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Mechanism of Host Plant Resistance in Rice Genotypes Against Brown Planthopper

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Abstract

In the present study, glasshouse screening was carried out to evaluate 532 rice genotypes to identify new sources of resistance against brown plant hopper, *Nilaparvata lugens* (Stal.) by following standard seed box screening technique (SSST) and standard evaluation system (IRRI, 1992). In mass screening replicated tests, 6 genotypes were identified as highly resistant and 36 as resistant. Moderate resistance was observed in 112 varieties. The remaining genotypes were categorized in susceptible and highly susceptible groups. Further the 10 selected genotypes having 5 resistant and susceptible lines including standard resistant and susceptible checks were screened by nymphal preference / non-preference test and feeding behavior test. Among the selected lines, resistant genotypes were relatively less preferred by nymphs against the susceptible genotypes. The lowest number of nymphs settled was recorded on resistant genotype PHHS17 (2.62) followed by Ptb 33 (3.67), BPT 2766 (3.83), RP 5995 Bphk17-5 (4.67), NHSH16 (4.83) compared to susceptible (12.36-16.33) at 72 hour of infestation. There was a positive correlation between the number of nymphs settled and the damage score. Likewise, the results of probing behavior test on selected genotypes indicated that resistant genotypes received more number of probing punctures than the susceptible genotypes even at different plant age (20, 40, 60, 80 days old). BPH produced highest number of probing marks on PHHS17 (55.67/plant). On resistant genotypes, feeding marks ranged from 30.64 to 36.17, 37.89 to 53.67, and 45.40 to 55.50 on 20, 40 and 60 day old plants, respectively which were significantly lower than susceptible genotypes (18.67 to 22.41 on 80 days old plants). There was no significant difference in probing marks between 60 and 80 days old plant. Plant resistance appeared to be increasing with increasing age irrespective of resistance level.

Keyword : *Nilaparvata lugens*, Antixenosis, Resistance, Rice

Rice (*Oryza sativa* L.) is one of the most important staple food crops and a primary source of energy to nearly half of the global population (>3 billions). Asian continent with 60 per cent of global population, contributes a lion share (>90 %) in global production and consumption of rice (Khush, 1992). It is planted on about 160 million hectares or around 11 per cent of the world's cultivated land with an annual production of 769.9 million tons (FAO, 2019). In India, area under rice is estimated to be 44 mha with a production of 118 mt (FAO, 2019).

Unfortunately, the unprecedented increase in global population has elevated an exponential rice demand for consumption, to meet this demand; there is need to scale-up an additional 40 per cent production by 2025 from the same land, with less water and labour in order to avoid disastrous consequences of biodiversity and water sheds (Khush, 1992 and FAO, 2009). In order to meet above challenges, FAO (2009) recommended to adopt rice varieties with higher yield and greater yield stability but unfortunately, the high yielding rice varieties and hybrids were linked to their higher susceptibility to pests and diseases those are the major constraints in rice production (Kumar *et al*., 2016). In India, a total of 221 insect species were reported to feed on rice (Arora and Dhaliwal, 1996). Among these, about 20 species were considered to inflict economic losses. The destructive species include, yellow stem borer, leaf folder, leaf hoppers, planthoppers, gall midge and ear head bug which occur every year in most of the rice growing areas of the world. Additionally, the sucking pests such as Rice hoppers, brown plant hopper (BPH) *Nilaparvata lugens* (Stal) and green leafhopper (GLH), *Nephotettix virescens* (Distant) has assumed greater importance in recent times as they contribute to greater loss with their direct and indirect (as vectors to few viral diseases) damage. These rice hoppers are considered as green revolution induced pests (Gunathilagaraj and Ganesh Kumar, 1997) and caused severe outbreaks in temporally in different countries including different states of India (Bijoy *et al.,* 2021).

Of these three species, BPH is the most destructive insect pest of rice causing serious threat to rice production. BPH is a piercing sucking pest which causes significant loss to rice yield by direct feeding. Both nymphs and adults settle in the lower part of plants and suck the phloem sap through stylet to cause direct damage. Heavy infestations of BPH will result in the death of rice plants, a condition commonly known as hopperburn. Apart from causing direct damage by feeding, it is a vector for diseases such as grassy stunt virus and rugged stunt virus.

Growing hybrid rice in environments under green revolution technologies (high yielding inbred varieties, synthetic fertilizer and huge amounts of pesticides) by the Asian farmers have already transformed planthoppers into a dangerous and destructive pest status posing a big challenge to achieve the desired and expected yield increments. The constraints further bring about other problems of pesticide usage like pollution, pesticide residues, resistance and resurgence in pests.

Of the various strategies, the host-plant resistance, one of the traditional yet popular IPM method has been recognized as the most practical, economical and environmentally friendly strategy to control planthoppers (Panda and Khush 1995). Studies on host-plant resistance have continued to be a major focus of research on planthoppers. Therefore, it is very important to identify resistant sources in rice genotypes against BPH. Hence, present study was conducted to evaluate reaction of rice genotypes against BPH.

Material and Methods

The research work described in the manuscript was carried out in the Greenhouses, Department of Entomology and All India Coordinated Rice Improvement Project on Rice (AICRP), V.C. Farm Mandya during 2019-2020 to identify new sources of resistance to BPH.

Insect Culture : Mass Rearing of BPH, *N. lugens*

Nilaparvata lugens was mass cultured in the green house on the susceptible rice variety Taichung Native 1 (TN1) and Jaya. Initially the field population of BPH was collected from unsprayed rice fields at college of agriculture, Mandya during 2019 *kharif*. The plants with BPH cultures were observed periodically to identify and remove natural enemies on regular basis along with wilted and dried plants. The adults were confined on 30-45 day old plants of TN1 raised in a plastic tray containing well puddled soil and placed in oviposition cages (45 x 45 x 60 cm) having wooden frames, wire-mesh side walls, glass top and door. After three days of release, the healthy gravid females were separated and released on fresh TN1 plants for further egg laying. The plants with eggs were taken out of cages, placed in separate cages with plastic tray for the nymphal emergence. Fresh plants were placed in the cages for nymphal feeding as and when required. There sulting second and third instar nymphs were used either for seedling screening purpose or for varietal resistance studies. The remaining nymphs were used for subsequent culturing on grown up susceptible plants.

Using this technique, a continuous pure culture of the *N. lugens* was maintained in the greenhouse during the period of study. The temperature and relative humidity in the greenhouse were ranged from 29^0 to 38^0C and 68-93 per cent, respectively.

Plant Materials

A set of 532 rice genotypes used for study were collected under the Germplasm Evaluation against Major Insect Pest (GEMP), National Hybrid

Screening Nurseries (NHSN), Multi Resistant Screening Trails (MRST), Plant Hopper Special Screening Trails (PHSS), Plant Hopper Screening Trials (PHST) and National Screening Nurseries (NSN) from Indian Institute of Rice Research (IIRR, Rajendranagar, Hyderabad) and were used to assess the level of resistance against *N. lugens* at seedling stage in glass house.

Mass Screening of Rice Genotypes against *N. lugens*

The main objective of mass screening was to identify the resistant lines and to eliminate the bulk of the susceptible lines rapidly. The test genotypes were screened in mass screening under glass house conditions for their level of resistance to BPH population. A standard seed box technique (SSST) suggested by IRRI was followed with suitable modifications as suggested by Jena *et al*. (2015).

Each test entries were sown in screening area inside glass house with a spacing of 5.0 × 2.5 cm rows and plants, respectively. For every 10 entries, 2 rows of both resistant (Ptb 33) as well as susceptible checks (TN 1) were sown. To provide sustainable humidity for insects to survive and to avoid disturbance of watering during screening, a constant depth of about 5 cm standing water was maintained. When the seedlings attained 10-15 days, the 2nd to 3rd instar nymphs were uniformly released. An average of 10-13 insects per seedling was released to maintain optimum population to differentiate the resistance level of the test genotypes. The seedlings were observed daily for damage symptoms. The per cent dead seedlings for each test genotype was recorded when >90 per cent of the plants in the susceptible check (TN1) gets wilted, burned and killed by BPH feeding, usually about 7-10 days after infestation. The percent dead seedlings in each genotypes was converted to different scores (Resistant and susceptible) using standard evaluation system (SES) scale, 0-9 rating provided by IRRI in the year 2013. The details of the scores are given in below Table 1 and 2.

TABLE 1

Score for BPH infestation/ damage on rice under green/glass house

Score	Symptoms	Reaction
0	No damage	Highly Resistant (HR)
1	Very slight damage	Resistant (R)
3	First and second leaves of most plants partially yellowing	Moderately Resistant (MR)
5	Pronounced yellowing and stunted or about 10to 25% of the plants wilting or dead and remaining plants severely stunted or dying	Moderately Susceptible (MS)
7	More than half of the plants	Susceptible (S)
9	All plants dead	Highly Susceptible (HS)

TABLE 2

Standard Evaluation System (SES) scoring for rice

Score	Per cent dead seedlings	Reaction
1	0-10	Highly Resistant (HR)
3	11-30	Resistant (R)
5	31-50	Moderately Resistant (MR)
7	51-70	Susceptible (S)
9	71-100	Highly Susceptible (HS)

Antixenosis Mechanism of Resistance.

Studies on Preference / Non-Preference Mechanisms

In this experiment, pre-germinated seeds of the selected rice genotypes were sown in rows, 5 cm apart, in seed box (70 cm × 40 cm × 10 cm). Each row contained 25 seeds. The susceptible check TN 1 was sown in two border rows of the box. The tray was kept in dark place to enhance seedling growth. Ten days after sowing of selected genotypes, approximately 350 first and second instar BPH nymphs was released by gently tapping over the seedlings in such a way that approximately five to seven nymphs settled on each seedling in the conventional seed box. The numbers of nymphs on each seedling were counted at 24, 48 and 72 hours after infestation. The seedling was disturbed after each count for reorientation of nymphs on seedlings. This procedure was done in seed box test described by Heinrichs *et al.* (1985).

Probing Marks

The selected test genotype was transplanted in plastic pots @ of 4 seedlings per pot and the plants were covered by using a plastic mylar tubes with ventilating windows covered by white muslin cloth. When the plants attain 20, 40, 60 and 80 days, a newly emerged 1st or 2nd instar female nymphs were released / introduced to each test genotype / pot @ of 1 female per tiller. After 24 hours of release the feeding marks were stained with 0.1 per cent safranine dye aqueous solution for 15 minutes and the number of stained stylet probes were counted for each genotype under microscope. The similar experiment was made for seedlings of age 20, 40, 60 and 80 days old separately. The means were worked out for interpretation and standard statistical procedures.

Statistical Analysis

Data were analyzed using two-way ANOVA and means were compared using a least significant difference test at 5 per cent level of significance with Microsoft Excel. The rates of nymphal preference and probing marks were square root transformed prior to analysis. Correlation analysis was performed using SPSS 25.0 (SPSS, IBM) and Pearson's correlation coefficient was used as a measure of the relationship between indicators.

RESULTS AND DISCUSSION

Mass Screening

Mass screening of 532 rice genotypes revealed that, six genotypes were found to be highly resistant with the damage Score <1 (PHSS-17, NHSN-16, BPT 2766, NSN2-10, NSN2-115, RP 5995 and Bphk 17-5), 37 genotypes were categorized as resistant (score 1-3) and 111 genotypes were moderately resistant (score 3-5) against BPH. Rest 378 accessions were found to be either susceptible or highly susceptible to BPH. The similar results were reported by Bhanu *et al.* (2014); Soumya & Jagadish (2017);

TABLE 3

Reaction of rice genotypes against BPH under glasshouse condition at Mandya, *kharif* 2019

Score	Per cent dead seedlings	Category	NPT	Genotypes
1	0-10	Highly Resistant (HR)	2	PHSS-17, NHSN-16, BPT 2766, NSN2-10, NSN2-115, RP 5995 Bphk17-5.Total. 6
3	11-30	Resistant (R)	2	NHSN 58, IC 76013*, PTB 33, NSN2-33, PTB 33, NHSN-23, NHSN-27, NHSN 40, BPT 910, PTB 33, NSN2-2, RMS-ISM-Bph 33-1, NSN2-132, NSN2-233, RP 65-9-2-2, NSN2-109, RNR 28389-1, WGL-1319 *, RP 251-10-3-2, NSN2-116, NHSN-1, RP 21-6-5-4, NSN2-106, NSN2-119, NSN2-134, NSN2-48, NHSN 115, NSN2-197, RP 068-18-3-5, NSN2-32, NSN2-8, PHSS-8,RP 165-7-2-B, NSN2-235, IR 73382-80-9-3-13-2-2-1-3-B (HWR-16), JS 1.Total. 37
5	31-50	Moderately Resistant (MR)	1	111
7	51-70	Susceptible (S)	1	144
9	71-100	Highly Susceptible (HS)	1	234

*Average plant damage score was based on 3 replications.

NPT-number of promising trials, Resistance category based on standard evaluation system (SES) for rice, IRRI, Philippines (2013)

Udayshree *et al*. (2018) and Pachauri *et al*., 2020. The detail of the genotypes with reaction to BPH is presented in Table 3. A total of six genotypes identified as promising with BPH resistance were taken up for further studies on different resistance mechanism and results are presented below.

Studies on Preference / Non-Preference Mechanisms

Nymphal Response

Based on results of mass screening study, we selected 5 resistant and 5 susceptible rice genotypes including standard checks to study the BPH preference response. Low number of BPH nymphs / seedling was observed on all resistant rice genotypes (Table 4) than susceptible ones. On resistant genotypes there was a decreasing trend in the number of nymphs settled between 1 day and subsequent observations while on the susceptible entries more number of nymphs were noted. On an average, 4.75 to 7 nymphs per seedling were recorded on resistant genotypes as against around 8.58 to 12.67 nymphs on the susceptible entries after 24 hr of release. The lowest number of nymph settling was found in genotype PHHS17 (4.75) while it was higher in TN 1 (12.67) followed by NHSN 20 (10.61). A similar trend of nymphal settling per seedling was observed on 48 hours and 72 hours after release (Table 4). Non-preference mechanism was reported to be a factor of resistance in rice against BPH as early as 1969. Kalode and Krishna (1979); Venugopala and Kalode (1985) reported that Ptb 33, Ptb 21, Leb Mue Nahng, ARC 6650 and CR 57-MR 1523 had less number of BPH nymphs as compared to TN1 and suggested the possibility of some attractants in the susceptible variety. And absence of feeding stimulants or presence of feeding repellents could be other possible reasons for non-preference in resistant genotypes.

In the present investigation, a positive correlation was observed with regard to the number of nymphs and damage score. Higher the number of nymphs settled greater was the damage and *vice-versa.* It appears that non-preference has a definite role in the manifestation of resistance in some of the varieties tested.

TABLE 4

Average number of BPH nymphs settled on selected rice genotypes

Genotypes	Category	Mean no. of nymphs/ seedling after*different times of infestation		
		24 hr	48 hr	72 hr
RP 5995 Bphk17-5	R	7.00 (2.74) cde	5.50 (2.45) d	4.67 (2.27) d
NHSH16		6.33 (2.61) ef	5.17 (2.38) de	4.83 (2.31) d
BPT 2766		6.83 (2.71) de	4.67 (2.27) de	3.83 (2.08) de
PHHS17		4.75 (2.29) f	3.67 (2.04) e	2.62 (1.77) e
Cul 3	S	8.58 (3.01) bcd	12.5 (3.61) b	12.36 (3.58) c
NHSN20		10.61 (3.33) ab	11.55 (3.47) bc	13.67 (3.76) bc
BPT 2863		8.67 (3.03) bcd	10.22 (3.27) c	13.92 (3.80) bc
PHHS3		9.00 (3.08) bc	12.33 (3.58) abc	14.00 (3.81) b
PTB 33 (RC)	RC	5.50 (2.45) ef	4.33 (2.20) de	3.67 (2.04) de
TN 1(SC)	SC	12.67 (3.63) a	14.33 (3.85) a	16.33 (4.10) a
S.Em (±)		0.07	0.06	0.06
CD @ p = 0.05		0.20	0.18	0.19

Mean of four replications; R- resistance; S- susceptible; RC- Resistance check; SC- susceptible check;
*Figures in parenthesis are square-root transformed values; Values in the column followed by common letters are non-significant at p=0.05 as per Tukey's HSD (Tukey, 1953)

TABLE 5

Plant age on the number of probing marks of N. lugens in rice genotypes

Genotypes	Category	Number of probing marks/plant* Plant age(days) 20	40	60	80
RP 5995 phk17-5	R	33.50 (5.83) b	44.44 (6.70) c	50.40 (7.13) b	50.08 (7.11) bc
NHSN16		30.64 (5.58) c	37.89 (6.20) d	45.4 (6.77) c	49.08 (7.04) C
BPT 2766		34.44 (5.91) ab	48.63 (7.01) b	52.50 (7.28) ab	49.81 (7.09) bc
PHHS17		36.17 (6.06) a	53.67 (7.36) a	55.67 (7.49) a	54.78 (7.43) a
Cul 3	S	15.88 (4.05) d	16.67 (4.14) e	21.33 (4.67) d	22.41 (4.79) d
NHSN20		15.22 (3.97) d	16.11 (4.08) e	16.02 (4.06) ef	20.18 (4.55) de
BPT 2863		14.39 (3.86) d	18.06 (4.31) e	17.27 (4.22) ef	20.15 (4.54) de
PHHS3		16.00 (4.06) d	19.49 (4.47) e	19.22 (4.44) de	20.97 (4.63) de
PTB 33	RC	35.22 (5.98) ab	52.44 (7.28) a	54.21 (7.4) a	53.29 (7.33) ab
TN 1	SC	13.89 (3.79) d	17.23 (4.21) e	16.00 (4.06) f	18.67 (4.38) e
SEm(±)		0.08	0.14	0.11	0.13
CD @ p=0.05		0.22	0.42	0.34	0.38

Mean of three replications; R- resistance; S- susceptible; RC- Resistance check; SC- susceptible check;
*Figures in parenthesis are square-root transformed values; Values in the column followed by common letters are non-significant at p=0.05 as per Tukey's HSD (Tukey, 1953).

Probing Marks

Number of feeding marks produced by the insect also differed significantly among different genotypes. The results of the probing behavior indicated that the resistant genotypes received more number of probing punctures than the susceptible ones even at different plant age of rice crop. The susceptible genotypes recorded the least number of probing punctures (13.89-15.22) whereas a greater number of probing punctures was observed on resistant entries (30.64-36.17) at the plant age 20.

On the 40 days old plant, the number of probing marks (Table 5) were ranging from 16.11 to 53.67. The highest number of probing marks was recorded on PHHS 17 and it was nearly 3.40 fold higher than the susceptible check TN1. This was followed by BPT 2766 (48.63), RP 5995 Bphk17-5 (44.43) and NHSN16 (37.89). Resistant check PTB 33 recorded 52.44 feeding marks / plant. Whereas the susceptible genotypes Cul 3, NHSN 20, BPT 2863 and PHHS 3 receive 16.11, 18.06, 19.49 and 16.67 marks / plant respectively, indicating that these varieties were suitable for feeding by the test insect.

A similar trend in number probing marks was noticed in case of 60 and 80 days old rice plant (Table 5) where PHHS 17 (55.67) recorded highest number of probing marks and TN 1 (18.67) got least marks per plant. A negative correlation was observed with regard to the number of probing punctures and damage score. Higher the number of punctures on the plant lower was the damage and *vice-versa.* Feeding varied from genotype to genotype and it determined insect food intake. It includes probing response or the application of proboscis and introduction of stylets into the food source and duration of feeding. In our observations, we found more restless behavior of BPH on resistant genotypes such as PHHS17, Ptb33, NHSH16, RP 5995 Bphk17-5 and BPT 2766 as insects moved all over the leaf sheath to find suitable feeding site. It suggested that the test

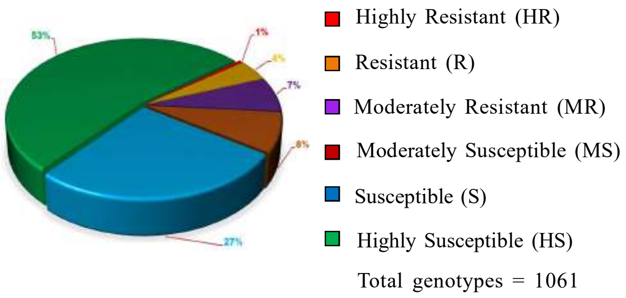

Fig. 1: Percentage of rice genotypes screened for BPH under different resistance category during, 2019-2020

plants presented some mechanical barrier to penetration for probing or plant sap was not palatable to the insects.

Feeding activity of *N. lugens* was high on the susceptible cultivars and reduced or nil feeding was noticed on resistant varieties (Paguia *et al.,* 1980; Cook *et al.,* 1987 and Pachauri *et al.*, 2020). Karim (1975) reported that the varieties xB5 and HR 12 received significantly higher number of punctures (59 and 48·2, respectively) which were about 2-10 times more than that received by other resistant varieties. The results indirectly revealed that non-preference of BPH to certain varieties may be gustatory rather than olfactory or visual.

Screening study showed that among the 532 genotypes screened against BPH for resistance, 6 were highly resistant with damage score <1 and 37 genotypes were resistant and remaining were categorized as susceptible. Antixenosis studies showed that among genotypes, PHHS17 and PTB33 performed better in terms of settling of nymphs, while it was on par with the rest of three, RP 5995 Bphk17-5, NHSN 16 and BPT 2766. In contrast, the susceptible genotypes performed significantly poor in avoiding nymphs to settle on them in comparison with all the resistant genotypes. However, in terms of number of probing marks resistant genotypes received significantly more number of probing marks than in susceptible ones. From this experiment, it can be concluded that host selection can affect BPH settling and feeding. The restless behavior of BPH on the resistant varieties also increases their vulnerability to the natural enemies. Rice genotypes PHHS17 and Ptb33 both displayed high levels of antixenosis and tolerance to BPH.

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