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NOTES

Field and greenhouse inoculation methods for assessment of sheath blight resistance in rice

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ABSTRACT - *Field and greenhouse inoculation methods were compared to determine the genetic variation for resistance to* Rhizoctonia solani *in 38 somaclones of rice cultivar Metica-1. Rice plants in pots were inoculated with isolate 4F1 at the age of sixty four days, with 2.0 g of the fungal culture, multiplied on rice grain and hull medium and placed on the soil surface around the plant. The differences among somaclones in relation to lesion height were significant and varied from 6.5 to 15.5 cm. In the field trial of artificial inoculation with fungal culture, 52 days after planting, the lesion height varied from 6.2 to 17.7 cm. The correlation between disease severity in the greenhouse and the field was positive and highly significant ($r=0.44$; $P\leq 0.01$), indicating the greenhouse inoculation as a safe method for screening germplasm for sheath blight resistance.*

Key words: *Rhizoctonia solani*, *Oryza sativa*, resistance, somaclones, disease evaluation.

INTRODUCTION

Rice sheath blight is caused by *Rhizoctonia solani* Kuhn [*Thanatephorus cucumeris* (Frank) Donk.] anastamosis group AG 1, intraspecific group IA and is ascribed economic importance in irrigated rice, mainly under tropical conditions. Soybean aerial blight, caused by the same intraspecific *R. solani* group, induces sheath blight epidemics in rice as well. In soybean-rice rotation in the southern states of U.S.A. for example, it increases the number of sclerotia in field soil during soy cultivation and serves as primary inoculum for the disease in the following rice crop (Belmar et al. 1987, Van Eeckhout 1991). A great number of rice and soybean cultivars are susceptible to *R. solani*, consequently the inoculum density increases over the course of time with soybean-rice rotation (Groth and Nowick 1992). Sheath blight is currently present on all rice farms in the State of Tocantins (Brazil) ranging from slight to high disease severities, swhere approximately 20,000 ha of rice is planted every year in rotation with soybean and the pathogen poses a great threat to the crop. Mean yield losses varying from 19 to 41% have been reported in a five-year study conducted in Beaumont, Texas (Marchetti and Bollich 1991). The search for sources of sheath blight resistance is a basic requirement for a

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resistance-breeding program for rice. The disease resistance of commercial rice cultivars and of the available exotic and native germplasm is usually evaluated in naturally infected fields. This evaluation method has the drawbacks of disease escape and simultaneous occurrence of other stem diseases. Most previous investigations in different countries have shown absence of immunity or a high degree of resistance in the available rice germplasm (Webster and Gunell 1992). However, some genotypes were identified as moderately resistant according to the lesion extension on the stem. Cultivars with a moderate resistance are required to reduce the losses disease caused by this disease to tolerable levels (Ou 1985). The test results for resistance are not consistent and vary among cultivars depending on the criteria applied for disease assessment (Sharma et al. 1990). In Brazil, sheath blight is evaluated by artificial inoculation in a greenhouse. The test cultivars presented differences based on the criterion lesion extension. The inoculation method with RGH (Rice grain and hull) medium in a greenhouse is rapid, uniform, safe and indicated for the evaluation of genotypes for resistance to *R. solani* (Prabhu et al. 2002). Based on lesion height as criterion for resistance determination, the rice cultivars differed significantly in artificial inoculation tests in greenhouses. These results indicated the need to compare this method with field tests to use it for large-scale germplasm screening for the identification of resistance sources.

Genetic variations in callus-regenerated rice plants are heritable and can be obtained both in monogenic and polygenic traits (Mandal 2000). In this study rice blast-resistant somaclones developed from calli of immature panicles of a susceptible rice cultivar Metica-1 (Araújo and Prabhu 2002) were used as germplasm source.

The objective of our study was to compare greenhouse and field test results in relation to genetic variation in Metica-1 somaclones for sheath blight resistance.

MATERIAL AND METHODS

Thirty-nine somaclones of rice cultivar Metica-1 and the commercial cultivar Metica-1 from which they were derived were used to test genetic variation for sheath blight resistance, by artificial inoculation in a greenhouse and on the field. Isolate 4F1 of R. solani was obtained from stems of Metica-1 plants of the rice cultivar BRS Rio Formoso do Araguaia with typical sheath blight symptoms, collected from rice fields in the state of Tocantins. The RGH medium for large-scale inoculum production was prepared by blending rice grain and hull 3:1 (v/v). The mixture was soaked in water overnight and then autoclaved in Erlenmeyer flasks (500 ml cap.) for 2 hours at 20 psi.

In the greenhouse experiment, sixty-four-day-old plants grown in aluminum pots (1.0 kg) were inoculated with 2.0 g of the inoculum multiplied on RGH medium placed on the soil surface in contact with the tillers. The inoculated plants in the greenhouse were maintained under continuous high relative humidity (90 to 100%) by a mist blower for six days until disease assessment. A complete randomized design with three replications was used. The disease severity was measured based on the lesion height in three main tillers.

In the field tests a randomized complete block design with three replications was used. The field plots consisted of five 3.0 m long rows spaced 0.2 m apart. Hundred grams of inoculum previously multiplied on RGH medium were spread over the standing water surface in the three central rows of each plot 52 days after planting. Fifty tillers collected randomly, thirty-nine days after inoculation, from two central rows of each plot were used for evaluation by the same criterion of lesion height. Non-infected tillers were also considered in the determination of mean lesion height. The lesion height data were analyzed using Anova and the mean separation by Tukey's test at 5% probability.

RESULTS AND DISCUSSION

The differences in disease severity based on lesion height measurements on stems were significant, both in the greenhouse and field evaluations of somaclones. The lesion heights varied from 6.5 to 15.5 cm in the greenhouse and from 6.2 to 17.7 cm in the field (Table 1). Somaclone CNAI 10421 did not differ from Metica-1 but differed significantly in lesion height from two (CNAI 10390 and CNAI 10416) and three somaclones (CNAI 10423, CNAI10397 and CNAI 10427), in greenhouse and field, respectively. The two somaclones CNAI 1039 and CNAI 10393 that presented vertical resistance to rice blast (Araújo and Prabhu 2002) did not differ from cultivar Metica-1 in lesion height, neither

Table 1. Sheath blight severity in Metica-1 somaclones inoculated with *Rhizoctonia solani* isolate 4F1 in greenhouse and field

Somaclone[1]	**Sheath blight severity** (Lesion height in cm)	
	Greenhouse	**Field**
CNAI 10416	15.49a[3]	8.52cde
CNAI 10390	15.05ab	10.63bcde
CNAI 10423	14.45abc	17.67a
CNAI 10408	14.26abc	7.66de
CNAI 10420	13.88abc	6.60de
CNAI 10392	13.81abc	7.45de
CNAI 10406	13.12abc	11.64bcd
CNAI 10391	12.14abc	8.23de
CNAI 10418	12.10abc	9.52cde
CNAI 10393	11.30abc	8.41cde
CNAI 10414	10.71abc	9.79bcde
CNAI 10415	10.43abc	9.36cde
CNAI 10417	10.37abc	9.03cde
CNAI 10419	10.32abc	9.42cde
CNAI 10405	10.02abc	9.71bcde
CNAI 10397	9.95abc	13.63abc
CNAI 10427	9.27abc	14.91ab
Metica-1[2]	9.12abc	9.65bcde
CNAI 10395	9.00abc	8.83cde
CNAI 10407	8.86abc	7.93de
CNAI 10402	8.80abc	8.28de
CNAI 10425	8.70abc	8.67cde
CNAI 10400	8.37abc	8.51cde
CNAI 10401	8.22abc	8.14de
CNAI 10403	8.00abc	6.64de
CNAI 10412	7.90abc	7.28de
CNAI 10399	7.80abc	7.94de
CNAI 10399	7.80abc	7.94de
CNAI 10411	7.66abc	7.10de
CNAI 10394	7.39bc	6.153e
CNAI 10410	7.30bc	7.00de
CNAI 10396	7.20bc	7.06de
CNAI 10409	7.16bc	6.67de
CNAI 10404	7.14bc	9.23cde
CNAI 10413	7.00bc	6.77de
CNAI 10426	7.00bc	7.05de
CNAI 10422	6.85c	6.97de
CNAI 10398	6.70c	7.33de
CNAI 10424	6.70c	6.87de
CNAI 10421	6.50c	6.62de

[1] Acession number in Active Germplasm Bank of Embrapa Rice & Beans
[2] Suceptible, non-regenerated check
[3] Means followed by the same letter do not differ significantly according to Tukey's test at 5% probability

in greenhouse nor in field tests. The correlation between greenhouse and field inoculation tests was significant (r=0.44; P≤0.01) (Figure 1). The low r values can however be attributed to disease escape from some of the tillers, sampled in the field inoculation test. The mean lesion height per tiller was computed based on a total number of 50 tillers, including the uninfected. The greenhouse method of testing was more precise than field evaluation and confirmed previous results regarding the efficiency of determining the genetic variation in resistance genotypes, based on lesion height (Prabhu et al. 2002) and is recommendable for preliminary screening of germplasm for sheath blight resistance under controlled uniform conditions. The resistance of the selected genotypes should however be further confirmed in experiments of artificial field inoculation.

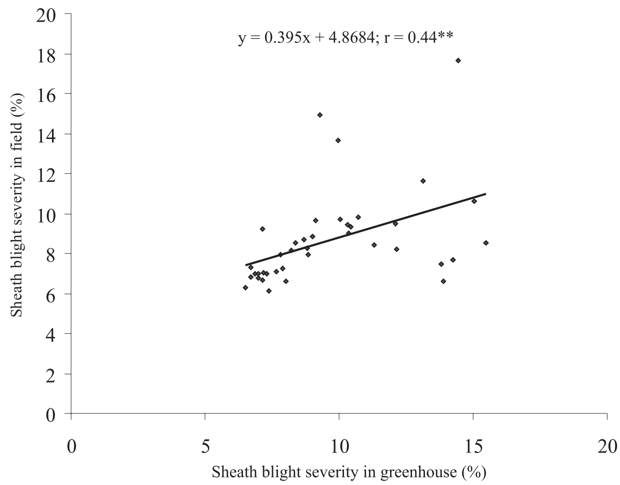

Figure 1. Relationship between sheath blight severity in somaclones, in greenhouse and field inoculation tests

Métodos de inoculações no campo e casa de vegetação para avaliação de queima de bainha em arroz

RESUMO - *Os métodos de inoculação de campo e casa de vegetação foram comparados para determinação da variação genética para resistência a queima da bainha, utilizando 38 somaclones da cultivar de arroz Metica-1. As plantas, aos 64 dias de idade em vasos, foram inoculadas com o isolado 4F1, utilizando 2,0 g do fungo multiplicado em meio de cultura grãos-casca de arroz, na base de planta. As diferenças entre os somaclones em relação a altura da lesão foi significativa, variando de 6,5 a 15,5 cm. O experimento de campo realizado com inoculação artificial com fungo, 52 dias após o plantio, a altura de lesão variou de 6,2 a 17,7 cm. A correlação entre severidade da doença em casa de vegetação e campo foi positiva e altamente significativa (r=0,44; P≤0,01), indicando que o método de inoculação de casa de vegetação pode ser utilizado com segurança para os testes de germoplasma visando resistência a queima de bainha.*

Palavras-chave: *Rhizoctonia solani*, *Oryza sativa*, resistência, somaclones, avaliação de doença.

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