Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(5): 2400-2405

Received: 08-06-2020
Accepted: 15-07-2020

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Evaluation of core rice germplasm accessions for resistance to brown planthopper (*Nilaparvata lugens* Stal) at seedling stage

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Abstract
Brown planthopper, *Nilaparvata lugens* is the major pest in rice production. Development of BPH-resistant varieties is an economical and effective way to control this pest. In present investigation 600 rice genotypes were screened in greenhouse along with resistant and susceptible checks *viz*., PTB 33 and TN1 respectively during *Kharif* 2016, *Rabi-Summer* 2017 and *Kharif* 2017 results were confirmed by probing mark test by BPH. Among the rice genotypes which showed the resistance to BPH were also studied for BPH honeydew excretion as indicator of antibiosis mechanism. Among the 600 genotypes five is shown resistant namely IC454040, IC454223, IC135425, IC301734 and IC301736. The selected resistance genotypes also exhibited significantly highest probing marks with resistant, IC454040 recorded lowest honeydew excreted area of 16.23mm2/2(♀) and followed by IC 454377, IC 301732, IC 301736, IC 460174X, IC 459199, and PTB 33 registered significantly lower honeydew excretion (17.22, 18.76, 19.49, 19.57 and 20.07 mm2 / 2 (♀) respectively). The rice genotype which has shown resistant to BPH can be used in breeding programmes for developing the BPH resistant varieties.

Keywords: Brown planthopper, genotypes, honeydew excretion, rice and screening

Introduction

Insects not only harm the plant by feeding on its tissue, some are also vectors of demoralizing rice viruses. All portions of the plant from root to panicle are attacked by various insects. All growth stages of the rice plant from the seedling in the nursery to the mature plant are vulnerable. Even after harvest, the grain which is stored is attacked. Of about 1,000 insect species known to attack rice, about 30 cause sufficient damage to require control.

Rice research from all over the world has made immense efforts to understand the mechanisms and response of brown planthopper resistance in rice research for recuperation. The frequent occurrence of insect attack has been identified as the key to the low rice productivity, however; brown planthopper, *Nilaparvata lugens* is one of the most destructive insect pest causing significant yield loss in most of the rice cultivars of India Kumar and Tiwari (2010) [10]. The brown planthopper causes serious yield reduction by directly sucking the plant sap and acting as a vector of various diseases. BPH draw nutrients from the phloem of rice plants Renganayaki *et al*., (2000) [15] and Park *et al*. (2008) [14]. High BPH population cause destroys a plant in a short period of time. Large number of plant hoppers causes the infested plants to become brown and dry. The condition is called hopperburn. Even if the planthopper population is not high enough to kill the plants. The present study undertook a screening evaluation to determine the reaction of core rice germplasm against BPH to identify resistant germplasm that can be used as donors in the rice breeding program and also identification and deployment of new genes for BPH resistance in rice varieties by host plant resistance mechanisms is the important strategy to reduce the damage caused by BPH to rice crop Kumar and Tiwari (2010) [10].

Materials and Methods

Six hundred Rice germplasm lines including eight checks *viz*. IR 64, Swarna, NDR-97, Danteshwari, Samleshwari, TN1 and Karma Mahsuri along with three resistant checks *viz.*, PTB33, IR-64 and IR 79538-1-1-1 were screened against brown planthopper population in glass hous Testing on screening of rice genotypes against brown planthopper, *Nilaparvata lugens* (Stal.) for identification of resistance donor was conducted in *Rabi* 2016, at the Glass house, Department of Entomology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh. The experimental material consisted of Six hundred genotypes along with TN1and PTB33 as susceptible and resistant check, respectively Rearing and maintenance of BPH Brown planthoppers initially collected from field were maintained throughout the year in the air cooled glasshouses at 32 ± 5ºC on forty day old TN1 seedlings in clay plots. The pots were placed inside rearing cages of 60x40x10 cubic centimetre which consist with of an iron frame with glass panels and small window on front side and fine wire mesh on top and other sides.

Screening of rice genotypes

Screening of rice genotypes was carried out by standard seed box screen testing method (Heinrich 1986) [6]. Mass screening tests were employed under controlled glasshouse conditions. The test and check material were pre germinated in Petridishes (10cm diameter) and these geminated seeds were transferred to wooden boxes of the size 60x40x10 cm containing well mixed homogeneous and sterilized soil. Each seed box contained 24 accessions with 20 seedlings of each including resistant check (PTB 33) in two middle rows and susceptible check (TN-1) four border and 2 middle rows. After sowing the tray were placed on cemented platform with7.5 cm water for maintaining the moisture level. When the seedling become 7 to 10 day old age first and second instar nymph were uniformly released on the seedlings, so that each seedling must be get infested with least 8 to 10 nymphs. The observations were recorded 7-10daysafterreleasinginsectswhenthe insect killed more than 90 percent of TN-1 seedlings. The reactions were recorded on a 0-9 scale suggested by Heinrichs (1986) [6].

Honeydew excretion test

Honeydew excretion test was suggested by Sogawa K. (2015) [16]. The assessed the area of honeydew excreted by the two female on the filter paper after 24 hours of confinement on the test genotypes. The females were confined on test plant with the help of inverted glass funnel. White filter paper (10 cm dia) used for soaking honeydew, were dipped in a solution of bromocresol green 2 gm per litter ethanol and allow to dry in sunlight, so that the filter paper turned yellowish orange initially and afterwards contact with honeydew secreted by female, blue spot appeared on the treated filter paper. As the concentration increased, the spot turned whitish in the canter. The spots were traced on transparent and later on measured by keeping on millimeter square graph. For getting more nitrogen from plant sap hopper generally used to feed voraciously and excrete out the honeydew. The amount of feeding by insect on the test genotype as well as susceptible (TN-1) and resistance check (PTB-33) expressed in term of honeydew excretion mm2 per two female. For our work, the filter paper technique was used. The plant at first tiller (40 days old) was located through two holes of the cup (up and down of the cup (5×5: H×R). The filter was placed at the base inside of the cup with a paper protecting it from humidity of the soil. For each plant to be screened, five female gravid hoppers were kept starving for 2 h 30 min. Then, the female hoppers were released on to plants to feed for 24 h, after which the filter papers were collected. Bromocresol green indicates phloem-based honey dew as blue-rimmed spots (indicate susceptible plants) and xylem-based honeydew as transparent (indicate resistant plants). The area of each spot on the bromocresol green-filter paper was mea-sured using a digital scanner and “Image J” software.

Results and Discussion

Detection and monitoring of functional resistance genes over the years

Six hundred Rice germplasm lines including eight checks *viz.* TN1 and resistant check., PTB 33, IR-64 and IR 79538-1-1-1 were screened against brown planthopper population in glass house condition with the aim of identifying potential resistant donors during *Kharif* 2016. Reactions of different genotypes against brown planthopper infestation are presented in Appendix I. Overall two genotypes exhibited highly resistant reaction, 27 genotypes exhibited resistant reaction, 38 genotypes exhibited moderately resistant reaction, and 58 genotypes showed moderately susceptible reaction and rest of the genotypes were showed susceptible reaction (Table 4.1). Resistant and susceptible plants are easy to classify because they fall into distinct classes. In horizontal resistance there is a continuous gradation between resistant and susceptible plants: the difference between resistance and susceptibility is not distinct. Vertical resistance is under the control of one (monogenic) or a few (oligogenic) major genes. Major genes have a strong effect and are easily identified.

Table 1: BPH reaction of 600 core rice genotype

Average plant damage score (Range)	Reaction	Screening of core rice for brown planthopper during the year			
		Kharif 2016	*Rabi-Summer* 2017	*Kharif* 2017	
		No. of Genotype	No. of Genotype	No. of Genotype	Check variety
0-1	Highly Resistant	5	5	5	PTB 33
1-3	Resistant (R)	77	42	31	IR64,IR79538-1-1
3-5	Moderate Resistant (MR)	224	63	52	
5-7	Moderate susceptible(MS)	145	214	59	
7-9	Susceptible & highly susceptible (HS)	149	276	453	Karma mahsuri, Samleshwari, Danteshwari, Swarna, Pusa Basmati 1, Jaya, TN 1
	Total	600	600	600	

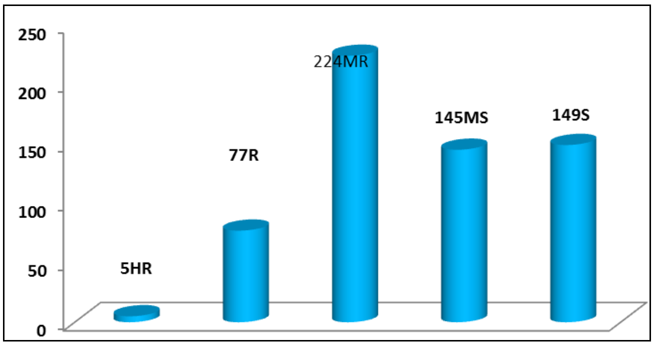

Fig 1: Kharif 2016

The graphical depiction of the reaction of genotype of screening in *kharif* 2016 for resistance to brown planthopper has been represented in the Fig. 1. From this graph it is clear that the scoring values obtained is skewed. As majority of the lines of the population (224 lines) are showing score values more than 3, we have obtained a slanted distribution towards susceptibility.

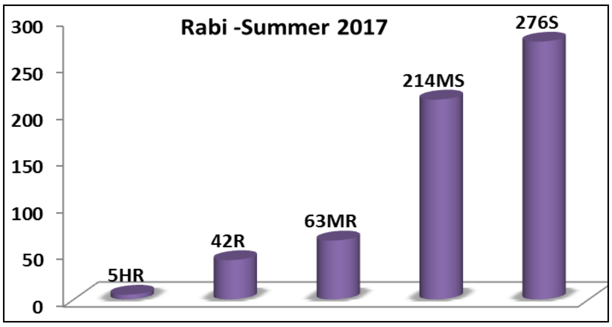

Fig 2: Rabi-Summer 2017

The graphical depiction of the reaction of genotype of screening in *Rabi* 2017 represented in the Fig. 2. From this graph it is clear that the scoring values obtained is skewed. As majority of the lines of the population was shown 5 genotypes highly resistance 42 genotypes resistance, 63 genotypes moderate resistance and 214 genotypes showed score values more than 7, we have obtained a slanted distribution towards susceptibility.

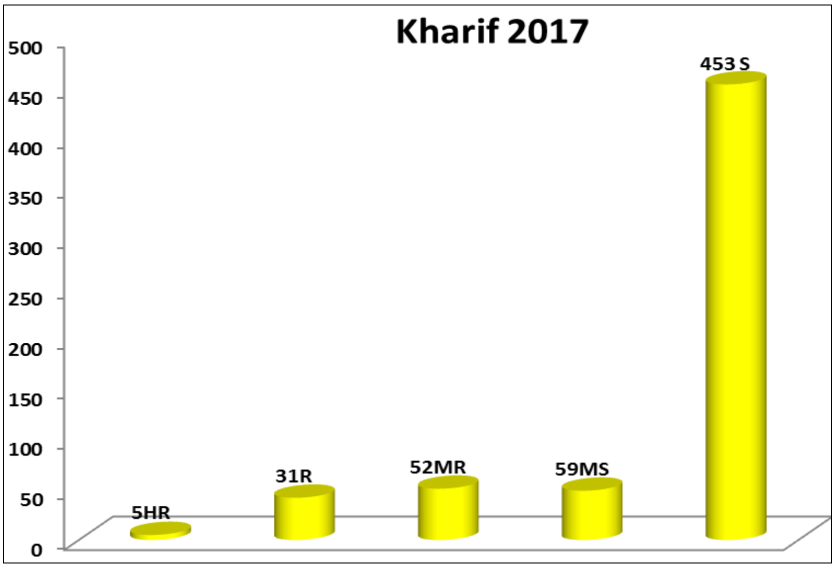

Fig 3: Kharif 2017

The graphical depiction of the reaction of genotypes of screening in *kharif* 2017 represented in the Fig. 3. From this graph it is clear that the scoring values obtained is skewed. As majority of the lines of the population was shown 5 genotypes highly resistance 36 genotypes resistance, 52 genotypes moderate resistance and 59 genotypes showed score values more than 7 categorised as susceptible and remaining 453 genotypes showed highly susceptibility genotypes get plant damage scored more than

Table 2: List of the Brown planthopper resistance promising rice accessions

S. No.	Accessions	Score	Reaction
1	IC453695	0.21	HR
2	IC454040	0.58	HR
3	IC454377	0.92	HR
4	IC454223X	0.85	HR
5	IC460174X	0.96	HR
6	IC459148	1.62	R
7	IC459199	1.16	R
8	IC459220	1.21	R
9	IC459797	1.71	R
10	IC460308	1.54	R
11	IC114326	2.42	R
12	IC114796	1.14	R
13	IC461616	2	R
14	IC124536	2.66	R
15	IC124546	2.33	R

16	IC124735	3	R
17	IC124743	2.28	R
18	IC124763	2.33	R
19	IC124989	1.62	R
20	IC126472	1.08	R
21	IC126596	1.67	R
22	IC135425	1.57	R
23	IC206606	1.54	R
24	IC206699	2.07	R
25	IC207184	3	R
26	IC207311	2.47	R
27	IC214133	1.58	R
28	IC124028	2.09	R
29	IC125139	2.88	R
30	IC86313	2.86	R
31	IC267449	2.68	R
32	IC300226	2.53	R
33	IC301732	1.23	R
34	IC301734	1.46	R
35	IC301736	1.27	R
36	IC386429	3	R
37	IR64 (Check)	1.03	R
38	IR79538-1-1-1 (Check)	1.00	R
39	PTB 33	1.00	R
40	TN1	9.00	HS

Assessment of genotypes under glasshouse condition for the brown planthopper resistance in the standard seed box screening technique was found to be perfect for classification of BPH resistance genotypes at seedling stage. The screening procedures standardized at IRRI and described by Heinrichs *et al*. (1985) [5] were adopted in this study. Performance of the accessions under glasshouse standard seed box screening technique (*kharif*-2016, *Rabi* 2017 and *kharif* 2017) during is depicted in Fig. 4.3 (a), 4.3 (b) at seedling stage. The main criteria to assess the performance of genotypes for brown planthopper resistance under glasshouse condition has to characterized by different parameters *i.e.*, highly resistance, resistance, moderate resistance, moderate susceptible and susceptible. Significant variation was found in resistance against brown planthopper during *kharif*-2017 and observed at 0-9 score as per SES 2014 (Table 4.3) along with checks. The accessions were identified for the reaction of the highly resistance was recorded in 5 accessions at score-0 namely namely IC454040, IC454223, IC135425, IC301734 and IC301736, however, 31 accessions identified for resistance having score-1 *i.e.*, IC453695, IC454377, IC459148, IC459199, IC459220, IC460174X, IC460308, IC124536, IC124546, IC124735, IC124743, IC125264, IC124763, IC124884, IC126313, IC134162, IC134850, IC134515, IC202398, IC202407, IC206606, IC206699, IC206890, IC99132, IC214133, IC214322, IC214546, IC124028, IC124867, IC125131, IC125139, IC125160, IC125368, IC125940, IC206586, IC86313, IC300226, IC301732, IC258895, IC377339, IC377996, IC386429,IC389453, IR64 and PTB33. Check variety Karma mahsuri, Samleshwari, Danteshwari, Pusa Basmati 1, Swarna, TN 1 and Jaya, showed highly susceptible and came under score-9. Although 51 accessions were observed moderately resistance 49 and 453 accession were recorded moderately susceptible and susceptible, respectively. These resistant varieties are compatible with biological control agents (predators, parasites, and pathogens) on which they have no direct adverse effect. The reduction in the brown planthopper (BPH) population on resistant and moderately resistant varieties improves the natural enemy to pest ratio in favour of biological control. The similar result were reported by Khush GS (2012) [9] Bhanu *et al*. (2014) [1] Gangaraju *et al* (2017) [3] Udayshree *et al*. (2018) [17].

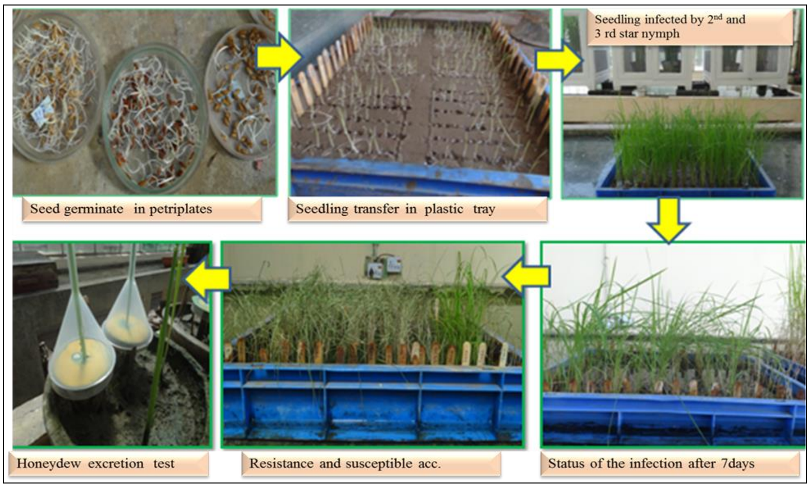

Fig 4: Stages of the core rice germplasm screening

Honeydew excretion test

The amount of honeydew excreted BPH female (♀) resistant genotypes the amount of honeydew excretion ranged from 16.23mm^2 to 36.54 mm^2. The results indicated that all the resistant rice genotypes showed significantly less amount of honeydew excretion as compared to susceptible check TN1 (86.28 mm^2) (Table 3.0) Among all the test genotype with resistant, IC454040 recorded lowest honeydew excreted area of 16.23mm2/2(♀) and followed by IC 454377, IC 301732, IC 301736, IC 460174X, IC 459199, and PTB 33 registered significantly lower honeydew excretion (17.22,18.76,19.49,19.57 and 20.07 mm2 / 2 (♀) respectively).

The resistances shown by rice genotypes were confirmed with honeydew excretion test which is regarded as antibiosis mechanism. This test is used for determination of the amount of sap ingested by the insect on resistant and susceptible rice genotype. So that the line IC 454377, IC 301732, IC 301734, IC 301736, IC 454223X, IC 460174X, IC 454040, IC 459199, IC 459220, IC 206606, IC 214133 and IC 38945 mm^2 per two female which showed less than 25 mm2 in the feeding test hence consider as resistance. However, the line IC460308, IC206699, IC124028, IC125139, IC86313 and IC300226 number with 27.5 and 34.2 mm^2 per two female were showed value more than 25 mm^2 so these was considered as moderate resistance at honeydew level. Honeydew excretion (♀) /2 damage score IC 454377, IC 301732, IC 301734, IC 301736, IC 454223X, IC 460174X IC 454040, IC 459199, IC459220 and IC459220 chess as compared PTB 33 (24.18).These accession identified as patented donors can be utilized in breeding programme for improvement as developed as resistance varieties.The breakdown of resistance in several varieties carrying major resistance genes have been reported from many Asian countries due to emergence of virulent biotypes Bhogali *et al*. (2015) [2].

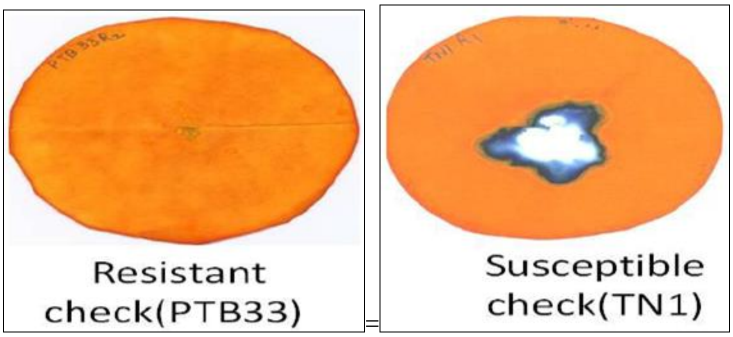

Fig 5: Amount of honeydew excreted by brown planthopper on susceptible and resistant checks.

Identification and deployment of new genes for BPH resistance in rice varieties by host plant resistance mechanisms is the important strategy to reduce the damage caused by brown planthopper to rice crop Kumar and Tiwari (2010) [10]. These findings are in close agreement with Watanabe *et al*. (2000) [18] who tested 178 rice genotypes, among five entries have showed resistant reaction to BPH damage, 28 entries showed the moderately resistant reaction. All other genotypes (12) were rated as moderately susceptible and susceptible (69) with damage score 7 and 9, respectively. The results are inconformity with other workers Jegadeeswaran *et al*. (2010) [8]. The standard checks PTB33 and TN1 has showed the resistant and highly susceptible reaction respectively and the study thus substantiates the findings of Harini *et al*. (2013) [4] Bhogadi *et al*. (2015) [2] and Pachauri *et al*. (2017) [12], Madurangi *et al*. (2011) [11].

Table 3: Honeydew excretion by brown planthopper on different rice genotypes

S. No.	Genotypes	Honeydew (mm$^{2/}$24 hrs)
1	IC 454377	17.22
2	IC301732	18.76
3	IC301734	18.09
4	IC 301736	19.49
5	IC454223X	19.34
6	IC460174X	19.57
7	IC454040	16.23
8	IC459199	20.07
9	IC459220	23.21
10	IC460308	34.22
11	IC206606	24.12
12	IC206699	29.41
13	IC214133	24.08
14	IC124028	33.51
15	IC125139	27.52
16	IC86313	36.54
17	IC300226	30.72
18	IC389453	25.56
19	PTB33	24.18
20	TN-1	86.28
	SEm±	1.05
	CD (5%)	3.26

Conclusion

The accessions were identified for the reaction of the highly resistance was recorded in 5 accessions at score-0 namely IC454040, IC454223, IC135425, IC301734 and IC301736, however, 42 accessions identified for resistance having score-1 *i.e.*, IC 453695, IC 454377, IC 202398, IC 202407, IC 206606, IC 206699, etc

The resistance shown by rice genotypes were confirmed with honeydew excretion test which is regarded as antibiosis mechanism. It was observed that the feeding activity on resistant lines were significantly less as compared to susceptible check. The resistance genotypes i.e., IC 454040, IC 454377, IC 301732, IC 301736, IC 460174X, IC 459199 were significantly differed over susceptible check *i.e.*, TN1 and resistance check *i.e.*, PTB-33. Those lines having honeydew excretion value less than 25 mm2 which is considered resistance category. The rice genotype which has shown resistant to BPH can be used in breeding programmes for developing the BPH resistant varieties.

Acknowledgement

Authors articulate honest gratitude to the Ministry of Science and Technology, Govt. Of India New Delhi for provided that the pecuniary support to the ICAR- Network Project "Consortium research Plateform on agrobiodiversity on germplasm characterization and evaluation" project and Director, NBPGR, New Delhi.

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