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METHODS OF SCREENING RICES FOR VARIETAL RESISTANCE TO CERCOSPORA LEAF SPOT

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METHODS OF SCREENING VARIETAL RESISTANCE TO *CERCOSPORA* LEAF SPOT OF RICE[1]

ABSTRACT

Autoclaved rice stem nodes with prune juice provide a favorable medium for the isolation of *Cercospora oryzae*, the fungus causing *Cercospora* leaf spot or narrow brown leaf spot, and for the mass production of spores spraying of spores in late afternoon resulted in good infection on plants in the field. As shown by artificial inoculation, rice at different stages of growth is susceptible to *C. oryzae*. An incubation period of 20 days is necessary before the first few lesions appear and 30 days before the maximum number of lesions is reached. The slow growth of the fungus causes the late appearance of the disease, generally observed in the field. A method of artificial inoculation for screening varieties for resistance and a disease scale of 0 to 9 units, based upon the number of lesions, are suggested.

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METHODS OF SCREENING VARIETAL RESISTANCE TO *CERCOSPORA* LEAF SPOT OF RICE

The rice disease *Cercospora* leaf spot, or narrow brown leaf spot, caused by the fungus *Cercospora oryzae* (=*Sphaerulina oryzae*), occurs practically anywhere the crop grows. Its occurrence or severity, however, may vary from place to place. In the 1930's and 1940's, it was a serious problem for rice farmers in the United States, particularly in Arkansas, Alabama, Louisiana, and Texas (Tullis 1937, Ryker 1947).

Some of the early investigators of *C. oryzae* concentrated on the identification of physiologic races and reported the occurrence of several races of the fungus (Ryker 1943, 1947). Other researchers advocated the development of *Cercospora*-resistant strains of rice through hybridization (Jodon 1954, Ryker 1941, Ryker and Cowart 1948), but most of the crosses produced were not resistant to all the races.

At the International Rice Research Institute (IRRI), the disease has become prevalent in many varieties and breeding lines (Fig. 1). Some resistance to *C. oryzae* has been observed in the field, but we feel it may not indicate a variety or line's true reaction to the disease because of the nonuniformity of the inoculum and the effect of environment. A method for judicious evaluation of varietal resistance to the disease is imperative.

We describe procedures for screening varieties for resistance to *Cercospora* leaf spot, including isolation, inoculation, and a scale for measuring disease intensity.

ISOLATION - THE NODE TECHNIQUE

C. oryzae is usually difficult to isolate because of its characteristic slow growth. Of several isolation methods tried, the *node technique* is satisfactory. The procedure involves the preparation of two kinds of media -- the rice stem node enriched with prune juice, and an agar medium. The stem node medium consists of fresh rice straws cut into 2.5 cm-long pieces with the node at the center. Each node is placed in a small test tube containing 0.5 ml prune juice and sterilized. The prune juice is prepared by boiling two dry prunes in 1 liter of water for 15 minutes and

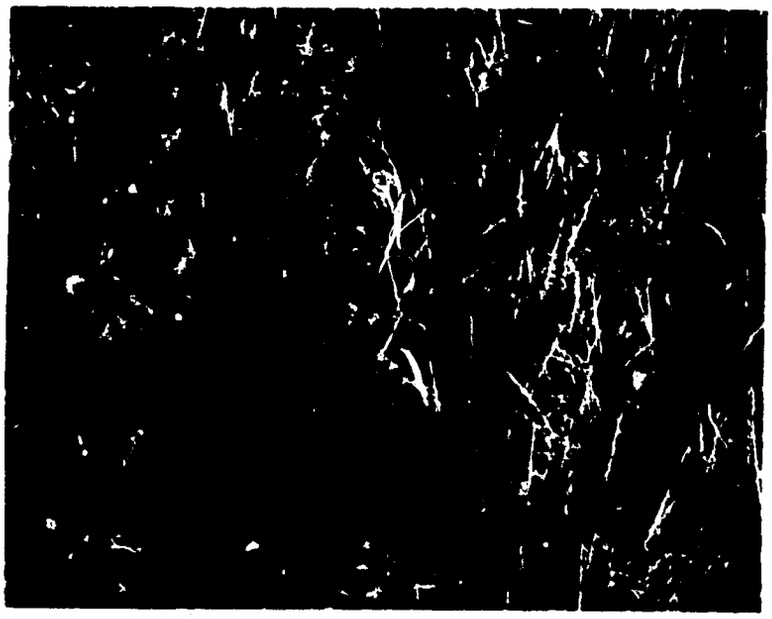

Fig. 1. *Cercospora* leaf spot infected plots, showing severely infected leaves (left) and susceptible and resistant breeding lines (above). IRRI, 1977.

adding 5 g sucrose and 1 g yeast extract. The agar medium is any one of the common agar medium, such as potato-dextrose, prune, V-8 juice, bean, or rice straw agar.

Fresh specimens of infected leaves are cut into sections (about 2 x 5 mm) and surface-sterilized for 10-20 seconds in a 5% calcium hypochlorite solution. Each section is placed on the surface of a node and incubated 4 or 5 days. Fungal growth usually appears on the surface of the node and sides of the straw. Most sections with profuse fungal growth on the second and third day are contaminants.

After 4 or 5 days, the sections showing slow mycelial growth are transferred with a needle or loop to a common agar slant. Growth is observed for another 4 or 5 days to check bacterial contaminants. Our trials indicated an average 50% chance of success for this isolation procedure.

Sporulation

Although *C. oryzae* grows and sporulates on bean (Ryker 1943), potato, soybean, and rice straw agar (Tasugi and Ikeno 1956), the spores produced

may not be sufficient to inoculate the large number of plants involved in screening rice varieties for disease resistance. In addition, sporulation may not occur at the right time.

The rice stem nodes used in the isolation technique induces sporulation. They are placed in 250-ml Erlenmeyer flasks filled with 10-15 ml of water and are sterilized for 1 hour. A suspension of spores and mycelial fragments is prepared by adding about 10 ml sterile prune juice to each test tube of pure culture slants and scraping the fungal growth off the surface of nodes with a sterile glass rod. The suspension is poured into each flask; the flask is shaken slightly to distribute the mycelial fragments in the medium. The seeded flasks are incubated at room temperature. Abundant spores are observed 4 days after seeding and spores are produced continuously for 2 weeks or more.

The prune-juice node medium produces spores earlier and in much greater quantity than the agar medium.

INOCULATION TESTS

In the field, *Cercospora* leaf spot occurs as the rice plants approach maturity. Two inoculation experiments were carried out to determine whether young plants, besides older ones, can be infected.

In the first test, 30-day-old seedlings of IR2307-64-2-2 grown in a seedbox in the screenhouse were sprayed with a spore suspension of a 12-day-old culture (Spore density: about 65 spores/microscopic field of low-power (10x) objective) and placed in a moist chamber overnight.

This inoculation test showed that the disease is not confined to maturing plants. Lesions appeared on test seedlings 21 days after inoculation. The incubation period was much longer than that of most fungal organisms.

Ryker and Jodon (1940), however, observed that the period of incubation (10-15 days) was shorter on susceptible US varieties, such as Blue Rose, Early Prolific, and Honduras and longer (19-40 days) on highly resistant varieties. Tullis (1937), on the other hand, reported a 14-day incubation period on Blue Rose, when the inoculation was done on detached leaves.

In second test, IR1487-372-1-1 plants, at 102, 81, 60 and 39 days after seeding in pots in the greenhouse, were inoculated with a spore suspension of a 12-day old culture (spore density: 65 spores per 10x microscopic field) outside the greenhouse at sundown. The procedure was similar that of Ryker (1943), but the plants were brought inside the greenhouse the following morning.

Rice plants of all stages of growth showed different numbers of lesions (Table 1). The severity of the disease did not differ much among plants of different ages, indicating more positively that *C. oryzae* can attack the rice plant at all growth stages. Our results tended to corroborate the

Table 1. Average number of lesions on IR1487-372-1-1 plants inoculated at four stages of growth.

Age of plant at inoculation	Lesions[a] (no./infected leaf per tiller)		
	23 DI	28 DI	33 DI
102 days	67.1[b]	160.5	196.0
81 days	80.7	163.7	199.6
60 days	59.6	161.3	182.3
39 days	52.9	-	-

[a] DI = days after inoculation. [b] Av. of 10 tillers.

findings of Ryker (1943) whose repeated inoculations failed to show any apparent difference in the reaction of seedlings and mature plants to the fungus. The maximum number of lesions was reached 30 or more days after inoculation in our tests. In the incubation period, regardless of the growth stage at which the plant was inoculated, was about 3 weeks. The long incubation and slow development of the lesions may account for the late appearance of the disease in the fields.

FIELD SCREENING TEST

In the field we tried the greenhouse inoculation technique to find method appropriate for screening large numbers of varieties and breeding lines for resistance to *C. oryzae*.

Two susceptible varieties, three moderately resistant, and, five resistant varieties (Table 2) were planted in the field during the 1976 dry season. At late booting stage, two rows of each variety were inoculated in the late afternoon with a spore suspension (60-70 spores per microscopic field of the low power (10x) objective) of a 12-day-old culture of *C. oryzae*; five rows were not inoculated. Lesions on flag leaves of inoculated plants were counted about 5 weeks after inoculation. Lesions on uninoculated plants with natural infection were likewise counted. Results of a comparison between inoculated and uninoculated plants of all the susceptible, moderately resistant, and resistant test lines showed the efficiency of the inoculation method (Table 2).

In the 1977 dry season, field screening of 76 varieties and elite breeding lines for *Cercospora* leaf spot resistance was started. Uninoculated rows for each variety or line were provided to check further the reliability of the inoculation method. The differences in percentages of resistant,

moderately resistant, and susceptible reactions on both inoculated and naturally infected entries clearly demonstrated the possibility of efficiently screening a large number of varieties (Table 3).

Methods of isolation, sporulation, and inoculation are illustrated in Figure 2.

Table 2. *Cercospora* lesion count (flag leaves) on artificially inoculated and naturally infected susceptible, moderate and resistant lines in the field.

Line	Inoculated		Natural infected	
	Number of lesions	Disease[a] scale	Number of lesions	Disease[a] scale
Susceptible				
IR1487-372-1-1	184.8	7	49.8	5
IR3184-62-1-2	219.5	9	19.4	3
Moderately resistant				
IR2805-72-5-3	58.5	5	21.2	3
IR2562-61-5-2-1	35.5	3	5.4	1
IR2798-99-3-2	30.2	3	7.1	1
Resistant				
IR2061-281-53-1	7.0	1	3.4	1
IR2071-843-1-1-6	5.6	1	2.1	1
IR2808-21-3-2	5.1	1	0.4	1
IR2070-132-2-2-5	2.5	1	0.9	1
IR2836-78-4-2	1.8	1	0.5	1

[a] Infection was rated on a scale of 0 to 9.0 = no lesions, 9 = leaf completely covered. See also Figure 3.

Table 3. Summary of reactions of 76 varieties and elite breeding lines to the *Cercospora* leaf spot inoculation test in the field. IRRI, 1977 dry season.

Reaction	Inoculated entries		Naturally infected entries	
	No.	%	No.	%
Resistant	40	52.6	51	67.1
Moderately resistant	12	15.8	23	30.3
Susceptible	24	31.6	2	2.6

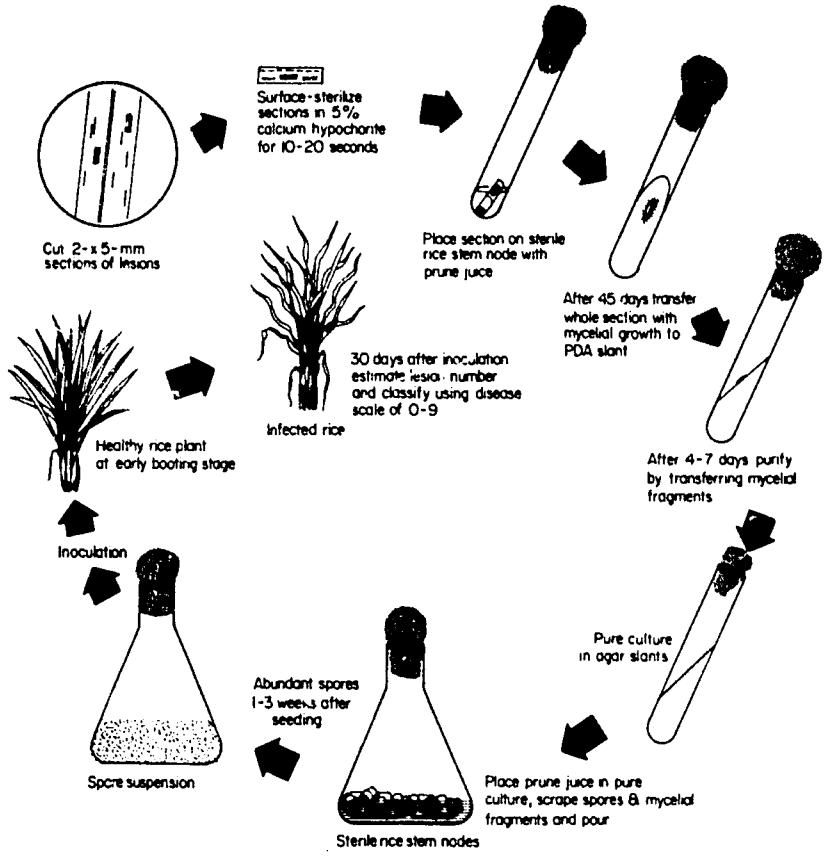

Fig. 2. *Cercospora oryzae* isolation and sporulation for mass screening for resistance.

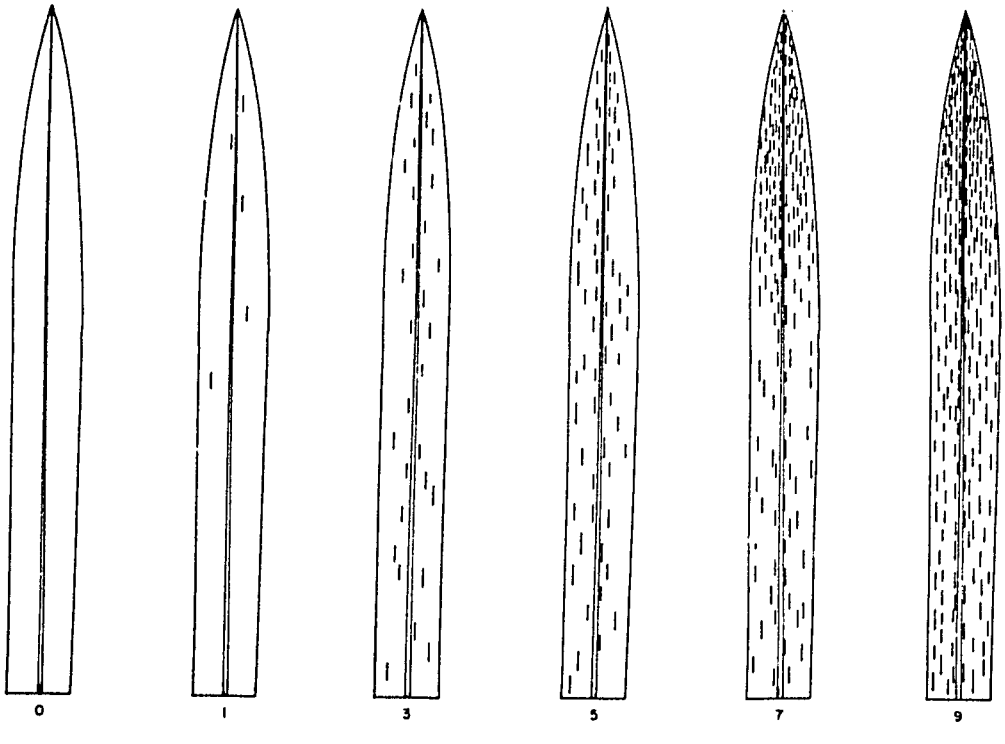

Fig. 3. Disease scale for scoring *Cercospora* leaf spot.

DISEASE SCALE

Varietal resistance to *Cercospora* leaf spot (disease intensity) may be expressed both by number and by type of lesions. Jodon et al (1944) used size of lesion and length of incubation period as the criteria for evaluating resistance and susceptibility. Chakrabarti (1964), who scored field infection on a scale of 1 to 3, used number of lesions.

Resistant varieties usually show darker brown, narrower, and shorter lesions than susceptible varieties but the differences are small and difficult to distinguish. Moreover, we observed different lesion types on the same leaf blades. Number of lesions is used, therefore, as the criterion for varietal resistance. The disease intensity may be scored on a scale of 0 to 9 (Fig. 3).

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