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Rapid *in vitro* Screening Method for Evaluation of Rice Genotypes against Blast Disease

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ABSTRACT

Through the use of standard assays, like Uniform Blast Nursery (UBN) it is difficult to screen the varieties for blast resistance round the year. It is also laborious and time consuming to screen the plants under natural conditions. Use of the spot inoculation technique on a detached leaf would allow simultaneous testing of multiple fungal isolates on the same plant in the laboratory. This could shorten the time for development of broad-spectrum blast resistant cultivars. The spot inoculation method allows determination of pathogenicity of *M. grisea* in a controlled environment. All operations, such as fungal culture, inoculation and observations of disease development, can be performed in the laboratory.

Key words : Inoculation, *Magnaporthe grisea*, Pathosystem, Phenotype, Rice Blast,Screening

Pyricularia grisea (Cooke) Sacc is one of the major fungal pathogens of rice *(Oryza sativa* L.) causing rice blast disease (Ou, 1985). The fungus can attack any aerial part of the rice plant, including seeds, in which the fungus may over winter for several years (Ito, 1932 and Manandhar, 1996). The most efficient way to manage rice blast is to grow resistant cultivars. Resistance genes have been identified by performing infection assays on rice plants in the greenhouse using local isolates of the blast pathogen (Bonman *et al.,* 1986). However, quarantine restrictions often prohibit the use of the greenhouse to carry out infection assays with foreign isolates because of the possibility that such isolates may escape into the nearby rice-growing area. Consequently, a pathogenicity assay in a closed-system laboratory situation is needed to estimate advanced breeding lines for a durable, broad-spectrum blast resistance to a wide range of isolates. The aim of this study was to develop a rapid screening method to obtain infection pattern by using well-characterized races of *M. grisea* on detached rice leaves under controlled environment.

MATERIAL AND METHODS

Eight International blast differential lines which are known to contain single or multiple blast resistance genes were tested against three predominant blast isolates (Table 1). The test was carried out *in vivo* in Uniform Blast Nursery (UBN) at DRR as well as *in vitro* under laboratory conditions to compare the efficacy of the methods.

In vitro evaluation

The seedlings were grown in a tray. The leaves of seedlings at four-leaf stage (2 weeks after sowing), were removed and cut into 5 cm segments. These leaf bits were immediately placed into petri dishes containing benzamidazole (40 ppm) solution. Each leaf bit was inoculated with three to four 5 µl droplets of conidial suspension containing $1x10^5$ conidia ml^{-1}. The petri dishes were placed in laboratory. Tween 20 (0.25%) was added to the spore suspension to increase the adherence of conidia to rice leaves. Three leaf bits were inoculated with individual fungal spore suspension with the help of a syringe and the plates were incubated at 28°C for seven days. Disease scoring was done (0-9 scale) based on the type of lesion(s) observed on the infected leaf bits considering 0 = no lesions; 1 = small brown specks pinhead size; 2 = brown specks slightly elongated necrotic spots, about 1-2mm in diameter with a distinct brown margin, lesions are mostly found on the lower leaves; 3 = lesion type is same as in scale 2, with grey centre lesions; 4 = typical sporulating blast lesions measuring about 3 mm in diameter, 5 = typical blast lesions actively sporulating with grey centre; 6 = blast lesions measuring 5mm in diameter; 7 = large lesions measuring >5mm; 8 = ideal spindle shape lesions grayish white centers surrounded with yellow halo ring; and 9 = dead.

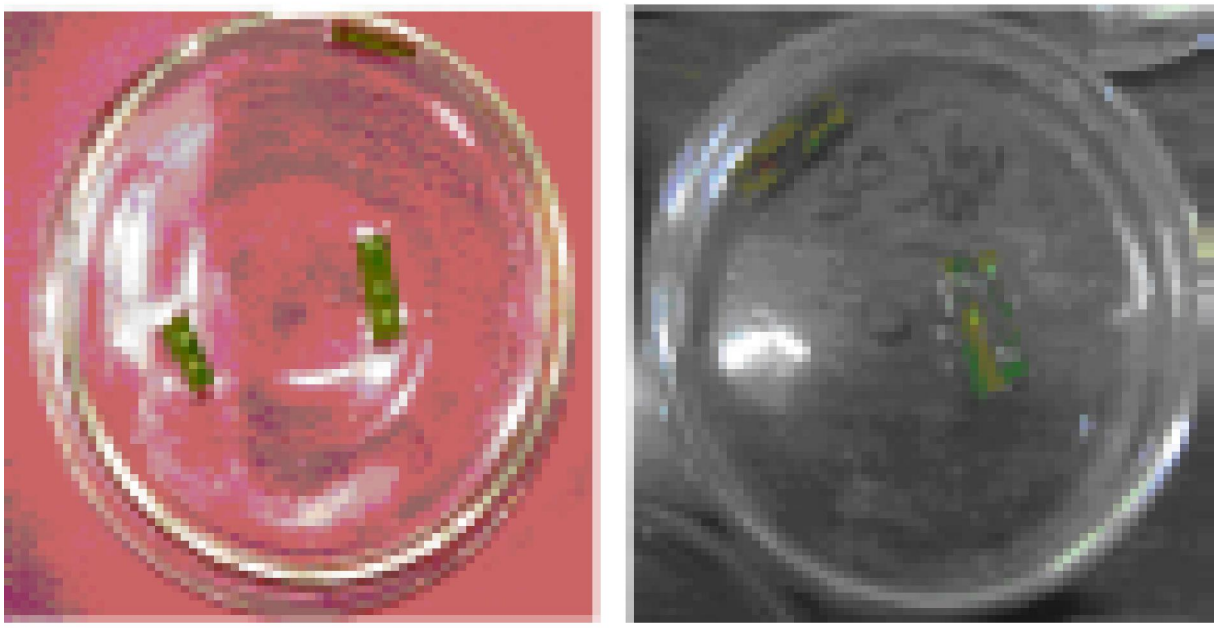

Table 1. Isolates used in the study

S.No	Isolate Code	Host	Place of Collection
1	SP 250	Raminad Str 3	Nellore
2	SP 262	Rasi	Maruteru
3	SP 267	HR12	DRR

Table 2. Performance of Co39 and *Japonica* varieties (LTH back ground) with resistance genes against blast isolates (*In vitro* method)

Differentials	Genes	SPI-250	SPI-262	SPI-267
Usen	Pi-a+	4	5	6
Dular	Pi-ka+	5	6	7
Kanto-51	Pi-k	3	2	8
Shia-tia-tsao	Pi-ks	4	4	7
Calaro	Pi-ks	7	2	8
Zenith	Pi-z+Pi-a+Pi-ii	7	7	7
Raminad Str3		3	4	5
NP125		8	6	7

0-3 – Resistant, 4-5 - Moderately Resistant, 6 - Moderately Susceptible, 7-9 – Susceptible (SES Scale, 1996).

Uniform Blast Nursery (UBN) method (*In vivo* method)

UBN was a 10x1m nursery bed and the soil was pretreated with FYM and NPK. The variety HR12 which is highly susceptible was sown as a border to act as an infector row. The test varieties like NIL's, RIL's, International blast differentials and monogenic *japonica* lines were sown in 10 cm apart on the bed. Relative humidity was maintained with water sprinklers. The UBN was covered with polythene sheets during night to maintain the humidity. Inoculation was made with the spore suspension of $1x10^5$ spores ml^{-1} concentration and sprayed on the 10-12 days old seedlings. Scoring was done after 10-15 days after inoculation depending on the severity of the infection on the susceptible check, using SES scale (IRRI, 1996): 0 = no lesions; 1 = small brown specks pinhead size without sporulating center; 2 = small round to slightly elongated necrotic grey spots, about 1-2mm in diameter with a distinct brown margin, lesions are mostly found on the lower leaves; 3 = lesion type is same as in scale 2, but significant number of lesions are on the upper leaves; 4 = typical sporulating blast lesions, 3-mm or longer infecting less than 2%; 5 = typical blast lesions infecting 2-10%; 6 = blast lesions infecting 11-25%; 7 =blast lesions infecting 26-50%; 8 = blast lesions infecting 51-75%; and 9 = more than 75% leaf area affected.

RESULTS AND DISCUSSION

When phenotyping of varieties for blast resistance with predominant 3 isolates was done under *in vitro* and *in vivo* conditions on international blast differentials (Table 2) similar type of reaction was obtained under both *in vitro* and *in vivo* conditions.

Spot inoculation of *M. grisea* on detached leaves has been well documented; as compared with needle-pricking method developed previously to inoculate rice with multiple fungal isolates (Inukai *et al.,* 1994). This method required additional wounding and disease reactions were evaluated in the greenhouse, where many variables such as wounding, light intensity and temperature can affect the disease development. Under *in vivo* conditions it is laborious and time consuming process to evaluate the disease. Due to unpredictable weather conditions it is difficult to develop the disease which requires continuous monitoring of relative humidity, temperature conditions etc.

A critical pathogenicity study with three characterized fungal isolates on rice cultivars was performed to validate the spot inoculation method. Similar host responses were observed with both the spot inoculation and the UBN. The response by spot inoculation was consistent with results obtained from the standard pathogenicity tests performed by Xia *et al.,* (2000).

The spot inoculation method allows determination of pathogenicity of *M. grisea* in a controlled environment. All operations, such as fungal culture, inoculation and observation of disease development can be performed in the laboratory. All materials can be destroyed upon completion of studies by steam sterilization or incineration. Because all processes are performed in a controlled laboratory setting, the risk of escape of pathogenic isolates into rice fields is reduced. Successful application of this technique would benefit investigators evaluating the pathogenicities of exotic isolates of *M. grisea*. Use of the spot inoculation technique would allow simultaneous testing of multiple fungal isolates on the same plant in the laboratory. This could shorten the time for development of broad-spectrum blast resistant cultivars.

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