Indian J. Genet., **67**(1): 28-30 (2007)

A novel method for simultaneously estimating yield parameters and disease incidence for neck blast using half-plant panicles inoculation approach in rice (*Oryza sativa* L.)

M. G. Vaishali, T. N. Girish, K. Nagabhushan, Hanamareddy Biradar and Shailaja Hittalmani

Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore 560 065

(Received: February 2005; Revised: February 2007; Accepted: February 2007)

Abstract

A new procedure is described to screen neck blast disease and simultaneously evaluate for grain yield within the same plant and estimate yield losses if any. Taking the example for neck blast resistance, fourteen rice (*Oryza sativa* L.) genotypes were evaluated for neck blast using the "half-plant panicles inoculation approach" to check the possibility of simultaneous screening for both yield parameters and neck blast resistance, thus avoiding loosing the valuable genetic material. In this approach half the number of panicles in each of the five selected plants of each genotype were inoculated with mixed blast isolates and the other half of the panicles of a plants were used as uninoculated control. The results from this approach were then compared with two other treatments where in all the panicles in the selected five plants were inoculated with the mixed isolate spores and untreated as control plants, respectively. The results indicated that no significant difference in the yield loss between the whole plant inoculated and whole plant uninoculated control and yield loss between the inoculated and uninoculated panicles of the same plant (half the number of panicles inoculated and rest was untreated control). This approach serves as a valuable method to simultaneously estimate yield parameters as well as neck blast incidence within the same plant and, thus helps to avoid having several treatments and progeny testing in segregating generations.

Key words: Rice, half-plant panicle inoculation, simultaneous selection, blast, grain yield

Introduction

Rice (*Oryza sativa* L.) blast disease caused by *Pyricularia grisea* (perfect stage *Magnaporthe grisea*) has been considered as a serious constraint to higher yields in all rice-growing areas [1]. A good and efficient disease management practice to be employed would depend on the accurate and precise information on the disease intensity and the relationship between disease intensity and yield loss [2]. Estimating yield loss for any disease requires a good knowledge of specific mechanisms of disease damage and pathways of disease-host interaction.

Rice blast causes yield losses at two stages, leaf blast at vegetative stage and neck blast at reproductive stage. Leaf blast causes reduction in plant height, number of tillers. The plant when reach adulthood may recover and develop resistance, thus keeping the losses in check [3]. Neck blast that affects the plant at vegetative stages, however, causes a higher yield reduction, which reduces number of mature fertile grains per panicle, 1000-grain weight, panicle weight and quality of the grains. When neck blast is severe, the panicle may break at the point of infection thus causing complete yield loss. Simultaneous screening of early segregating generation for both neck blast resistance and yield parameter is a difficult task and leads to misrepresentation and loss of genetic material. In order to save the plants from complete loss, a procedure to save the otherwise valuable plant material is proposed. To overcome this problem, screening process was improvised by adopting the half-plant panicles inoculation method which is proposed below. The objective of the study was to detect whether there was significant differences in whole plant inoculated, half plant inoculated and whether the procedure is viable for simultaneously evaluate neck blast disease and percent grain yield losses due to the disease in the same season and in the same plant.

Materials and methods

A set of fourteen genotypes (Table 1) was evaluated for reaction to neck blast using half-plant panicle approach. The seedlings were grown in the nursery and 21-day-old seedlings were transplanted to main field in augmented RCBD design and evaluated for neck blast at flowering. The genotypes evaluated included checks C 101A51 (resistant), CO 39 and HR 12 (susceptible) rice varieties.

The experiment comprised of three treatments. In the first treatment at flowering stage, only half the number of panicles in each of the selected five plants of each genotype was inoculated with mixed isolate

spores of neck blast and the other half of the panicles were used for uninoculated control within that plant. In the second treatment all the panicles of the five plants were inoculated with the mixed isolate spores (whole plant inoculation). In the third treatment all the panicles were un-inoculated (control plants). Each panicle was considered as single observation unit. Data on number of grains per panicle, number of filled grains per panicle, panicle weight and grain weight per panicle were recorded on all the plants. The disease components like the number of infected panicles and lesion length on the neck (cm) were scored. T-test was carried out based on mean panicle observations in each treatment for percent fertile grains to detect whether there was any significant difference for neck blast resistance (in terms of yield loss in infected panicles of) and yield parameters (from the healthy panicles) in all the three treatments.

Preparation of the inoculum: Thirty neck blast isolates from Southern Karnataka were selected based on the molecular characterization using RAPD markers [4]. All the 30 isolates were revived on PDA slants and kept in the incubator at 28°C for eight days. Ten to twelve days prior to inoculation, 10 ml of distilled water was put in previously revived culture and the spores were dislodged using a sterilized inoculation loop. The dislodged spores and mycelial suspension were then poured on to cold oatmeal agar (OMA) plates. The plates were then incubated for 4-5 days at 28°C until mycelial growth was observed. The mycelial surface in the plates was disturbed with sterilized glass slides and exposed to fluorescent light continuously for 5-7 days to induce sporulation. About 10 ml of sterilized water mixed with a few drops of Tween-80 was poured on to each culture plate. Using glass slides the mycelial surface was scraped and filtered through two-layered cheesecloth. The spore concentration was adjusted to 5×10^4 conidia/ml for inoculation. The 30 isolates selected for inoculation were mixed in equal quantities.

Inoculation of plants with spore suspension: Plants were grown until flowering. A piece of cotton was wrapped around the panicle node to retain the inoculum and to maintain a moist microenvironment. Five ml of blast spore suspension of the mixed isolates was pierced with a syringe on to each neck of the panicle. After inoculation the plants were covered by polythene sheets to maintain a humid condition until disease developed. After the symptoms appeared on the neck after 30 days the yield and diseased parameters were measured and evaluated using standard parameters.

Results and discussion

The mean data of inoculated and control panicles with respect to grains/panicle and filled grains/panicle are presented in Table 1. Though grain yield is influenced by several yield parameters, it is the fertile grains/panicle and number of panicles that finally determine the grain yield. Hence fertile grains/panicle was considered for

Table 1. Mean performance of different rice genotypes for neck blast inoculation using half-plant panicles approach

Sl. No.	Genotype	1st treatment						2nd treatment			3rd treatment		
		Infected panicles			Control panicles								
		Grains/ panicle	Filled grains/ panicle	%filled grains	Grains/ panicle	Filled grains/ panicle	%filled grains	Grains/ panicle	Filled grains/ panicle	%filled grains	Grains/ panicle	Filled grains/ panicle	%filled grains
1	IR 36	126.3	78.9	62.47	134.8	122.0	90.50	152.0	92.3	60.72	139.0	126.0	90.65
2	IR 64	186.3	100.2	53.78	206.4	195.3	94.62	192.3	108.2	56.27	208.2	196.3	94.28
3	IR 72	156.3	92.3	59.05	169.0	129.0	76.33	162.0	100.0	61.73	156.2	119.3	76.38
4	CO 39 (Check)	109.8	25.2	22.95	123.5	95.2	77.09	113.2	28.0	24.74	134.2	100.2	74.66
5	HR 12 (Check)	195.3	45.9	23.50	186.2	157.4	84.53	165.5	23.6	14.26	192.3	165.2	85.91
6	IR 50	146.9	28.0	19.06	136.3	120.0	88.04	163.2	42.3	25.92	126.9	110.3	86.92
7	*Pi-2* (C 101A51 - Check)	102.3	88.3	86.32	129.5	116.2	89.73	100.6	92.1	91.55	136.5	120.6	88.35
8	*Pi-2/4* (C 101A51/C 101PKT)	100.6	84.5	83.99	109.3	99.3	90.85	112.6	85.2	75.67	100.9	90.6	89.79
9	*Pi-1/2* (C 101LAC/C 101A51)	138.2	100.0	72.36	108.9	96.2	88.34	123.5	89.2	72.23	96.5	88.9	92.12
10	*Pi-1/2/4* (C 101LAC/ C 101A51) (C 101PKT)	112.6	94.0	83.48	113.2	100.0	88.34	96.5	85.3	88.39	126.9	110.5	87.08
11	*Pi-1* (C 101LAC)	100.3	66.0	65.80	120.6	110.2	91.38	90.5	56.0	61.88	116.9	109.2	93.41
12	*Pi-4* (C 101PKT)	106.5	85.0	79.81	115.2	96.0	83.33	100.5	80.1	79.70	120.3	102.4	85.12
13	*Pi-1/4* (C 101LAC/C 101PKT)	96.5	64.0	66.32	102.5	100.0	97.56	95.3	62.0	65.06	103.8	99.3	95.67
14	Azucena	136.2	100.3	73.64	129.0	110.0	85.27	164.3	123.5	75.17	120.6	100.8	83.58

1st treatment = Half the number of panicles in each of the five plants were inoculated; 2nd treatment = Whole plant inoculated; 3rd treatment = Whole plant uninoculated

T-test (Table 2) in the experiment. T-test showed that there was no significant difference in the number of filled grains/infected panicle between half-plant and whole-plant inoculations and in the number of filled grains/healthy panicle between half-plant uninoculated and whole-plant uninoculated treatments. (Table 1 and 2). The T-test carried out between the differences in yield losses (between half the number of panicles inoculated within a plant and whole plant inoculated treatments), clearly showed no significant difference between the two approaches in the assessment of yield loss interms of number of filled grains per panicle.

The results of the experiment thus revealed that there is no need to grow separate plants for control and inoculation. Evaluation for neck blast resistance by artificial inoculation through half-plant panicles approach offers an added advantage in that it facilitates in approximately determining the yield potential of a genotype through the estimation of it's yield parameters. The approach would thus benefit in artificial inoculations of segregating rice plants like F_2 and other valuable plant material with low seed quantity for both grain parameters and disease reaction in the same season in the same plant. This method there by saves one generation as the progeny testing can be avoided. The approach is most appropriate for neck blast as it is possible to localize neck blast infection to the desired panicles within a plant.

Table 2. T-test of percent filled grains between half and whole rice plant panicles inoculated with blast isolates

Sl. No.	Genotype	1/2 panicle inoculated	Whole plant inoculated	t-test	1/2 panicle uninoculated	Whole plant uninoculated	t-test
1.	IR 36	62.47	60.72	ns	90.50	90.65	ns
2.	IR 64	53.78	56.27	ns	94.62	94.28	ns
3.	IR 72	59.05	61.73	ns	76.33	76.38	ns
4.	CO 39 (Check)	22.95	24.73	ns	77.09	74.66	ns
5.	HR 12 (Check)	23.50	14.26	ns	84.53	85.91	ns
6.	IR 50	19.06	25.92	ns	88.04	86.92	ns
7.	*Pi-2* (C 101A51 - Check)	86.31	83.00	ns	89.73	88.35	ns
8.	*Pi-24* (C 101A51/C 101PKT)	84.00	84.67	ns	90.85	89.79	ns
9.	*Pi-12* (C 101LAC/C 101A51)	72.36	72.23	ns	88.34	92.12	ns
10.	*Pi-124* (C 101LAC/C 101A51) (C 101PKT)	83.48	87.08	ns	88.34	99.39	ns
11.	*Pi-1* (C 101LAC)	65.80	61.88	ns	91.38	93.41	ns
12.	*Pi-4* (C 101PKT)	79.81	79.70	ns	83.33	85.12	ns
13.	*Pi-14* (C 101LAC/C 101PKT)	66.32	65.06	ns	97.56	95.66	ns
14.	Azucena	73.64	75.17	ns	85.27	83.58	ns

ns = non-significant

Acknowledgement

The authors are thankful to the Rockefeller Foundation, USA for the financial support to Shailaja Hittalmani in the form of Research Project FS031 # 2000-2003.

References

1. **Ou S. H.** 1985. Rice diseases. 2nd edn. Commonwealth Mycological Institute, Kew, UK, pp 380.
2. **Nutter F. W. Jr.** 1990. Generating plant disease epidemics in yield loss experiments. *In*: Crop loss assessment in rice. IRRI, Los Banos, Philippines, p 139-150.
3. **Goto K.** 1965. Estimating losses from rice blast in Japan. *In*: The rice blast disease. Johns Hopkins Press, Baltimore, Maryland, p 195-202.
4. **Srinivasachary, Shailaja Hittalmani, Girish Kumar K., Vaishali M. G. and Shashidhar H. E.** 2002. Genetic analysis of rice blast fungus of southern Karnataka using DNA markers and reaction of popular rice genotypes. Curr. Sci., **82**: 133-135.