lesion lengths of the F_2 populations

Table 10-1: Mean lesion lengths (cm) of F_1 plants and parent cultivars ($\overline{P}$) clip inoculated with three isolates of *Xanthomonas campestris* pv. *oryzae* at maximum tillering stage.

Cross type	Parents	Isolate IRN812		PXO99		PXO86	
		F_1	$\overline{P}$	F_1	$\overline{P}$	F_1	$\overline{P}$
SxS[1]	TN1 x IR9101	34.6	37.1	26.1	24.8	24.6	24.7
MxS	BR51 x TN1	30.9	32.0	24.9*	20.0	22.8	22.1
	BR51 x IR9101	31.0	32.6	23.6*	18.5	21.7	20.0
	TN1 x IR28	33.4	30.0	27.8*	20.1	25.1	23.3
	IR28 x IR9101	32.4	30.5	25.4*	18.6	23.4	20.5
	IR40 x TN1	31.7	30.5	27.3*	22.3	21.0	21.4
	IR40 x IR9101	31.6	31.0	24.3	20.8	20.0	19.3
	Cisadane x TN1	34.6	31.8	28.2*	23.3	25.5	22.6
	Cisadane x IR9101	37.3	32.4	27.4*	21.8	23.2	20.5
MxM	BR51 x IR28	29.7*	25.4	20.6*	13.8	22.2	18.4
	Cisadane x IR28	31.4*	25.2	21.1*	17.1	23.6	18.9
	Cisadane x BR51	30.2*	27.3	21.4*	17.0	21.5	17.9
	BR51 x IR40	25.3	25.9	15.7	16.0	17.2	16.9
	IR40 x Cisadane	28.8	25.7	20.9	19.2	16.8	17.2
	mean	32.4	30.5	24.8	19.8	22.9	20.8
Parent cultivars	TN1	36.6 ± 1.05		26.3 ± 2.31		26.8 ± 2.19	
	IR9101	37.7 ± 2.78		23.3 ± 1.21		22.6 ± 0.37	
	BR51	27.5 ± 1.07		13.7 ± 0.41		17.4 ± 1.23	
	IR28	23.4 ± 0.60		14.0 ± 1.78		19.4 ± 1.14	
	IR40	24.4 ± 3.10		18.2 ± 0.31		16.1 ± 0.05	
	Cisadane	26.0 ± 1.70		20.2 ± 0.98		18.4 ± 2.31	

[1]) S = Susceptible cultivar; M = Moderately resistant cultivar.

*) F_1 mean differs significantly from the mean parent value (P = 0.05, t-test for differences). Values represent the mean of two replications.

for the three series were 10.0, 8.5 and 7.1 cm, respectively. Because of differences between series each test of 100-200 F_2 plants per cross was analyzed separately, using the values for parent plants located in the same F_2 plot.

F_2 population means for lesion lengths were significantly higher than the midparent values in about half the tests (Table 10-2). Especially the means of crosses between two moderately resistant parents remained significantly higher than the parent means, as was found in the F_1 test. Crosses between moderately resistant cultivars also showed significant positive skewness (Table 10-2). Considering the great range of lesion lengths within these populations and the relatively small number of highly susceptible individuals recovered, it appears likely that the dominance for susceptibility is incomplete. Homozygous individuals are probably more susceptible than heterozygous individuals. The long "tail" found in the crosses between moderately resistant cultivars, seen by their significant positive skewness, was felt to be a scale effect, as susceptible genotypes often show greater variation between individuals than less susceptible genotypes.

Significant deviations from the zero value for the adapted scaling test were found in several cases (Table 10-2). However, only in one population, Cisadane x IR28, was the deviation uniformly positive and significant in all three F_2 replications. Four other populations were found with uniformly positive or negative deviations of considerable size. Considering the large number of tests carried out, and the known scale effect of the highly susceptible individuals, it appears that there is little evidence that factors other than additivity and dominance were affecting resistance of the progeny to Xco.

Individual broad-sense heritabilities per replication varied from zero to 0.88, with mean heritabilities per cross varying from 0.20 to 0.69 (Table 10-3). Crosses between moderately resistant parents showed heritabilities as high as those between susceptible and moderately resistant parents. This is probably due to the larger variance of the susceptible parent cultivars. Because of this relationship between cultivar means and variances a square root transformation was felt necessary. The heritability of the square root transformed data varied between crosses, but the range was similar to that of the original data. Heritabilities varied from 0 - 0.90 per replication, with a mean heritability per cross from 0.23 - 0.71. Heritabilities of crosses between susceptible and moderately resistant cultivars were as high or higher than those between moderately resistant cultivars, with the exception of the cross IR28 x IR9101. The

Table10-2: Lesion lengths (cm) and variances of the means (var/N) of F_2 populations and parent cultivars, skewness of the F_2 distribution, differences between the F_2 population and the mid-parent values, and values for a scaling test of F_2 data, parent values and the adjusted F_1 means.

Cross type	Parents	Planting series	Parents P1 mean	P1 var/N ($x10^{-1}$)	P2 mean	P2 var/N ($x10^{-1}$)	F_2 mean	F_2 var/N ($x10^{-2}$)	skewness	F_2 - (P1+P2)/2	$4F_2$-$2F_1$-(P1+P2)
SxS	TN1 x IR9101	1	16.8	11.3	14.9	12.8	17.2	10.7	- 0.01	1.35	3.76
		2	12.1	7.4	12.4	3.2	11.6	14.6	- 1.59*	- 0.65	- 3.84*
		3	15.1	3.5	12.9	2.5	13.2	8.1	- 0.01	- 0.80	- 4.78*
SxMR	BR51 x TN1	1	5.1	1.2	16.8	13.4	12.0	8.6	- 0.22	1.05	- 1.06
		2	3.6	0.9	12.1	16.4	7.1	5.7	1.56*	- 0.75	- 6.59*
		3	3.3	1.3	15.1	3.5	8.0	7.4	0.79*	- 1.24*	- 9.47*
	BR51 x IR9101	1	5.1	1.7	9.4	12.6	11.3	9.0	0.15	4.05*	12.09*
		2	3.3	1.2	11.3	4.8	6.9	7.8	2.30*	- 0.40	- 4.95*
		3	3.1	0.8	13.7	3.4	8.3	6.9	0.99*	- 0.10	- 5.20*
	TN1 x IR28	1	17.0	13.5	2.9	1.5	11.6	22.4	- 0.07	1.65*	- 1.07
		2	14.9	9.4	3.4	1.1	10.8	20.3	0.32	1.65*	- 0.36
		3	13.6	1.6	3.8	5.2	8.2	8.7	0.46*	- 0.58	- 8.97*
	IR28 x IR9101	1	3.5	2.4	10.9	6.6	11.8	7.9	- 0.13	4.60*	13.11*
		2	2.7	0.8	18.3	3.0	11.8	8.5	- 0.32	1.30*	- 2.50
		3	3.4	0.9	10.5	2.7	7.4	4.8	0.09	0.45	- 3.39*
	IR40 x TN1	1	8.4	2.9	16.7	24.2	13.9	10.3	0.13	1.35	- 0.35
		2	5.5	2.1	14.5	14.2	11.7	23.1	0.12	1.70*	1.96
		3	3.9	0.9	13.9	2.7	8.4	3.3	0.63*	- 0.50	- 6.16*
	IR40 x IR9101	1	4.7	0.7	17.9	2.5	11.0	13.7	0.16	- 0.30	- 5.08*
		2	6.4	1.8	13.7	6.9	10.4	8.8	- 0.04	0.35	- 2.28
		3	3.6	1.2	13.6	2.8	8.3	8.5	0.09	- 0.30	- 4.13*

Table 10-2 (continued).

Cross type	Parents	Planting series	Parents P1 mean	P1 var/N ($x10^{-1}$)	P2 mean	P2 var/N ($x10^{-1}$)	F_2 mean	F_2 var/N ($x10^{-2}$)	skewness	F_2 - (P1+P2)/2	$4F_2-2F_1$ - (P1+P2)
	Cisadane	1	6.0	1.8	17.6	15.1	14.1	18.8	1.37*	2.32*	4.28
	x TN1	2	4.8	0.8	19.1	66.7	13.2	36.9	0.53*	1.25	- 0.09
		3	4.1	1.5	12.6	6.4	9.1	12.4	- 0.28	0.75	0.54
	Cisadane	1	5.0	2.3	17.6	4.5	12.6	12.4	0.26	1.30*	- 0.48
	x IR9101	2	5.9	1.1	14.8	2.3	11.9	9.0	0.25	1.55*	0.77
		3	5.3	1.5	13.6	1.9	10.6	4.4	0.24	1.15*	- 0.45
MRxMR	BR51 x IR28	1	3.3	0.5	2.3	0.5	4.9	3.3	1.70*	2.10*	5.57*
		2	3.4	1.1	2.3	0.9	5.5	7.5	1.38*	2.65*	7.86*
		3	3.8	1.4	3.6	1.8	4.4	2.8	1.51*	0.70*	- 0.96
	IR28 x IR40	1	6.1	2.1	6.7	1.8	7.9	4.1	0.45*	1.50*	-
		2	2.9	1.2	7.4	2.0	6.1	2.1	0.74*	0.95*	-
		3	2.5	0.7	4.0	0.8	3.6	5.6	0.91*	0.35	-
	Cisadane	1	3.8	1.5	2.6	0.9	7.0	5.7	0.20	3.80*	13.59*
	x IR28	2	5.2	1.1	2.3	0.3	5.3	5.9	0.82*	1.55*	4.37*
		3	3.4	1.5	2.4	0.4	4.1	2.3	0.95*	1.20*	3.36*
	Cisadane	1	5.1	1.8	3.0	0.3	4.8	4.8	4.85*	0.75*	0.95
	x BR51	2	5.8	2.2	3.8	1.3	4.4	2.2	0.69*	- 0.40	- 4.22*
		3	4.5	1.5	3.9	1.0	5.7	4.4	1.40*	1.50*	3.83*
	BR51 x IR40	1	3.6	1.0	5.3	1.3	4.6	2.8	1.48*	0.15	0.85
		2	3.3	0.4	5.1	9.7	4.2	1.3	1.17*	0.00	0.13
		3	2.2	0.4	3.3	3.3	3.0	0.7	1.47*	0.25	1.21*
	IR40	1	5.5	1.6	3.2	4.4	5.1	3.5	1.41*	0.75*	2.29*
	x Cisadane	2	7.7	2.4	6.9	3.5	7.1	7.6	0.29	- 0.20	- 1.88
		3	5.1	1.7	4.5	7.5	4.6	2.1	1.09*	- 0.20	- 1.74*

*) Value significantly different from zero (P=0.05, test for skewness or t-test).

cross between the two susceptible parents showed a low heritability (0.26), as was expected considering the greater variance of the parent cultivars (Table 10-3).

The range of plants selected for high and low resistance within each population was larger in crosses between moderately resistant and susceptible cultivars than in crosses between two moderately resistant cultivars (Table 10-4). A surprisingly large range of lesion lengths was found in the cross between the two susceptible cultivars. This indicates that some resistance factors are present in both parents. F_2 plants as susceptible as the most susceptible plants from the SxS cross were selected from four

Table 10-3: Broad sense heritability[1] (h^2) for resistance to lesion development following clip inoculation with *Xanthomonas campestris* pv. *oryzae* on F_2 populations of 15 crosses between six rice cultivars.

Cross type	Parents	h^2	
		A	B
SxS	TN1 x IR9101	0.22	0.26
SxMR	BR51 x TN1	0.20	0.40
	BR51 x IR9101	0.54	0.43
	TN1 x IR28	0.61	0.53
	IR28 x IR9101	0.47	0.23
	IR40 x TN1	0.43	0.50
	IR40 x IR9101	0.64	0.71
	Cisadane x TN1	0.54	0.61
	Cisadane x IR9101	0.69	0.42
MRxMR	BR51 x IR28	0.66	0.46
	IR28 x IR40	0.40	0.39
	Cisadane x IR28	0.66	0.56
	Cisadane x BR51	0.49	0.42
	BR51 x IR40	0.49	0.40
	IR40 x Cisadane	0.45	0.39

[1]) Broad sense heritability calculated according to $h^2 = (varF_2 - (varP1+varP2) / 2) / varF_2$
In column A the lesion length values were used; in column B the square root transformed lesion lengths were used.

Table 10-4: Mean lesion lengths (cm) of plants selected from the extremes of F_2 populations of fifteen crosses between six rice cultivars inoculated with *Xanthomonas campestris* pv. *oryzae* and the mean lesion lengths of F_3 lines descending from these plants.

F_2								
Cross type	Parents	F_2 plant selections					Parent means	
		R[1]	M	S	S-R	N[2]	P1	P2
SxS	TN1 x IR9101	8.3	13.9	20.5	12.2	356	14.7	13.4
SxMR	BR51 x TN1	3.7	8.4	17.3	13.6	425	4.1	13.5
	BR51 x IR9101	2.7	7.7	20.2	17.5	454	3.8	11.5
	TN1 x IR28	2.8	10.3	19.9	17.1	326	14.9	3.4
	IR28 x IR9101	2.8	10.6	17.6	14.8	414	3.2	16.2
	IR40 x TN1	5.4	11.4	17.7	12.3	255	5.9	17.0
	IR40 x IR9101	2.3	9.2	15.8	13.5	448	4.2	15.1
	Cisadane x TN1	5.0	11.0	20.6	15.6	260	5.0	16.4
	Cisadane x IR9101	3.4	11.3	20.9	17.5	497	5.4	15.3
MRxMR	BR51 x IR28	1.5	11.8	12.6	11.0	479	3.5	2.7
	IR28 x IR40	2.1	5.5	11.3	9.2	385	3.8	6.0
	Cisadane x IR28	1.9	3.8	11.0	9.1	408	4.1	2.4
	Cisadane x BR51	1.6	3.5	14.1	12.5	485	5.1	3.6
	BR51 x IR40	1.6	3.1	8.9	7.3	406	3.0	4.6
	IR40 x Cisadane	2.3	4.6	11.7	9.4	533	6.1	4.9

F_3								
Cross type	Parents	F_3 lines				Test	Parent means	
		R	M	S	S-R	series[4]	P1	P2
SxS	TN1 x IR9101	24.7[3]	28.1	28.1	4.8	4	19.1	18.3
SxMR	BR51 x TN1	10.5	11.8	13.9	3.4	2	4.7	19.4
	BR51 x IR9101	10.4	13.0	13.6	3.2	1	5.8	18.9
	TN1 x IR28	5.0	6.2	6.9	1.9	3	2.3	10.2
	IR28 x IR9101	10.2	12.5	12.8	2.6	2	6.3	17.4
	IR40 x TN1	12.0	13.2	13.5	1.5	1	9.2	17.4
	IR40 x IR9101	3.6	5.4	7.8	4.2	3	3.2	11.9
	Cisadane x TN1	10.4	13.7	16.3	5.9	1	9.5	17.2
	Cisadane x IR9101	15.6[3]	21.9	26.4	10.8	4	8.0	17.0
MRxMR	BR51 x IR28	2.4	3.2	5.0	2.6	3	2.5	2.3
	IR28 x IR40	6.2	7.1	9.2	3.0	1	5.1	6.5
	Cisadane x IR28	3.9	4.7	5.9	2.0	3	5.6	3.0
	Cisadane x BR51	4.4	4.5	5.3	0.9	3	4.9	3.1
	BR51 x IR40	5.8	6.1	7.0	1.2	1	4.9	6.4
	IR40 x Cisadane	11.1[3]	13.1	17.3	6.2	4	14.5	12.0

[1]) R=10 most resistant F_2 plants and F_3 lines descending from these plants, M = 20, plants with lesion lengths at or close to the population mode, S = 10 most susceptible F_2 plants and F_3 lines decending from these plants.

[2]) Total number of F_2 plants tested.

[3]) F_3 lines tested in the screenhouse.

[4]) Crosses of the same test series were simultaneously assessed.

other crosses with TN1 or IR9101 as a parent. Very susceptible individuals were not found in the F_2 populations of the MRxMR crosses, with the exception of the cross Cisadane x BR51. The smaller range of lesion lengths of these MRxMR crosses indicates some overlap of resistance factors between MR cultivars.

F_3 generation

The F_3 generation was grown in three planting series, with each population present in one of the three series only. Plants were heavily infected with tungro virus and in some cases fewer than eight healthy plants per F_3 line per replication remained for BB assessment. A second sowing of the three most heavily infected populations, TN1 x IR9101, Cisadane x IR9101 and IR40 x Cisadane, was assessed in the screenhouse. Correlations between field and screenhouse assessment of mean lesion lengths of the forty lines per cross were 0.72, 0.80 and 0.65, respectively. This indicates that results from the other, less heavily infected populations, were probably not too seriously affected by the virus.

Means of lines descending from selected F_2 plants with means at or near the F_2 population mode were in all cases between the means for the lines descending from the selected susceptible and resistant F_2 plants (Table 10-4). Mean within-line variances and ranges of these "mode" lines were equal to those of the lines selected from resistant and susceptible F_2 plants and usually greater than within-plot variances of the parent cultivars (Table 10-5). F_2 plants in the mode group were therefore not more heterozygous than plants chosen from the population extremes. This is clear evidence for the presence of several resistance factors in most crosses, rather than one (additive) factor.

The response to the selection carried out in the F_2 population was fairly low. Differences of 7.3 to 17.5 cm between selected F_2 plants were reduced to differences of 0.9-5.9 cm (Table 10-4). When expressed relative to the F_2 and F_3 plot means per population, these differences gave selection heritabilities between 0.07 and 0.56 (Table 10-6).

F_3 line means transgressing the most susceptible parent were found in the SxS cross and in all MRxMR crosses (Tables 10-7 and 10-8), but not in most SxMR crosses. Transgression for susceptibility strengthens the evidence that the dominance of susceptibility seen in the F_1 generation is incomplete. If the selected susceptibile plants were highly heterozygous then a greater loss of susceptibility is to be expected.

Line means transgressing the most resistant parent were found in

Table 10-5: Mean within-line variance and range (cm) of F_3 lines descending from selected resistant (R), mode (M) and susceptible (S) F_2 plants and of subplots of the parent cultivars ($\bar{P}$).

Parents	F_3 line variance				F_3 line range[1]			
	R	M	S	$\bar{P}$	R	M	S	$\bar{P}$
TN1 x IR9101	12.53[2]	12.74	10.18	10.62	10.9	11.3	10.2	10.5
BR51 x TN1	7.29	7.84	7.84	3.63	8.4	8.5	8.4	5.0
BR51 x IR9101	5.62	8.07	4.54	5.48	7.0	8.9	6.8	6.9
TN1 x IR28	2.25	6.76	5.76	2.93	4.7	7.7	7.4	5.0
IR28 x IR9101	7.84	9.61	6.25	7.25	8.2	9.3	7.5	8.5
IR40 x TN1	6.10	7.51	8.58	5.46	7.4	7.7	8.4	6.8
IR40 x IR9101	1.69	4.00	7.29	4.99	3.8	6.1	8.4	6.1
Cisadane x TN1	10.37	11.16	11.36	7.79	10.1	9.9	10.7	8.9
Cisadane x IR9101	16.00[2]	12.96	16.00	8.15	12.2	11.0	12.4	9.0
BR51 x IR28	0.53	0.94	3.35	0.36	2.3	3.0	5.7	1.8
IR28 x IR40	4.88	5.15	5.90	2.66	6.6	7.2	7.4	6.1
Cisadane x IR28	1.64	2.46	2.69	1.42	3.8	4.9	5.0	3.6
Cisadane x BR51	1.28	1.51	2.19	1.21	3.4	3.8	4.5	3.5
BR51 x IR40	4.00	4.41	5.29	2.79	6.0	6.2	7.0	5.1
IR40 x Cisadane	7.73[2]	7.29	12.32	7.12	8.5	8.4	11.2	8.3

[1]) Mean of the maximum - minimum lesion lengths of plants, determined for each F_3 line.

[2]) Populations tested in the screenhouse.

the SxS cross and in two SxMR crosses, but not in the tests of the MRxMR crosses, with the exception of the screenhouse test of IR40 x Cisadane. This one-sided transgression among the MRxMR crosses reflects the differences in difficulty distinguishing extremes in skewed populations. The extreme plants in the "tail" are easily identified while the extremes closer to the mode are almost indistinguishable from the remainder. This problem of distinguishing small differences between resistant types was more difficult in field tests than in the screenhouse, as field-grown plants are relatively more resistant than screenhouse-grown plants.

Table 10-6: Heritabilities (h^2) of resistance to *Xanthomonas campestris* pv. *oryzae* based on the ratio of the response to selection and the selection range. This is calculated as the ratio of the difference between F_3 lines descending from susceptible and resistant F_2 plants and the difference between the selected F_2 plants relative to population means, (F_3(S-R)/F_3mean)/(F_2(S-R)/F_2mean), and as the ratio of the difference between F_4 lines descending from selected F_3 plants and the difference between the selected F_3 plants, (F_4(S-R)/F_4mean)/(F_3(S-R)/F_3mean). Both line and plant selection was carried out in the F_3 generation.

Cross type	Parents	h^2		
		F_3/F_2	F_4/F_3 line	plant
SxS	TN1 x IR9101	0.19	0.34	0.27
SxMR	BR51 x TN1	0.18	0.40	0.22
	BR51 x IR9101	0.13	0.38	0.16
	TN1 x IR28	0.18	-	-
	IR28 x IR9101	0.15	0.64	0.32
	IR40 x TN1	0.11	0.79	0.26
	IR40 x IR9101	0.56	0.36	0.34
	Cisadane x TN1	0.33	0.64	0.47
	Cisadane x IR9101	0.29	-	-
MRxMR	BR51 x IR28	0.33	0.11	0.09
	IR28 x IR40	0.25	0.27	0.26
	Cisadane x IR28	0.25	0.33	0.26
	Cisadane x BR51	0.07	-	-
	BR51 x IR40	0.09	-	-
	IR40 x Cisadane	0.11	0.07	0.03

F_4 generation

The F_4 field was grown outside the IRRI farm to avoid infection with tungro virus. Because of this race 2 isolate IRN812 was used for assessment instead of race 6 isolate PX099. Isolate-specific interactions between PX099 and IRN812 for the selected lines cannot be completely excluded from explanations of results of the F_4 tests. Four crosses were not tested in the F_4 as heavy virus infection in the F_3 delayed or destroyed the harvests of these populations.

Table 10-7: Mean lesion lengths (cm) of selected resistent (R) and susceptible (S) F_3 plants based on line means and individual plant means, and lesion length of F_4 lines descending from selected F_3 plants.

F_3

Cross type	Parents	Line selection			Plant selection			F_3		
		R	S	S-R	R	S	S-R	mean	P1	P2
SxS	TN1 x IR9101	3.7	20.8	17.1	2.6	25.9	23.3	14.9	19.1	18.3
SxMR	BR51 x TN1	5.2	17.8	12.6	3.1	22.1	19.0	12.0	4.7	19.4
	BR51 x IR9101	6.4	18.1	11.7	4.8	22.8	18.0	12.5	5.8	18.9
	IR28 x IR9101	5.5	17.6	12.1	3.0	21.9	18.9	12.0	6.3	17.4
	IR40 x TN1	6.6	17.0	10.4	3.2	22.2	19.0	13.0	9.2	17.4
	IR40 x IR9101	1.4	12.0	10.6	6.4	16.7	10.3	5.8	3.2	11.9
	Cisadane x TN1	6.2	19.8	13.6	4.0	23.8	19.8	13.4	9.5	17.2
MRxMR	BR51 x IR28	2.0	10.6	8.6	1.0	12.2	11.2	3.5	2.5	2.3
	IR28 x IR40	3.3	17.1	13.8	1.7	20.2	18.5	7.4	5.1	6.5
	Cisadane x IR28	2.8	8.7	5.9	1.3	12.1	10.8	4.8	5.6	3.0
	BR51 x IR40	3.5	12.0	8.5	1.9	16.3	14.4	6.2	4.9	6.4

F_4

Cross type	Parents	Line selection			Plant selection			F_4		
		R	S	S-R	R	S	S-R	mean	P1	P2
SxS	TN1 x IR9101	12.1	18.3	6.2	13.2	19.9	6.7	16.0	17.4	15.7
SxMR	BR51 x TN1	10.1	15.8	5.7	9.1	13.9	4.8	13.5	10.3	18.2
	BR51 x IR9101	10.9	15.5	4.6	11.1	14.1	3.0	13.0	10.8	14.2
	IR28 x IR9101	7.7	14.7	7.0	8.2	13.8	5.6	11.2	6.1	16.2
	IR40 x TN1	8.9	16.5	7.6	8.7	14.1	5.4	12.0	10.0	16.0
	IR40 x IR9101	7.3	14.9	7.6	7.2	14.1	6.9	11.5	12.2	16.5
	Cisadane x TN1	7.7	15.5	7.8	7.7	16.0	8.3	12.0	9.6	16.6
MRxMR	BR51 x IR28	9.4	12.4	3.0	9.4	12.5	3.1	10.9	10.1	7.2
	IR28 x IR40	8.8	13.9	5.1	8.4	14.9	6.5	10.1	9.3	9.7
	Cisadane x IR28	7.2	10.8	3.6	6.9	12.1	5.2	8.8	10.9	6.7
	BR51 x IR40	11.9	13.0	1.1	11.5	12.4	9.0	12.2	11.7	12.7

The results of the line and plant selections for resistance and susceptibility carried out in the F_3 generation are shown in Table 10-7. Response to plant selection was lower than for line selection, despite the greater difference between selected groups of plants based on plant selection. The absolute range between lines descending from susceptible and resistant plants was larger for the SxMR crosses than for the MRxMR crosses. Selection heritabilities are shown in Table 10-6.

Transgression for susceptibility was found in the SxS cross, and a line was found with 7% longer lesions than the most extreme subplot of IR9101 (Table 10-8). Selection for resistance resulted in reduced lesion lengths in F_4 lines. The most resistant lines were recovered from the cross between Cisadane x IR28, where a line with lesions 30% shorter than those of the most resistant parent subplot was obtained, a reduction of 1.4 cm in absolute lesion length. The most resistant lines of this cross had lesions only 25% of those of the susceptible cultivar TN1 tested in this planting series. Significant increases in resistance were also found among lines selected from the crosses IR40 x TN1 and IR40 x IR9101.

Number of resistance factors

An estimate of the number of resistance factors and difference between cultivars for resistance factors, can be gained by studying the extremes found in a segregating population. Extremes more resistant and more susceptible than both parent cultivar extremes give evidence that the parents have different resistance factors. Transgression in segregating populations is determined by the observed extremes in the parent cultivars. Due to environmental variation and the relatively large experimental error, it was not possible to obtain precise estimates of the number of transgressing genotypes, or of the exact size of the difference between the parent cultivars and the extreme genotypes.

TN1 x IR9101-46 (SxS)

The F_1 and F_2 plants of this cross between the two susceptible cultivars were as susceptible as both parents (Tables 10-1 and 10-2). Some evidence of transgression for resistance and susceptibility was found in the three F_2 series (Table 10-8). In the screenhouse test of the F_3 generation of this cross three and five lines were found, respectively, for transgression for susceptibility and resistance. Plants selected from these transgressing lines resulted in six F_4 lines with increased suscep-

tibility beyond TN1 and 14 F_4 lines with increased resistance beyond IR9101. This is clear evidence that both cultivars contained factors for resistance, and that these factors are not the same.

Among the 14 F_4 lines showing transgression were three lines, originating from a single F_2 plant, with levels of resistance equal to that of the moderately resistant cultivars IR40 and Cisadane when tested with the same isolate, IRN812. The most resistant line had a mean lesion length of 8.3 cm (Table 10-8). While the possibility cannot be altogether excluded that this F_2 plant originated from outcrossing in the F_1 or seed mixture, another F_2 plant produced F_4 progeny lines with mean lesion lengths only 1-2 cm longer.

If each cultivar contained a single resistance factor their combination would then result in a decrease in lesion length of about 45%, or some 7.5 cm, in relation to the mid-parent value, to produce such a line. Considering the large number of F_4 lines recovered with lesion lengths between the parent values and the most resistant lines it seems likely that at least one of the cultivars had two or more small resistance factors, and that the recombination of these factors can lead to varying levels of resistance.

Six F_4 lines originating from six different F_2 plants had lesions longer than TN1, the more susceptible of the two susceptible parents (Table 10-8). The most susceptible of these had a lesion length of 21.1 cm, almost 2 cm longer than the most susceptible subplot of TN1. These lines are probably without resistance factors.

BR51-282-8 x TN1 and BR51-282-8 x IR9101-46

BR51 was crossed with the two susceptible cultivars TN1 and IR9101. The F_1, when tested with PX099, was nearly as susceptible as the susceptible parent, indicating recessive inheritance of resistance factors (Table 10-1). When the F_1 was tested with PX086 and IRN812 the mean was intermediate between the two parents. However, BR51 itself appears more resistant with PX099 than with PX086 and IRN812, and it is possible that the recessive factor is not effective against PX086 and IRN812.

The F_2, tested with PX099, showed some transgression beyond the susceptible parent in all three series for both crosses. Transgression beyond the resistant parent is very difficult to show clearly, considering the highly resistant reaction of BR51 with PX099. However, when selected susceptible plants from the F_2 generation were grown in the F_3, only two lines were found with means clearly more extreme than the parent culti-

Table10-8: Number of resistant (R) and susceptible (S) F_2 plants and F_3 and F_4 lines with lesion lengths transgressing the parent extremes and the mean lesion length (cm) of the most extreme F_2 plant, F_3 and F_4 lines, and the most extreme plants or subplots of the parent cultivars, of 15 crosses between six rice cultivars.

F_2		Number of transgressing plants[1]		Lesion length of extremes among			
				F_2 plants[1]		Parent plants[1,2]	
Cross type	Parents	R	S	R	S	R	S
SxS	TN1 x IR9101	4.3	2.0	4.8	22.2	6.3	21.8
SxMR	BR51 x TN1	1.3	3.0	1.6	20.7	1.6	17.4
	BR51 x IR9101	0.3	7.7	2.1	23.1	1.7	16.5
	TN1 x IR28	0	1.3	2.1	23.7	1.4	21.1
	IR28 x IR9101	1.0	1.3	1.9	18.8	1.6	18.4
	IR40 x TN1	0	2.3	3.8	20.1	3.5	19.3
	IR40 x IR9101	5.3	0.7	1.3	19.7	2.7	20.1
	Cisadane x TN1	0.3	4.0	3.2	21.8	2.8	20.7
	Cisadane x IR9101	1.0	5.0	2.5	23.4	2.7	20.4
MRxMR	BR51 x IR28	0.3	34.0	1.0	15.6	1.0	6.5
	IR28 x IR40	2.3	12.0	1.3	12.9	2.0	9.4
	Cisadane x IR28	0	25.0	1.6	12.7	1.1	8.4
	Cisadane x BR51	5.0	11.0	1.3	13.5	1.9	9.1
	BR51-282-8 x IR40	0.7	7.7	1.3	11.3	1.4	7.3
	IR40 x Cisadane	1.0	9.0	1.7	13.2	2.0	9.7

F_3		Number of transgressing F_3 lines		Lesion length of extremes among			
				F_3 lines		Parent subplots	
Cross type	Parents	R	S	R	S	R	S
SxS	TN1 x IR9101	5[3]	3	13.1	38.4	22.5	32.5
SxMR	BR51 x TN1	1	0	3.8	17.9	4.2	22.7
	BR51 x IR9101	0	1	7.1	17.3	4.2	16.9
	TN1 x IR28	0	0	3.1	8.8	1.2	16.8
	IR28 x IR9101	1	0	5.1	15.6	5.2	19.1
	IR40 x TN1	0	0	6.8	17.6	6.8	19.1
	IR40 x IR9101	5	0	1.7	10.7	2.1	15.3
	Cisadane x TN1	1	0	7.6	18.5	8.3	21.0
	Cisadane x IR9101	0[3]	0	6.0	18.1	10.2	31.0

Table 10-8 (continued)

F_3 Cross type	Parents	Number of transgressing F_3 lines R	S	Lesion length of extremes among F_3 lines R	S	Parent subplots R	S
MRxMR	BR51 x IR28	0	22	1.8	11.4	1.7	2.7
	IR28 x IR40	0	5	4.1	19.6	4.1	8.7
	Cisadane x IR28	0	3	2.7	10.0	2.3	6.6
	Cisadane x BR51	0	10	2.7	6.2	2.4	5.2
	BR51 x IR40	0	2	4.3	11.9	4.0	8.2
	IR40 x Cisadane	6[3]	9	6.9	19.6	11.0	16.9

F_4 Cross type	Parents	Number of transgressing F_4 lines R	S	Lesion length of extremes among F_4 lines R	S	Parent subplots R	S
SxS	TN1 x IR9101	14	6	8.3	21.1	14.6	19.8
SxMR	BR51 x TN1	1	0	8.8	17.7	8.9	18.6
	BR51 x IR9101	0	7	9.4	16.9	6.1	15.2
	IR28 x IR9101	0	0	6.1	16.5	4.6	16.8
	IR40 x TN1	6	0	5.4	17.1	8.8	17.2
	IR40 x IR9101	13	0	5.1	17.1	9.2	18.2
	Cisadane x TN1	5	2	5.9	19.7	7.9	19.3
MRxMR	BR51 x IR28	0	3	6.8	16.5	6.3	13.4
	IR28 x IR40	3	9	5.6	20.1	6.3	11.9
	Cisadane x IR28	2	3	3.2	15.7	4.6	12.6
	BR51 x IR40	5	1	8.2	15.3	9.8	14.7

[1]) Mean of three series.

[2]) Maximum and minimum lesion lengths for both parent cultivars.

[3]) F_3 assessment carried out in the screenhouse.

vars. F_4 lines from the most resistant F_3 plants showed no transgression. Seven F_4 lines were found which were more susceptible than TN1, but the most susceptible of these, with a mean lesion length of 16.9 cm, is still not as susceptible as those lines found in the cross between TN1 and IR9101.

The transgression above the susceptible parent in the F_2 would indicate some differences between BR51 and the two susceptible cultivars, but the experimental error in the F_2 is fairly large. These plants produced a few F_4 lines which were more susceptible than IR9101 but the most resistant F_4 line was not more resistant than BR51 itself. While this one-sided transgression is difficult to explain, BR51 seems to have multiple resistance factors. One or more of these factors is different from that in IR9101, but perhaps the same as the factor in TN1.

TN1 x IR28 and IR28 x IR9101-46

The F_1 means of the cross TN1 x IR28 resembled those of the susceptible parent with all three test isolates (Table 10-1). The F_1 of IR28 x IR9101 gave similar results with PX086 and PX099 but the mean was midway between the two parents when tested with IRN812. The resistance factors of IR28 are therefore largely recessive.

No evidence of transgression was found in the F_2 of both crosses (Table 10-8). F_3 lines from extreme F_2 plants did not show transgression, although the parent types were recovered. The F_4 of IR28 x TN1 was not tested; the F_4 of IR28 x IR9101 did not contain any transgressing lines, although many lines similar to both parents were recovered.

From these results it appears that IR28 contains both the resistance factors in TN1 and IR9101, but may contain more resistance factors.

IR40 x TN1 and IR40 x IR9101-46

The F_1 of IR40 x TN1 resembled the mid-parent value with all three test isolates (Table 10-1); the F_1 of IR40 x IR9101 also resembled the midparent values when tested with PX086 and IRN812 but was more susceptible when tested with PX099. The F_2 of both crosses showed no positive transgression, but slight evidence of negative transgression was found in the cross with IR9101 (Table 10-8). The resistant F_2 plants resulted in five F_3 lines more resistant than IR40. Selection in the F_3 resulted in clear negative transgression in both the cross with TN1 and with IR9101, although more lines were found in the cross with IR9101 than with TN1.

The one-sided transgression of these crosses is difficult to explain but may be due to recessive resistance factor(s), making selection of homozygous susceptible plants in the F_2 more difficult. The large negative transgression shows that IR40 contains other resistance factors than those in TN1 and IR9101. Addition of the factors of TN1 and IR9101 to those of IR40 resulted in an increase in resistance of 40-55% (3-4 cm shorter lesions). The relatively large number of transgressants would indicate that IR40 does not have too many factors, possibly only one or two, but that each has a large effect.

Cisadane x TN1 and Cisadane x IR9101-46

The very susceptible F_1 means with all three isolates in both crosses indicates that Cisadane contains other resistance factors than both susceptible parents, and that these factors are recessive (Table 10-1).

A few highly susceptible F_2 plants were found in the crosses with TN1 and with IR9101 (Table 10-8). Plants of both populations were heavily infected with tungro virus. This may have affected the ability to identify extreme individuals. With one exception, no transgressants were found in the F_3, even in the second test in the screenhouse. However, many highly resistant lines were found in the F_4 of Cisadane x TN1; the most resistant line was 25% more resistant than the most resistant subplot of Cisadane. Two highly susceptible lines were also found. The F_4 of Cisadane x IR9101 could not be tested due to tungro damage in the F_3.

The F_1 and F_4 evidence indicate that Cisadane has different resistance factor(s) than TN1. The F_1 and F_2 evidence, although this latter must be viewed with caution due to the large experimental error, also indicates that the resistance factor(s) in Cisadane are not the same as in IR9101. Cisadane itself is a fairly variable cultivar, sometimes appearing highly resistant and sometimes only moderately susceptible, although the seed used was descended from a single plant. This could explain the difficulty in identifying extreme types in the F_2 and F_3 generations.

BR51-282-8 x IR28

The F_1 mean exceeded the parental values, but was somewhat lower than TN1 when tested with all three isolates, indicating some overlap of resistance factors in both parent cultivars. The F_2 showed large transgression for susceptibility, but no F_2 plants were found to be as susceptible as TN1

(Table 10-8). This pattern was repeated in the F_3 and F_4 generations, where many lines were found more susceptible than the more susceptible of the two parent cultivars. The most susceptible line was 23% longer than the most susceptible parent subplot. No lines were found clearly more resistant than the more resistant parent. The increase in susceptibility indicates that the two cultivars differ for one or more resistance factors but have at least one factor in common.

IR28 x IR40

The F_1 of this cross could not be tested due to a shortage of F_1 seed. The F_2 showed considerable transgression above the more susceptible parent, but no plants were as susceptible as TN1 (Table 10-8). Some evidence for negative transgression was also seen in the F_2. The F_3 and F_4 showed transgression in both directions. F_3 and F_4 lines were found at least as susceptible as TN1. The very wide segregation in the F_4 suggests that these two cultivars have completely different resistance factors and that each probably has more than one resistance factor.

Cisadane x IR28

The F_1 of this cross was considerably more susceptible than both parents with PX086, resembled the more resistant IR28 with IRN812 and resembled the more susceptible cultivar Cisadane with PX099 (Table 10-1). Dominance or recessiveness of the factor(s) therefore seems isolate-dependent.

The F_2 showed considerable transgression for susceptibility, but no plants were found as susceptible as TN1 (Table 10-8). Three F_3 lines were more susceptible than Cisadane, but the most susceptible F_3 line had a mean lesion length of only 10.0 cm, which is still considerably more resistant than TN1. The most susceptible F_4 line had a mean lesion length of 15.7, 19% more susceptible than Cisadane. Two F_4 lines more resistant than IR28 were also found; the most resistant had a mean lesion length 70% of the most resistant subplot of IR28.

The considerable gain in resistance shows that these two cultivars have one or more resistance factors different. However, the failure to recover lines as or more susceptible than the susceptible cultivar TN1 and IR9101 shows that they also have one or more resistance factors in common.

Cisadane x BR51-282-8

The F_1 plants were more susceptible than both parents, but less susceptible than TN1 when tested with all three isolates, indicating that the two cultivars have some resistance factors in common (Table 10-1). The F_2 had considerable positive transgression but no highly susceptible plants were recovered (Table 10-8). F_3 lines selected from these F_2 plants were more susceptible than the parents, but still moderately resistant; the mean lesion length of the most susceptible F_3 line was only 6.2 cm. No F_3 lines were found clearly more resistant than BR51. The F_4 could not be tested due to the heavy tungro infection in the F_3 generation in the field.

The narrow range of F_3 lines indicates that the resistance factors in these cultivars are largely the same. However, the F_1 clearly showed some differences. Both cultivars therefore have more than one resistance factor.

BR51-282-8 x IR40

The F_1 of this cross was similar to both parents and to the midparent value when tested with all three isolates (Table 10-1). The F_2 showed some transgression for susceptibility, but no highly susceptible plants were recovered (Table 10-8). Two F_3 lines showed positive transgression, but the more susceptible of these lines was still considerably more resistant than TN1. Lesions of F_4 lines reached a maximum of only 5% longer than the most susceptible subplot of the two parents. The lesions of the most resistant F_4 line was 16% shorter than those of the most resistant subplot of the parents.

These two cultivars therefore appear to have largely the same resistance factors, but each has at least one factor different.

IR40 x Cisadane

The F_1 was similar to both parents and to the midparent values, indicating that the cultivars have the same resistance factors, or that the factors are additive (Table 10-1). The F_2 showed some transgression for susceptibility, but no plants as susceptible as TN1 were recovered among the 406 F_2 plants tested. Selected F_2 plants yielded nine F_3 lines with mean lesion lengths longer than the most susceptible parent subplot and six lines with lesions shorter than the most resistant parent subplot.

The F_4 could not be tested due to the heavy tungro infection in the F_3 generation in the field.

Considering the modest positive transgression in the F_2 and F_3 and the considerable transgression for resistance in the F_3, it seems that these cultivars have at least one resistance factor in common, but also differ for at least one factor.

Residual effects of Xa-4

The F_2 populations of five crosses were found to segregate for resistance when inoculated with race 1 isolate PX061. Lesion lengths of F_2 plants inoculated with this isolate varied from shorter than 1 cm to longer than 30 cm (Table 10-9). All populations showed a bimodal distribution for lesion length following inoculation with PX061, and a division of the plants into resistant and susceptible was made. Mean lesion lengths of F_2 plants classified as resistant to PX061, based on the F_2 distribution, were longer than the lesion length of the resistant parent cultivar. This reflects the incompletely dominant character of the Xa-4 allele for resistance.

Distribution of four of the five crosses fit a 3:1 ratio for resistant:susceptible reactions based on this division (Table 10-9). The F_2 population of Cisadane x TN1 did not fit a 3:1 ratio and the distribution of the F_2, while bimodal, less clearly allowed division of the F_2 plants into resistant and susceptible groups. A number of F_3 lines from this population were tested to ascertain the presence or absence of the Xa-4 allele for resistance in the F_2 plants in question.

When inoculated with the race 6 isolate PX099 mean lesion lengths of plants with the Xa-4 allele for resistance were found to be significantly shorter than lesions of plants without Xa-4. The difference in lesion lengths between the two groups of plants ranged from about 3 cm to greater than 7 cm. This indicates that the Xa-4 allele for resistance to isolate PX061 of Xco, or a closely linked gene, affects resistance to race 6 isolate PX099.

Correlations between the paired lesion length measurements ranged from 0.38** for Cisadane x TN1 to 0.66** for BR51 x TN1. While these correlations are significant it is clear that less than half of the variance in lesion length following inoculation with PX099 can be explained by the reaction of the plant to inoculation with PX061. The maximum lesion length, following inoculation with PX099, was about equal for both groups of plants with and without the Xa-4 allele. However, plants with

Table 10-9: Lesion length (cm) of F_2 plants and parent cultivars of five cross populations clip inoculated with two isolates of *Xanthomonas campestris* pv. *oryzae*.

TN1 x IR28

Cultivar/F_2 group	Isolate PXO61	PXO99	N
IR28 (Xa-4)	1.6	11.0	20
TN1 (xa-4)	9.7	20.3	21
F_2 total	4.7	17.8	146
R (< 5.5 cm)[1]	2.2	16.0	109
S (> 5.5 cm)	11.9	23.1	37

P χ^2 3:1 for resistance to PXO61: 0.1<P<0.25

P (S-R = 0, for resistance to PXO99): P<0.001

BR51 x IR9101

Cultivar/F_2 group	Isolate PXO61	PXO99	N
BR51 (Xa-4)	2.6	12.0	33
IR9101 (xa-4)	27.7	33.5	31
F_2 total	9.7	23.2	362
R (< 15 cm)	5.1	21.0	274
S (> 15cm)	24.0	30.1	88

P χ^2 3:1 for resistance to PXO61: 0.75<P<0.9

P (S-R=0, for resistance to PXO99): P<0.001

BR51 x TN1

Cultivar/F_2 group	Isolate PXO61	PXO99	N
BR51 (Xa-4)	0.7	6.5	14
TN1 (xa-4)	15.0	25.3	14
F_2 total	4.7	16.6	123
R (< 6 cm)	2.3	14.8	91
S (> 6 cm)	11.7	21.7	32

P χ^2 3:1 for resistance to PXO61: 0.75<P<0.9

P (S-R=0, for resistance to PXO99): P<0.001

Cisadane x TN1

Cultivar/F_2 group	Isolate PXO61	PXO99	N
Cisadane (Xa-4)	3.3	17.2	58
TN1 (xa-4)	27.2	32.7	10
F_2 total	14.6	30.7	236
R (< 18 cm)	8.3	29.6	159
S (> 18 cm)	27.6	32.9	77

P χ^2 3:1 for resistance to PXO61: P<0.01

P (S-R=0, for resistance to PXO99): P=0.0013

IR28 x IR9101

Cultivar/F_2 group	Isolate PXO61	PXO99	N
IR28 (Xa-4)	3.4	15.6	32
IR9101 (xa-4)	23.7	25.7	26
F_2 total	11.5	25.0	363
R (< 18.5 cm)	6.3	23.6	275
S (> 18.5 cm)	27.9	29.5	88

P χ^2 3:1 for resistance to PXO61: P=0.75

P (S-R=0, for resistance to PXO99): P<0.001

[1]) R = plants resistant to PXO61, S = plants susceptible to PXO61.

the Xa-4 allele for resistance often were moderately resistant to PX099 while plants without this allele were usually susceptible.

Within the group of F_2 plants resistant to PX061 significant correlations were found for the lesion lengths when inoculated with both PX061 and PX099. Correlations ranged from 0.35** to 0.57**. This indicates that the factors affecting moderate resistance to PX099 have some influence on the lesion length of a resistant reaction, although this influence is not very large.

The F_3 lines of three crosses were tested to further investigate the influence of Xa-4 on resistance to PX099. Lines could clearly be differentiated into resistant, segregating, or susceptible when inoculated with PX061. Mean lesion length of lines segregating for resistance was between the lesion lengths of the uniformly resistant and uniformly susceptible lines (Table 10-10). The mean variance of lines segregating for resistance to PX061 was much higher than for lines with a uniform reaction to this isolate. When inoculated with PX099 lines uniformly resistant to PX061 had significantly shorter lesions (P=0.01) than segregating lines and lines uniformly susceptible to PX061. Segregating lines had significantly shorter lesions (P=0.01) than lines uniformly susceptible to PX061. This reconfirmed the effect of the Xa-4 allele, or a closely linked gene, on the level of resistance to PX099.

Based on the F_3 line reactions the parent F_2 plants could be designated as homozygous dominant, heterozygous, or homozygous recessive for the Xa-4 gene for resistance. The lesion lengths of the homozygous dominant plants were not significantly different from the heterozygous plants.

DISCUSSION

Selection for resistance to Xanthomonas campestris pv. *oryzae*

Selection was in most cases successful in producing lines with lesion lengths longer or shorter than the parent cultivars. Highly resistant lines were not found, but lines significantly more resistant than the parents were recovered, especially from crosses between moderately resistant cultivars. Lines more susceptible than the most susceptible cultivars were also recovered; these are probably without resistance factors.

Broad-sense heritability of resistance factors in the F_2 ranged from 0.2-0.7, and slightly higher when the lesion lengths were transformed to the square root values. These values are similar to those found by Wasano

Table 10-10: Mean lesion lengths (cm) and variances of F_3 lines, parent cultivars and original selected F_2 plants of three crosses between four rice cultivars following clip inoculation with two isolates of *Xanthomonas campestris* pv. *oryzae*.

TN1 x IR28

F_3 line reaction to PXO61	N	PXO61 mean	PXO61 var	PXO99 mean	PXO99 var	Selected F_2 plants mean	Selected F_2 plants var
Resistant	16	2.7	2.00	16.3	32.33	15.6	22.09
Susceptible	7	23.3	18.45	29.3	17.78	21.8	15.98
Segregating	27	9.6	71.75	22.3	52.15	16.9	33.64
IR28 (Xa-4)		2.0	0.30	11.4	16.50		
TN1 (xa-4)		20.2	-	34.4	-		

BR51 x TN1

F_3 line reaction to PXO61	N	PXO61 mean	PXO61 var	PXO99 mean	PXO99 var	Selected F_2 plants mean	Selected F_2 plants var
Resistant	14	2.9	1.74	17.0	19.90	17.2	17.38
Susceptible	10	21.0	16.83	28.1	17.00	24.2	9.25
Segregating	26	7.1	43.75	21.3	35.50	14.7	20.47
BR51 (Xa-4)		1.9	0.40	11.1	9.31		
TN1 (xa-4)		26.8	10.20	30.7	18.40		

Cisadane x TN1

F_3 line reaction to PXO61	N	PXO61 mean	PXO61 var	PXO99 mean	PXO99 var	Selected F_2 plants mean	Selected F_2 plants var
Resistant	11	1.8	0.53	22.2	16.60	23.8	19.47
Susceptible	7	25.9	8.97	29.6	11.92	34.1	25.03
Segregating	15	9.4	93.05	26.5	20.99	25.2	51.40
Cisadane (Xa-4)		1.2	0.90	14.2	5.85		
TN1 (xa-4)		24.7	12.44	29.9	0.61		

and Dhanapala (1982) but much lower than those found by Yamada (1984, 1986a). Both Wasano and Dhanapala (1982) and Yamada (1984, 1986a, 1986b) used the 0-7 scale of Ezuka and Horino (1974b) for assessment (see Chapter 2, this thesis). This is not a linear scale and makes greater distinctions between highly resistant and highly susceptible reactions than between moderate reactions. This has the result of transforming the data in a roughly arc-sin fashion. Dispite this, highly resistant and highly susceptible reactions still have lower variances than moderate reactions with the scale used. Wasano and Dhanapala (1982) studied a cross between cultivars with scores between 4 and 5, and did not further transform the data. Yamada (1984) studied a cross between a cultivar with a score of less than 2 and a cultivar with a score of 7 and used a square root transformation. The much lower variances for the parent cultivars recorded by Yamada resulted in the higher broad-sense heritability values.

The results of the line selection in the F_3 was better than the results in the F_2. Values for the heritability of resistance to Xco based on the two-way selection in the F_2 were slightly lower than those of seven crosses studied by Wasano and Dhanapala (1982). Heritabilities based on plant selections in the F_3 were lower than those based on line selections. Selection is therefore most effective in later generations, when the plants are more homozygous.

The extra effort involved in carrying out line selection can be an objection. However, line selection can be combined with selection for other purposes. While this may not result in the greatest increase in resistance, it should improve moderate resistance significantly. A sign that improvement can be expected would be that the F_1 is less resistant then the parent cultivars. Wasano and Dhanapala (1982) also recommended selection in the F_4 or later generations.

Number of resistance factors

TN1 and IR9101 were found to each have at least one small resistance factor, but possibly more than one. IR28 is suspected to contain both these factors but IR40 and Cisadane probably do not. BR51 may have the resistance factor of TN1 only.

BR51 and Cisadane seem to have resistance factors in common with IR28. This implies that IR28 has at least three factors. BR51 and Cisadane have at least one factor in common, but also differ for resistance factors, so each cultivar must have at least two resistance factors. IR40 had factors common with BR51 and Cisadane, so IR40 must also have at least

two factors for resistance. This would imply that at least five, but probably more resistance factors are present in the six cultivars studied. All MR cultivars seem to have two or more factors while the two susceptible cultivars TN1 and IR9101 each have at least one resistance factor.

Resistance factors of relatively small individual effects are therefore commonly found in rice cultivars. While there appears to be a considerable overlap in resistance factors among the moderately resistant cultivars tested, selection in crosses between two rice cultivars will almost always result in some increase in resistance. Higher levels of quantitative resistance seem to imply that three or more resistance factors are present.

Wasano and Dhanapala (1982) calculated that only one factor determined the quantitative resistance of the cultivar Nipponbare. The susceptible cultivar used in the test, Norin 22, was in fact itself not very susceptible, having a score of only 4.7 on the scale of 0-7. Nipponbare had a score of 4.0. Significant transgression for both resistance and susceptibility appears to have been found in the F_2 and F_3 generations (Wasano and Dhanapala, 1982), which would imply that the cultivars contained different resistance factors. Wasano and Dhanapala calculated that the two cultivars differed for only one factor. However, the procedure used by Wasano and Dhanapala to calculate gene number is based on the assumption that all factors come from one parent only. It is therefore doubtful whether his conclusion as to the number of resistance factors is correct.

Effect of factors for resistance

Resistance factors appeared to be incompletely recessive, based on the susceptibility of the F_1 and some F_2 populations, relative to the mid-parent values. Wasano and Dhanapala (1982) also reported that quantitative resistance factors were recessive.

No clear indication was found that factors other than additivity and dominance also influenced measured lesion lengths in the tested crosses, although the experiment was not designed to test this rigorously, and conclusions are tentative. Yamada (1984) reported evidence for epistasis or interaction for environmental factors in two crosses between IR28 and susceptible cultivars, since the square-root transformation of his data did not completely eliminate the significance of the scaling test results. As previously mentioned, the assessment scale used by Yamada changes the distribution of the population because the class sizes are not equal.

This may have affected his results.

The effect of each resistance factor is difficult to estimate and it is not expected that the effect of a single gene will be the same in all genetic backgrounds, or that all genes for quantitative resistance will have equal effects. However, some estimates can be made.

If the assumption is made that TN1 and IR9101 each had one gene for resistance, then the lesions of the most susceptible lines, presumably without a resistance gene, were only 2-3 cm longer than TN1, indicating that each factor reduced lesion length by 3 cm. The addition of the resistance factor from TN1 or IR9101 to those of the MR cultivars also decreased lesion length by about 3 cm in the most resistant F_4 lines.

When the effect of a factor is 3 cm, crosses between MR cultivars with at least partly differing resistance factors should have resulted in F_4 lines with lesions 3 cm shorter or longer than the respective parent cultivars. The cross between IR28 and Cisadane produced F_4 lines with lesions 2 cm shorter than the more resistant parent and lesions 2.5 cm longer than the more susceptible parent. Several crosses showed a slightly different pattern. For example, the cross between IR28 and IR40, which probably do not have common resistance factors, produced resistant F_4 lines with lesions at least 3 cm shorter than IR28, but the most susceptible lines had lesions 8 cm longer than IR40. Transgression for susceptibility in the cross between BR51 and IR28 produced lines with lesions 4 cm longer than BR51 but no lines were recovered which were more resistant than IR28. BR51 and Cisadane, when crossed, did not show transgression for resistance, and transgression for susceptibility was only about 1 cm.

There is therefore some evidence that the effect of a loss or gain of a gene for quantitative resistance differed with the level of resistance, i.e. with the number of remaining factors. Loss of a resistance gene in a susceptible cultivar, which has only one factor, resulted in about 3 cm longer lesions. The addition of an extra factor to an already moderately resistant cultivar resulted in lesions 0-3 cm shorter than the moderately resistant parent. Loss of a gene for resistance in moderately resistant cultivars, which have two or three factors, seemed to result in greater increases in lesion length, up to 8 cm.

Residual effect of Xa-4

A residual effect of the Xa-4 allele or a closely linked resistance factor, was shown to affect the resistance of F_2 plants and F_3 lines to race 6 isolate PX099. Lesion lengths of plants with one or more Xa-4

allele appeared to be 4-7 cm shorter than those of plants without an Xa-4 allele. Reactions of plants with the Xa-4 allele ranged from moderately resistant to susceptible while plants without the Xa-4 allele (xa4xa4) were moderately to highly susceptible. The broad range of lesion lengths in both groups indicates that other factors besides the Xa-4 gene were important in determining the reaction of the plant to inoculation with PX099. The correlation between reactions of plants with an Xa-4 allele for both isolates shows that these other factors can also affect the expression of the resistance to PX061 to a minor degree.

Ogawa et al. (1988) found evidence for a residual effect of the Xa-4 allele, or a closely linked gene, on Philippine race 2 isolate PX086 and race 3 isolate PX079 during development of near-isogenic lines. Near-isogenic lines were 3-4 cm shorter than the recurrent parent in two genetic backgrounds, but not in a third. When near-isogenic lines were developed for the Xa-10 gene for resistance no residual effect was found. The residual effect of other Xa genes were not tested in the Philippines. Yamada (1986b) demonstrated evidence of a residual effect of Xa-1 and Xa-kg on representative races of japanese races II, III and IV. Plants resistant to race I, when inoculated with isolates of virulent races II, III and IV, had lesions of 1.5 on the scale of Ezuka and Horino, while plants susceptible to race I isolates had lesions of 2 or more.

The Xa-4 allele is incompletely dominant when tested with race 1 isolate PX061 (Olufowote et al., 1977). The residual effect is therefore also expected to be incompletely dominant. However, the F_1 of crosses between lines susceptible and moderately resistant to PX099 were more susceptible than the mid-parent value. This tendency was more noticeable with PX099 than with race 2 isolates PX086 and IRN812 (Table 10-1). Factors for quantitative resistance appear recessive, especially in crosses between moderately resistant parents. Since it is unlikely that the residual effect of Xa-4 is recessive, it appears that other factors for quantitative resistance were more important than the Xa-4 gene in determining the final lesion length when inoculated with PX099.

In crosses between two moderately resistant cultivars, both homozygous for the Xa-4 gene for resistance, no segregation for Xa-4 is expected. The F_1 of these crosses were more susceptible than either parent, but none were as highly susceptible as the susceptible cultivar TN1, indicating a resistance factor in common. This could have been the Xa-4 gene, or a closely linked gene. However, IR28 appeared not to have factors common with IR40, based on wide transgression in the F_3 and F_4 generation, while both IR28 and IR40 have the Xa-4 gene. Further research is needed

to test whether the residual effect of Xa-4 is expressed in the specific genetic background of this cross and to test the interaction of other factors for resistance to PX099 with the Xa-4 gene.

CHAPTER 11

GENERAL DISCUSSION

DURABILITY

Physiological specialization to major genes for resistance to bacterial blight in rice has been repeatedly shown in *Xanthomonas campestris* pv. *oryzae* (Xco). Such specialization is most evident when high levels of resistance due to the action of a single gene are reduced to very low levels. The large cultivar x isolate interactions found between rice cultivars with major genes Xa-4, xa-5 and Xa-10 and Philippine isolates of Xco demonstrate that Xco can largely overcome these resistance genes. Little evidence was reported in this thesis (Chapter 9) for interactions other than those based on major genes. This would indicate that quantitative resistance may be durable. However, such tests of a number of cultivars and isolates are only of limited value in predicting the durability of the resistance. The isolates used in this study had not necessarily been exposed to the cultivars before collection. There is therefore no reason to expect specialization for such quantitative resistance factors, although virulence factors may have been present at a low level in the local population. Sampling for specific virulence to quantitative resistance factors was further limited by the restriction to Philippine isolates of Xco only. In addition, the large variations within cultivar reactions for symptom development made clear demonstration of small race-specific effects (less than 6 or 7 cm) difficult.

The question therefore remains whether the combination of quantitative factors for resistance will create a resistance which is both sufficiently effective and durable, even if used on a large scale and for longer periods. This can perhaps only be determined in retrospect (Johnson, 1981).

Virulence of Xco to the Xa-4 gene for resistance, or to a closely linked factor, was shown in this report to be incomplete in the Philippine race 6 isolate, and probably in some isolates of races 2 and 3 as well. As this gene has been used in the Philippines for 20 years, it can therefore be said that this residual effect of Xa-4 is relatively durable. The gene Xa-4 shows durability when considered as a minor gene for quantitative resistance, not as a gene for high levels of resistance (Pederson and

Leath, 1988).

The manner with which one uses factors for quantitative resistance may affect the durability of these factors. Within a crop species uniformity for a major gene for resistance to important pathogens has been created by selection. This uniformity in turn exerts a strong selection for new virulent pathogen isolates. Multilines or mixtures of cultivars have been suggested as a method of reducing the uniformity in improved cultivars to increase the durability of genes for resistance (Browning and Frey, 1969). Uniformity of minor genes for resistance is less likely, as selection is not as rigorous or not present at all. Homogeneity for minor genes for resistance is only to be expected in self-pollinating species when the cultivar is descended from a single homozygous plant selection. Heterogeneity for minor genes is expected when more plants are used as the basis of a cultivar, as when negative selection or recurrent selection is used (Parlevliet, 1989) or when selection was done in segregating material. Uniformity for minor genes is not likely in cross-pollinating crops, unless these are single-cross hybrids.

Rice is a self-pollinating species which has been considerably improved in the last 25 years. Strong monogenic resistance in rice to Xco has been used intensively and cultivars uniform for a single resistance gene are continuously present over large areas. It is not surprising then that races virulent to these resistance factors have developed. As selection techniques improve and the interest in hybrid rice cultivars increases, the chance grows that the rice crop will become more uniform for minor genes for resistance to bacterial blight. Specialization for virulence to such resistance factors is then more likely. To avoid this, selection procedures which maintain heterozygosity while raising the general level of resistance in a crop are advised.

OTHER XANTHOMONADS

Because durability can only be determined in retrospect it is useful to compare the results found here with those of other host-parasite systems with similar characteristics in an effort to discover some general trends.

The speed with which specialization develops can be affected by:

1. the nature and genetic basis of the resistance,
2. the local diversity of resistance factors in the host(s), and
3. the inherent diversity of virulence factors in the pathogen population.

These three factors can together or separately affect the host-pathogen relationship.

We can see great differences for these three factors among plant pathogenic *Xanthomonads*.

Symptoms of black rot in cabbage caused by *Xanthomonas campestris* pv. *campestris* (Xcc) are similar to those in rice. Symptoms range from small (1-3 mm) lesions restricted to the tissue surrounding the hydathodes of resistant plants to large, coalescing lesions and systemic infection in very susceptible plants. Inoculation by "notching" at the leaf edge shows that bacteria can multiply and spread in resistant plants, but at a lower rate than in susceptible plants, especially at temperatures lower than 24 °C (Staub and Williams, 1970).

Xcc, however, is a fairly general pathogen with a wide host range and brassicas are a very heterogeneous, cross-pollinating group. A non-specialized pathogen will almost always be confronted with a very heterogeneous host population, as it moves between species. Physiological specialization is not expected in such pathogens. Only one major gene for resistance, the recessive gene "f", seems to have been identified in cabbage. The level of the resistance of the heterozygote is strongly influenced by modifier genes (Williams et al., 1972), which can also by themselves impart a moderate resistance in FF plants. The "f" gene for resistance does not seem to have been used in commercial cultivars and moderate resistance has been developed by accumulating minor genes. This moderate resistance is apparently durable and no race differentiation of Xcc has been reported (Yuan and Alvarez, 1985).

The genetics of resistance to bacterial blight in cotton is similar to that of rice. A diversity of genes for resistance to *Xanthomonas malvacearum* has been used in improved cultivars of cotton, often in oligogenic gene complexes. Up to sixteen major genes have been identified, although it is not clear if resistance in leaf, stem and cotton boll are controlled by the same or different factors (Ebbels, 1976). The expression of the resistance varies with the environment; in some environments individual genes give near-immune reactions while in others resistance appears as a quantitative trait (Innes and Brown, 1969). Minor genes for resistance to *X. malvacearum* were also identified in a highly discriminat-

ing environment. Minor genes have been shown to influence the effect of major genes, and as such may be combined with major genes to increase the levels of resistance. Pyramiding of minor gene complexes also resulted in plants with a moderate level of resistance (El-zik and Bird, 1970).

However, virulence factors are evidently very common in *X. malvacearum*, to such an extent that race definitions are considered useless in some areas (Brinkerhoff, 1970). The variation in this pathovar is so great that it is evidently able to adapt quickly to a resistance. Reductions in the level of resistance were noticed already one year after introduction of a new cultivar (Brinkerhoff, 1970). Cultivars with quantitative polygenic resistance apparently did not prove more durable than other cultivars in India (Brinkerhoff, 1970).

Bacterial leaf spot of sweet pepper caused by *Xanthomonas campestris* pv. *vesicatoria* is a disease of the mesophyll of the leaves, rather than of the vascular system, as in rice. Hypersensitive major gene resistance results in a few, small flecks. The multiplication of avirulent bacteria is reduced, but not completely inhibited, in leaves of such resistant plants (Stall and Cook, 1966). The resistance has proven to be race-specific (Hibberd et al., 1987). A moderate resistance, reducing lesion numbers and size, was also reported (Hibberd et al., 1988). This was felt to be an incomplete form of monogenic hypersensitivity, as the rate of bacterial multiplication in a line with this resistance resembled the rate of multiplication of an avirulent isolate of the bacteria in the same line. If this quantitative resistance is monogenically inherited races virulent to the gene may be expected to form relatively quickly.

Bacterial pustule disease in soybean is also a disease of the leaf mesophyll. Resistance to *Xanthomonas campestris* pv. *glycines* in soybean is imparted by the recessive "rxp" locus. This moderate resistance reduces lesion incidence rather than lesion size or bacterial multiplication within leaf tissue. Artificial inoculation by wounding shows that the bacteria can multiply within the leaf, resulting in severe symptoms. This resistance has been widely used in commercial cultivars in the Southern USA and has proven durable for more than 30 years (Groth and Braun, 1986).

Bacterial leaf streak of rice, caused by *Xanthomonas campestris* pv. *oryzicola*, is different from bacterial blight in that it is a disease of the leaf mesophyll rather than the vascular system. The bacteria enter through the stomates and lesions are limited in size (Mew and Vera Cruz, 1986). Genes for resistance to bacterial leaf blight do not impart resistance to bacterial leaf streak. This disease is not considered very damaging and resistance has not been systematically sought.

Comparisons between pathogenic *Xanthomonads* reveal that large differences between the different hosts and the different pathogens exist. A combination of specific factors in each system determines the final level of resistance. High levels of resistance, when used, have not proven durable at all. Moderate levels of resistance have proven very durable in soybean and brassicas, but not in cotton or sweet pepper.

COMPONENTS OF RESISTANCE

Infection by Xco in rice can be divided into a number of steps following arrival of the bacteria on the plant surface. These are:

1. epiphytic survival and/or multiplication on the leaf surface,
2. entrance into the hydathodes,
3. entrance into the vascular system,
4. multiplication in the vascular system,
5. movement in the vascular system, and
6. release of inoculum.

Step 2 is bypassed when infection is through wounds. Factors for resistance can be expected to affect the disease progress at one or more steps of the infection process. However, the potential maximum effect of resistance factors on each step of the infection process may vary. In addition, some steps of the infection process are more easily assessed for differences between cultivars than other steps, and one therefore tends to concentrate on factors influencing these steps.

The clipping and pricking methods used for most selection work is an assessment of steps 4 and 5, factors limiting multiplication and movement of the bacteria in the vascular system, plus possible differences in sensitivity of the tissue to bacterial presence. Large differences exist between cultivars.

Data presented in Chapter 8 of this thesis indicate that resistance affecting stages 1, 2 and/or 3 also exists. This can be found by spray inoculation. Factors for resistance limiting lesion number in rice may be affecting step 2, the entrance into the hydathodes. Horino (1984) has shown that the structure of the hydathode of *Leersia japonica* prevents invasion by Xco. Similar variation in the structure of hydathodes may exist between rice cultivars. Hydathode and water pore number can also vary between cultivars. Other mechanisms for resistance may also be

possible. Host genotype can influence step 1, the colonization of pathogenic bacteria on host leaf surfaces (Hirano and Upper, 1983; Mew and Vera Cruz, 1986). Crosse (1963) reported that fewer pathogenic bacteria grew on the leaf surfaces of a resistance cherry cultivar than of a susceptible cultivar.

Factors limiting lesion number were also reported in soybean, against *X. campestris* pv. *glycines*, and in sweet pepper, against *X. campestris* pv. *vesicatoria*. Groth and Braun (1986) reported that the resistance factor "rxp" in soybean was distinct from factors limiting bacterial multiplication inside the leaf. This factor has proven extremely durable. The resistance factor in sweet pepper affected both lesion number and bacterial multiplication (Hibberd et al., 1988). However, both pustule disease of soybean and leaf spot of pepper are diseases of the leaf mesophyll, not of the vascular system, and comparisons with Xco in rice are of limited value.

MECHANISMS OF RESISTANCE

This study has shown a general relationship between lesion size and bacterial presence in rice leaves. The effective dose needed to cause active lesion development in 50% of the inoculated leaves was shown to be negatively correlated with the final lesion lengths of the cultivars (Chapter 7), indicating that more bacteria are needed to cause infection in resistant cultivars than in susceptible cultivars. Bacterial populations increased equally in resistant, moderately resistant and susceptible cultivars for the first two to three days after inoculation, but populations in resistant cultivars stabilized at a level 10-100fold lower than in susceptible cultivars. Populations of bacteria in moderately resistant cultivars were also shown to stabilize at a lower level than in highly susceptible cultivars (Chapter 4). Both high levels of resistance due to the presence of the xa-5 gene for resistance and moderate resistance therefore appear to quantitatively affect bacterial establishment and multiplication.

Specific interactions affecting bacteria in plant tissues have been found by histological examination of leaf tissue (Horino et al., 1981). Avirulent bacteria appeared irregular in shape three days after inoculation and the bacterial cells were surrounded by fibrillar material originating from the walls of the plant xylem vessels in which the bacteria were located. Cells of virulent isolates were not irregular in shape and no

fibrillar material was found to develop. Whether similar changes take place in moderately resistant plants following inoculation remains to be investigated.

Inoculation by avirulent isolates inhibits subsequent infection by a virulent isolate (Horino, 1976). Antibacterial substances have been isolated from leaves following inoculation with avirulent isolates (Nakanishi and Watanabi, 1977). The presence of these substances has been found negatively correlated with the rate of bacterial multiplication.

Evidence therefore indicates that resistance in rice to Xco is an active reaction by the plant in response to the presence of avirulent bacteria in xylem tissue (Mew, 1987). The effect is probably dose-dependent, since low inoculum concentrations result in smaller or inactive lesions in otherwise susceptible plants. Both highly resistant and moderately resistant plants appear to affect bacterial growth in the same way, and vary quantitatively rather than qualitatively in effect.

CHOICE OF ASSESSMENT SYSTEM

It is important that the right assessment system is used, i.e. a system which is as representative for the resistance of the plant as possible. Lesion size following clip inoculation has been shown in Chapter 4 to be correlated with the resistance of the plant, as measured by the number of bacteria present in a leaf. In Chapter 8 it was shown that the additional measurement of lesion numbers following a spray inoculation can give extra information about relative resistance of cultivars which is otherwise not obtained by clip inoculation.

In Chapter 2 various assessment systems were discussed in relation to clip or prick inoculation and it was concluded that assessment based on the percentage diseased leaf area was very appropriate for comparisons of highly resistant cultivars, but was less appropriate for moderately resistant cultivars. A lack of relationship between leaf length and lesion length for 50 cultivars was shown in Chapter 3. This justified the use of lesion length as a parameter for assessing moderate levels of resistance. In Chapter 3 lesion length measurements were compared to the lesion length/leaf length (percentage leaf length infected) measurements. Only 20-40% of the variance in the percentage leaf length infected was explained by the differences in lesion length between cultivars; the rest of the variance was due to differences in leaf length between cultivars. Differences in leaf length of a segregating population will not normally

vary as much as in a screening, where cultivars of varying origin are compared. However, it is clear that variations in leaf size become confounded with those for lesion size, and this can have an effect on conclusions of a genetic study when this is assessed using the percentage diseased leaf area or other relative measurement. When the plants differ greatly for leaf length, conclusions concerning inheritance of quantitative resistance based on percentage diseased leaf area measurements are highly questionable.

The situation can be further distorted when an assessment scale is used. As was discussed in Chapter 10, genetic studies of quantitative traits use the variance of a population and the distribution of individuals within this population as indicators for the degree and type (additive, dominant, epistatic) of genetic variation within the population. The divisions of an assessment scale will affect the number of individuals in each class. Equal class sizes will not distort this distribution but unequal class size, as is the case with the scale of Horino et al. (1974b), will change the apparent distribution to a great extent. The variance of a population will also be greatly affected by the class sizes. One must therefore be aware of the consequences of such a scale and view the conclusions of a genetic analysis of a quantitative trait with the necessary caution (Yamada, 1984).

The analyses for this thesis were carried out without transformation of the absolute values for lesion length unless this was found to be required due to a significant correlation between treatment variances and treatment means. As all treatments per replication could be assessed within a single day, it was felt that the absolute values for measurements allowed good comparisons.

Use of absolute values for symptom development in fact implies an additive model for host-pathogen interactions, whereby the assessed symptoms are considered the sum of all factors influencing symptom development. Implicit in an additive system is the assumption that the effect of a resistance factor in a very susceptible system is equal to the effect of the same factor in a less susceptible system.

In Case 1 lesion lengths (cm) of highly susceptible younger plants are compared with those of older, less susceptible plants. No cultivar x age interactions are found.

Case 1: Hypothetical values for lesion length (cm) of two cultivars of two plant ages following inoculation with *Xanthomonas campestris* pv. *oryzae*.

Cultivar	Plant age: Young	Old	
1	50	20	Case 1
2	35	5	

In an effort to improve comparisons between experiments, data are often expressed relative to the most susceptible treatment. For example a highly susceptible cultivar. When such a comparison is made, one is then using a multiplicative system to describe the effect of resistance factors. This means that a decrease in susceptibility of, for example, 50%, is seen as an equal effect, regardless of whether this actually is a decrease from 10 to 5 cm or from 30 to 15 cm (Cases 2 and 3).

Case 2 and 3: Percentage transformation of the lesion lengths presented in case 1. Case 2 (upper) compares plants of two ages, for each cultivar, Case 3 (lower) compares cultivars within each age class.

Cultivar	Plant age: Young	Old	
1	100	40	Case 2
2	100	14	
1	100	100	Case 3
2	70	25	

Use of such a percentile (or other) transformation results in a significant interaction value, found with the same set of measurements. In the example given here, one would conclude, using the absolute values of Case 1, that both cultivars decrease equally in susceptibility with age. Susceptibility and resistance are considered additive and screening can be done equally well at both ages. When the analysis is done using the relative values one cultivar appears to decrease in susceptibility faster than the other. Susceptibility and resistance are considered

multiplicative. One then concludes that screening can better be carried out in older plants (Case 3), or that physiological changes leading to decreased susceptibility take place faster in some cultivars than in others (Case 2).

Conclusions based on the significance of such interactions can therefore only be made when one has evidence to prefer one transformation system above another. Detailed genetic studies of cultivars of varying levels of quantitative resistance are needed for this evidence. Since we do not have extra information which would indicate the appropriateness of an additive or a multiplicative system, it seems correct to use the absolute values for lesion length for analysis, but to consider the conclusions in the light of both additive and multiplicative systems.

GENETICS OF RESISTANCE

Quantitative resistance (QR) is defined by Yamada (1984) as a resistance which results in a continuous distribution of individuals for BB symptoms in the F_2 population. Quantitative resistance can be due to the oligogenic or polygenic background of the resistance, but can also be a monogenic resistance which inherits additively, or with a very large experimental error. Evidence presented in Chapter 10 indicated the presence of at least five factors for resistance, each with a small effect. A moderate level of resistance is achieved when at least two, but probably more of these factors are present. These QR factors appear to be common in rice cultivars, and extreme susceptibility is the exception rather than the rule. Selection for quantitative differences in lesion length is therefore expected to result in some increase in resistance in most cases, and can be easily coupled with a breeding program for improvement of other agronomic traits. Selection for high levels of bacterial blight resistance based on minor genes will necessitate a crossing program specifically directed to this goal.

The estimates of the number of genetic factors affecting moderate resistance were determined based on the number of transgressing lines in the F_3 and F_4 generations, and on the range (in cm lesion length) between the most resistant and the most susceptible lines recovered in each population. This procedure was followed as the two susceptible parent cultivars both appeared to have at least one resistance factor each. Some F_4 lines of the cross between these two cultivars were significantly more susceptible than both parent cultivars, and may be without resistance

factors against Philippine isolates. These lines should be further tested, using several isolates, to confirm this hypothesis. If a line is found which is confirmed to be without minor genes for resistance to bacterial blight it should be used in further genetic studies of quantitative resistance.

A residual effect of the Xa-4 gene, or a closely linked factor for resistance, was shown using the race 6 isolate PX099. Ogawa et al. (1988) showed evidence for a similar residual effect of the Xa-4 gene to race 2 and 3 isolates. Some evidence for a residual effect against another race 2 isolate was presented in Chapter 9. It is not clear how general residual effects of major genes for resistance to bacterial blight are. Many improved cultivars have one or more major genes effective to some isolates in the Philippines or Japan. The two susceptible cultivars used in this study, for example, both had one or more known major genes, although they are very susceptible to virulent isolates both in the Philippines and in Japan. A more systematic study of this aspect of resistance is needed for all major resistance genes.

In literature concerning many bacterial pathogen systems mention is made of the difficulties encountered in determining the dominant or recessive nature of individual alleles for resistance, even when these have a large effect. Homozygous F_2 plants usually give a stable reaction and can easily be assigned to a resistant or a susceptible group. The reactions of heterozygotes are often not as unequivocally determined. The reaction of these plants may be found to fall between the extremes of the resistant and susceptible parents, in which case the trait can be considered to be predominantly additive. However, it has been shown that the reaction of such heterozygotes can vary from nearly completely resistant to nearly completely susceptible under different test conditions.

Hibberd et al. (1988) found that a gene for reduced lesion number in sweet pepper against *X. vesicatoria* could be classified as recessive to race 1 but dominant to race 3. Race 1 was a highly aggressive isolate which was presumably able to overcome the resistance of the single allele in the heterozygote which the weakly aggressive race 3 isolate presumably could not overcome. As the inoculum concentration has also been shown to affect the final lesion size (Chapter 7), an increase in inoculum concentration is expected to cause a similar change in reaction.

Siddhu and Khush (1978) report a similar phenomenon, referred to as a "reversal of dominance", in rice when inoculated with Xco. Crosses were made between a susceptible cultivar and a cultivar with the Xa-6 gene for

adult plant resistance in the booting and flowering stage. Only 1/4 of the F_2 plants were resistant at booting and the gene was considered recessive. At flowering 3/4 of the plants were resistant, so that the gene was then considered dominant. It was felt that the resistance of heterozygotes, which had only one dose of the allele for resistance, was less developed at booting stage than the homozygote, resulting in a susceptible classification of the heterozygote. At flowering the resistance was fully developed and the heterozygotes were scored as resistant.

El-zik and Bird (1970) remarked on the effect the scoring procedure will have on the designation of allelic action. Division of plants into resistant and susceptible groups, needed for determination of genetic ratios, implies a clear difference between these two types and the absence of intermediate types. With a quantitative trait, such as the quantitative resistance to bacterial blight in cotton or rice, a predetermined range for resistance and susceptibility may be used when there is a good separation of the two types. If this is not the case a predetermined range can artificially impose such a ratio on a continuous distribution (Murty and Khush, 1972). When a predetermined range is used, changes in the population mean, such as those caused by differences in temperature or plant age, can also influence the conclusions. A lowered population mean, without a change in the relative distribution of the individual F_2 plants, could be interpreted as an apparent increase in resistant types, when these fall below a predetermined limit for resistance (Innes and Brown, 1969).

CHAPTER 12

HOW TO SELECT FOR QUANTITATIVE RESISTANCE TO BACTERIAL BLIGHT

INTRODUCTION

Quantitative resistance (QR) to bacterial blight (BB) is here defined as a difference found between lines for symptom development (lesion length, percentage diseased leaf area (%DLA)), without any clear cut division between resistant and susceptible plants. Such a clear cut division is not usually expected when screening cultivars but may become apparent in an F_2 analysis of a cross between a highly resistant and a highly susceptible parent. High QR can be caused by the combined action of minor genes but can also be largely due to an incomplete effect of a major gene. While QR shows few race-specific interactions, implying apparent horizontal resistance (van der Plank, 1968) and thus perhaps durability, the apparent race-nonspecificity may be due to the relatively low level of resistance in comparison to major gene resistance (MGR), and the associated greater difficulty in detecting small interactions (Parlevliet and Zadoks, 1977). However, relatively high levels of QR have been found which appear effective over a broad range of isolates within a country (Yamada, 1984; Chapter 9, this thesis) and QR therefore is felt to have an important role in protecting rice from BB damage.

In areas where the potential for disease is low, the disease incidence is low and the disease severity low to moderate. In these areas moderate to high QR may be sufficient to protect a crop from significant yield loss, especially when combined with a strict attention to eliminating potential sources of infection in the field.

In areas with a greater potential for epidemics, the disease incidence and severity are high and the disease appears earlier in the season, or after a heavy storm (Ou, 1985). In these areas high QR may not be sufficient to protect a crop fully and major gene resistance (MGR) supported by high QR is needed. The expression of MGR, when combined with high QR, gives a considerably higher level of resistance, limiting symptoms to few and tiny lesions. MGR protected by QR may last longer because the inoculum present in the field will be much lower and the chance that a new race arises will be considerably reduced. If such a new race should

occur, the damage will be restricted due to the QR still effective.

The history of BB of an area should be studied. Where MGR cultivars are being grown or are expected to be used widely in the near future a survey for the prevalence of virulence to the MGR in use should be made. A decison can then be made to select either for high QR alone, a task which is fairly straightforward, or to incorporate both MGR and QR. This last approach may require more work during selection but a method is described by which this may not be necessary.

SCREENING FOR QUANTITATIVE RESISTANCE ONLY

Screening of cultivars or lines

Promising cultivars or lines must be first screened to assess their QR. While the effect of growth stage on resistance is not very critical (Chapter 5) lines with large differences in development (>20 days) should be grouped and tested separately. In this case repeated sowing of the susceptible check cultivar will be needed in order to have a proper comparison. Symptoms on plants grown in a screenhouse are more pronounced than in field or greenhouse, and this allows greater chances to distinguish small differences between cultivars. If uniform material is available 10-20 plants per line are sufficient; more plants should be tested if the material is heterogeneous.

Choice of the isolate for testing is very critical. An isolate virulent against as many major genes as possible is needed. Highly aggressive isolates give long lesions on susceptible cultivars earlier after inoculation than less aggressive isolates. Highly aggressive isolates better distinguish small differences between cultivars. Isolates must be pretested against the most commonly found resistance genes. Virulence is usually defined as the ability to produce lesions of a size greater than a predetermined limit. However, this does not exclude low level residual avirulence to a major resistance gene. Such residual avirulence, which may be quite common, can best be detected by analysis of an F_2 progeny of a cross between a highly susceptible cultivar and a cultivar with the major gene in question. Residual avirulence can also be detected, although less accurately, by comparing symptom development of a group of cultivars with a particular major gene with that of a group of cultivars without the gene in question. Where all cultivars with a particular major gene show a reduced lesion length in relation to the other cultivars when

inoculated with a particular isolate, residual avirulence should be suspected. If a major gene occurs in the cultivars tested, the use of such an incompletely virulent isolate can lead to the choice of cultivars with a residual major gene instead of those with QR.

Plants should be clip inoculated with a concentrated ($1x10^8$ bacteria/ml or more) inoculum suspension and scored when lesions in the susceptible check cultivar almost reach the leaf sheath, but not later than 21 days after infection. Scoring should be done by measuring lesion length, especially if cultivars of different leaf lengths are being tested simultaneously. This would be the case when both tall traditional cultivars and improved short stature cultivars are being tested. Even when all cultivars are of about equal stature, small differences between cultivars may not be detectable when the percentage diseased leaf area (%DLA) is estimated, even if more measurements are made, as visual estimates are least precise between 25 and 75 %DLA.

Lines with mean lesion lengths less than 50% of the lesions of the susceptible check may be held for potential use; where many such lines are found a lower limit may be used. Lines with very short lesions (<10% of the susceptible check) should be viewed with caution as the resistance is possibly due to a major gene. If the test isolate is known to be avirulent or incompletely virulent against the known adult plant resistance gene Xa-3 a second test at booting stage is advisable in order to better distinguish lines with high QR from those with adult plant resistance. The Xa-3 gene may have only been partially active at the time of testing, and so will not give a clear resistant reaction (Zhang and Mew, 1985).

Additional tests

Two additional tests should be carried out to give critical extra information useful for the breeder. These are:

1. a comparison of the reactions of selected cultivars with a more varied set of isolates, especially differential isolates known to be avirulent against individual major genes, and
2. a test for quantitative resistance following spray inoculation.

Testing with differential isolates will indicate if any known major genes are present in the promising lines. If a known major gene is found to be present in a line extra tests with isolates truly virulent against this gene may be needed to distinguish QR based on residual avirulence

from QR based on polygenes.

A spray inoculation test for lesion number should also be carried out. This is especially useful for areas where low levels of infection frequently occur, indicating that the bacteria are endemic in the field. In such areas the apparent extra resistance to bacterial entrance in intact leaves, described in Chapter 8, can increase field resistance of a cultivar. This factor will be less important where bacterial entrance is largely through wounds in the leaf tissue caused by storm damage.

The spray test can best be carried out in a greenhouse as close examination of the leaves is needed. Plants should be spray inoculated with a concentrated inoculum suspension. At maximum tillering 20-25 ml suspension per plant was found to be sufficient to wet all leaves, using a hand spray device. Leaves must be examined regularly until the first lesions appear along the leaf margins. The lesion incidence should be assessed one or two days later, before the lesions have coalesced into a single long lesion. Cultivars which develop relatively large numbers of lesions per leaf should be discarded. As this test carries a large variation it may be repeated if time allows, either using the same plant material at a later growth stage or using new plant material.

Selection of individuals

Beginning with the F_3, assessment of individual plants for increased QR can be similar to the first screening procedure described above. A single virulent isolate can be clip inoculated on three or four large, wellformed leaves of plants at or after panicle initiation stage, and scored 14-21 days later. Measurement of lesion length is advisable for maximum distinction between individuals and to avoid the confounding effects of varying leaf sizes.

SCREENING FOR QUANTITATIVE RESISTANCE COMBINED WITH MAJOR GENE RESISTANCE

In areas where high QR alone cannot adequately protect a crop from significant yield loss MGR and QR should be combined. The three screening procedures described above can be followed. However, the most efficient isolate for selection is one which is incompletely virulent to the MGR in question, but which gives long, fast-growing lesions on a highly susceptible check cultivar. Such an isolate allows selection of individuals with both resistance factors or with only very high QR following a single

inoculation.

Where no such isolate can be identified two isolates, one avirulent and one virulent to the MGR must be individually inoculated to plants of each line and the scores of each isolate treated as a separate screening. Spray inoculations can also be carried out with two isolates but care must be taken that the plants are well isolated from each other. During selection in early segregating generations plants must be assessed individually for their reactions. In this case separate tillers of each plant must be individually inoculated and scored. This requires more preparation, as each tiller must be individually labelled for isolate.

SUMMARY

The effectiveness of the resistance in rice (*Oryza sativa* L.) cultivars to *Xanthomonas campestris* pv. *oryzae*, the causal organism of bacterial blight, has been greatly reduced by new races of the organism. Interest developed in the quantitative resistance (QR) of moderately resistant and moderately susceptible cultivars, in the hope that the combination of multiple, perhaps race-nonspecific, factors for resistance would prove more durable than the major gene resistance found in highly resistant cultivars.

ASSESSMENT OF QUANTITATIVE RESISTANCE

QR can best be assessed by measuring lesion length following clip or prick inoculation. Lesion length appeared independent of leaf length, leaf width and diameter of the main leaf xylem vessel. Scoring of the percentage diseased leaf area, commonly used to study resistance to this pathogen, is faster than scoring of lesion length, but less accurate in the range found among moderately resistant cultivars. In addition, the percentage diseased leaf area confounds differences in leaf size with differences in resistance.

Younger plants were more susceptible than older plants and unfolding leaves were slightly more susceptible than completely unfolded leaves. However, QR was evident at all growth stages and leaf ages as a reduction in lesion length relative to lesion lengths in susceptible cultivars. Assessment of QR is best done on fully developed leaves of plants just after maximum tillering.

The daily rate of lesion extension (cm day^{-1}) was slower in moderately resistant cultivars than in susceptible cultivars. The daily rate of lesion extension was not constant, and changes in this rate (cm day^{-2}) deviated significantly from zero. Small but significant differences for this parameter were found between the isolates tested, but were not found between the cultivars tested. This indicates that differences between cultivars will become more evident with increasing days after inoculation and would imply later assessment. Since isolate aggressiveness, leaf senescence and the increasing variance with increasing lesion length also affect the ability to accurately distinguish levels of QR at a specific

moment, the optimum time for assessment will vary with isolate choice, plant age and the mean level of resistance of the cultivars being tested.

NATURE OF QUANTITATIVE RESISTANCE

The nature of QR was investigated in several cultivars. Lesion length following clip inoculation was correlated (r=0.54**-0.85**) with ^{10}log number of bacterial cells per leaf. Leaves of highly resistant cultivars contained up to 100fold fewer bacterial cells than leaves of susceptible cultivars. Leaves of moderately resistant cultivars contained about tenfold fewer bacterial cells than susceptible cultivars. A negative correlation was also shown between lesion length and the inoculum dosage needed to cause active lesions in 50% of inoculated leaves of ten cultivars (r=-0.76*). QR therefore affects the ability of bacterial cells to establish and multiply within a leaf following clip inoculation.

The ability of bacterial cells to enter intact leaves via the hydathodes is not tested using the clip inoculation method. Differences between cultivars for lesion number following spray inoculation were shown. Certain cultivars had fewer lesions following spray inoculation than would be expected based on their lesion length following clip inoculation. Incidence of disease in the first nine weeks after transplanting was better predicted by both lesion length following clip inoculation and lesion number assessment following spray inoculation than by lesion length assessment only. This implies an extra component of resistance limiting bacterial entry into intact leaves.

Race-specificity of QR to diverse Philippine isolates was tested in cultivars varying from highly resistant to susceptible. Large variation within reactions limited detection of race-specific effects to effects greater than about 7 cm. No confirmed evidence of minor interactions based on unknown resistance factors was found, although incomplete resistance due to known race-specific major genes was found in several cases. Such incomplete resistance was difficult to distinguish from other quantitative resistance factors.

GENETICS OF QUANTITATIVE RESISTANCE

Multiple resistance factors were shown to be present in moderately resistant and susceptible cultivars based on F_3 line variances and on

transgression in F_2, F_3 and F_4 generations of crosses between six cultivars. A minimum of five factors was indicated. One of these factors was probably Xa-4 gene for resistance, or a closely linked gene, which showed a residual effect (effect remaining when attacted by a virulent race) with the isolate used. Selection using one isolate was effective in improving the level of QR to another isolate.

SAMENVATTING

De resistentie in rijst (*Oryza sativa* L.) tegen *Xanthomonas campestris* pv. *oryzae*, die "bacterial blight" veroorzaakt, wordt soms doorbroken door nieuw fysio's van het pathogeen. Deze niet duurzame vorm van resistentie wordt bepaald door een enkel resistentiegen met een groot effekt. De belangstelling voor de verschillen in symptoomontwikkeling tussen matig resistente, matig vatbare en volledig vatbare rassen is toegenomen. Men hoopt dat een kwantitatieve resistentie gebaseerd op de kombinatie van meerdere genen met elk een klein effekt duurzamer zal blijken te zijn. In dit proefschrift worden diverse eigenschappen van de kwantitatieve resistentie in rijst tegen *Xanthomonas campestris* pv. *oryzae* beschreven.

METEN VAN KWANTITATIEVE RESISTENTIE (KR)

Een goede maat voor het niveau van KR in een plant is de lesielengte in het blad na "clip" of "prik" inoculatie. Proeven tonen aan dat de lesielengte onafhankelijk is van bladlengte, bladbreedte en diameter van het grootste xyleemvat in de middennerf. Schatting van het percentage aangetast bladoppervlak is een een tot nu toe veel gebruikte en snelle methode ter bepaling van hoog niveaus van resistentie. Echter het meten van de lesielengte geeft veel nauwkeuriger informatie bij de bestudering van matig resistente rassen met KR. Bovendien, de schatting is gebaseerd op de verhouding tussen lesie- en bladlengte, waardoor de verschillen in bladlengte verstrengeld worden met die van de lesielengte. Dit kan leiden tot foute beoordelingen van resistentie wanneer planten met sterk uiteenlopende bladlengtes vergeleken worden.

Jongere planten zijn vatbaarder dan oudere planten en zich ontvouwende bladeren zijn enigzins vatbaarder dan volledig ontvouwen bladeren. In alle groeistadia en bladleeftijden was de KR aantoonbaar als een reduktie van de lesielengte vergeleken met de lesielengte in vatbare rassen. KR kan het beste bepaald worden aan volledig gestrekte bladeren na maximum uitstoeling (50-60 dagen na zaaien). De dagelijkse toename in lesielengte (cm dag^{-1}) was geringer in matig resistente rassen dan in vatbare rassen. De dagelijkse toename was niet geheel linear tussen de dag van verschijnen van de lesie en de laatste dag van meting, kort voor bladsterfte. Deze veranderingen in de dagelijkse toename (cm dag^{-2}) bleken niet te verschil-

len tussen de onderzochte rassen maar wel tussen isolaten. Dit betekent dat verschillen tussen rassen meestal in de tijd duidelijker worden en het zou ervoor pleiten dat men na inoculatie langer wacht met meten om de verschillen tussen rassen het beste aan kunnen te tonen. Daarentegen hebben isolaat aggressiviteit, bladafsterving en de toenemende variantie gekoppeld aan toenemende bladlengte ook een belangrijk effekt op de metingen. Isolaat keuze, plant leeftijd en de gemiddelde niveau van resistentie van de onderzocht rassen zullen de tijdstip beinvloeden wanneer KR de meest nauwkeurig te bepalen is.

EIGENSCHAPPEN VAN KWANTITATIEVE RESISTENTIE

KR werd onderzocht in verschillende rassen. Lesiegroote na "clip" inoculatie is gekorreleerd (r=0.54**-0.85**) aan de ^{10}log hoeveelheid bakteriën per blad. Bladeren van zeer resistente rassen bevatten acht dagen na inoculatie tot 100 maal minder bakteriën dan bladeren van vatbare rassen, terwijl de bladeren van matig resistente rassen tot 10 maal minder bakteriën bevatten dan de vatbare rassen. Een negatieve korrelatie (r=-0.76*) werd gevonden tussen lesielengte en de koncentratie inoculum nodig om aktieve lesies te produceren in 50% van de geïnoculeerde bladeren. KR lijkt dus de kolonisatie en vermeerderingen van bakteriën in de rijstbladeren na "clip" inoculatie te beïnvloeden.

De mogelijkheid dat bakteriecellen binnen dringen via de waterporiën van intakte bladeren wordt niet door "clip" inoculatie getoetst. Na bespuiting met een bakteriesuspensie werden verschillen tussen rassen voor lesie aantal waargenomen. Bepaalde rassen vertoonden minder lesies na bespuiting met een bakteriesuspensie dan verwacht op grond van hun reaktie na "clip" inoculatie. De relatieve resistenties van rassen, bepaald door het percentage geïnfecteerde planten in de eerste negen weken na uitplanting, werd beter verklaard door een index die bestond uit zowel lesielengte als lesie aantal dan door lesielengte alleen. Bovengenoemde relaties wijzen op een extra komponent van resistentie die het binnendringen van bakteriën in intakte bladeren beïnvloedt.

De fysiospecificiteit van KR werd getoetst door rassen variërend van zeer resistent tot vatbaar te toetsen met diverse Filippijnse isolaten. Door de grote variantie binnen een behandeling waren verschillen in lesielengte kleiner dan ongeveer 7 cm niet significant. Er werd geen duidelijk bewijs gevonden voor interakties die op onbekende resistentiefaktoren berusten. Reakties van twee hoofdgenen voor resistentie tegen

bepaalde isolaten bleek soms onvolledig te zijn, en gaf een matige resistente of matige vatbare beeld. Deze onvolledige resistentie is moeilijk te onderscheiden van andere KR faktoren.

OVERERVING VAN KWANTITATIEVE RESISTENTIE

De aanwezigheid van meerdere recessief overervende resistentiefaktoren werd aangetoond in matig resistente en vatbare rassen op grond van de variantie van F_3 lijnen en de waargenomen transgressie in F_2, F_3 en F_4 generaties van kruisingen tussen zes rassen. Minimaal vijf verschillende resistentiefaktoren waren aanwezig in deze rassen. Waarschijnlijk is één van deze faktoren het Xa-4 gen, of een nauw gekoppelde gen. Een residu-effekt van het Xa-4 gen werd gevonden bij inoculatie met het isolaat PX099. Selektie voor een hoger niveau van KR met isolaat PX099 resulteerde ook in een hoger niveau van KR tegen een andere isolaat.

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CURRICULUM VITAE

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