

Effect of Plant Age and Leaf Maturity on the Quantitative Resistance of Rice Cultivars to *Xanthomonas campestris* pv. *oryzae*

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ABSTRACT

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Resistance of rice (*Oryza sativa*) cultivars to bacterial blight, caused by *Xanthomonas campestris* pv. *oryzae*, as assessed by lesion length, increased considerably with plant age. The fastest increase occurred between 30 and 50 days after sowing. Quantitative resistance was evident at all growth stages as a reduction in lesion length relative to susceptible cultivars. Lesions of all cultivars decreased about equally in absolute length with plant age, but the relative decrease in lesion length was greater with moderately resistant cultivars than with highly susceptible cultivars. The change in quantitative resistance with increasing plant age is different from that of adult plant resistance to *X. c. oryzae* caused by the *Xa-3* gene for resistance. Immature leaves that were still extending were more susceptible than adjacent mature, fully extended leaves, but immature leaves of moderately resistant cultivars were less susceptible than those of highly susceptible cultivars. To compare entries of different maturation dates, lesion length should be assessed when plants are between maximum tillering and flowering.

Previous studies on rice (*Oryza sativa* L.) have shown that plant age greatly influences symptom expression after clip inoculation with *Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye, the causal organism of bacterial blight (6,10). Although leaves of young plants of a susceptible cultivar become quickly blighted, lesions in leaves of older plants expand more slowly. Reactions of resistant plants are also known to be affected by plant age, and this has been used to distinguish types of resistances (8).

Highly resistant reactions in adult plants are related to the presence of one or more specific genes for resistance, called *Xa* genes (2,8). Reactions caused by these major genes have been divided into seedling and adult plant resistances (8). Seedling resistance is stable over the entire growth cycle and in adult plants results in lesions of 0-15% of the length of the lesions on the most susceptible cultivar (3,8). Cultivars with adult plant resistance based on the major gene *Xa-3* (11) are similarly resistant in the adult plant stage but are susceptible in the seedling stage (13). Both resistances are race-specific (2).

Cultivars with moderate levels of resistance to *X. c. oryzae* are also common

(8). Such cultivars develop lesions that vary from 16 to 66% of the length of those on lesions of highly susceptible cultivars (10). These cultivars are usually considered susceptible in younger plants but may be fairly resistant in adult plants (9). The moderate resistance of cultivar IR1545-339 to strain PXO86 was caused by the incomplete reaction of the recessive *xa-5* gene for resistance (15). In other reports, moderate resistance was not related to known genes for resistance and appears quantitative (7). Although there is some evidence that moderate resistance in this and other host-parasite relationships may be strain-specific (12,15), the interactions are much smaller than those associated with the known major genes and may indicate a degree of race-nonspecificity of this type of resistance (8).

Ezuka and Horino (1) and Mew et al (10) observed that disease symptoms in mature plants of susceptible cultivars are less severe than in seedlings, although the disease score usually does not drop by more than one or two points of the Standard Evaluation Scale (4). This change can influence whether a given level of resistance is likely to protect a crop during the whole cropping season. This change can also affect the comparisons made between cultivars, as a previous study has indicated a superior ability to distinguish intermediate levels of resistance in older plants rather than in younger ones.

The relationship between mean lesion length and plant age on moderately resistant plants with quantitative resistance, therefore, needed to be compared with the general relationship on susceptible cultivars. If a similar relationship exists, the increase in resistance of mod-

erately resistant cultivars with increased plant age can be attributed to factors influencing symptom development in all rice plants. If the general pattern in susceptible cultivars is different from that in moderately resistant cultivars with quantitative resistance, genes for quantitative resistance may influence other factors in the resistance process.

Changes in symptom expression of several cultivars with intermediate levels of resistance known to be quantitative (7) were studied to determine the extent to which the expression of the resistance varies with plant age. The relative levels of resistance of several cultivars were also compared on both immature, extending leaves and mature, fully extended leaves to determine if leaf maturity influences resistance expression. When factors affecting resistance are already effective in immature leaves, differences between cultivars should be evident. All comparisons were made with a single measurement of lesion length, because this has been shown to be as effective as determinations of the daily rate of lesion expansion for comparing cultivars for levels of resistance (6).

MATERIALS AND METHODS

Plant age tests. The rice cultivars and breeding lines Cisadane, BR51-282-8 (BR51), IR28, IR40, TN1, and IR9101-46 (IR9101), were used. The first four cultivars show intermediate reactions and the last two cultivars are susceptible checks. The chosen entries were all short statured.

Seeds were sown on days 1, 8, 15, 22, 29, 36, and 47 and plants were transferred to 18-cm-diameter clay pots on greenhouse tables 1 wk after germination, six plants per pot. Pots were filled with lowland rice paddy soil. Ammonium sulfate was applied twice (175 kg/ha total) and normal insect control (monocrotophos) was applied when needed. All side tillers were regularly trimmed away so that only the main tiller could develop.

The experimental design was a split-split plot with three replications. Sowing dates were main plots, cultivars were subplots (three pots per subplot), and bacterial strain used for inoculation was the sub-subplot factor. Three of the six plants in each pot were inoculated with one strain and three with the other strain.

Inoculum of virulent single-colony derivatives of race 2 strain PXO86 and race 6 strain PXO99 was stored and pre-

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pared as described previously (6). The top two fully developed leaves of all plants were inoculated by clipping 2–3 cm from the leaf tip with scissors dipped into the bacterial suspension (5). Pots were inoculated on the same date, which corresponded to 77, 70, 63, 56, 49, 42, and 30 days after the different sowing dates. Lesion lengths were measured 14 days after inoculation.

In a second experiment, seeds were sown at intervals to synchronize maximum tillering, late booting, and post-flowering of all six cultivars. Experimental design and culture were as described earlier, except that there were eight plants in each of four pots, with four plants inoculated with each strain, and the side tillers were allowed to develop. Plants were inoculated as described earlier; in the last two growth stages, only flag leaves were inoculated. Lesion lengths were measured 14 days after inoculation.

Leaf maturity tests. The difference in reaction of immature leaves and fully developed leaves was tested on plants of Cisadane, BR51, IR40, and TN1. Plants were grown in the greenhouse in 18-cm-diameter clay pots, three plants per pot. The experimental design was a complete randomization with three replications (three pots) per cultivar. At 60 days after sowing, tillers were selected with a top-most immature leaf not longer than half of the length 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 earlier with strain PXO86 and lesion lengths were measured 14 days after inoculation. The experiment was repeated once.

Data from the first experiment with seven sowing dates were used to compare leaves of the same leaf position but of two leaf maturities. Leaf position refers to the order in which a leaf is produced. Leaf one is the first true leaf, and the flag leaf has the highest leaf position. Leaf position was regularly noted during growth, and 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 and already approaching senescence by the time lesion length was scored.

Data analysis. Lesion lengths were subjected to analyses of variance, and cultivar means were separated by Bonferroni's test for differences. The difference between lesion lengths on immature and mature leaves was similarly analyzed to determine the effect of leaf maturity on lesion length. A linear regression of lesion length on sowing date was fitted to assess the effect of sowing date on observed resistance.

RESULTS

Plant age tests. Only the oldest plantings of IR28 and TN1 were flowering at the time of inoculation, whereas all plants of the oldest planting group except those of Cisadane had flowered by the end of the test. Lesion lengths on the top two leaves were averaged because there was only a very slight difference between the mean lengths of lesions on the two leaves (0.2 cm), and this observation was similar across cultivars. A significant difference between the strains was found; strain PXO99 was more aggressive than PXO86 (Table 1).

Lesion length decreased with increasing number of days after sowing (Table 1). The linear correlations (R^2) between lesion length and days after sowing were significant ($P < 0.01$); values were 0.89 and 0.82 for strains PXO86 and PXO99, respectively. No significant interaction between days after sowing and strain was found. The linear regressions relating lesion length to the number of days after sowing included: lesion length = $-0.19(\text{days}) + 20.0$ cm for strain PXO86; and lesion length = $-0.18(\text{days}) + 22.6$ cm for strain PXO99. Estimated regression coefficients were found significantly different ($P < 0.01$) from zero in both cases, and the standard error of the line was 1.21 and 1.50, respectively, for strains PXO86 and PXO99.

Significant differences among cultivars were observed at all plant ages. The difference in centimeters between the intermediate and the susceptible cultivars was larger with PXO99 than with PXO86. This cultivar \times strain interaction was largely attributable to the increased lesion lengths on the susceptible cultivars TN1 and IR9101. No significant age \times cultivar interaction was found; mean lesion length of plants inoculated 77 days after sowing for all cultivars was 10 cm shorter than those of plants inoculated 30 days after sowing (Table 1).

The second test of the last stages of development showed significantly shorter lesions on flag leaves of booting and postflowering plants than on leaves at maximum tillering for all cultivars tested (Table 2). There was no significant difference between lesion lengths on leaves of plants at the booting and the postflowering stages. The two highly susceptible cultivars were again clearly distinguishable from the other four cultivars in all stages. The relative resistance of the four moderately resistant 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 cultivar \times age or cultivar \times strain interactions were found.

Leaf maturity tests. Lesion averaged 2.2 cm longer on immature, still unfolding leaves compared with lesions on mature, fully extended leaves directly below ($P = 0.05$). Significantly longer lesions were recorded for immature leaves of TN1 than for immature leaves of the three moderately resistant cultivars. No significant cultivar \times maturity interaction was found; the difference in lesion length between immature leaves and mature leaves was similar for all cultivars.

When data from the test of seven sowing dates was used to compare the lesion lengths on leaves of the same leaf position, no significant difference was found between recently matured leaves and those already approaching senescence.

Table 1. Lesion lengths (cm) of the upper two leaves 14 days after clip inoculation^y of six rice cultivars of seven ages using two strains of *Xanthomonas campestris* pv. *oryzae* (PXO86 and PXO99)

Days from sowing	PXO86							PXO99						
	Cultivar							Cultivar						
	Cisadane	BR51-282-8	IR40	IR28	IR9101-46	TN1	Mean	Cisadane	BR51-282-8	IR40	IR28	IR9101-46	TN1	Mean
30	15.9	14.8	13.6	15.4	20.4	20.7	16.8	13.6	18.2	15.8	17.3	24.6	26.1	19.3
42	6.8	8.3	7.0	12.5	14.0	15.8	10.7	8.1	12.2	10.3	13.1	21.0	22.6	14.6
49	5.9	6.4	9.4	9.6	14.4	14.2	10.0	7.2	7.0	12.5	10.9	19.5	20.8	13.0
56	4.0	6.7	7.0	10.9	9.6	10.8	8.2	7.6	7.1	9.0	9.7	15.6	16.3	10.9
63	4.0	4.8	7.4	7.1	9.9	11.1	7.4	6.3	6.0	7.8	8.0	15.5	16.9	10.1
70	3.9	6.0	6.5	9.0	10.7	16.2	8.7	4.8	7.1	7.1	9.8	18.4	20.4	11.3
77	3.1	5.6	4.1	4.6	9.0	11.2	6.2	3.8	6.3	4.9	7.5	16.9	18.8	9.7
Mean	6.2 c ^z	7.5 c	7.8 c	9.9 b	12.6 a	14.3 a	9.7	7.3 d	9.1 cd	9.7 c	10.9 c	18.5 b	20.3 a	12.7

^y Inoculum concentration = 1×10^9 cfu/ml.

^z Standard error of an average 0.38. Cultivar means within each strain group followed by the same letter are not significantly different ($P = 0.05$), as determined by Bonferroni's test for differences. Values are means of three replications, nine plants per replication mean.

Table 2. Lesion lengths (in centimeters, average of the upper two leaves) measured 14 days after clip inoculation^w of six rice cultivars at three growth stages with two strains of *Xanthomonas campestris* pv. *oryzae* (PXO86 and PXO99)

Growth stage ^x	PXO86							PXO99						
	Cultivar							Cultivar						
	Cisadane	BR51-282-8	IR40	IR28	IR9101-46	TN1	Mean	Cisadane	BR51-282-8	IR40	IR28	IR9101-46	TN1	Mean
MT	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	14.3 a
B/F ^y	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	7.9 d
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	7.9 d
Mean	6.0 d ^z	8.4 c	8.2 c	8.6 c	14.2 b	16.0 a	10.2	5.8 d	8.9 c	9.0 c	9.0 c	12.4 b	15.0 a	10.0

^wInoculum concentration = 1×10^9 cfu/ml.

^xMT = maximum tillering, B/F = booting/flowering, and PF = 2 wk postflowering.

^y Flowering is for each cultivar, respectively, 103, 85, 88, 68, 82, and 75 days after sowing.

^z Standard error of the mean for cultivars is 0.351, for growth stages is 0.248. Values are means of three replications, eight plants per replication mean.

DISCUSSION

Differences in resistance of six rice cultivars to *X. c. oryzae* were detectable at all plant ages tested. The cultivars could be clearly divided into two groups, those that were susceptible and those with a moderate level of resistance. The difference between these two groups was more apparent with strain PXO99, the more aggressive of the two strains, than with strain PXO86. Differences among cultivars of the moderately resistant group were difficult to ascertain at all plant ages, and there was no evidence that differences were significantly easier to detect in older plants than in younger plants. The group of cultivars tested here varied in maturity by about 2 wk. The relative levels of resistances of the six cultivars did not change in the period when some cultivars were flowering and some were not.

In all cultivars, susceptibility to *X. c. oryzae* decreased as the plant increased in age. The largest decrease in lesion length occurred fairly early, between 30 and 42 days after seeding. The decrease was less evident after maximum tillering, but flag leaves were more resistant than leaves at booting stage.

This decrease was uniform for all cultivars when comparisons were made with the measured lesion lengths. However, the decrease can also be expressed relative to the length of the lesions in the most susceptible stage; in this case, the plants tested 30 days after sowing. When expressing the lesion length of the most resistant stage, 77 days after sowing, as a percentage of this susceptible stage, symptoms on the intermediate cultivars were reduced to 20–40% of the original values, whereas symptoms on the two susceptible cultivars remained at 50–80% of the original values. This manner of assessing resistance assumes that the relative importance of a decrease of 1-cm lesion length varies with the original level of resistance. Assessment of results using the measured lesion lengths does not make this assumption.

A similar trend was found by Kauffman et al (5) and Ezuka et al (3) for susceptible and intermediately resistant

cultivars. Mew et al (10) also found that lesions on susceptible cultivars inoculated 90 days after sowing were shorter than those on the same cultivars inoculated 30 days after sowing. They referred to this as a “shift from very susceptible to susceptible” and did not consider this related to race-specific seedling resistance to *X. c. oryzae*, which is stable over the entire growing season (8).

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 attributable to the adult plant resistance reported by Mew et al (10) and Qi and Mew (13,14). This adult plant resistance is typified by a change from a susceptible reaction in the seedling stage to a resistant to highly resistant reaction at booting. In cultivars with adult plant resistance (monogenically inherited), the shift occurs between 60 and 80 days after sowing (10,13), although in certain cultivars with presumptive adult plant resistance (unknown inheritance), the change occurs earlier and is more gradual (13).

The somewhat longer lesions on immature compared with mature leaves are probably attributable to factors other than the quantitative resistance reported here. The quantitative resistance in moderately resistant cultivars was already expressed in the leaves before they reached their fully extended size. No difference was found between the reaction of fully extended leaves shortly after extension and leaves of the same leaf position but already closer to senescence on older plants.

Mew et al (10) reported that immature leaves gave inconsistent reactions. They also reported that lesions on the second and third leaf from the top were sometimes shorter than lesions on the top leaf. They confused leaf maturity with leaf position; a test reported here is a more precise indication of the effect of leaf maturity on susceptibility. The results of Mew et al (10) go against the general trend of decreasing lesion length with both increasing plant age and leaf maturity and may indicate that the top

leaves inoculated by them were not completely extended at the time of inoculation.

It appears that plant age does not greatly affect our ability to distinguish among intermediately resistant cultivars. This would indicate that screening for quantitative resistance to *X. c. oryzae* can be done at all stages of growth. In case where cultivars differ greatly in dates of maturity, it is better to test sometime between 50 and 70 days after sowing, because changes in susceptibility are small in this period. The strain chosen should be both virulent and highly aggressive on all cultivars tested.

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