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Green house screening of rice (*Oryza sativa* L.) germplasm against rice leaffolder (*Cnaphalocrocis medinalis* G.)[1]

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Abstract:

Rice leaffolder (Cnaphalocrocis medinalis G.) is a major insect pest of rice and distributed all over the rice growing areas of the world including Pakistan. It attacks the rice crop on vegetative and reproductive stage resulting in huge yield losses. It was imperative to exploit the sources of resistance to cope with the threat the pest and its environmental friendly control. Present study was carried out in greenhouse of Plant Genetic Resource Institute, NARC. Fifty genotypes of rice included local and exotic germplasm were screened in pot experiment during crop year 2011. TNI was used as susceptible check. The damage results revealed that two wild species, O. rufipogon and O. brachyantha were resistant, 14 genotypes were moderately resistant, 10 genotypes found moderately susceptible, 14 genotypes were susceptible and 10 genotypes were highly susceptible. Dendrogram was prepared to show the varietal clusters and their relationship on the basis of damage rating. The present study will facilitate the breeders in the development of leaffolder resistant varieties in future.

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Key words: Rice, *Oryzae sativa,* Leaffolder, *Cnaphalocrocis medinalis,* Screening, Germplasm, Dendrogram, Resistance, Susceptibility

Introduction

Rice (*Oryzae sativa* L.) is one of the most important staple foods of the world and reduces the food security issue of almost 112 countries of the world. Around 90% of the world rice is produced and consumed in Asian countries (Hassian and Narciso, 2004; Rao *et al.*, 2010). In Pakistan, rice is also second major staple food after wheat and second export commodity after cotton. Rice is planted on more or less 2.7 million hectares and annual production of 6.7 million tones. It accounts for 3.1 percent of value added in agriculture and 0.7 % in GDP (Economic Survey of Pakistan, 2013-14).

Despite much advancement in rice research and development, the rice yield in Pakistan is very low as compared to other rice growing countries. One of the major yield limiting factors is the attack of insect pests (Behura *et al.*, 2011). Of these, rice leaf folder (LF) *C. medinalis* is one of the most important pest (Ezuka and Kaku, 2000; Rani *et al.*, 2007) and cause substantial yield losses every year (Rehman, 2003; Salim *et al.*, 2003). Yield loss estimation due to leaf folder is 30-80% (Nanda and Bisoi, 1990). Pandya *et al.* (1987) reported that leaf folder cause damage more at early crop stage, medium at tillering stage and low at the milky seeds stage. In some cases rice leaffolder caused more yield loss as compared to blast disease, (Wu and Zeng, 2004; Xu and Jiang, 2003).The damage is cause by larvae of LF. The caterpillar of LF ties the edges of leaves together with silken threads by forming a folded protective cavity within which the larva feeds on by scraping the green leaf surface prohibiting photosynthesis.

Before 1980, RLF was considered a minor and sporadic pest but now it became a major insect pest of rice crop throughout the world including Pakistan (Teng *et al.,* 1993). In fact, changes in physical environment, cultural practices, imbalance use of fertilizer particularly high usage of nitrogenous fertilizer, multiple cropping pattern, non-judicial uses of insecticides and lack of resistance sources in high yielding varieties. (Shah *et al*., 2008). Over dependence on insecticides is not sound and sustainable pest control strategy as it results in resistance against insecticides, reduction in biocontrol agents, outbreak of secondary and sporadic pests and contamination of environment and food product. (Rehman, 2001). Realizing the importance of varietal resistance in insect control strategies, the present study was initiated. The growing of insect resistant rice varieties is a major tactic in the IPM. Farmers do not have to bear extra cost, easy to use, do not disturb the natural balance, economically viable and environmental friendly. Therefore, it is vital to investigate and find out the resistance souses against rice leaffolder. The present study was conducted to screen the rice germplasm to find out resistance sources against rice leaf folder.

Materials and Methods

The experiment was conducted in a green house of Plant Genetic Resource Institute, NARC Islamabad during the rice growing season of year 2011. Fifty lines of rice germplasm were acquired from International Rice Research Institute (IRRI) Philippines. In addition, three unapproved elite basmati varieties along with susceptible check TN1 were used in the experiment (Figure 2).

The procedure for screening of rice germplasm was used according to Henrichs *et al.* (1985). Clay pots were filled with soil. 10 seeds per entry were sown in each pot and seeds were covered with fine soil. The pots were placed in a metal try filled with water. Thinning of seedlings was done to 5 per pot at 10 days after seeding. Nitrogen was applied for the better growth and creates succulence in the seedlings. After 14 days, potted seedlings were covered with a nylon cloth net cage and introduced 5 larvae per plant. Cloth net was removed to provide natural conditions for better growth of rice seedlings.

For screening, infestation was maintained at least 60% leaf damage to susceptible check. After 21 days of insect infestation, data on the extent of damage was recorded. For each entry, all the leaves were examined and each leaf was rated (0-3) based on the extent of damage as given below.

Grade	**Damage grade**
0	No damage
1	Upto 1/3 leaf area scraped
2	More than 1/3 to ½ of leaf area scraped
3	More than ½ of leaf area scraped

Based on the number of leaves with each damage grade, damage rating (R) was computed as follows:

$$A = \frac{\text{(No. leaves with damage grade of 1*100) 1}}{\text{Total No. of leaves observed}}$$

$$B = \frac{\text{(No. leaves with damage grade of 2*100) 2}}{\text{Total No. of leaves observed}}$$

$$C = \frac{\text{(No. leaves with damage grade of 3*100) 3}}{\text{Total No. of leaves observed}}$$

$$\text{Damage rating (R)} = A + B + C \div 6$$

The damage rating (R) was calculated for each entry including susceptible check. Adjusting damage rating (D) was also determined for each entry as given below:

$$D = \frac{\text{R of test entry}}{\text{R of susceptible check}} \times 100$$

Adjusted damage rating (D) was converted into following 0 - 9 scale.

Damage Rating %	Scale	Status
0	0	Highly resistant (HR)
1-10	1	Resistant (R)
11-30	3	Moderately resistant (MR)
31-50	5	Moderately susceptible (MS)
51-75	7	Susceptible (S)
More than 75	9	Highly susceptible (HS)

Dendrograph

The dendrogram was constructed on the basis of adjusted damage rating (D) and were subjected to cluster analysis by Euclidean distance Statistica software 0.7.

Results

Resistant varieties play important role in the management of insect pest of any crop as compared to any other control measures. Therefore, 50 rice lines including (local and exotic) were screened against rice leaf folder. Results of the present study showed that two wild rice species, *O. rufipogon* and *O. brachyantha* were found to be resistant (R), 14 were moderately resistant (MR), 10 were moderately susceptible (MS), 14 were susceptible (S) and 10 were highly susceptible (HS). The results also showed the infestation by the rice leaf folder was found to range from 12.15% to 95.54%, respectively (Table 1). Resistant wild species *O. rufipogon* and *O. brachyantha* registered the leaf folder infestation 8.71% and 8.95%, respectively.

Among the highly susceptible lines, maximum leaf infestation (95.54 %) was found on rice line, IR03W133 (Table 1).

According to dendrogram rice varieties were classified into five major groups. Each group was further divided into two sub-groups except class B. The remaining sub-groups were designated as A (sub-group A1 and A2), group C (sub. group C1 and C2), group D (sub group D1 and D2) and group E(sub group E1 and E2). Group A includes 10 rice genotypes; 6 fall in sub group A1and 4 fall in sub group A2. According to the adjusted damaging rating (D) values, these sub-groups were consisting of highly susceptible material. The highest D value was 86.17% of IR73382-85-9-1-2-3-B-1-B in sub-group A1. In sub-group A2, the highest D value was 95.54% of rice line, IR03W133, which was very close to the susceptible check variety TN1 (100%).

Group B consists of two rice species (*O. rufipogon* and *O. brachyantha)* because of almost similar damaging rating. *Oryza rufipogon* has 8.71% adjusted damage rating (D) value whereas *O. brachyantha* has 8.95% D value.

Group C consisting of 14 rice lines, out of which, 8 were belongs to sub-group C1 and 6 were sub-group C2 on the basis of damage rating. In group C1, the D value of IR73382-7-12-1-4 was 12.15%; 12.41% of IR65483-104-11-4-23-B; 13.21% of IR03W127; 13.07% of ARC 10982; 13.62% of IR 56 and 16.21% of IR65483-104-13-13-12-1-b. Similarly in group C2, the D value of IR02W113 was 25.06%; IR02W104 23.63%; IR65483-118-5-7-14-13-B 23.05%; IR69502-6-SRN-3-UBN-1-B 20.43%; IR03W134 21.28%; IR02W113 28.37% and 28.37% of IR75870-8-1-2-B-6-1-1-B (Figure 1).

Group D comprises of 14 rice lines; out of which 5 were belong to sub group D1 and 9 were belong to sub group D2 on the basis of damage rating. In sub group C1, the highest D value was 55.61% for IR80340-23-B-13-1-B-B. In group D2, the highest adjusted damage rating value was 76.4% of rice line, IR65483-31-5-18-1.

Similarly group E contains 10 rice lines; 6 belong to sub-group E1 and 4 falls into sub-group E2. According to the adjusted damaging rating values, these groups fall in moderately susceptible rice genotypes (Figure 1).

Discussion

The concept of host plant resistant is quite old, therefore, the development and use of resistant varieties against insects and offers promising possibility and becomes inevitable under different conditions. To identify pest resistance sources, it is very important to have such type of genetically diverse crop materials. Therefore, the current study was under taken to identify the leaffolder resistance sources.

The results revealed that only two species of wild rice, *O. rufipogon* and *O. brachyantha* were found resistant to leaf folder. These results are in conformity with Rehman, 2003; Shah *et al.*, 2008, who also reported wild species resistant to leaf folder. The remaining tested rice lines were either moderately resistant, moderately susceptible, susceptible or highly susceptible. Rehman *et al.* (2005) reported that all the cultivated rice varieties lack the resistance against leaf folder and are susceptible to this pest in Pakistan.

Henrich *et al.* (1985) reported that rice varieties, TKM6, GEB24, CO7, PTB33, ARC10982, Shete, Bir-Me-Fen, Kaohsiung Sen Yu 169, *O. rufipogon* and *O. brachyantha* are resistant against rice leaf older. Similarly, Rekha *et al.* (2001); Nadarajan and Nair (1983); and Punithavalli *et al.* (2013) also reported varieties GEB 24 (a mutant of Konamani) and TKM6 as resistant to leaf folder. However, the present study revealed that rice varieties, TKM 6 and GEB24 were found to be moderately susceptible to leaf folder. The difference in results may be due to the different accession of TKM 6 used, which is susceptible to local strain of leaf folder. Similarly, our results vary with Punithavalli *et al.* (2013) who reported PTB33 as a resistant variety contrary to our results which showed it as a susceptible variety. Punithavalli *et al.* (2013) and Tripathi *et al.*

(2013) reported rice variety, CO36 as a susceptible, which was found to be moderately susceptible according to our findings. ARC10982 is moderately resistant and Kaohsiung Sen Yu 169 is susceptible. The present study was based on the initial screening of rice germplasm against leaf folder to find resistant sources. Since in the present study, the mechanism of resistance was not reported to reveal that the rice varieties/lines lacked the characteristics of host for insect oviposition, feeding, shelter and ultimately suitable/unsuitable for leaffolder development. Therefore, it is suggested that studies on mechanism of resistance need to be conducted to confirm either a tested rice variety/line is resistant or susceptible to the insect pest. Development of resistant varieties to leaf folder is vital to reduce the production cost of resource poor farmers of the country.

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Table 1: Reaction of rice genotypes against leaffolder.

Entry No	Designation	Leaves with damage grade			Damage rating (R)	Adjusted damage (D)	Scale	Response
		1%	2%	3%				
1	IR64	15.78	26.31	23.68	11.78	40.12	5	MS
2	IR07W101	0	19.36	116.12	22.57	76.68	9	HS
3	IR07W102	10.71	42.85	42.85	16.06	54.70	7	S
4	IR73382-85-9-1-2-3-B-1-B	12.50	33.33	106.24	25.30	86.17	9	HS
5	IR07W105	18.75	54.16	93.75	27.47	93.56	9	HS
6	IR07W104	18.91	32.43	24.32	12.61	42.94	5	MS
7	IR00W101	20.00	17.14	28.57	24.76	84.33	9	HS
8	IR06W102	10.00	33.33	79.99	20.55	69.99	7	S
9	IR73382-7-12-1-4	11.90	9.52	0	3.57	12.15	3	MR
10	IR73680-3-1-14-1-4	17.39	17.39	32.62	11.23	38.24	5	MS
11	IR55423-01	4.16	25.00	124.99	25.69	85.43	9	HS
12	IR03W132	10.41	50.00	85.71	24.40	83.10	9	HS
13	IR03W133	31.03	41.37	12.44	28.73	95.54	9	HS
14	IR02W110	22.22	37.03	33.33	15.43	52.55	7	S
15	IR02W112	9.30	13.95	20.93	7.36	25.06	3	MR
16	IR02W113	8.33	35.71	0	8.33	28.37	3	MR

17	IR69502-6-SRN-3-UBN-1-B	8.00	16.00	24.00	6.00	20.43	3	MR
18	IR03W134	5.00	10.00	22.50	6.25	21.28	3	MR
19	IR03W127	10.00	13.33	0	3.88	13.21	3	MR
20	IR75870-8-1-2-B-6-1-1-B	31.81	18.18	0	8.33	28.37	3	MR
21	IR80340-23-B-13-1-B-B	6.00	20.00	72.00	16.33	55.61	7	S
22	IR80351-25-B-2B	26.92	46.15	0	12.17	41.45	5	MS
23	IR56	18.00	0	6.00	4.00	13.62	3	MR
24	IR02W104	10.41	25.00	6.24	6.94	23.63	3	MR
25	IR65483-118-25-31-7-1-5-B	20.00	0	15.00	5.83	19.85	3	MR
26	IR65483-104-13-13-22-1-B	19.24	9.52	0	4.76	16.21	3	MR
27	IR02W107	15.21	30.43	110.86	26.80	91.28	9	HS
28	IR65483-104-11-4-23-B	15.62	6.25	0	3.64	12.41	3	MR
29	IR65483-106-8-2-19-B	23.52	11.76	88.24	19.44	66.21	7	S
30	IR65483-113-5-2-18-B	15.21	13.04	32.60	10.14	34.53	5	MS
31	IR65483-118-5-7-14-13-B				6.77	23.05	3	MR
32	IR65483-6-4-21-B-B-1	8.33	8.33	75.00	15.28	52.04	7	S
33	IR65483-6-31-5-18-1	16.66	20.00	80.00	20.14	76.40	9	HS
34	IR36	33.33	38.10	42.86	18.35	62.50	7	S
35	BIR ME FEN	9.09	9.09	40.90	9.84	33.51	5	MS
36	TKM6	30.30	36.36	0	11.11	37.84	5	MS
37	GEB24	34.37	18.75	0	8.85	30.15	5	MS
38	CO7	24.24	30.20	18.18	12.12	41.28	5	MS
39	ARC 10982	23.07	0	0	3.84	13.07	3	MR
40	CO36	13.63	36.36	40.90	15.15	51.60	7	S
41	SHETE	24.00	40.00	24.00	14.66	49.93	5	MS
42	KAOSHIUNG SEN YU 169	11.42	34.28	68.75	19.04	64.85	7	S
43	PTB33	32.25	58.06	19.35	18.27	62.22	7	S
44	O.rufipogon	15.33	O	0	2.56	8.71	1	R
45	O.brachyantha	15.78	0	0	2.63	8.95	1	R
46	Supri	10.00	10.00	90.00	18.06	61.51	7	S
47	Qaiynat	18.75	25.00	75.00	18.75	63.86	7	S
48	PK.386	32.14	14.29	53.57	17.56	59.80	7	S
49	BAS.515	30.43	17.39	0	20.02	68.18	7	S
50	TNI (CONTROL)	7.89	26.31	142.10	29.36	100	9	HS

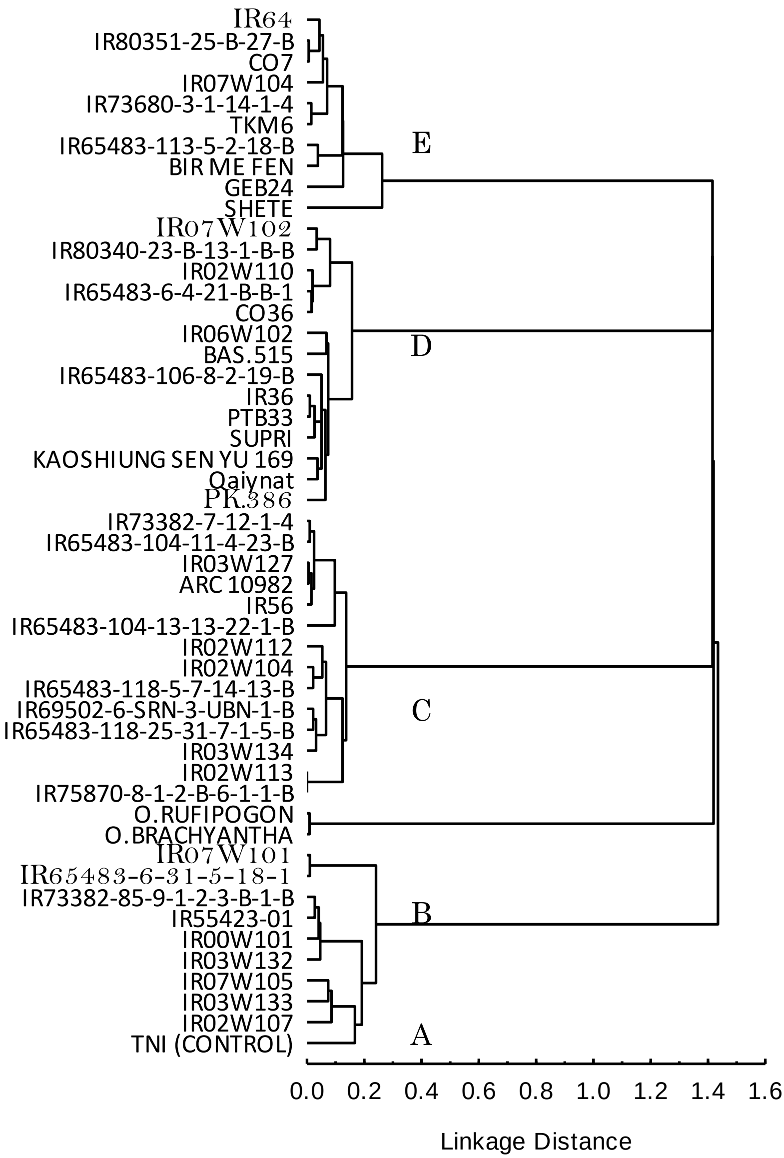

Figure 1. Dendrogram of 50 rice genotypes on the basis of adjusted damage rating (D).

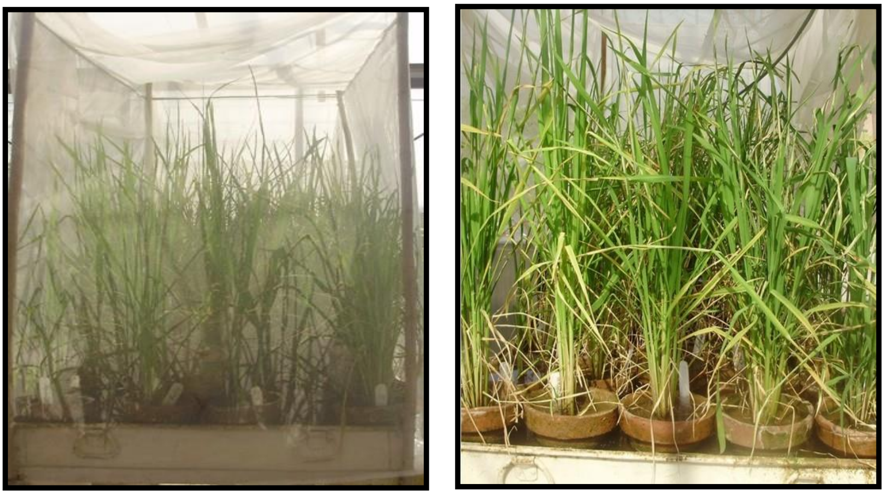

Figure 2. Showing the screening of rice germplasm against rice leaf folder.