537-541.

AN IMPROVED TECHNIQUE FOR EVALUATING RESISTANCE OF RICE VARIETIES TO XANTHOMONAS ORYZAE

H. E. Kauffman, A. P. K. Reddy,
S. P. Y. Hsieh, and S. D. Merca

Plant Pathologist, IRRI, P.O. Box 583, Manila, Philippines. Plant Pathologist, AICRIP, Rajendranagar, Hyderabad-30, A. P., India. Plant Pathologist, National Chung Hsing University, Taichung, Taiwan. Research Assistant, IRRI, P.O. Box 583, Manila, Philippines.

ABSTRACT

A new clipping technique for inoculating rice (Oryza sativa) with Xanthomonas oryzae, the causal organism of bacterial blight, consists of clipping off the tips of rice leaves with a pair of scissors whose blades have been dipped in bacterial suspension. Five 60- to 80-day-old plants can be successively inoculated after dipping the scissors into a bacterial suspension containing 10^9 cells/ml. Although the technique primarily measures tissue susceptibility, good correlation exists between tissue susceptibility and natural infection. Compared with earlier methods, the clipping method is more efficient, allows diseased leaf extract to be more easily used as inoculum, and gives more consistent results from field trials.

Plant Dis. Reptr. 57: 537-541.

Bacterial blight of rice, caused by Xanthomonas oryzae (Uyeda & Ishiyama) Dowson, has become a serious disease in Asia during the past few years since the introduction and widespread growing of the nitrogen-responsive, high-yielding, semidwarf varieties. Chemical control of this disease has not been effective in tropical Asia, and it has been only moderately effective in Japan. Research efforts for controlling the disease have centered on the development of resistant varieties.

In addition to field screening under natural conditions, three artificial methods have been used to evaluate varietal resistance to the disease: 1) immersing seedlings in a bacterial suspension (1, 4, 7), 2) spraying bacterial suspensions on seedlings (7, 9), and 3) pricking the leaves with a pin or needle (1, 2, 5, 8), or combinations of the above (6, 9). The pinprick method is the one most frequently used.

MATERIALS AND METHODS

In the greenhouse studies, single rows of varieties Zenith (moderately resistant) and JC 70 (highly susceptible) were sown in 20-mm pots. The seedlings were inoculated 25 to 30 days after seeding. In the field tests, varieties IR8 (susceptible) and IR20 (resistant) were grown in seedbeds and transplanted to the field 21 days after seeding. They were fertilized with 75 kg/ha nitrogen at planting and with an additional 25 kg/ha at panicle initiation. Spacing of these varieties in the field was 20 cm both within and between rows.

Xanthomonas oryzae isolate Pxo 25 was used in most studies. This isolate was collected from the IRRI farm 1 month before these studies were conducted. Tests showed it to be the most virulent of 40 isolates collected from the IRRI farm and from surrounding farmers' fields. The isolate was maintained on Wakimoto's medium (11). We prepared 48-hr cultures of bacterial suspensions by adding sterile distilled water to the surface of the agar media and adjusting the bacterial population to 10^9 cells/ml. All inoculations were made within 1 hr after preparing the bacterial suspension. Scissors used in clipping the leaves were surgical scissors with 3-inch blades.

In the greenhouse the inoculation method consisted of dipping the scissors in the bacterial suspension, grasping the seedlings in the pot with one hand, and clipping simultaneously. In the field each plant with 20 to 30 leaves was grasped with one hand and the tops of all leaves were clipped off simultaneously. A score chart was devised to evaluate leaves inoculated by the clipping method (Fig. 1). The score chart of Ou, et al. (5) was used to evaluate those inoculated by the pinprick method.

RESULTS

Symptom development: Disease symptoms first appeared 4 to 5 days after inoculation. The initial symptoms were leaf curling near the cut-off portion. The curling was much more pronounced on susceptible varieties than on resistant varieties. Water-soaked lesions soon developed from the cut surface and advanced down the leaf. By 14 days the lesions on leaves of resistant plants had usually advanced 1 to 2 mm from the cut surface; on susceptible varieties they had advanced 15 to 20 cm. On highly susceptible varieties the lesion advanced down the leaf sheath and the new emerging leaves were pale yellow.

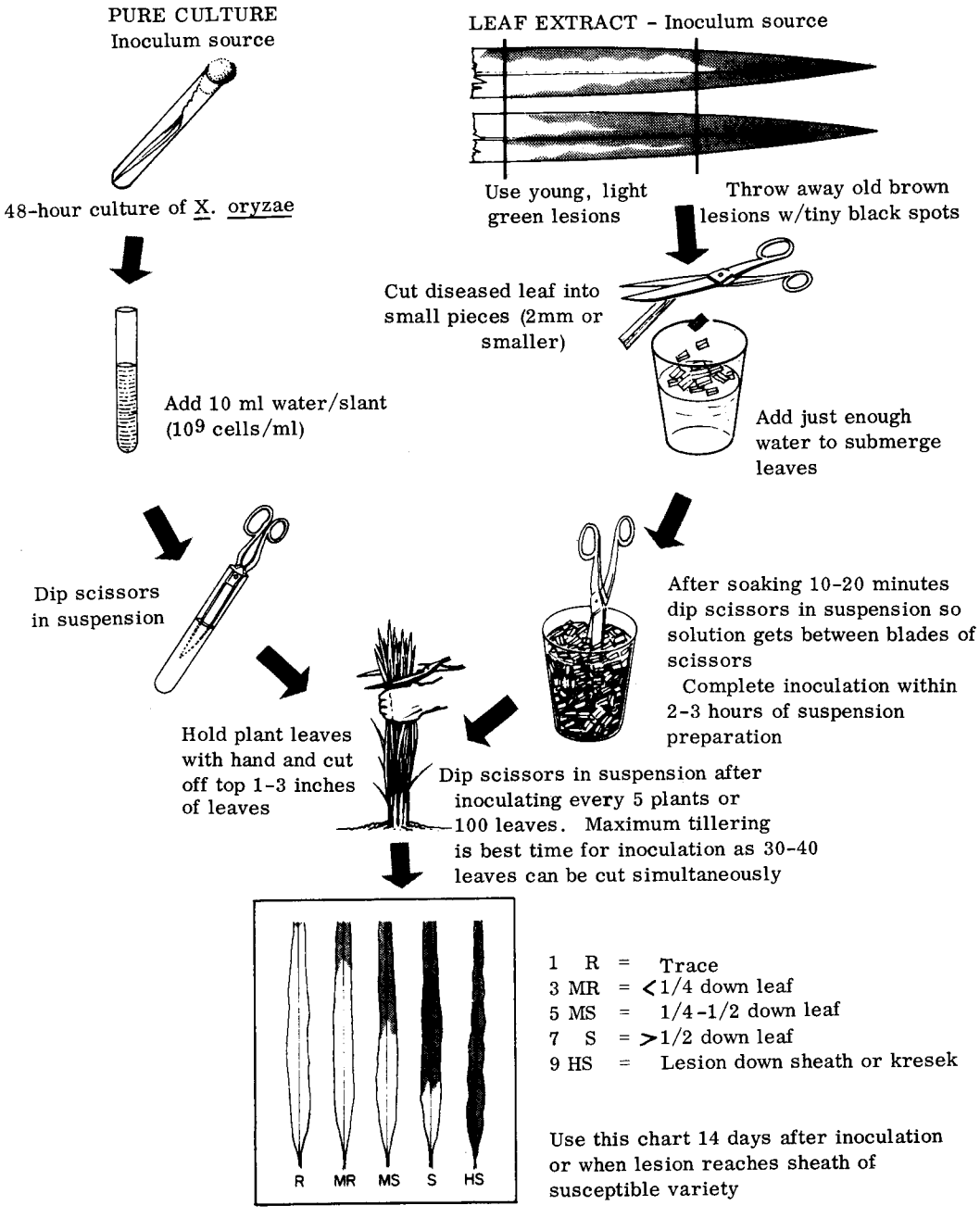
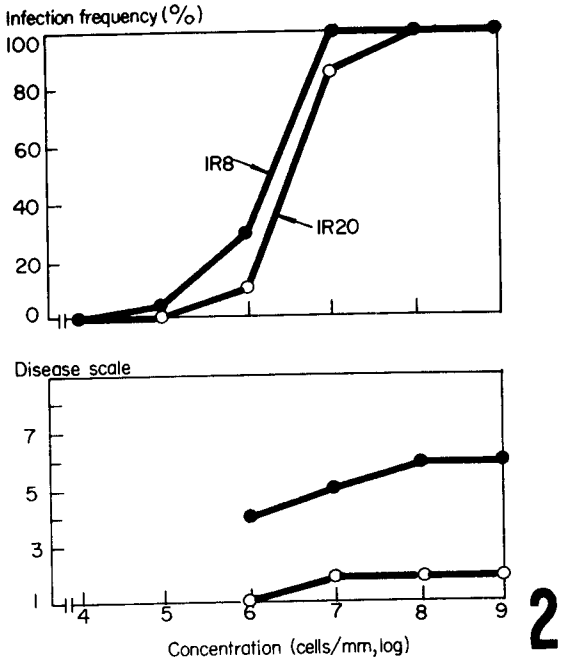


FIGURE 1. BACTERIAL LEAF BLIGHT INOCULATION: CLIPPING METHOD



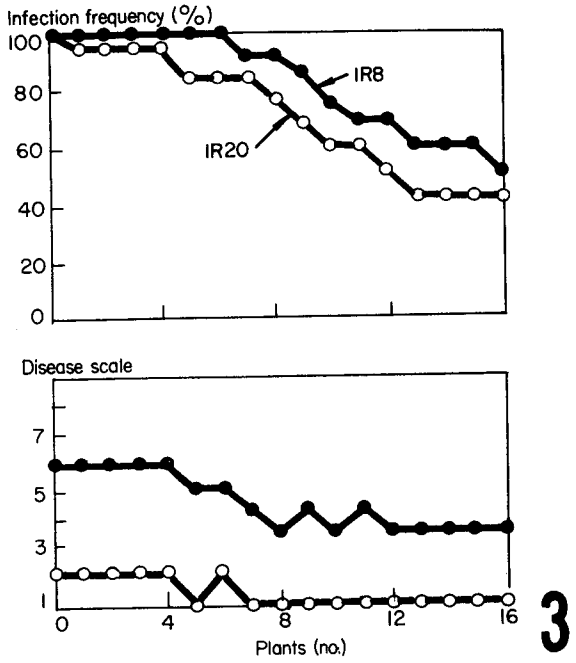
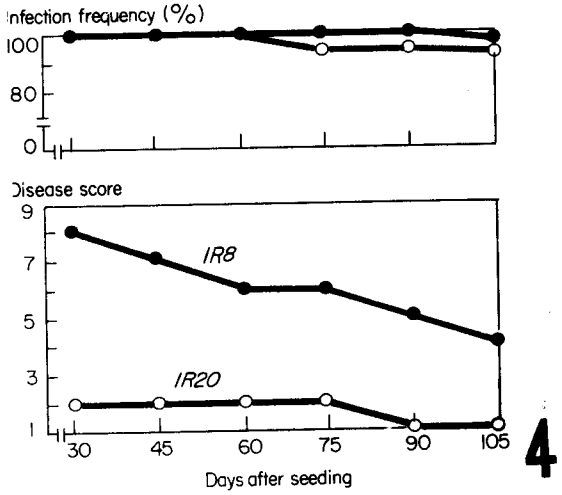


FIGURE 2. Effect of bacterial concentration on infection frequency and disease score on IR20 (resistant) and IR8 (susceptible) at 90 days after sowing.

FIGURE 3. Effect of serial inoculation on infection frequency and disease score of IR8 (susceptible) and IR20 (resistant) inoculated at 90 days after seeding.

FIGURE 4. Effect of plant age on infection frequency and disease score of IR8 (susceptible) and IR20 (resistant).



Clipping versus pinprick inoculation method: Because the pinprick method is frequently used for screening varieties and studying bacterial virulence, we compared it with the clipping method in greenhouse tests. In plants inoculated by the clipping method, symptoms usually developed 2 to 3 days earlier than in those inoculated by the pinprick method; however, because infection was established in the center of pinprick-inoculated leaves, the lesions had a short distance to move to the leaf sheath. Thus, scores from both inoculation methods were similar. The clipping method, however (Table 1), gave significantly lower variance than the pinprick method on both JC 70 (susceptible) and Zenith (moderately resistant). The position of the inoculated leaf did not significantly affect the disease score in the two varieties when inoculated by either method.

A weakness of the pinprick inoculation tests has always been the variable results obtained by different persons doing the inoculation and scoring. Comparative tests were run by two individuals to see which method gave the least variation. With the clipping method the two individuals obtained similar disease scores. From pinprick inoculation tests, however, they obtained significantly different disease scores on the susceptible variety but similar scores on the resistant variety (Table 1).

Table 1. Comparison of variations of disease reaction on a susceptible and a moderately resistant rice variety caused by inoculation method, leaf position, and the personnel factor in a greenhouse experiment.

Inoculation method and leaf position	Experiment no. 1[a]			Experiment no. 2		
	Avg score	S	CV(%)	Avg score	S	CV(%)
Clip inoculation			JC 70 (susceptible)			
1st leaf[b]	8.85	0.250	6	8.87	0.100	4
2nd leaf	8.85	0.250	6	8.87	0.100	4
Pinprick inoculation						
1st leaf	7.40	2.525[c]	22	8.73	0.367	7
2nd leaf	7.95	1.100	13	8.80	0.167	5
			Zenith (moderately resistant)			
Clip inoculation						
1st leaf	3.40	0.225	14	3.47	0.433	19
2nd leaf	3.70	0.900	26	4.00	0.563	19
Pinprick inoculation						
1st leaf	3.07	1.167	19	3.30	0.767	26
2nd leaf	3.00	4.067	67	3.67	0.633	22

Significantly different from corresponding value of Exp. no. 2 at 5% and 1% levels, respectively.

[a]Experiments no. 1 and 2 conducted by two different individuals.

[b]1st leaf equals top inoculated leaf.

[c]Underlined values are significantly higher than corresponding value of other inoculation method.

Effect of bacterial concentration: Bacterial populations of 10^7 cells/ml or greater in the inoculum suspension were adequate to give nearly 100% leaf infection on resistant and susceptible varieties (Fig. 2). Below 10^7 cells/ml, infection frequency declined rapidly. The disease scores on both IR8 and IR20 decreased and the incubation period was slightly longer with lower inoculum concentrations.

Effect of serial inoculations: IR8 and IR20 were used as test varieties to determine how many plants could be successively inoculated between dippings in the bacterial suspension (Fig. 3). We found that five plants could be successively inoculated before the infection frequency began to decline. Disease score and incubation period were rather uniform for the first five plants, but the following plants had lower disease scores and longer incubation periods.

Effect of plant age: When plants of IR8 and IR20 were simultaneously inoculated at six different growth stages, infection frequencies were nearly 100% at all ages (Fig. 4). Disease scores on IR20 were only one point less at the flag leaf stage (105 days) than at the seedling stage (30 days). Thus, the disease score on the resistant variety was not greatly influenced by plant age. On IR8, however, the disease score was strongly influenced by plant age. The flag leaf (105 days) score was 4 and the seedling (30 days) score was 9, when the seedling was killed by the disease. Other resistant and susceptible varieties were inoculated and, in general, all varieties tended to become slightly more resistant toward maturity, with the greatest change occurring in the susceptible plants.

Clipping inoculation technique and natural infection: In a replicated yield trial 135 entries were inoculated 60 days after seeding on one side of each plot. Disease scores on the leaves were taken 14 days after inoculation. The other side of each plot was inoculated 80 days after seeding. Disease scores on the secondary spread were taken 4 weeks later to assess the natural infection visually on the basis of percentage of leaf area showing natural infection.

The clipping inoculation score and the natural disease score were highly correlated. All entries susceptible in the first inoculation test were susceptible to the secondary disease spread except for four entries which showed only limited disease spread. All varieties resistant to the clipping inoculation test were also resistant to the secondary spread of the disease.

Pure cultures versus leaf extract for inoculum: Leaf extract from infected leaves readily caused infection when used as the inoculum source. Young developing lesions, however, gave higher populations of X. oryzae and better infection than old lesions, because competition with saprophytic bacteria was minimized. Populations of 10^9 cells/ml of X. oryzae were readily obtained by cutting infected leaves and submerging the pieces in an equal volume of water. Suspensions of leaf extract gave disease scores equal to those of pure cultures, although they usually had a slightly longer incubation period.

DISCUSSION

Although the clipping technique effectively induces infection by various bacterial diseases (3, 11, 12), it has not been generally used on a large scale to screen for varietal resistance. In our studies the clipping inoculation technique had many advantages over the pinprick technique for inoculating rice with X. oryzae. Its inoculation procedure was more efficient and resulted in more reliable evaluation of infection. Disease scores from leaf to leaf and from plant to plant also varied less for the plants inoculated by clipping than for those inoculated by the pinprick method.

The studies indicated that, for mass inoculations, bacterial concentrations of 10^9 cells/ml should be used and that up to five plants can be successively inoculated before the scissor blades are redipped in the bacterial suspension. It was not necessary to inoculate single leaves inasmuch as inoculating whole plants gave high leaf infection frequency. Plants could be inoculated at any age, but the optimum time appeared to be at maximum tillering (60- to 80-day-old plants) when 20 to 30 leaves could be simultaneously inoculated. A large number of leaves gave ample replication, resulting in valid disease readings.

Plant age influenced disease scores of the susceptible variety more than of the resistant variety, although the susceptible variety was distinctly more susceptible than the resistant variety at all ages. The most dramatic differences between the two, however, resulted when the plants were inoculated at 30 to 75 days after seeding. Age has also been reported to influence development of bacterial diseases in other crops (10, 12).

Varietal reaction induced by the clipping method and that induced by natural infection were highly correlated. The few entries that did not give high correlation were susceptible in the early stages of growth but tended to show resistance at later stages.

The simplicity and reliability of the clipping technique has special significance for rice workers in Asia, as screening trials and virulence studies can now be conducted in many locations. Research workers on small experimental stations can use leaf extracts for screening trials even though they do not have facilities for isolating and maintaining pure cultures of the bacterium. They must, however, first test and adapt the procedures to best suit their local conditions.

Literature Cited

1. CHIEN, C. C., and Y. C. HUNG. 1970. Studies on the bacterial leaf blight of rice plant. I. Discussion on the inoculation methods and the selection of resistant rice varieties. J. Taiwan Agr. Res. 19(1): 46-50.
2. KAUFFMAN, H. E., and R. S. K. V. S. PANTULU. 1972. Virulence patterns and phage sensitivity of Indian isolates of Xanthomonas oryzae. Ann. Phytopathol. Soc. Japan 38: 68-74.
3. McCARTER, S. M., and C. A. JAWORSKI. 1969. Field studies on spread of Pseudomonas solanacearum and tobacco mosaic virus in tomato plants by clipping. Plant Dis. Reptr. 53: 942-946.
4. MIZUKAMI, T. 1967. Resistance of rice plant to bacterial leaf blight and strains of causal organism. Japan Agr. Res. Q. 1(3): 6-11.
5. OU, S. H., F. L. NUQUE, and J. P. SILVA. 1971. Varietal resistance to bacterial blight of rice. Plant Dis. Reptr. 55: 17-21.
6. PADMANABHAN, S. Y. 1969. A mass screening technique against bacterial blight of rice caused by X. oryzae. Indian Phytopath. 22: 396-398.
7. REITSMA, J., and P. S. J. SCHURE. 1950. "Kresek" and bacterial disease of rice. Contrib. Gen. Agr. Res. Sta. Bogor No. 117: 17.
8. SAKAGUCHI, S., S. TAKASHI, and N. MURATA. 1968. Studies on the resistance to bacterial leaf blight, Xanthomonas oryzae (Uyeda et Ishiyama) Dowson, in the cultivated and wild rice. Bull. Nat. Inst. Agr. Sci. D(18): 1-29.
9. SRIVASTAVA, D. N., Y. P. RAO, J. C. DURGAPAL, J. K. JUNDAL, and W. SINGH. 1967. Screening rice varieties for resistance to bacterial blight. Indian Far. 16: 25-28.
10. STRIDER, D. L. 1970. Tomato seedling inoculations with Corynebacterium michiganense. Plant Dis. Reptr. 54: 36-39.
11. WAKIMOTO, S. 1967. Strains of Xanthomonas oryzae in Asia and their virulence against rice varieties. In Proc. Symposium Trop. Agr. Res., Ministry Agr. and Forest. pp. 19-24.
12. WINSTEAD, N. N., and A. KELMAN. 1952. Inoculation techniques for evaluating resistance to Pseudomonas solanacearum. Phytopathology 42: 628-634.