



Species:	<i>Rhizoctonia solani</i>
Product Class(es):	SBI (DMI) fungicides
Method type described:	radial growth test
Date of protocol:	2022-01
Version	1
comments	This work instruction establishes the procedure for Rice Sheath Blight in-vitro testing.

Sample Collection

Symptoms on rice typically start to develop at or above water level, or above soil level in upland conditions. Pale, greenish-grey, ellipsoidal lesions appear which about 2-3 cm long are initially and have a dark brown margin, but then enlarge and coalesce in combination with a change in color to fawn or off-white with a brown or purplish brown margin. Lesions may be found anywhere on the leaf sheath, initially appearing on the outer sheath but then extending to the inner sheath, and under favorable conditions in humid warm climates they can even be detected on the leaf blade.

Sheath samples showing sheath blight symptoms are collected from the field.

- Best time to collect samples are around 70-80 DAT
- Clean the samples from mud
- Wrap the clean samples with paper towel and insert them into brown bags/envelopes.
- Once in the lab, put them directly in refrigerator (4°C).
- These samples are commonly useable up to 1 month after collection.

Sample Processing and Propagation

Make small size samples by cutting from the middle of the lesion to the nearest green area. These cut samples should not be larger than 1 cm. Wash the cut samples by putting them in a sequence of ddH₂O - ddH₂O - 70% alcohol. Each step is about 15 seconds. Dab them in sterile paper towel. Place samples on semi selective medium (alkaline water agar medium) or water agar and incubate for 24-48 hours. The

confirmation of the right pathogen can be done by observation under microscope. The media will be fully covered with fungal mycelium for 5-7 days after inoculation and then sclerotia will be formed. Once sclerotia or the mycelium is ready, harvest and transfer to PDA in petri dish to enable a single colony culture. Mycelium can be harvested by using a 5 mm cork borer.

Sensitivity Tests

Active Ingredient solution

- Prepare a 1% concentration stock liquid by adding 1 ml difenoconazole into 99 ml ddH₂O.
- Prepare a 1000 ppm AI by adding 40 ml of stock solution into 60 ml ddH₂O
- Transfer 10 ml of this 1000 ppm AI solution into 90 ml ddH₂O to make a 100 ppm AI solution.
- Do the sequential steps to get 10, 1, 0.1 and 0.01 ppm.

Test media

- Prepare 45 ml PDA agar to test sensitivity to difenoconazole.
- Transfer 5 ml from each of the AI solution into PDA to make a 10% dilution.
- The total of 50 ml test solution is then transferred to sterile petri dishes (disposable, d: 90 mm) in 3 replications.

Evaluation and Data Processing

Using a 5 mm cork borer, prepare 21 punches of the isolated pathogen Put one punch in the middle of test media in petri dish for all concentrations, including control (0 ppm). Seal the petri dishes individually by using plastic wrap. Evaluation is performed when fungal growth in control media has reached about 90%, observed by unaided eye. Measure the diameter of the growth. Percentage inhibition calculation is calculated as follow:

$$\% \text{ Mycelial Growth Inhibition (\%MGI)} = \frac{(\text{diameter of control} - \text{diameter of treated})}{\text{diameter of control}} \times 100\%$$

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