

PHYTOPATHOLOGICAL NOTES

An Improved Method for Evaluating Rice Sheath Blight

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ABSTRACT

A new reliable stem-tape-inoculation (STI) method to screen rice cultivars for resistance to sheath blight has been developed and found successful. The pathogen had no effect on seed germination of rice. It does not need any injury for infection on rice plants. *Phytopathology* 65:214-215

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Rice sheath blight was reported for the first time from Japan in 1910 (4). The disease has since been reported from Taiwan, China, India, and Sri Lanka (5). Wu in Taiwan (8) estimated loss between 14-17%. Kozaka (3) reported that 30-40% of the cultivated rice area in Japan was affected by sheath blight. Ezuka et al (2) had inoculated seedlings by inserting agar culture inside the sheath at 7-8th leaf stage. They (2) also inoculated field-grown plants by placing culture on rice straws around the stem. Chang (1) inoculated nursery plants during the tillering stage by placing culture on straws between the tillers. Wu (8) sprayed culture inoculum on the basal parts of 30- to 40-day-old transplanted plants. Tu (7) inserted the sclerotium from one-month-old potato-dextrose agar (PDA) culture to the inner side of the leaf sheath. A seedling screening similar to uniform blast nursery was used at The International Rice Research Institute (IRRI) (5). The purpose of this investigation was to evaluate several inoculation methods, and to evolve an efficient and reliable method for mass screening.

The pathogen was conveniently isolated as described here. Diseased specimens, natural or inoculated, were washed in water for 10 minutes and rinsed with distilled water for 5 minutes. About 2-4 mm rectangular pieces of healthy sheath tissue adjoining diseased area were cut with a sterilized blade, immersed in absolute alcohol for 1-2 minutes, then, surface sterilized in aqueous 1.25% sodium hypochlorite for 2 minutes. Surface-sterilized pieces were transferred to petri plates containing 2% water agar without any wash and incubated for 40 hours. Hyphal tips of fast-growing mycelium which grew from the tissue were aseptically transferred to peptone-sucrose-agar (PSA) slants (peptone 10 g, sucrose 10 g, sodium glutamate 0.5 g, agar 15 g, grams per liter distilled water, pH 6.8).

The rice sheath blight pathogen grew very rapidly on PSA, PDA, and other common laboratory media at 25-30 C, and entirely covered 10-cm diameter petri plates within 48 hours. On PSA at 25-30 C, the mycelium initially was white, then slowly turned brown. The septal pore apparatus was observed clearly in older cells under phase contrast microscope. The mycelium became entangled, formed loose spherical white sclerotia that gradually turned a salmon (brown) color. The sclerotia were 4-5 mm in diameter and irregular in shape. The pathogen has been identified under the broad concept discussed by Parmeter and Whitney (6) as *Rhizoctonia solani* Kühn, and it has been deposited with the Commonwealth Mycological Institute, Kew, England, under IMI 165563.

For pathogenicity tests and inoculation methods, inoculum was either grown on PSA or on autoclaved rice stem pieces in petri plates. About 1-cm-long stem pieces were immersed in 1.0% dextrose solution for about 5 minutes, then transferred to Erlenmeyer flasks. Rice stem pieces which occupied ~ 30% air space of flasks, plugged with cotton and autoclaved at 1 atmosphere pressure (15 psi) for 20 min. These were inoculated by stock culture of *R. solani* and incubation of 10-12 days at 25-30 C was sufficient to produce satisfactory inoculum.

Soil infestation tests in plastic pots (15 × 15 × 20 cm) containing autoclaved soil in greenhouse and in nonautoclaved nursery soil under field conditions were carried out by placing infested rice straw inoculum between the rows of sown surface-sterilized seeds of several cultivars of rice. Seeds germinated quite well even though the pathogen was near by. Typical brown sclerotia were developed on the lower sheaths of the seedlings. About 14% of the seedlings died, but disease expression in the seedlings condition was not satisfactory.

Soil infestation around transplanted 6-week-old seedlings (3 weeks after transplanting) of rice cultivars Padma, IR 22, Jaya, Cauvery, Vijaya, Pusa 2-21, Bala, and BJ-1 was done by placing inoculum near the stems of 10 test plants per cultivar under greenhouse conditions. High humidity was provided in a chamber for 6 hours per day for 3 days. The disease development was very slow and one month after inoculation the average lesion length in 10 plants of each of the cultivars tested was: Padma, 6.5; IR22, 8.1; Jaya, 5.6; Cauvery, 8.0; Vijaya, 5.8; Pusa 2-21, 8.8; Bala, 10.0; and BJ-1, 9.3. Similar tests under field conditions were also carried out on many other cultivars with less success.

Eight cultivars used in the previous experiment were grown in Hoagland's solution with 2.0% agar in 50-ml glass vials. Fourteen-day-old seedlings (three per vial) were inoculated by placing an 8-mm diameter disk of inoculum on PSA around the base of the stem. Controls were not inoculated. Hoagland's solution was replenished as required. Under high humidity, the pathogen was able to grow very well on all cultivars. Mycelial threads were developed between the stems. Leaf tips dried first and progressed toward the base of the leaf base. General drying was from older to younger leaves. Ramifying mycelium with branching typical of *Rhizoctonia* was observed under microscope on small brown necrotic lesions. Although the technique was convenient for determining pathogenicity, it was not suitable for

screening large numbers of cultivars.

Germination of rice seeds in the presence of the pathogen on PSA in petri plates at 10, 15, 20, 25, 30, and 35 C was not affected. However, compared to controls, infected seedlings were dehydrated and stunted. Small brown necrotic lesions on sheaths and leaves of malformed seedlings were not suitable for estimation of the resistance/susceptibility status of cultivars.

The upper portions of stems were cut, inoculum on PSA was placed over the cuts and covered with the Scotch® (3-M Corporation, St. Paul, Minnesota) brand 202-2 masking tape. Brown necrotic lesions were developed on the cut edges of stem, but due to the inherent nature of rice plants inner whorls of stem and inoculum with tape were pushed up. Since the inoculum with the tape was pushed up, the technique was not desirable.

A stem-tape-inoculation (STI) method was developed in which inoculum grown on PSA or on autoclaved stem pieces was placed directly on noninjured sheaths of 6-week-old rice plants either grown in greenhouse or field at about 6-10 cm above the water line. The pathogen did not require a wound to cause infection. The inoculum was covered with Scotch brand 202-2 masking tape or cellophane tape. The tapes were nontoxic to plants. Usually 10 or more plants per cultivar were inoculated. Control plants were treated similarly without the pathogen. Inoculated and noninoculated greenhouse-grown plants were placed in the humidity chamber for 6 hours per day for 3 days, whereas field-grown plants had overhead sprinkler watering for 4 hours per day. Green color of the sheath at the point of inoculation was gradually lost due to dehydration and it developed grey-green oblong lesions (2-3 cm) with definite reddish-brown borders. Lesions coalesce, overlap, and formed typical cobra-skin pattern on inoculated sheaths (Fig. 1). Buff-white to brown sclerotia were developed on inoculated sheaths and leaf blades. Disease development was much faster with this technique than other techniques tried. Probably the chances of survival of the pathogen on the host plants are greater with the STI technique than in standing irrigation water and soil. The STI method was found most convenient for screening of 4,390 entries under field conditions.

The pathogen was reisolated from infected plants which were inoculated by different methods. It was easy to reisolate whenever sclerotia developed on host plants. Reisolated cultures of *R. solani* were tested and found pathogenic on rice plants.

An improved method of sheath blight screening was required because the introduction of dwarf, compact, and high-yielding cultivars has resulted in the disease becoming severe in Kerala, Uttar Pradesh, and Kashmir. The difference in resistance and susceptibility was better in tests conducted on mature plants than in seedling test (1, 2) because the seedlings do not manifest the typical symptoms of disease expression. The STI technique described here, used with overhead sprinkler irrigation has given uniform and consistent results in our screening and epidemiological investigations.

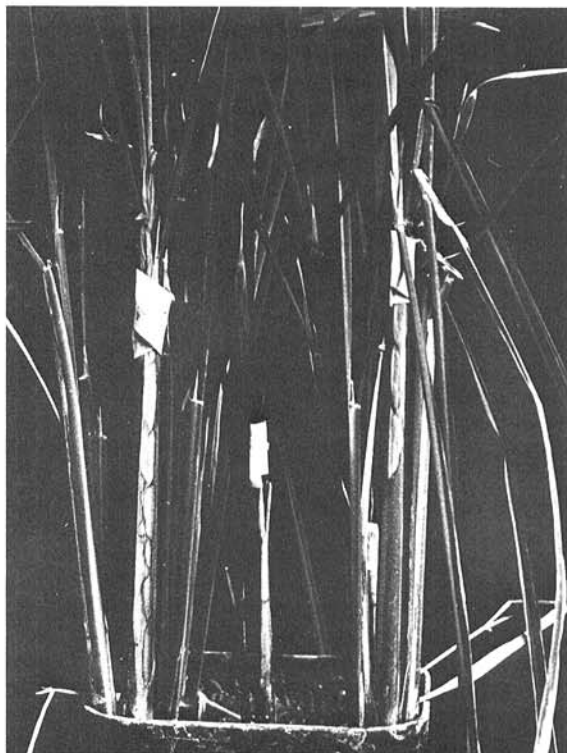


Fig. 1. Overlapping necrotic sheath blight lesions with definite reddish brown borders induced by stem-tape-inoculation (STI) method on greenhouse-grown plants.

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