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Evaluation of germplasm accessions for resistance to rice Brown planthopper, *Nilaparvata lugens* (Stål)

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Abstract

A total of 1003 germplasm accessions collected from different parts of India were mass screened for their reaction to brown planthopper, *Nilaparvata lugens* (Stål) by standard seed box technique using TN1 and PTB 33 as susceptible and resistant checks respectively during 2015-16 at Indian Institute of Rice Research, Hyderabad. Out of 1003 entries screened, 37 entries exhibited a damage score (DS) ranging from 0-5 and were designated as highly resistant, resistant and moderately resistant to BPH, and the remaining 966 entries were susceptible with a damage score of 5.1-9.0. Out of 37 accessions, two accessions *viz.*, IC 75975 (DS-0.77), IC 216750 (DS-(0.80) were highly resistant, 21 accessions were resistant (DS-1.0-3.0) and 14 accessions were moderately resistant (DS- 3.1-5.0). The selected resistant entries were assessed for their feeding preference by brown planthopper by measuring feeding marks. They exhibited more number of probing (feeding) marks by BPH ranging from 5.2–31.6/seedling indicating the non-suitability of the accessions for feeding by the insect. Resistant check PTB-33 recorded 18.4 probing marks and susceptible TN1 recorded 3.1 probing marks/ seedling. The identified resistant germplasm accessions can be used in the breeding programmes to develop BPH resistant varieties.

Key words: Brown planthopper, germplasm accessions, host plant resistance, mass screening, *Nilaparva lugens*, probing marks.

Introduction

Rice is one of the world's most important staple food crops. There are many constrains in the rice production among which insect pests remain a constant problem in all rice growing areas. One of the most economically important insects is the brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) which can cause huge damage where both nymphs and adults suck the plant sap directly and indirectly transmits viral diseases such as ragged stunt and grassy stunt (Jena *et al*., 2006). Due to the infestation, plants turn yellow and dry up rapidly. At early infestation, yellow patches appear, which soon turn brownish due to the drying up of the plants resulting in 'hopper burn', and could result in 30-100% yield loss (Park *et al*., 2008). The control of BPH with chemical insecticides not only results in insecticide resistance development, but also has detrimental impact on natural enemies (Jhansi Lakshmi *et al*., 2010a and c and b; BalaKrishna and Satyanarayana, 2013). Host plant resistance is the most important measure to keep the insect pests under control. It is considered, that a resistant plant variety that reduces the insect population by 50 per cent in each generation is sufficient to eliminate an insect of economic importance within few generations (Painter, 1951). The necessity to identify suitable new resistant donors for brown planthopper from different sources is important in order to combat the pest and develop varieties resistant to BPH. It is also necessary to understand the mechanisms responsible for manifesting resistance into the selected cultures with desirable characters, so that these can be utilized effectively in the breeding programme. Keeping this in view, present investigation was planned to evaluate the germplasm accessions for their resistance to brown planthopper and to study the antixenosis mechanism of resistance for feeding.

Materials and methods

Mass rearing of brown planthopper: BPH was mass reared on the susceptible rice variety TN1 as described by Jhansi Lakshmi *et al*., 2010c. BPH population was initially collected from rice fields and pure culture was maintained in the greenhouse at a temperature of 30±5°C

with a relative humidity of 60±5% on 60 day old potted rice plants. Mass rearing was done in the cages of 70 cm x 62 cm x 75 cm dimension with glass panels on one side and wire mesh on all other sides. Twenty adult gravid female hoppers were collected with an aspirator and were released on pre-cleaned potted plants and were placed in oviposition cages. After four days of egg laying, the gravid females were collected and released on fresh batch of TN1 plants for further egg laying. Plants with eggs were taken out of cages and placed in separate cages for the nymphs to hatch. Fresh plants were placed in the cages with nymphs as and when required. The hatched nymphs were utilized for experiments as and when they attained the desired age. Necessary precautions were taken to keep the culture free from predators such as mirid bugs, spiders, other natural enemies and other hoppers like WBPH and GLH. Using this technique, a continuous pure culture of BPH was maintained during the period of study.

Mass screening of germplasm accessions: In order to identify the sources of resistance to BPH, 1003 germplasm accessions were mass screened under controlled greenhouse conditions as per the technique described by (Kalode *et al.*, 1975). The entries were pre-germinated in petridishes and sown individually with the help of forceps in screening trays (50cm x 40cm x 8cm) filled with fertilizer enriched puddled soil. Each screening tray contained 20 test lines with about 15 -20 seedlings per line, one row of resistant check (PTB 33) in the middle and two rows of susceptible check (TN1) in the border. Each row of susceptible and resistant check contained 30-40 seedlings. After planting, the screening trays were placed in fibre trays (60cm x 180cm x 8cm) filled with water. The screening trays were covered with mylar cages when the plants were 12-13 days old to prevent escape of the nymphs. First and second instar nymphs of BPH were released on the seedlings by tapping heavily infested plants from oviposition cages on the screening trays, ensuring that each test seedling was infested with at least 6-8 nymphs. The infested trays were monitored regularly for plant damage. When TN1 plants on one side showed damage, the tray was rotated by 180^{O} for even reaction on both the sides. When more than 90 per cent plants in the susceptible check were killed, the test entries were scored for the damage reaction, based on the 0-9 scale of International Standard Evaluation System (SES, 2013) (Table 1). All the 1003 germplasm entries were screened in two replications and the identified resistant accessions were screened in 5-7 replications.

Table 1: Criteria for BPH damage score in greenhouse screening

Resistance score	Plant state	Rating
0	No damage	Highly Resistant
1	Very Slight damage	
3	Lower leaf wilted with two green upper leaves	Resistant
5	Two lower leaves wilted with one green upper leaf	Moderately resistant
7	All three leaves wilted but stem still green	Moderately susceptible
9	All plants dead	Susceptible

Feeding behaviour of adult brown planthopper on 50 selected germplasm accessions based on probing marks: The highly resistant, resistant and moderately resistant entries along with some susceptible accessions, susceptible and resistant checks were selected to find out the feeding behaviour of one day old adult and third instar nymphs of brown planthopper expressed in terms of feeding marks or probing marks on the leaves and stems of the rice entries (Naito 1964). For this purpose, a single one day old adult female, third instar was caged for 24 hours on seven day old test entry in a test tube and this was replicated five times. After 24 hours, the insect was removed and the test plant was stained by dipping for one hour in one per cent aqueous erythrosine solution to distinguish the feeding marks from the test entries. The feeding marks were counted and the data were analysed statistically in completely randomized block design and the means were separated using DMRT.

Results and discussion

Germplasm accessions resistant to BPH

Results pertaining to screening of 1003 germplasm accessions are presented in Table 2.

Out of these 1003 germplasm accessions, 37 accessions exhibited a damage score (DS) ranging from 0-5 and were designated as highly resistant, resistant and moderately resistant to BPH, and the remaining 966 accessions were found susceptible with a damage score of 5-9. Out of 37 accessions, two accessions *viz*., IC 75975 (DS-0.77) and IC 216750 (DS-0.80) were highly resistant, 21 accessions *viz.*, IC 76013, IC 76057, IC 216735, IC 216974, IC 540644, IC 216759, IC 216553, IC 75961, IC 76010, IC 216636, IC 75990, IC 216737, IC 216585, IC 216602, IC 216788,

IC 217492, IC 216618, IC 215054, IC 216680, IC 218166 were resistant (DS-1.0-3.0) and 14 accessions viz., IC 217610, IC 218053, IC 75797, IC 76000A, IC 217507, IC 76033, IC 216650, IC 216605, IC 216651, IC 216944, IC 217750, IC 217309, IC 216678 were moderately resistant (DS-3.1-5.0) (Figures 1 a, b and c).

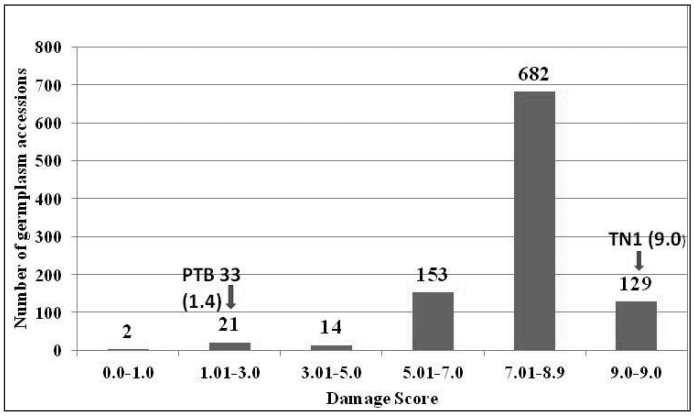
The frequency distribution graph (Figure 2) shows that in the remaining 964 germplasm accessions, 153 accessions were moderately susceptible with a damage score of 5.1 to 7.0, 682 accessions were susceptible with a damage score of 7.1 to 8.9 and the remaining 129 accessions were highly susceptible with a damage score of 9.0. The resistant check PTB 33 recorded a damage score of 1.4 and the susceptible check TN1 recorded a damage score of 9. Host plant resistance is the most economical and desirable method for the management of crop pests (Sharma, 2002). Screening for resistance to brown planthopper is a continuous process to identify new sources of resistance. In India, host plant resistance to BPH is being exploited in several research centres and very important sources of resistance have been identified.

Figure 2: Frequency distribution of damage score of germplasm accessions

Table 2: Damage Score and reaction of germplasm accessions to brown planthopper

Sr.No	Germplasm accessions	Damage score	Reaction	Sr. No	Germplasm accessions	Damage score	Reaction
1	IC75975	0.77	HR	21	IC215054	2.63	R
2	IC216750	0.8	HR	22	IC216680	2.64	R
3	IC76013	1.08	R	23	IC218166	2.99	R
4	IC76057	1.08	R	24	IC217610	3.07	MR
5	IC216735	1.27	R	25	IC218053	3.18	MR
6	IC216974	1.5	R	26	IC75797	3.2	MR
7	IC540644	1.53	R	27	IC76000A	3.24	MR
8	IC216759	1.61	R	28	IC217507	3.59	MR
9	IC216553	1.62	R	29	IC76033	3.84	MR
10	IC75961	1.64	R	30	IC216650	3.86	MR
11	IC76010	1.75	R	31	IC216566	3.87	MR
12	IC216600	1.93	R	32	IC216605	3.91	MR
13	IC216636	2.06	R	33	IC216651	3.99	MR
14	IC75990	2.1	R	34	IC216944	4.01	MR
15	IC216737	2.26	R	35	IC217750	4.21	MR
16	IC216585	2.32	R	36	IC217309	4.56	MR
17	IC216602	2.37	R	37	IC216678	5	MR
18	IC216788	2.38	R	38	TN1	9	HS
19	IC217492	2.39	R	39	PTB 33	1.4	R
20	IC216618	2.6	R	40	M0-1	4.86	MR

HR: Highly Resistant; R: resistant; MR: Moderately Resistant; MS: Moderately Susceptible; S: Susceptible; HS: Highly Susceptible



Figures 1a, 1b and 1c: Screening trays with germplasm accessions

Ramulamma *et al.* (2015) reported that out of 400 germplasm accessions tested, 2 were resistant and 13 were moderately resistant to BPH. Nagendra Reddy *et al*. (2016) screened 620 entries, out of which four entries viz., IET 23620, IET 23660, IET 23739 and IET 23771 were resistant and eleven entries were moderately resistant and remaining entries were susceptible. Akanksha *et al*. (2017) evaluated nine hundred and twenty rice germplasm accessions for their reaction to brown planthopper, out of which twelve accessions were resistant while 23 accessions were moderately resistant and others were susceptible. Reeta Lakra *et al*. (2016) screened 260 wild rice germplasm lines out of which 13 were highly resistant, 30 were resistant, 38 were moderately resistant, 5 were moderately susceptible and others susceptible. Ritu and Ravi Saxena (2009) screened 198 rice germplasm accessions for BPH resistance and of them 12 were resistant, 14 were moderately resistant and 178 were susceptible.

Feeding behaviour of brown planthopper on selected germplasm accessions based on probing marks:

BPH adults: The results on number of probing marks by BPH adults are presented in Table 3.

Table 3: Probing marks of adults of brown planthopper on germplasm accessions

S No	Germplasm accession Number	Probing Marks Adult		S No	Germplasm accession Number	Probing Marks Adult
1	IC75975	17.4±2.9 $^{d-o}$		27	IC76000A	14.4±2.6^{j-p}
2	IC216750	14±1.5 $^{j-p}$		28	IC217507	23.4±1.3^{b-d}
3	IC76013	22.8±1.0 $^{b-e}$		29	IC76033	17.2±2.8e-m
4	IC76057	13±2.4 $^{k-p}$		30	IC216650	26.2±0.8^{bc}
5	IC216735	14.4±0.9 $^{i-p}$		31	IC216566	19.6±2.2^{c-j}
6	IC216974	31.6±6.3^{b}		32	IC216605	19.8±1.1^{c-j}
7	IC540644	17.6±1.4^{d-m}		33	IC216651	19.8±2.2^{c-j}
8	IC216759	22.4±1.9^{c-f}		34	IC216944	21.2±2.5^{c-g}
9	IC216553	15.6±2.0^{g-o}		35	IC217750	21.6±1.6^{c-g}
10	IC75961	5.2±0.9^{s}		36	IC217309	18.6±1.8^{d-k}
11	IC76010	21±3.0^{c-i}		37	IC216678	13±1.2^{k-p}
12	IC216600	12.4±0.8^{l-p}		38	IC217107	18.6±1.7^{d-k}
13	IC216636	17.4±1.5^{d-m}		39	IC218002	13±1.6^{k-p}
14	IC75990	10.2±0.4^{p-r}		40	IC218085	8±2.5^{rs}
15	IC216737	16.2±0.9^{e-o}		41	IC216822	9.4±0.7^{p-r}
16	IC216585	12.2±1.1^{m-p}		42	IC75786	4.5±0.5^{s}
17	IC216602	20.8±1.2^{c-h}		43	IC218011	7.6±2.2^{q-s}
18	IC216788	14.8±2.0^{h-p}		44	IC216841	16.8±2.1^{e-m}
19	IC217492	19.4±2.4^{c-j}		45	IC218658	13±1.5^{k-p}
20	IC216618	18.4±2.5^{d-l}		46	IC217452	16.6±1.4^{e-m}
21	IC215054	19.6±2.5^{c-i}		47	IC75966	15.8±1.5^{f-m}
22	IC216680	19±1.3^{d-j}		48	IC218062	17.6±1.3^{d-l}
23	IC218166	16.2±3.1^{g-o}		49	TN1	3.1±0.7^{t}
24	IC217610	9.4±0.8^{p-r}		50	PTB 33	18.4±1.1^{e-k}
25	IC218053	20±2.5^{d-j}		51	M0-1	14.6±1.9^{i-p}
26	IC75797	12±2.4^{o-q}			SEd	0.3851
					CD(.05)	0.7592

Note: The means in a column followed by same letter are not significantly different from each other

The results indicated that there was a significant difference among the germplasm accessions with regard to probing marks. The resistant accession IC 216974 recorded maximum number of probing marks (31.6) while susceptible check TN1 has recorded lowest number of marks (3.1) by adult brown planthopper. The resistant entries recorded more number of probing marks compared to susceptible entries. Maximum number of probing marks were recorded in the resistant accession IC 216974 (31.6) followed by IC 216650 (26.2), IC 217507 (23.4), IC 76013 (22.8), IC 216759 (22.4), IC 217750 (21.6). The resistant check PTB 33 has more number of probing marks (18.4). The susceptible accessions recorded less number of probing marks (7.6-18.6) (Figure 3).

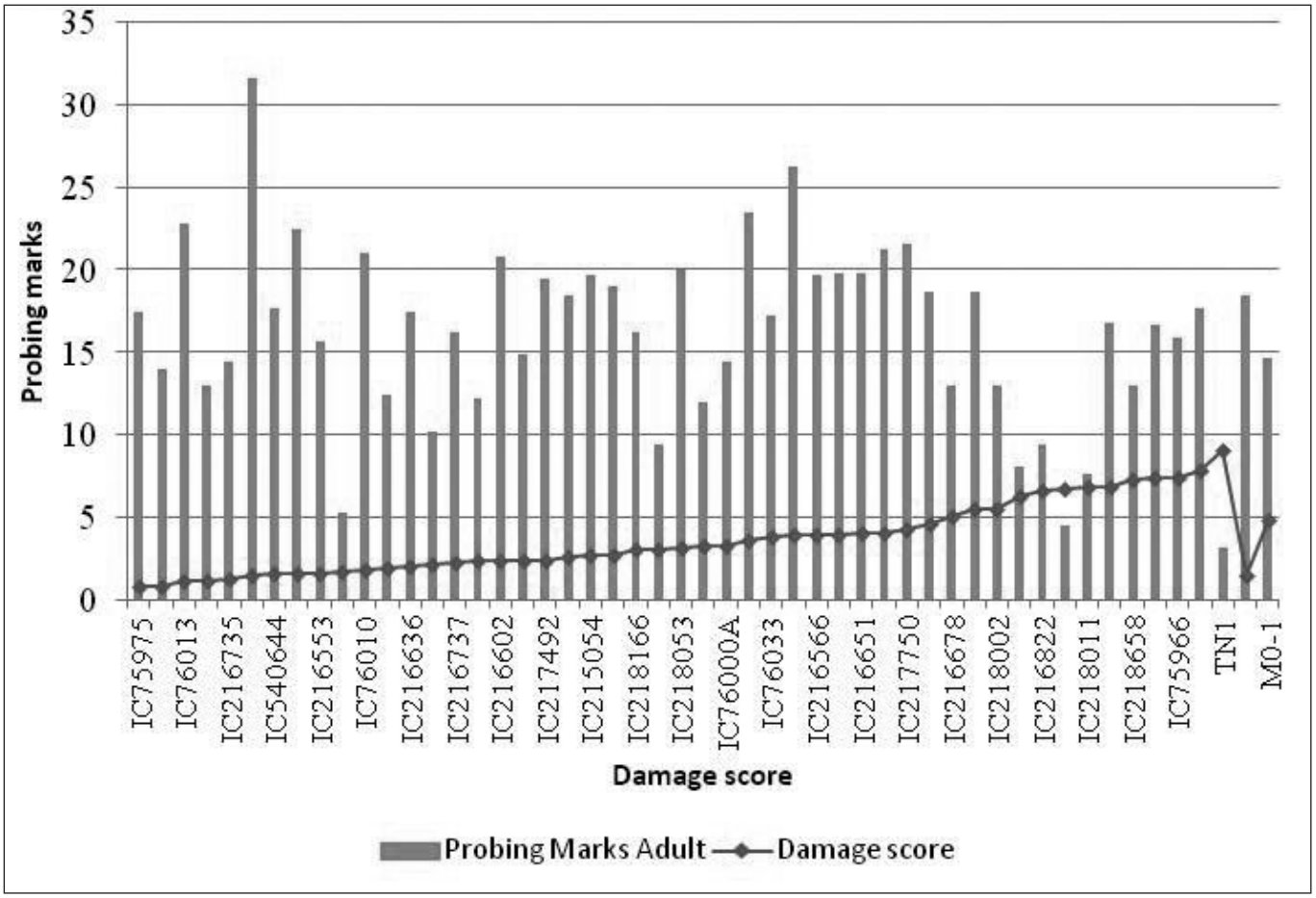


Figure 3: Relation between damage score and probing marks of BPH adults on germplasm accessions

BPH nymphs: BPH nymphs probed more number of times on the resistant germplasm accessions compared to susceptible accessions (Table 4). The resistant germplasm accession IC 216680 was probed maximum number (19.5) of times followed by IC 216974 (18), IC 76013 and IC 216650 (16.8), IC 217750 (16.4) and IC 216735 (16.2) and the resistant check PTB 33 received 12 feeding marks. The susceptible entries were probed less number of times (average 13.7 probing marks/seedling) and the susceptible check TN1 received the least number of probing marks (2.8) (Figure 4).

Table 4: Probing marks of brown planthopper nymphs on germplasm accessions

S No.	Germplasm accession Numbers	Probing Marks of Nymphs	S. No.	Germplasm accession Number	Probing Marks of Nymphs
1	IC75975	$8.8\pm1.5^{l-n}$	27	IC76000A	$13.4\pm1.4^{a-i}$
2	IC216750	$14.2\pm2.0^{a-i}$	28	IC217507	7 ± 0.7^{mn}
3	IC76013	$16.8\pm1.4^{a-d}$	29	IC76033	$12.8\pm2.8^{b-l}$
4	IC76057	$9.1\pm2.2^{k-n}$	30	IC216650	$16.8\pm2.1^{a-c}$
5	IC216735	$16.2\pm1.4^{a-h}$	31	IC216566	$13.1\pm3.4^{a-j}$
6	IC216974	18 ± 1.3^{a}	32	IC216605	$12.8\pm2.0^{a-k}$
7	IC540644	$12.5\pm2.2^{a-e}$	33	IC216651	$11.4\pm1.2^{d-m}$
8	IC216759	$10.8\pm1.1^{f-m}$	34	IC216944	$10.6\pm1.6^{f-m}$
9	IC216553	$15.2\pm2.6^{a-g}$	35	IC217750	$16.4\pm1.9^{a-e}$
10	IC75961	$14.8\pm0.9^{a-g}$	36	IC217309	$15\pm2.0^{a-g}$
11	IC76010	4.8 ± 0.2^{no}	37	IC216678	$13.8\pm2.6^{a-i}$
12	IC216600	$14\pm1.3^{a-i}$	38	IC217107	$11.8\pm1.4^{d-l}$
13	IC216636	$10.8\pm1.7^{f-m}$	39	IC218002	$15.6\pm0.5^{a-f}$
14	IC75990	$11.4\pm1.1^{d-m}$	40	IC218085	$12.1\pm1.0^{a-k}$
15	IC216737	$10.2\pm1.3^{g-m}$	41	IC216822	$11\pm0.8^{e-m}$
16	IC216585	$9.6\pm1.4^{i-m}$	42	IC75786	$9\pm2.2^{j-n}$
17	IC216602	$12.4\pm1.8^{c-i}$	43	IC218011	$11\pm1.9^{j-i}$
18	IC216788	$14.6\pm2.4^{a-i}$	44	IC216841	$12.4\pm3.2^{d-l}$
19	IC217492	$12.8\pm1.6^{a-k}$	45	IC218658	$10.8\pm1.6^{f-m}$
20	IC216618	$10.2\pm1.2^{h-m}$	46	IC217452	$16.6\pm1.4^{a-d}$
21	IC215054	$14.2\pm2.1^{a-i}$	47	IC75966	7.7 ± 0.7^{mn}
22	IC216680	19.5 ± 0.9^{ab}	48	IC218062	$13.2\pm2.3^{a-j}$
23	IC218166	$13.8\pm1.5^{a-i}$	49	TN1	2.8 ± 0.4^{o}
24	IC217610	$13.6\pm1.6^{a-i}$	50	PTB 33	$12.2\pm1.7^{c-l}$
25	IC218053	$12.4\pm1.1^{a-k}$	51	M0-1	$14.2\pm5.8^{a-k}$
26	IC75797	5.6 ± 1.0^{no}		SEd	0.372
				CD(.05)	0.7334

Note: The means in a column followed by same letter are not significantly different from each other

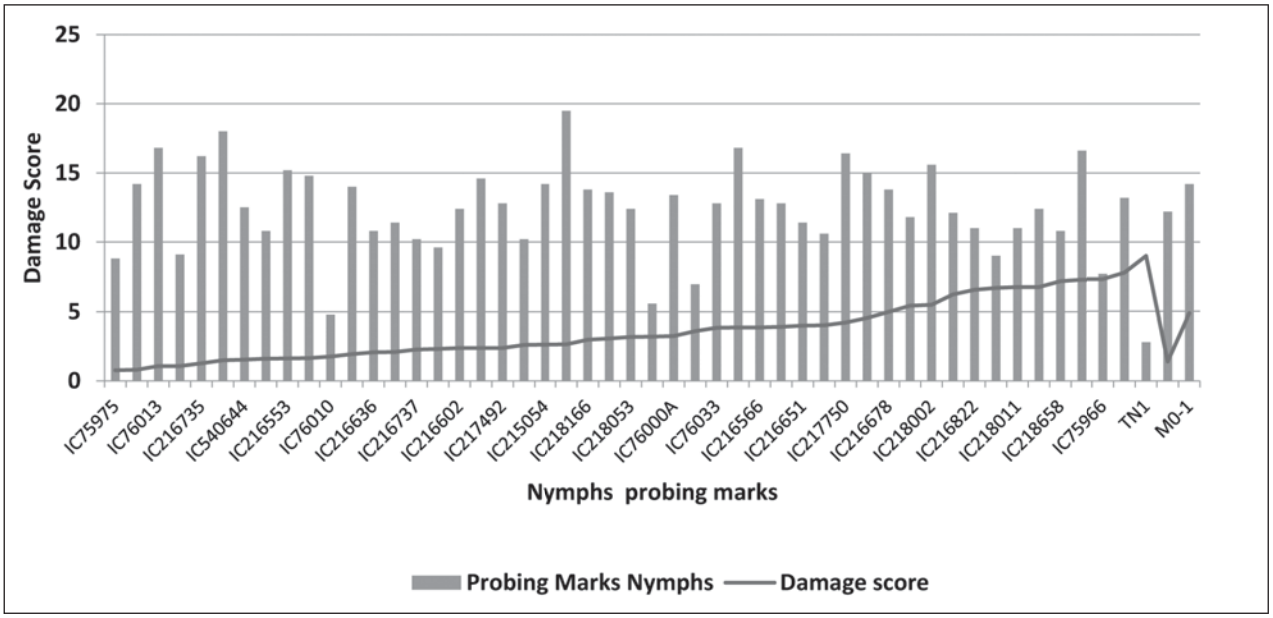


Figure 4: Relation between damage score and probing marks of BPH nymphs on germplasm accessions

In general nymphs probed less number of times than adults. More number of feeding punctures in the resistant and moderately resistant entries might be due to the reason that, these resistant and moderately resistant entries did not sustain prolonged feeding due to the presence of certain feeding deterrents or toxic chemicals or absence of feeding stimulants. Hence, the insect had to probe more on the resistant genotypes to locate feeding sites (Sogawa, 1982). Our results corroborate with the findings of several workers (Sogawa and Pathak, 1970; Karim 1975; Reddy and Kalode, 1985; Li *et al.* 1991; Pophaly *et al.* 2001; Alagar *et al.*, 2007; Kale *et al.* 2007; Anitha *et al*., 2015) who reported that the number of probing marks were more on resistant varieties compared to susceptible ones. Udayababu *et al.* (2011) also reported that average probing marks on resistant plants ranged between 30.4 to 42.9 whereas resistant and susceptible checks have recorded 22.1 and 6.7 probing marks, respectively. Bhanu *et al.* (2014) observed that brown planthopper probed more number of times on the resistant cultures like MTU 1075 (128.1 probing marks), MTU IJ 206-7-4-1 (112.8 probing marks) and MTU PLA 99-1-3-1-2 (110.2 probing marks) compared to susceptible ones. Nagendra Reddy *et al.* (2016) reported that the resistant entries including IET No. 23620 (26.5) and IET No. 23660 (22.3) and moderately resistant entries including IET No. 23661 (25.0), IET No. 23705 (23.3) and IET No. 23702 (23.2) were probed more number of times which were on par with resistant check, Ptb 33 (26.5 feeding punctures). Nanda *et al.* (1999) recorded that PTB 33 had a maximum of 110 probing marks on the leaf sheaths on 10-day old plants compared to 22 probing marks on TN1. The rest of the test varieties had 35 to 85 probing marks. Our studies corroborate with the findings of above authors.

Correlation between damage score and probing marks

Correlation analysis between damage score and probing marks of adults (R^2=-0.3575) and nymphs (R^2=-0.20879) indicated negative correlation eventhough it is non-significant. More number of probing marks were observed on the germplasm accessions which are resistant and vice versa (Table 5).

Table 5: Correlation between Damage Score and Probing marks

	Damage score	Probing Marks Adult	Probing Marks Nymphs
Damage score	1		
Probing Marks-Adult	-0.3575	1	
Probing Marks-Nymphs	- 0.20879	0.3209	1

When the data were subjected to linear regression analysis (Table 6 and Figure 5), a negative relation was observed between damage score and number of probing marks of nymphs and adults. In the adults, probing marks (non-preference for feeding) is able to explain 12.7 percent of variation in damage score and for each unit increase in the probing marks, the damage score is decreased by 0.14 units. In the nymphs, probing marks is able to explain 4.3 percent of variation in damage score and for each unit increase in probing marks the damage score is decreased by 0.135 units. In addition to probing marks i.e non-preference for feeding, the varietal resistance is dependent on other parameters also. In the present study, the germplasm accessions resistant to BPH and with more number of probing marks which are not preferred for feeding can be used in the breeding programme to develop brown planthopper resistant varieties.

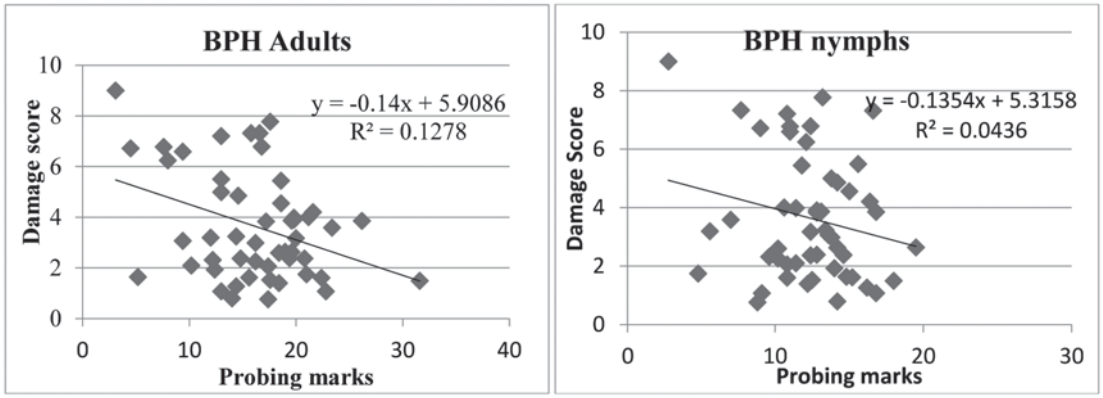


Figure 5: Regression between probing marks in BPH adults and nymphs and damage score

Table 6: Linear Regression analysis between damage score and probing marks

Variable	No of obser-vations	Regression equation	Standard Error	R2
Probing marks of nymphs	51	y = -0.135x + 5.315	1.157002764 0.090589566	0.0436
Probing marks of adults	51	y = -0.14x + 5.908	0.89088068 0.052254111	0.127

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