

PROBING BEHAVIOUR OF BROWN PLANTHOPPER *NILAPARVATA LUGENS* (STAL.) IN THE RESISTANT GERMPLASM ACCESSIONS

ABSTRACT: A total of 1110 ENRRD rice germplasm accessions were evaluated for their resistance to brown planthopper, BPH in glasshouse by adopting internationally accepted standard seed box screening technique and scored on 0-9 scale by following the IRRI method of standard evaluation system for rice. Among 1110 rice germplasm accession tested, 3 were categorized as highly resistant, 24 as resistant, 19 as moderately resistant, 192 as moderately susceptible, 509 as susceptible and rest of other 363 genotypes were highly susceptible to BPH. The studies on antixenosis mechanism of resistance for feeding by probing marks revealed that resistant entries, were least preferred by BPH nymphs and adults with more number of probing marks. The results indicate that the germplasm accessions viz., IC 343457, IC 300168 and IC 377051 were highly resistant and possess non-preference mechanism of resistance and are not preferred by BPH for feeding. The identified resistant entries can be used in the breeding programmes to develop BPH resistant varieties.

KEYWORDS: *Nilaparvata lugens*, germplasm accessions, host plant resistance, probing marks, feeding behavior, rice

INTRODUCTION

Cultivation of rice is the major activity and source of staple food and income for million of households round the globe. In India, rice is cultivated in an area of 42.96 million hectares with a total production and productivity of 158.75 million tonnes and 3.69 metric tonnes per hectare, respectively. Paddy, is attacked by about 800 species of insect pests in both field and storage. Of all these, brown planthopper (BPH),

Nilaparvatalugens (Stal) (Homoptera: Delphacidae) is economically important, 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 (Khush and Brar, 1991). BPH damage results in appearance of round and yellow patches, which soon turn brownish due to the drying up of the plants which is called as 'hopper burn', and could result in causing yield loss ranging from 10-75%. Different chemical pesticides were advised to manage BPH, however their negligent use resulted in their revival, the development of insecticide resistance, and a negative influence on natural enemies (Chelliah and Heinrichs, 1980., Jhansi Lakshmi *et al*, 2010a, 2010b). An alternate strategy for controlling brown planthopper is to improve host plant resistance. Growing pest-resistant cultivars offers farmers free pest control and can work well with other pest control strategies in an integrated pest management programme. The most significant benefit of plant resistance is its particular, cumulative, and long-lasting impact on insect populations. The necessity to identify suitable new resistant donors for BPH from different sources is utmost important in order to combat this insect and to develop material resistant to different biotypes. It is necessary to understand the mechanism and factors which are responsible for manifesting the resistance into the selected cultures with desirable characters, so that these can be utilized effectively in the breeding programme. Keeping this in view, the present investigation of identification of resistant sources from the ENRRD (Establishment of National Rice Resource Database) germplasm accession to brown planthopper was carried out and the anti-xenosis mechanism of resistance for feeding in terms of probing marks was studied.

MATERIALS AND METHODS

Mass Rearing of Brown Planthopper

The BPH was mass reared on susceptible variety TN1 in the Entomology greenhouse of ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad. Twenty adult gravid female hoppers were released on pre-cleaned potted TN1 plants in oviposition cages. Plants with eggs were taken out and placed in separate cages for hatching. The hatched nymphs were utilized as and when they obtain the desired age.

Mass Screening of ENRRD Entries

In order to identify the sources of resistance to BPH, 1110 ENRRD (Establishment of National Rice Resource database) germplasm accessions supplied by ICAR-National Bureau of Plant Genetic Resources, New Delhi and multiplied and maintained by ICAR-Indian institute of Rice research, Rajendranagar, Hyderabad were mass screened. Screening was carried out by adopting mass screening test under controlled greenhouse conditions as per the technique described by (Kalode *et al*, 1975, Nagendra Reddy *et al*, 2016). Theseed of the ENRRD germplasm entries was pre-germinated in petridishes and sown individually in the 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 (PTB33) in the middle and two rows of susceptible check (TN1) in the borders. Each entry was screened in two replications. After sowing, the screening trays were placed in fibre trays (60cm x 180cm x 8cm) filled with water. The screening trays were covered with mylar cages and first and second instar BPH nymphs were released on to 12-13 day old seedlings. When more than 90 per cent plants of the susceptible check, TN1 were killed, the test entries were scored for the damage reaction, based on a 0-9 scale of International Standard Evaluation System (IRRI, 2013) as described in table 1. ENRRD germplasm accessions showing damage score below 5.0 were retested with two replications to confirm the consistency of the reaction to BPH.

Feeding/probing behaviour of third instar BPH nymphs and adults on selected ENRRD

germplasm accessions

A total number of 43 ENRRD germplasm accessions including highly resistant, resistant moderately resistant and susceptible accessions along with susceptible and resistant checks were selected to find out the feeding/probing behaviour of third instar nymphs and adults expressed in terms of feeding or probing marks on the stems of rice plants. Prior to insertion of the stylets, the planthopper secretes a small amount of coagulable saliva while pushing the labial tip onto the plant epidermis. This makes a tight connection between them leaving characteristic circular marks at the point of stylet insertion. The salivary deposit on the plant epidermis is called a feeding mark. The non-preference of the BPH for food was studied by counting probing marks. Number of probing marks made by a single one day old female and third instar nymph during one day feeding on resistant and susceptible rice cultures was recorded. For this purpose, a single one day old adult female insect was allowed to feed on seven day old test entry in a test tube for 24 hours and this was replicated five times. After 24 hours, the insect was removed and the test plant was stained by dipping in one per cent aqueous Erythrosin-B solution for one hour to distinguish the feeding marks on the test entries (Naito, 1964 and Ponnada, 2011). The feeding marks were counted by using magnifying hand lens. The experiment was also conducted with third instar nymphs. The data were analyzed statistically in Completely Randomized Design (CRD) and the means were separated using LSD.

Correlation and regression analysis:

Pearson correlation analysis and linear regression analysis

between the damage score and probing marks of nymphs and adults was carried out using OPStat software to understand their relationship and interdependence.

RESULTS AND DISCUSSION

ENRRD germplasm accessions resistant to BPH

Among 1110 rice germplasm accessions tested, Three entries *viz.*, IC Nos. 343457 (DS 0.3), 300168 (DS 0.6) and 377051 (DS 0.9) were highly

resistant with a damage score of 0.1 to 1.0; while, 24 entries viz., ICN Nos. 319799 (DS 1.1), 343394 (DS 1.2), 301181 (DS 1.3), 449821 (DS 1.3), 343392 (DS 1.3), 464944 (DS 1.4), 450041 (DS 1.4), 341334 (DS 1.4), 300166 (DS 1.6), 377527 (DS 1.7), 300167 (DS 1.8), 319350 (DS 2.1), 346927 (DS 2.1), 343515 (DS 2.2), 300202 (DS 2.4), 577624 (DS 2.5), 545441 (DS 2.5), 497079 (DS 2.7), 461801 (DS 2.7), 377423 (DS 2.8), 554787 (DS 2.8), 354787 (DS 2.9), 321833 (DS 2.9) and 252243 (DS 3.0), were resistant with a damage score of 1.1 to 3.0; whereas 19 entries were identified as moderately resistant with a damage score of 3.1 to 5.0 (Table 2 and Fig 1). Among the remaining 1110 ENRRD entries, 192 entries were moderately susceptible (DS 5.1 to 7.0), 509 entries were susceptible (DS 7.1 to 8.9) and remaining 363 entries were highly susceptible (DS 9.0). PTB 33 was resistant (DS 1.4) and TN1 was susceptible (DS 9.0).

Screening for resistance to brown planthopper is a continuous process to identify

new sources of resistance. International Rice Research Institute (IRRI) has initiated screening of thousands of rice varieties and lines including worldwide germplasm collections and breeding lines of rice in which a large number of varieties were identified as resistant (Fernando *et al.*, 1979). In India, host plant resistance to BPH is being exploited in several research centres and important sources of resistance have been identified their use in breeding programme has been attempted (Alagar *et al.*, 2007, Deen *et al.*, 2010, Ramulamma *et al.*, 2015, Anupama *et al.*, 2018 and Anjali *et al.*, 2022).

Feeding/probing marks

A total number of 41 ENRRD germplasm accessions including highly resistant and resistant accessions along with susceptible and resistant checks were selected to find out the feeding/probing behaviour of third instar nymphs and adults expressed in terms of feeding or probing marks on the stems of rice plants.

BPH adults

There was a significant difference among the entries with regard to probing marks

(Table 3 and Fig 2). The resistant accessions recorded more number of probing marks compared to susceptible accessions. Among the resistant accessions, the resistant accession, IC 377051 had maximum number of feeding punctures (20.8) while IC145400 received minimum number of feeding punctures (4.0). The susceptible check TN1 had 6.8 probing marks while the resistant check, PTB33 recorded the maximum number of probing marks (44.0). The resistant germplasm accessions viz., IC 377051 (20.8), IC497079 (19.4), IC464944 (18.6), IC300167 (17.8), IC319799 (16.8), IC377527 (16.4) recorded more number of feeding marks compared to other resistant and susceptible entries. The germplasm accessions viz., IC145400 (4.0), IC252243 (4.4), IC300683 (4.4), IC301172 (4.4), IC301181 (4.8), IC449821 (4.8), IC319350 (4.8), IC377746 (4.8), IC343392 (5.2), IC300378 (5.4) recorded very less number of feeding punctures. The highly resistant germplasm accessions were probed more number of times (14.8) by the BPH adults compared to the resistant (12.1), moderately resistant (8.1) and susceptible accessions (7.8).

BPH nymphs

There was a significant difference among the entries with regard to probing marks by nymphs (Table 2 and Fig 1). In the resistant accessions, highest number of probing marks by nymphs were observed on IC464944 (29.4) followed by IC 461801 (15.4), IC497079 (12.2), IC319799 (10.6) and IC450382 (11.2), PTB33 had the maximum number of probing marks (38.6). Lowest number of probing marks were observed on IC300163 (2.0), followed by IC319350 (4.0), IC145400 (4.2), IC343392 (4.4) and IC301172 (4.4) and TN1 had 6.8 probing marks. Nymphs probed more number of times on the highly resistant germplasm accessions (10.6) compared to resistant (10.5), moderately resistant (6.4) and susceptible entries (6.3). More number of feeding punctures were observed when the plant is fed with adult BPH (10.4) compared to the

nymphs(8.6).More number of feeding punctures in the resistant entries might be due to the reason that, these resistant and moderately resistant entries did not sustain prolonged feeding due to presence of certain feeding deterrents or toxic chemicals or absence of feeding stimulants. Hence, the insect had to probe more number of times on the resistant genotypes to locate feeding sites (Sogawa and Pathak, 1970). The results corroborate with the findings of several workers (Ponnada *et al* 2011, Anupama *et al.*, 2018, 2022, Nagendra Reddy *et al*, 2016 and Priyadarsini *et al*, 2021) who reported that resistant varieties received more probing marks than susceptible ones.

Correlation and regression between damage score and probing marks

Correlation analysis between damage score and probing marks by nymphs (-0.290^{NS}) and adults (-0.303*) indicated a negative relation which is significant in adults. More number of probing marks were observed in the germplasm accessions which are resistant and vice versa (Table 3 and Fig 2). A negative relation between damage score and probing marks made by brown planthopper and white backed planthopper in rice germplasm accessions was suggested by Ramesh *et al*, 2014 and Anupama *et al*, 2018, Priyadarsini *et al*, 2021, Anupama *et al*, 2022. There was a negative relation between damage score and probing marks and the probing marks could explain 19% variation in the damage score and for each unit increase in the probing marks, the damage score is decreased by 0.328 units (Table 4 and Fig 3).

CONCLUSIONS

In the present investigation, ENRRD germplasm accessions viz., IC343457, IC300168 and IC377051 were highly resistant to brown planthopper with less damage score (0.0 to 1.0) and with more number of probing marks which indicate that they are not preferred for feeding and these accessions can be used in the breeding programmes to develop brown planthopper resistant varieties.

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Table 1. Classification of resistance based on damage reaction.

Plant state	Damages core	Resistance classification
None of the leaves yellow or dead	0	Highly resistant
One bottom leaf yellow	1	Resistant
One or two leaves yellow or leaf dried	3	Moderately resistant
One or two leaves dried or one leaf healthy	5	Moderately resistant
All leaves dried or yellow but stem green	7	Susceptible
Plant dead	9	Highly susceptible

Table 2. Damagescore and probing marks of brown planthopper in ENRRD germplasm accessions

S.No	Germplasm accessions (IC No)	DS	Probing marks made by		S.No.	Germplasm accessions (IC No)	DS	Probing marks made by	
			Nymphs	Adults				Nymphs	Adults
1	IC 343457	0.3	10.4(3.2) ^{c-g}	8.8(2.9) ^{g-m}	23	IC 461261	3.2	6.0(2.4) ^{e-n}	8.4(2.9) ^{g-n}
2	IC 300168	0.6	10.6(3.1) ^{c-g}	14.8(3.8) ^{b-g}	24	IC 450072	4.0	10.4(3.2) ^{c-f}	9.2(2.9) ^{f-m}
3	IC 377051	0.9	10.8(3.3) ^{c-e}	20.8(4.4) ^b	25	IC 413638	4.1	11.0(3.2) ^{c-f}	7.4(2.7) ^{h-n}
4	IC 319799	1.1	8.2(2.8) ^{d-m}	16.8(3.9) ^{b-f}	26	IC 377746	4.5	4.8(2.2) ^{i-o}	4.8(2.1) ^{mn}
5	IC 343394	1.2	6.2(2.5) ^{e-n}	6.0(2.3) ^{j-n}	27	IC 332672	4.6	4.8(2.2) ^{i-o}	9.2(2.9) ^{f-m}
6	IC 301181	1.3	6.4(2.5) ^{e-n}	4.8(2.1) ^{l-n}	28	IC 145400	4.6	4.2(2.0) ^{m-o}	4.0(1.9) ⁿ
7	IC 343392	1.3	4.4(2.1) ^{l-o}	5.2(2.2) ^{k-n}	29	IC 300378	4.7	7.6(2.7) ^{d-n}	5.4(2.3) ^{j-n}
8	IC 449821	1.3	6.6(2.6) ^{e-n}	4.8(2.1) ^{l-n}	30	IC 145402	4.7	5.2(2.2) ^{i-o}	9.8(3.1) ^{e-k}
9	IC 341334	1.4	10.4(3.2) ^{c-g}	11.2(3.2) ^{d-j}	31	IC 300683	4.8	4.6(2.1) ^{k-o}	4.4(2.1) ^{mn}
10	IC 464944	1.4	29.4(5.2) ^b	18.6(4.1) ^{b-d}	32	IC 300991	4.8	5.0(2.2) ^{i-o}	10.4(3.2) ^{d-j}
11	IC 300166	1.6	9.2(2.9) ^{d-k}	7.6(2.7) ^{h-n}	33	IC 450382	4.9	11.2(3.3) ^{c-e}	9.1(2.9) ^{g-m}
12	IC 377527	1.7	5.2(2.3) ^{i-o}	16.4(4.0) ^{b-e}	34	IC 17037	5.0	6.2(2.4) ^{f-n}	13.0(3.6) ^{b-h}
13	IC 300167	1.8	9.0(2.9) ^{d-j}	17.8(4.2) ^{bc}	35	IC 145419	5.0	8.6(2.9) ^{d-l}	10.3(3.2) ^{d-j}
14	IC 319350	2.1	4.0(1.9) ^{no}	4.8(2.2) ^{k-n}	36	IC 301172	5.0	4.4(2.1) ^{l-o}	4.4(2.1) ^{mn}
15	IC 343515	2.2	6.6(2.5) ^{e-n}	11.0(3.3) ^{c-i}	37	IC 300163	5.0	2.0(1.4) ^o	11.0(3.2) ^{d-j}
16	IC 300202	2.4	7.8(2.8) ^{d-n}	7.0(2.6) ^{h-n}	38	IC 577781	9.0	5.9(2.3) ^{g-n}	7.4(2.6) ^{h-n}
17	IC 545441	2.5	9.4(3.0) ^{c-i}	9.6(3.1) ^{f-l}	39	IC 577803	9.0	7.1(2.7) ^{d-n}	8.0(2.8) ^{h-n}
18	IC 577624	2.5	10.4(3.1) ^{c-h}	12.2(3.5) ^{b-i}	40	IC 577833	9.0	6.4(2.5) ^{e-n}	7.0(2.6) ⁱ⁻ⁿ
19	IC 461801	2.7	15.4(3.9) ^c	11.6(3.4) ^{c-i}	41	IC 577922	9.0	5.2(2.3) ^{h-n}	7.4(2.7) ^{h-n}
20	IC 497079	2.7	12.2(3.5) ^{cd}	19.4(4.1) ^{b-d}	42	PTB 33	1.4	38.6(6.1) ^a	44.0(6.4) ^a
21	IC 321833	2.9	5.2(2.3) ^{h-n}	8.8(2.9) ^{g-m}	43	TN1	9.0	6.8(2.6) ^{d-n}	9.0(2.9) ^{g-m}
22	IC 252243	3.0	5.8(2.3) ^{g-n}	4.4(2.1) ^{mn}					
	S.Em(±)		0.3071	0.339				0.3071	0.339
	C.V.(%)		24.84	25.07				24.84	25.07
	C.D.(0.05)		0.8573	0.9462				0.8573	0.9462

Note: Means in a column followed by same letter are not significantly different from each other.
Figures in parenthesis are square root transformed values

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Table3.Correlationmatrixbetweendamagescoreandprobingmarksof BPH

Componentsofresistance	Damagescore	probingmarksny mphs	probingm arksadults
Damagescore	1		
probingmarksny mphs	-0.290 ^{NS}	1	
probingmarksadults	-0.303 [*]	0.404 ^{**}	1

Table4.Linearregressionanalysisbetweendamagescoreandprobingmarksof BPH

Parameter	No.observati ons	Regressionequation	StandaradError	R ²
probingmarksNymphs	43	$y=-0.1627x+12.175$	0.09529726	0.1026
probingmarksAdults	43	$y=-0.1652x+14.006$	0.08864272	0.0915

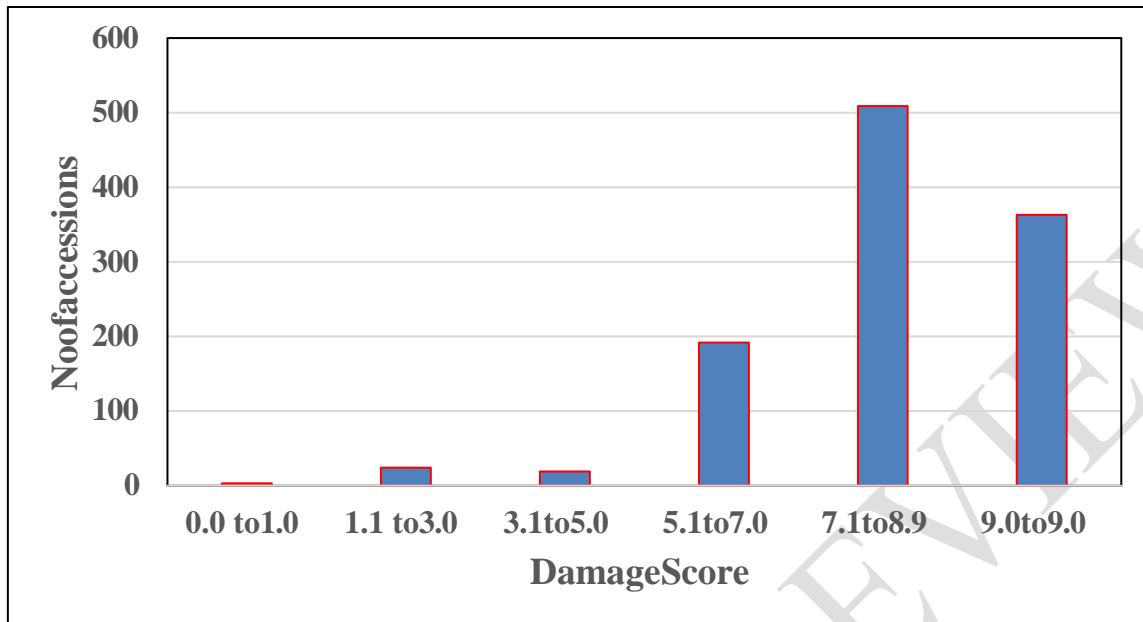


Fig1.Frequencydistribution of damagescorein theENRRDgermplasm accessions

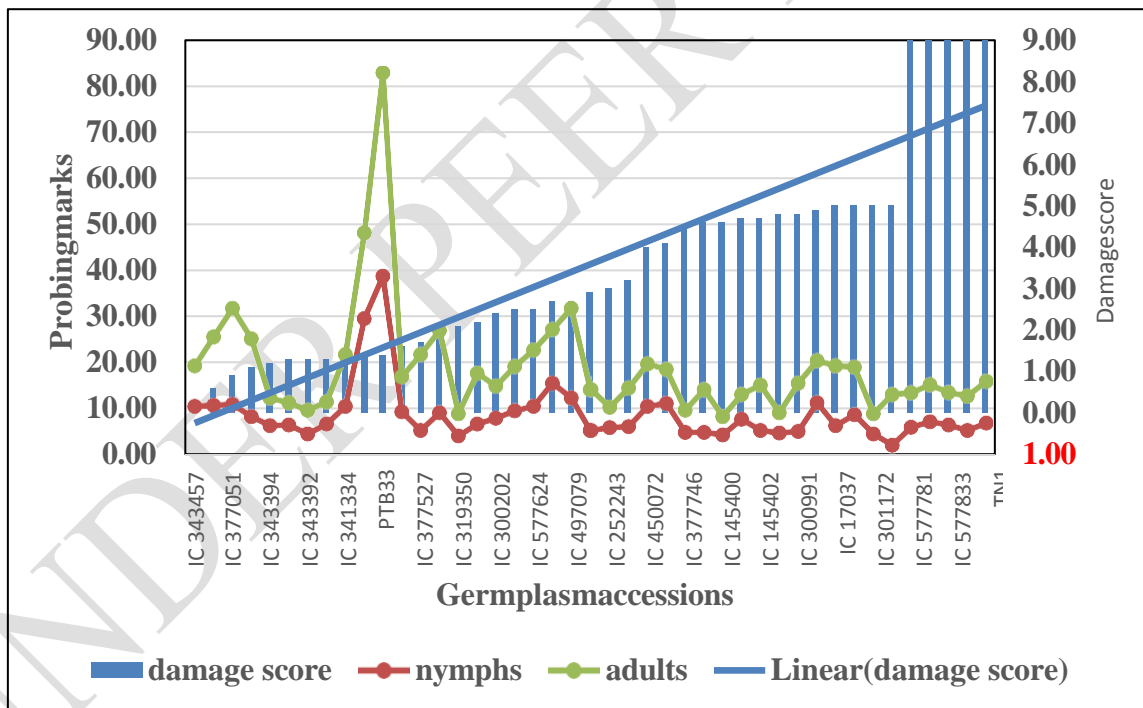


Fig2.Relationbetween damagescoreand probingmarks of BPH nymphs and adults

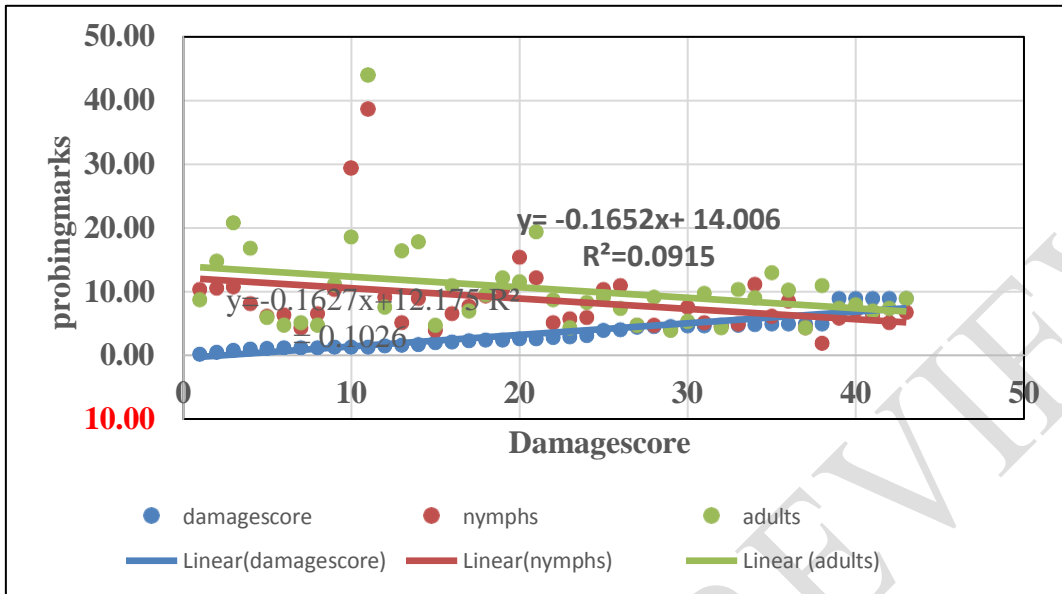


Fig3. Regression equation between damagescore and probingmarks of BPH

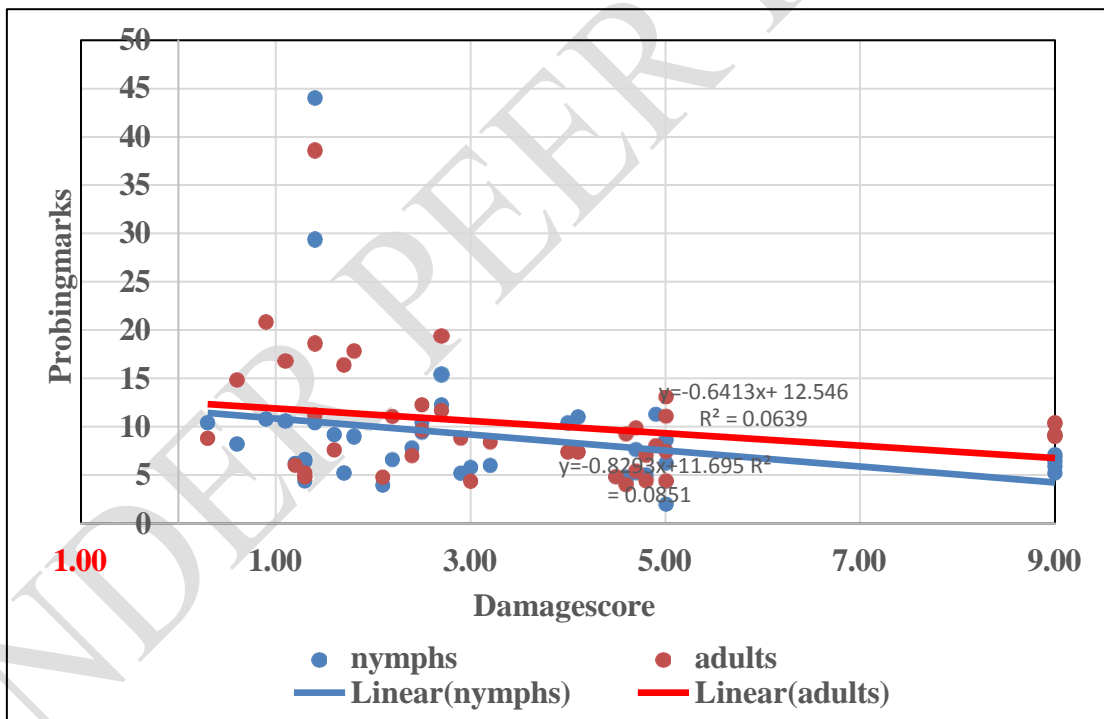


Fig4. Regression graph between damagescore and probingmarks of BPH nymphs and adults



Plates 1a and 1b. Mass screening of ENRRD germplasm accessions for BPH resistance



Plate 2 Experiment on Probing marks