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A Rapid Field Screening Method for Evaluation of Resistance to Leaffolder, *Cnaphalocrocis Medinalis* Guenee in rice

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Abstract

Rice leaffolder, *Cnaphalocrocis medinalis* (Guenee) is one of the major foliage feeding insects found in Asia and all the important rice growing regions in India. Identification of resistant sources plays a major role in the eco-friendly management of leaffolders, for which continuous screening of various germplasm lines and breeding material is essential. In the present study, a rapid field screening method was developed for evaluating large number of rice germplasm lines. It involves release of third instar larva on to the rice plants at 30-45 days after transplanting, allowing it to feed for 48 hrs and assessment of damaged leaf area by ImageJ program. It helps in reliable and faster identification of resistant sources. The method is also useful in precise phenotyping of mapping populations in breeding programs for the development of leaffolder resistant varieties.

Keywords: Leaffolder, screening, resistance, damaged leaf area, damaged leaves

Introduction

Rice is the most important staple food crop grown in India in an area of about 41 million hectares with a production of 104.32 million tonnes (Directorate of Economics & Statistics, Government of India, 2016). Rice production is limited by a number of biotic constraints, of which insect pests play a major role causing 25 -30% yield losses. Among the foliage feeders, leaf folder, *Cnaphalocrocis medinalis* (Guenee) has become a major threat to rice production in many Asian Countries including China, Sri Lanka, Vietnam Pakistan, Japan, Korea, Malaysia and India. Larvae fold the leaves by stitching with silken threads and feed on the green mesophyll tissue resulting in white membranous patches that are visible from a distance in the rice field. Rice plants have ability to compensate for the leaf folder damage during tillering stage. However, larval densities at more than three larvae per hill at the maximum tillering stage resulted in 20% unfilled grains. At flowering stage, flag leaf damage of more than 25% resulted in 50% unfilled grains (Padmavathi *et al*, 2013). During the outbreak period, yield reduction of 30–80% was reported from severely damaged fields (Kushwaha, 1988). So far, chemical control is the only practical method available for the farmer for its management and as the damage caused by leaf folder is highly visible to farmers, it triggers them to go for toxic insecticide application.

Growing resistant variety plays a major role in the management of insects especially in low input farming situations of India. It is also highly compatible with other methods of pest management. Screening for insect resistance under natural field conditions is a long term process. At the same time, it is difficult to identify reliable and stable sources of resistance due to variation in insect populations in space and time. To overcome these problems, it is essential to develop and standardize multi or no-choice screening techniques through artificial releases of the pest populations. Keeping this in view, the present study was undertaken to develop a modified feeding test that helps in screening large number of genotypes for resistance to leaffolder in the field.

Materials and Methods

Field experiments were conducted during *Kharif* 2014 at the research farm, Indian Institute of Rice Research, Hyderabad, India. The climate in this region is predominantly semi-arid, with mean temperatures in the range of 22–42° C, and an average annual rainfall of 896 mm.

In the first method, field screening was done as per the standard evaluation system (SES) for rice (IRRI, 2014). Forty eight genotypes were grown in the nursery and after 25 days, transplanted in the main field in rows at spacing of 20 x 10 cm. Taichung Native 1 (TN 1) was grown as susceptible check and W 1263 as resistant check after every 10 rows. The susceptible TN 1 variety was also grown as border and higher doses of nitrogen were given to increase leaffolder populations. All the other recommended agronomic practices were followed in raising the crop. At 25 days after transplanting (DAT), the genotypes were covered with nylon net and leaf folder adults were released

inside the net from greenhouse reared population or by collecting from the neighbouring fields (Plate.1). Adults were released two times, once at 30 DAT and second at 40 DAT @ 100 adults per release. Cotton dipped in 20% honey solution was placed inside the net as food for adults. Adults were allowed to remain in the net for a week and then the net was removed. Observations were then recorded after 30 days on ten randomly selected plants in each genotype. At each observation, total number of leaves and leaf folder damaged leaves were recorded to calculate per cent damage in each genotype. Leaf was considered to be damaged by the leaffolder only when one-third or more of its area showed symptoms. The per cent damaged leaves were converted to adjusted damaged leaves rating (ADLR) using the following formula, which was then converted to 0 to 9 scale. A test was considered valid when damaged leaves in the susceptible check averaged at least 50%.

$$\text{\% damaged leaves in each entry} = \frac{\text{Number of damaged leaves in a hill/plant x 100}}{\text{Total number of leaves in a hill/ plant}}$$

$$\text{Adjusted damaged leaves rating (ADLR)} = \frac{\text{\% damaged leaves in test entry x 100}}{\text{\% damaged leaves in susceptible check}}$$

Based on the adjusted values, entries were rated as follows:

Scale	ADLR
0	No damage
1	1-20%
3	21-40%
5	41-60%
7	61-80%
9	81-100%

In the present study, forty eight rice genotypes were grown in the nursery and after 25 days, transplanted in the main field in rows of 45 hills each at spacing of 20 x 10 cm. Taichung Native 1 (TN 1) was grown as susceptible check and W 1263 as resistant check after every 10 rows, similar to the SES method. In each genotype, three plants/ hills were selected at random and screened, each plant representing one replication. Leaves of each plant in a genotype were covered with a nylon mesh bag and tied at the bottom. A single third instar larva was released on to the leaves from the top of the bag and allowed to feed for 48 hours on the most susceptible stage of the crop, *i.e.*, 30 – 45 DAT (Plate.2). Larvae from the leaf folder culture maintained at IIRR greenhouse as per the standard procedure were used for releases (Padmavathi *et al*, 2013). After 48 hours of feeding, larva was collected and the number of damaged leaves were counted, collected and preserved to estimate the damaged leaf area. Damaged leaves were scanned with Cannon MF 4320-4350 scanner at colour mode with 300dpi image quality. Leaf area fed was measured by using imagej software (Rasband 1997-2016; http://imagej.nih.gov/ij/). The damaged area recorded was converted to adjusted damaged area rating (ADAR) using the following formula:

$$\text{Adjusted damaged area rating (ADAR)} = \frac{\text{Damaged area (mm}^2\text{) in test entry}}{\text{Damaged area (mm}^2\text{) in susceptible check}} \times 100$$

These percentages were converted to 0 to 9 scales as follows:

Scale	ADAR
0	no damage
1	1 to 10%
3	11 to 30%
5	31-50%
7	51-75%
9	more than 75%

In both the methods, genotypes with mean scale score of 0 to 3 were considered resistant, 5 as moderately resistant and 7 to 9 as susceptible.

Results & Discussion

In the first method, the damaged leaves varied from 10.13 to 64.52% with maximum damage in MTU 1160 and minimum damage in W 1263. Adjusted damaged leaves rating ranged from 22.14 to 146.47% in different genotypes with eight entries having damage more than the susceptible check TN1. Based on the scores, six genotypes were found resistant with 3 score including resistant check W1263 and ten were moderately resistant with 5 score. Remaining 32 genotypes were susceptible with 7 and 9 scores (Table 1).

In the special screening method, damaged area varied from 68.41 to 428.81 mm^2 with minimum damaged area in IET 22449 and W 1263 and maximum damaged area in RP Bio 4918-50-13. In the susceptible check TN1, mean damaged area was 268.24 mm^2. Adjusted damaged area rating ranged between 27.06 and 158.08% in various genotypes with four genotypes recording more than 100% damage, higher than the susceptible check, TN1. Based on the scores, six genotypes were found resistant with ≤ 3 score and 19 were moderately resistant with ≤ 5 score. Rest of the 23 genotypes were susceptible showing score range of 7 - 9 (Table 1).

Development of a resistant variety involves continuous effort of screening large number of plant populations and germplasm lines for resistance to rice leaffolder. Leaffolder was considered as a minor and sporadic pest before 1990's and hence, much attention was not given for screening and identification of resistant cultivars. Later during 1985 onwards, Heinrichs *et al* (1985) emphasised the need of identification and breeding of resistant cultivars to combat this pest menace in Asia. Initially, identification of resistant sources was done based on field screening with natural pest populations (Velusamy and Chellaiah,

Table 1. Screening of rice genotypes for resistance to leaffolder, *Cnaphalocrocis medinalis*

Genotype	Method 1		Method 2			Genotype	Method 1		Method 2	
	% ADLR	DS	% ADAR	DS			% ADLR	DS	% ADAR	DS
IET 21850	65.54	7	85.79	9		JGL 21126	81.66	9	101.05	9
IET 22222	75.26	7	95.91	9		JGL 21133	69.21	7	94.27	9
IET 22568	48.41	5	41.27	5		JGL 21794	83.39	9	66.51	7
IET 22552	41.80	5	36.54	5		JGL 21820	90.32	9	38.03	5
IET 22155	22.14	3	29.96	3		JGL 21828	99.77	9	88.35	9
IET 22199	88.53	9	35.50	5		JGL 21851	54.10	5	48.14	5
IET 22223	69.06	7	31.61	5		JGL 21868	65.70	7	57.40	7
IET 22439	67.89	7	137.61	9		JGL 21883	45.52	5	50.88	5
IET 22449	33.48	3	27.06	3		JGL 23634	52.95	5	50.68	5
IET 22486	103.22	9	33.34	5		JGL 23640	49.97	5	59.90	7
IET 22489	33.70	3	50.22	5		JGL 23666	78.29	7	84.78	9
JGL 19621	110.25	9	51.77	7		MTU 1140	43.26	5	50.60	5
JGL 20122	95.81	9	56.07	7		MTU 1153	48.97	5	50.89	5
JGL 20171	110.45	9	59.29	7		MTU 1155	42.24	5	46.43	5
JGL 20624	75.69	7	32.34	5		MTU 1159	68.56	7	158.08	9
JGL 20769	82.56	9	61.64	7		MTU 1160	146.47	9	64.38	7
JGL 20776	47.97	5	72.17	7		MTU 1162	27.68	9	97.55	9
JGL 20777	86.40	9	40.95	5		MTU 1163	36.02	3	27.55	3
JGL 20779	103.39	9	46.53	5		RP 4918-228(S)	62.61	7	62.04	7
JGL 21002	144.37	9	58.01	7		RP Bio 4918-236	129.66	9	100.09	9
JGL 21041	78.62	7	91.68	9		RP Bio 4918-24K	35.68	3	29.20	3
JGL 21066	64.78	7	39.89	5		RP Bio 4918-142	111.76	9	29.10	3
JGL 21075	81.88	9	67.11	7		RP Bio 4918-50-13	72.14	7	49.86	5
JGL 21078	99.41	9	49.55	5		TN 1	100.00	9	100.00	9
JGL 21099	64.43	7	55.01	7		W 1263	23.92	3	27.32	3

ADLR = Adjusted damaged leaves rating; ADAR = Adjusted damaged area rating
Method 1 = SES for rice IRRI method based on damaged leaves
Method 2 = Rapid screening method based on damaged area

1985, Heinrichs *et al*, 1985). However, non- uniform pest pressure and unpredictability of field populations restricted the reliability of the field evaluation. To overcome these limitations, a greenhouse screening method was developed (Waldbauer and Marciano, 1979; Heinrichs *et al.*, 1985). This method involves growing potted plants in the greenhouse and allowing larvae to feed for a prolonged time. Later, Bentur and Kalode (1990) proposed a feeding test to rapidly identify varieties resistant to rice leaffolder in the greenhouse. However, this test has few drawbacks including pupation of the fifth instar larva in case of unsuitable host plant as well as difficulty in accurately assessing the damaged area.

Subsequently, many rice researchers screened germplasm lines in the field under natural populations through SES and identified few cultivars with resistance to rice leaffolder (Heinrichs *et al*, 1985; Velusamy and Chellaiah, 1985; Uthamasamy, 1985; Khan *et al*, 1988; Rekha *et al*, 2001; Anil Verma *et al*, 2015).

However, SES method evaluation of resistance is based on the number of damaged leaves wherein even a slight feeding by the leaffolder larva is considered without taking into account the severity of damage, leading to overestimation of damage. Whereas in the present study, the severity of damage was considered by taking into account only those leaves showing one-third area or more of damage. This gives more accurate estimation of damage due to feeding by leaf folder. Hence, in the present study, more genotypes were found moderately resistant while some of these were graded as highly resistant by SES method. Also, the SES method does not account for insufficient pest pressure as during times of pest escapes whereas the present method offers a reliable alternative in ensuring confirmed pest damage effect through feeding by most damaging third instar larva, through artificial release.

Conclusions

Growing resistant variety is an important tactic accepted by the farmers for the effective management of insect pests. In the present study, an accurate and precise method for rapid field assessment of resistance to rice leaffolder was developed as a reliable alternative to the standard SES method for the identification of resistant sources

and for phenotyping studies in breeding programs for the development of resistant rice varieties.

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Plate 1. Field screening of germplasm lines for resistance to rice leaffolder- SES method

Plate 2. Rapid field screening method for evaluation of resistance to rice leaffolder; A) Field view of screening method B) Covering the leaves of each genotype with a net bag; C) Release of 3rd instar larva inside the net bag; D) Larva and leaf damage after 48 hrs; E) Damaged area measurement with ImageJ