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Tolerance against brown planthopper (*Nilaparvata lugens*) in wild rice (*Oryza sativa*) accessions

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ABSTRACT

Brown planthopper, *Nilaparvata lugens* (Stål), is a major insect pest of rice in all the rice growing areas of the world. In the present study, the tolerance mechanism of resistance in wild rice accessions belonging to different *Oryza* species such as *O. nivara* (IRGC104646, CR100204), *O. australiensis* (IRGC105275, IRGC105270), *O. punctata* (IRGC99577) along with resistant check, Ptb33 and susceptible check, TN1 has been studied. The experiments were carried out on 30-days old plants under glasshouse conditions during *kharif* 2017 and 2018. IRGC99577 (22.80 and 24.00) took maximum days to hopper burn condition during both seasons. Functional plant loss index (%) was minimum in IRGC99577 (18.10%) followed by Ptb33 (20.96%) and IRGC104646 (29.47%). Least plant dry weight loss index (mg) was observed in Ptb33 and highest in TN1 during both the seasons. Overall, results indicated that rice accessions, IRGC99577 and IRGC104646 showed high levels of tolerance as compared to other wild rice accessions that were tested. The identified accessions thus could serve as potential sources of resistance in breeding BPH resistant varieties.

Keywords: Brown planthopper, Hopper burn, Host plant resistance, Wild rice accessions

Rice (*Oryza sativa* L.) is classified as one of the most economically important cereal foods. *Oryza* genus includes only two cultivated species, viz. *O. sativa* L. (Asian rice) and *O. glaberrima* Steud. (African rice) and approximately 20 wild species (Nayar 2014). The demand for rice is on hike in the international market and is estimated to reach about 555 MT by 2035 (Oana 2017).

Main constraints in rice production are associated with several biotic and abiotic stress factors. Approximately 52% of the global production of rice is lost annually owing to the damage caused by biotic stress, out of which, about 25% are caused by insect pests (Bhogadhi and Bentur 2015). Among the various insect pests, brown planthopper (BPH), *Nilaparvata lugens* (Homoptera: Delphacidae) is one of the most destructive insect pests causing significant yield losses in most of the rice cultivars of Asia. BPH nymphs and adults injure the plants by directly sucking the plant sap and causing plant drying/wilting or 'hopper burn' symptoms when it becomes serious. The losses to rice caused by BPH in Asia have been reported up to $300 million per year (Min *et al.* 2014). The blanket use of insecticides to control this pest is already creating problems especially for the environment.

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Nearly 50 years of research has produced considerable information on planthopper-rice interaction, including extensive information on the levels of host-plant resistance in rice (Fujita *et al.* 2013). Overall, research since 1970s has produced a number of resistant varieties, some with known resistance genes, viz. *Bph6, Bph14, Bph15, Bph18, Bph20, Bph33* (Huang *et al.* 2001, Rahman *et al.* 2009, Hu *et al.* 2018). Understanding the mechanism of resistance is important before evolving resistant varieties (Horgan 2009, Cheng *et al.* 2013, Bhanu *et al.* 2014). The tolerant lines have a higher economic threshold level than susceptible lines which can save farmers' money spent on insecticide sprays to manage BPH. Hence, the present study was conducted to evaluate tolerance mechanism of resistance as well as measure the level of tolerance in wild rice accessions against BPH.

MATERIALS AND METHODS

Plant material: Five wild rice accessions, viz. IRGC99577 (*Oryza punctata*), IRGC105270, IRGC105275 (*O. australiensis*), IRGC104646, CR100204 (*O. nivara*) along with susceptible check (TN1) and resistant check (Ptb33) were used to study different tolerance parameters. Plants were grown in small pots (10 cm diameter) and kept under glasshouse conditions at a temperature of 28±2°C and 75±5% relative humidity. Experiments were conducted on 30-days old plants.

BPH population: The source BPH insects were collected from rice fields and were reared on 30-days old

TN1 plants under greenhouse conditions at Rice Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30°54' latitude and 75°48' longitude, 247 m above mean sea level) according to the method given by Heinrichs *et al.* (1985).

Tolerance paramaters

Days to cause hopper burn symptoms: The pre-germinated seeds of the test accessions were sown in small pots containing well puddled soil during *kharif* 2017 and 2018, and kept in water trough in five replications in completely randomized design. Seedlings of each accession were covered with cylindrical mylar cages. On 30-days old plants, 2nd–3rd instar nymphs @25 nymphs/seedling were released and allowed to feed till plants started showing hopper burn symptoms (wilting and drying of leaves). The day on which the plant showed wilting and drying symptoms (hopper burn) was recorded.

Functional plant loss index: This experiment was in continuation of experiment on days to hopper burn symptoms. After the plants wilted, dried completely, they were uprooted from the pots carefully, washed properly to remove any adhering soil. The plants were oven-dried at 70°C for 72 h and then weighed. Similarly, uninfested plants were maintained for each accession and weight of uninfested plant was also taken in the same manner after oven drying. Functional plant loss index was calculated using the formula given by Panda and Heinrichs (1983).

$$\text{Functional Plant Loss Index} = 1 - \frac{\text{Dry weight of infested plant}}{\text{Dry weight of uninfested plant}} \times 100$$

Plant dry weight loss index: Dry weight of infested and uninfested plants was taken as mentioned above. The 2nd–3rd instar nymphs, @25 nymphs, released on each plant at the start of experiment, were also collected (live and dead) from wilted/dried seedlings, ovendried for 48 h at 55°C and weighed. Plant dry weight loss index was calculated by using formula given by Panda and Heinrichs (1983).

Plant Dry weight Loss Index (PDWLI, mg)

$$= \frac{[(\text{Dry weight of uninfested plant})-(\text{Dry weight of infested plant}])}{\text{Dry weight of BPH progeny on infested plant}}$$

The data obtained from tolerance experiments were statistically analysed in a completely randomized design using analysis of variance (ANOVA) following statistical software SPSS v 20.0. Means and standard errors were calculated for comparison purpose at 5% level of significance. Data which lacked normality were transformed before statistical analysis and the arc sine (√percentage) and square root transformations were used for different data in order to meet the requirement of normalization.

RESULTS AND DISCUSSION

Days to cause hopper burn symptoms, functional plant loss index and plant dry weight loss index are the parameters to identify tolerance among plants against insect pests.

Days to cause hopper burn symptoms: The time period taken for hopper burn symptoms (wilting/drying) by BPH infested plants was used as a measure of tolerance for different accessions. All the accessions differed significantly in time taken for hopper burn symptoms after BPH infestation during 2017 (Table 1, $F_{6,28} = 430.62$, $P \leq 0.001$). The susceptible check, TN1 took minimum days to dry completely, whereas, the resistant accession IRGC99577 took maximum time to dry which was at par with resistant check Ptb33 followed by IRGC104646.

During 2018, all the accessions differed significantly in time taken for hopper burn after BPH infestation (Table 2, $F_{6,28} = 384.69$, $P \leq 0.001$). Based on number of days taken to dry (9.40 to 22.80 days), the test accessions were ranked in order from maximum to minimum days as IRGC99577, Ptb33, IRGC104646, IRGC105270, IRGC105275, CR100204 and TN1 (Table 2). In this study, the feeding by BPH was less on resistant accessions hence the plants could withstand drying as compared to susceptible TN1. Resistant accessions, viz. IRGC99577 (22.80 days) and IRGC104646 (21.60 days) took more time to dry as compared to susceptible check TN1 (9.40 days). Since, BPH feeds more on susceptible plants as compared to resistant ones, early hopper burn symptoms were observed on TN1 than on wild accessions. The results of our studies have been corroborated by the research work of earlier scientists on different germplasms (Jhansi Lakshmi *et al.* 2012, Reddy *et al.* 2016, Dharshini and Siddegowda 2015). Similarly,

Table 1 Different tolerance parameters to *Nilaparvata lugens* in wild rice accessions during 2017

Accession	Days to hopper burn symptoms (days)*	FPLI (%)#	PDWLI (mg)*
IRGC99577	22.80±0.20a (4.88)	18.53±0.50a (25.48)	12.12±0.35a (3.62)
IRGC105270	20.60±0.24c (4.65)	31.80±1.00b (34.30)	22.50±0.56b (4.85)
IRGC105275	18.20±0.37d (4.38)	34.59±0.33c (36.00)	25.59±0.52c (5.16)
IRGC104646	21.60±0.24b (4.75)	30.18±0.45b (33.31)	25.94±0.41c (5.19)
CR100204	12.40±0.24e (3.66)	37.98±0.39d (38.03)	26.11±0.22c (5.21)
Ptb33	22.40±0.24a (4.84)	19.13±0.52a (25.91)	11.21±0.45a (3.49)
TN1	9.40±0.24f (3.22)	79.88±0.75e (63.33)	131.53±0.72d (11.51)
CD (P=0.05)	(0.90)	(1.26)	(0.14)

FPLI, Functional Plant Loss Index; PDWLI, Plant Dry Weight Loss Index.

*Figures in parentheses are the means of √n+1 transformations; #Figures in parentheses are the means of arc sine √percentage transformations. Means within a column followed by the same letter are not significantly different at P≤0.05 according to DMRT.

Table 2 Different tolerance parameters to *Nilaparvata lugens* in wild rice accessions during 2018

Accession	Days to hopper burn symptoms (days)*	FPLI (%)[#]	PDWLI (mg)*
IRGC99577	24.00±0.32[a] (4.99)	18.10±0.52[a] (25.16)	11.27±0.35[b] (3.50)
IRGC105270	21.00±0.32[c] (4.69)	30.93±0.49[d] (33.77)	23.44±0.26[c] (4.94)
IRGC105275	19.00±0.32[d] (4.47)	32.92±0.12[e] (34.99)	24.96±0.37[d] (5.09)
IRGC104646	22.00±0.32[b] (4.79)	29.47±0.44[c] (32.87)	24.25±0.58[cd] (5.02)
CR100204	13.20±0.20[e] (3.77)	38.06±0.33[f] (38.07)	25.05±0.30[d] (5.10)
Ptb33	23.40±0.24[a] (4.94)	20.96±0.49[b] (27.23)	9.32±0.46[a] (3.21)
TN1	10.40±0.24[f] (3.38)	80.24±0.27[g] (63.58)	125.53±0.57[e] (11.25)
CD (P=0.05)	(0.92)	(0.79)	(0.13)

FPLI, Functional Plant Loss Index; PDWLI, Plant Dry Weight Loss Index.

*Figures in parentheses are the means of √n+1 transformations; [#]Figures in parentheses are the means of arc sine √percentage transformations. Means within a column followed by the same letter are not significantly different at P≤0.05 according to DMRT.

Soundrarajan *et al.* (2018) also confirmed that resistant cultivars have more tolerance index than susceptible ones. More time taken to hopper burn indicates that the plants are able to tolerate a greater number of BPH on them, thus are more resistant to BPH.

Functional plant loss index: The functional plant loss index (FPLI) also differed significantly among tested accessions during 2017 ($F_{6,28}$ = 852.58, P≤0.001) and 2018 ($F_{6,28}$ = 2195.57, P≤0.001). It was minimum in IRGC99577 and maximum in TN1 during 2017 (Table 1). Similarly, during 2018, minimum FPLI was observed in IRGC99577 followed by Ptb33. Resistant accessions showed low FPLI values as compared to susceptible ones (Table 2). The present findings are in conformity with the results of Dharshini and Siddegowda (2015) who reported more FPLI in TN1 (30.98%) and lowest in Ptb33 (5.97%) showing that landraces of rice exhibited high levels of tolerance against BPH. More feeding on susceptible accessions resulted in more FPLI as compared to resistant ones (Baehaki and Iswanto 2017, Prahalada *et al.* 2017). Varieties with high tolerance level are able to provide resistance for a longer period of time as this resistance functions as second line of defence which is difficult to overcome by insects. Accessions with less FPLI values suggest that BPH feeding caused less plant loss in them and these are able to survive for longer period of time.

Plant dry weight loss index: During 2017, all the accessions differed significantly in plant dry weight loss index ($F_{6,28}$ = 3384.80, P≤0.001). It was lowest in Ptb33 followed by IRGC99577 and IRGC105270 which were significantly less than susceptible check TN1 (Table 1). All the tested accessions showed significantly less PDWLI than TN1. During 2018, PDWLI ranged from 9.32 mg to 25.05 mg among tested accessions and it was significantly lower than TN1 (Table 2, $F_{6,28}$ = 3469.18, P≤0.001). All the test accessions ranked from minimum PDWLI to maximum as Ptb33, IRGC99577, IRGC105270, IRGC104646, IRGC105275, CR100204 and TN1. All the tested wild rice accessions showed significantly less PDWLI than TN1. It was apparent that PDWLI was less in resistant accessions, thus confirming the presence of tolerance mechanism in these accessions. PDWLI represents the plant dry weight loss per mg of insect dry weight produced. So, more feeding results in more loss of plant dry weight and vice-versa. This explained the comparatively more value of PDWLI in TN1 and less value in Ptb33. The BPH population development on susceptible plants causes reduction in plant dry weight (Sarao and Bentur 2016). In the present study, we also recorded more BPH dry weight on susceptible plants which resulted in more reduction in dry weight of plants as in TN1 and vice-versa.

The less plant loss is the evidence of the high level of tolerance in resistant genotypes. Bhanu *et al.* (2014) observed high tolerance in resistant genotypes led to lesser damage to plants. The tolerance resistance mechanism is a stable and moderate level of resistance which is difficult to overcome by insects (Prahalada *et al.* 2017).

Host plant resistance is one of the key pest management tactics and is an important tool of integrated pest management. It does not pose selection pressure on insect pests, and thus prevents the build-up of biotype of insect pests. This component is less explored as compared to antixenosis and antibiosis. The findings of present study indicate that accessions of *O. punctata* IRGC99577 and *O. australiensis* IRGC105270 have high levels of tolerance against brown planthopper. These accessions could be further explored to develop tolerant varieties against BPH with durable resistance.

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