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Rice Brown Planthopper, *Nilaparvata lugens* (Stål) Feeding Behaviour in Resistant NAGINA22 Rice Mutants

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ABSTRACT

Background: Rice (*Oryza sativa* L.), is an important staple food and an excellent source of calories in India. Of the different insect pests attacking rice, brown planthopper, BPH, *Nilaparvata lugens* (Stål) is an important sucking pest causing upto 100% yield losses. Host plant resistance is the most important method in keeping BPH under control and understanding the underlying mechanisms of resistance is necessary to breed the resistant varieties with desirable characters.

Methods: Selected EMS mutant lines of Nagina22 (N22) variety, which were previously mass screened under controlled greenhouse conditions were assessed for their antixenosis mechanism to brown planthopper feeding by measuring probing marks and honeydew excretion area by following standard procedures and statistical analysis was done by using Statistix 8.1 software.

Result: Both the brown planthopper nymphs and adults probed more in the resistant mutants compared to the susceptible mutants whereas the BPH adults probed more times (24.4) compared to the nymphs (22.2). The recorded honeydew excretion area by the resistant mutants was lower than that of susceptible mutants. In general, the BPH adults fed more and excreted more honeydew (79.5 mm^2) compared to the nymphs (59.0 mm^2). The damage score has a negative correlation with the probing marks of adults (-0.207) and nymphs (-0.411); and a positive correlation with honeydew excretion of adults (0.547) and nymphs (0.200). In this study, the EMS N22 mutants resistant to BPH, with a greater number of probing marks and less honeydew excretion area can serve as best donors in the breeding programmes to develop brown planthopper resistant varieties.

Key words: Antixenosis mechanism, Honeydew excretion, Nagina22 mutants, *Nilaparvata lugens*, Probing marks, Rice.

INTRODUCTION

Rice (*Oryza sativa* L.), is an important staple food and an excellent source of calories for 33% of world's population. The brown planthopper (BPH), *Nilaparvata lugens* (Stål) a Hemipteran belonging to Delphacidae family is the most damaging rice insect pest in temperate and tropical regions of East and South Asia (Satturu *et al*., 2020). BPH is a phloem sap-sucking insect pest that damages rice plants by causing "hopper burn" and stunted growth. Infested plants immediately wilt and turn yellow. The incidence of BPH has been rising in recent years and management of the pest has been more challenging (Sunil *et al*., 2017 and Ishwarya Lakshmi *et al*., 2022). Rice cultivars susceptible to BPH typically experience yield reductions of up to 60%. The ragged stunt and grassy stunt disease causing viruses are also carried by brown planthopper. In addition to threatening natural enemies, the use of harmful chemicals for insecticidal management leads to the reduced susceptibility of BPH to insecticides (Jhansi Lakshmi *et al*., 2010a and Reddy *et al*., 2022). Host plant resistance is the most crucial mechanism in keeping insect pests under control. A resistant plant variety that reduces the insect population by 50% every generation is thought to be able to exterminate an economically significant pest within a few generations.

Plants are capable of withstanding insect damage through three different mechanisms: antixenosis, antibiosis and tolerance. A non-preference type of resistance called antixenosis deters or repels insects, which lowers colonization, feeding, or oviposition. The non-preference mechanism was recognized as a source of resistance in BPH as early as 1969. It has been identified in majority of BPH resistant rice accessions. Finding suitable novel resistant donors and figuring out the mechanisms driving the manifestation of resistance into these cultures are crucial for controlling the brown planthopper and breeding BPH-resistant cultivars. In light of this, the current study was designed to investigate the antixenosis mechanism of resistance to feeding in the selected EMS mutant lines of Nagina22 (N22) variety, which were previously mass screened by following Standard

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Seedbox Screening Test (SSST) under controlled greenhouse conditions as per the technique described by (Kalode *et al*., 1975; Anjali *et al*., 2022).

MATERIALS AND METHODS

This experiment was conducted in the greenhouse of Department of Entomology, ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad during 2021-22. In accordance with Jhansi Lakshmi *et al*. (2010b), BPH was mass reared on the highly susceptible rice variety TN1. Initial BPH populations were collected from rice fields and pure cultures were kept on 60-day-old potted rice plants in cages inside the greenhouse at a temperature of 30°C and a relative humidity of 70±5%. Twenty female hoppers were collected with aspirator, released in oviposition cages on clean potted plants. The gravid females were removed after four days and reintroduced on fresh TN1 plants for subsequent egg laying. Plants with eggs were removed from cages and put in separate cages so the nymphs could hatch. New plants were kept in the cages as and when necessary and as soon as the hatched nymphs reached the desired age, they were taken out to use for the required studies.

The non-preference/ antixenosis mechanism of BPH to feeding was studied by counting probing marks and measuring honeydew excretion by brown planthopper.

The number of feeding marks/probing marks made by a single one-day old female insect and third instar nymph during one day feeding on 12 resistant mutants along with parent line N22, resistant check, PTB 33, MO1 and susceptible check, TN1 was recorded. For this purpose, a single one-day-old adult female insect or a third instar nymph was allowed to feed on a seven-day old test entry in a test tube for 24 hours and it was replicated five times. The insect was removed after 24 hours and the test plant was stained by being dipped in a 1% aqueous Erythrosin-B solution for an hour in order to view the feeding marks on the test entries. Utilizing a magnifying hand lens, the feeding marks were counted. The data was statistically analysed using completely randomized design (CRD) and means were separated by LSD.

The amount of honeydew excreted indicates the extent of feeding by BPH on different test mutants. It was measured using the method described by Nanthakumar *et al*. (2012) in mutants, resistant and susceptible checks in terms of honeydew area (mm^2) excreted by the constant number of insects. In 1000 ml plastic pots, seeds of susceptible check, TN1, resistant check PTB 33, MO 1, wild type, N22 and selected twelve N22 mutants were sown. After 10 days, only one healthy seedling was retained.

Nine cm diameter circles of Whatman number 1 filter papers which were dipped in Bromocresol green-ethyl alcohol solution and dried were placed over the cardboard piece at the base of the stem of thirty day old plants. A tiny plastic cup was placed on the filter paper. Five one day old BPH females, or third instar nymphs were released into the cup over the filter paper. The area of honeydew droplets excreted by the BPH which fall onto the filter paper and change into blue spots was measured in millimeters (mm^2) using the graphical method after the insects were allowed to feed for 24 hours. Based on the mean value from 3 replications, various entries were statistically compared. Regression and correlation analysis were done among damage score, probing marks and honeydew excretion area by using Statistix 8.1 software.

RESULTS AND DISCUSSION

The number of probing marks made by the adult female BPH in the selected N22 mutants ranged from 12.2 to 45.4 (Table 1, Plate 1, Fig 1). The highest number of feeding punctures were reported in the mutant NH 4535 (45.4) which was on par with the resistant check PTB 33 (45.8) and is notably distinct from other entries. NH 663 and NH 4632 (12.2) recorded lowest number of probing marks. The susceptible check TN1 recorded 10.0 probing marks, MO1 and parent line Nagina22 recorded 37.0 and 19.2 probing marks respectively. The resistant entries (24.9 No. of probing marks) differed significantly from susceptible entries (22.1 No. of probing marks) with regard to number of probing marks by adults.

In case of BPH nymphs, the number of feeding marks ranged from 15.6 to 34.2 (Table 1, Plate 1, Fig 1). Highest number of feeding punctures were found in the entry, NH 663 (34.2) which was comparable with PTB 33 (33.2). Whereas the lowest number of probing marks were observed in NH 4856 and NH 4614 (15.6). Susceptible check TN 1 recorded 8.0 probing marks only. In resistant entries, number of probing marks were greater (23.2) than that in the susceptible entries (17.7). The BPH adults probed more number of times (24.4) compared to nymphs (22.2).

Phloem sap is the main food source for brown planthopper, a vascular feeder. When the stylets are inserted and the labial tip is pressed against the plant epidermis, the BPH secretes a little amount of coagulable saliva. They form a strong bond as a result, leaving distinct circular imprints where the stylet is inserted. A salivary deposit on the plant epidermis is referred to as a “feeding mark”. The frequency of probing over time can be calculated by counting the number of feeding marks on various plant materials (Sogawa, 1982).

More feeding punctures in the resistant entries may be because these resistant entries didn’t sustain prolonged feeding because of specific feeding deterrents, hazardous substances, or a lack of feeding stimulants present. 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 *i.e*., Reddy *et al*. (2016) and Anupama *et al*. (2018) who reported that resistant varieties received a greater number of probing marks than susceptible ones. Ponnada *et al*. (2011) also reported that average probing marks on resistant plants ranged between 30.4 to 42.9 whereas



Plate 1: Probing marks made by brown planthopper adults and nymphs on N22 mutants. A- Adults; N- Nymphs.

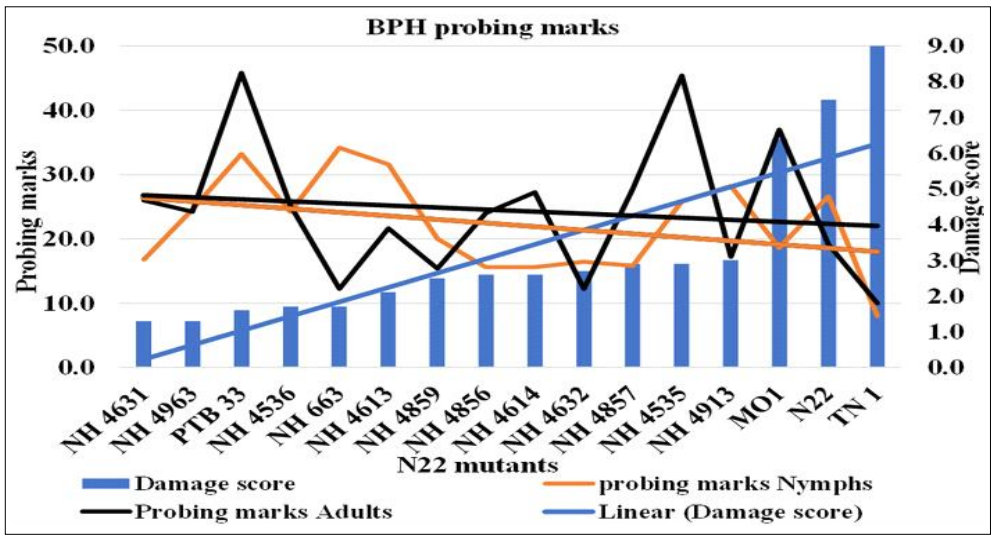


Fig 1: Relation between damage score and probing marks in brown planthopper nymphs and adults in N22 mutants.

resistant and susceptible checks have recorded 22.1 and 6.7 probing marks, respectively. Resistant cultures like MTU 1075, MTU IJ 206-7-4-1 and MTU PLA 99-1-3-1-2 have received more number of feeding marks which were 128.1, 112.8 and 110.2, respectively. The total honeydew excreted area by the adult BPH females ranged from 31.3 mm^2 to 113.3 mm^2 (Table 1, Plate 2, Fig 2). The total honeydew excreted area (mm^2) was highest in the mutant NH 4613 (113.3 mm^2) which was on par with N22 (140.0 mm^2) while the lowest area was recorded in NH 4536 (31.3 mm^2). The susceptible check TN 1, MO 1 and resistant check PTB 33 recorded 209.0 mm^2, 74.3 mm^2 and 14.7 mm^2 honeydew excretion area respectively. The honeydew excretion was less in resistant entries (65.3 mm^2) compared to that on susceptible entries (141.1 mm^2).

The total honeydew area by BPH nymphs ranged from 30.3 mm^2 to 148.0 mm^2 (Table 1, Plate 2, Fig 2). NH 663 showed the highest honeydew excretion area (148.0 mm^2).

Table 1: Probing marks and honeydew excretion (mm^2) in brown planthopper nymphs and adults in N22 mutants.

Mutant	Damage score	Probing marks		Honeydew excretion (mm^2)	
		Nymphs	Adults	Nymphs	Adults
NH 4631	1.3	16.80 (3.94)^{de}	26.00 (5.04)^{bc}	51.33^{c-f}	71.66^{b-d}
NH 4963	1.3	24.60 (4.93)$^{a-e}$	24.20 (4.86)$^{b-d}$	32.33^{d-f}	84.06^{a-d}
NH 4536	1.7	24.20 (4.85)$^{a-e}$	25.20 (4.97)$^{b-d}$	30.33^{ef}	31.33^{cd}
NH 663	1.7	34.20 (5.78)^{a}	12.20 (3.47)^{ef}	148.00^{a}	70.00^{b-d}
NH 4613	2.1	31.60 (5.46)^{ab}	21.60 (4.63)$^{c-e}$	113.33^{ab}	113.33^{a-d}
NH 4859	2.5	20.00 (4.45)$^{b-e}$	15.40 (3.83)$^{d-f}$	46.00^{c-f}	101.00^{a-d}
NH 4856	2.6	15.60 (3.90)^{e}	24.00 (4.81)$^{b-d}$	53.00^{c-f}	64.00^{b-d}
NH 4614	2.6	15.60 (3.93)^{e}	27.20 (5.19)^{bc}	63.66^{b-e}	71.33^{b-d}
NH 4632	2.7	16.40 (3.98)^{de}	12.20 (3.47)^{ef}	46.66^{c-f}	47.00^{b-d}
NH 4857	2.9	15.80 (3.88)^{e}	27.80 (5.21)^{bc}	43.66^{c-f}	63.66^{b-d}
NH 4535	2.9	25.80 (5.00)$^{a-d}$	45.40 (6.45)^{a}	45.33^{c-f}	45.33^{b-d}
NH 4913	3.0	28.20 (5.29)$^{a-c}$	17.20 (4.11)$^{c-f}$	49.33^{c-f}	71.33^{b-d}
PTB 33	1.6	33.20 (5.69)^{a}	45.80 (6.70)^{a}	7.00^{f}	14.66^{d}
MO 1	6.4	18.60 (4.24)$^{c-e}$	37.00 (5.97)^{ab}	41.66^{c-f}	74.33^{b-d}
N22	7.5	26.58 (5.48)^{ab}	19.20 (4.28)$^{c-f}$	88.66^{bc}	140.00^{a-d}
TN 1	9.0	8.00 (2.77)^{f}	10.00 (3.12)^{f}	83.33^{b-d}	209.00^{a}
S.Em (±)		0.3793	0.423	18.057	43.545
C.V. (%)		18.42	19.86	20.03	83.55
C.D. (0.05)		1.0716	1.1951	52.015	125.44

Note: The means in a column that have the same letter after them don't differ significantly by LSD (P=0.05). Square root converted values are represented by figures in parenthesis.

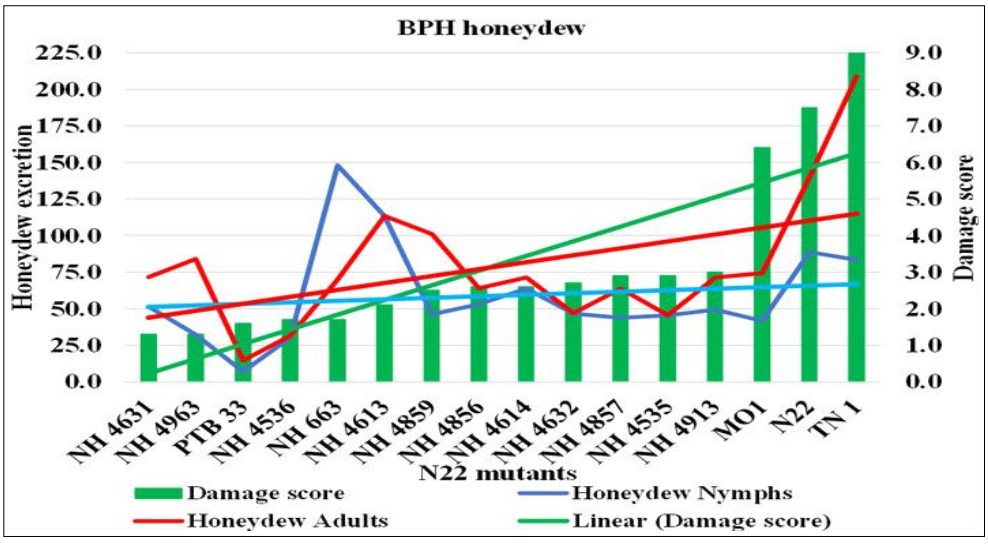


Fig 2: Relation between damage score and honeydew excretion in brown planthopper nymphs and adults in N22 mutants.

The lowest area was reported in NH 4536 (30.3 mm^2). TN 1, N22, MO 1 and PTB 33 reported honeydew excretion area of 83.3 mm^2, 88.7 mm^2, 41.7 mm^2 and 7.0 mm^2 respectively. The recorded honeydew excretion area by the resistant entries (56.2 mm^2) was lower than that of susceptible entries (71.2 mm^2). In general, the BPH adults fed more and excreted more honeydew (79.5 mm^2) compared to the nymphs (59.0 mm^2).

As sap feeders, homopteran insects absorb the highly water-containing plant sap. The filter chamber mechanism expels extra water to prevent the digestive enzymes from becoming diluted. This excretory product, known as honeydew, is rich in sugars, amino acids, waxes, lipids and other nutrients. The plant sap that BPH consumes directly correlates with the honeydew quantity it excretes.

The amount of honeydew excretion by female adults, when fed on resistant cultivars was less whereas the amount of honeydew excreted was greater when the insects fed on susceptible than on resistant varieties (Reddy *et al*., 2016). These differences in the amount of honeydew excretion indicate differences in the relative amount of sap intake. In this study, resistant entries showed significantly less amount of honeydew excretion than that of susceptible check TN1. The feeding rate of the brown planthopper was attributed as the capability to differentiate between susceptible and resistant entries.

The correlation analysis among the damage score, probing marks and honeydew excretion (Table 2) indicated a negative correlation between damage score and probing marks of adults (-0.207) and nymphs (-0.411) eventhoug it is non-significant. The brown planthopper probed more number of times on the entries with less damage score (resistant) and vice versa. There was a significant positive correlation between damage score and adult total honeydew excretion (0.547) and nymphal total honeydew excretion (0.200) which is non-significant.

When the data were subjected to linear regression analysis (Table 3, Fig 3 and 4), a negative relationship was

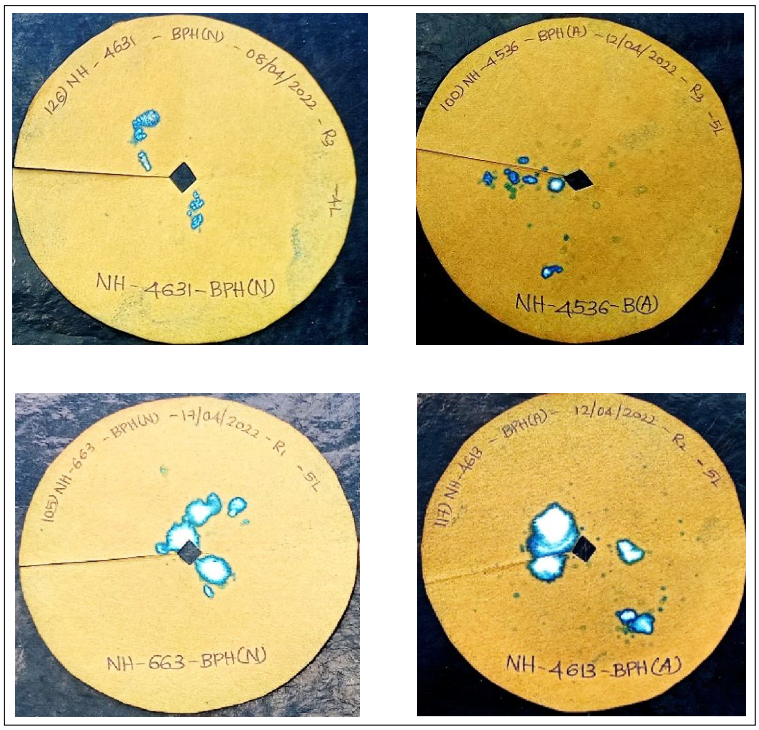


Plate 2: Honeydew excretion in brown planthopper nymphs and adults in N22 mutants.

Table 2: Correlation matrix among resistance components of brown planthopper in N22 mutants.

Resistance component	Damage score	Probing marks nymphs	Probing marks adults	Honeydew nymphs	Honeydew adults
Damage score	1				
probing marks nymphs	-0.411^{NS}	1			
probing marks adults	-0.207^{NS}	0.228^{NS}	1		
Honeydew nymphs	0.200^{NS}	0.225^{NS}	-0.553*	1	
Honeydew adults	0.547*	-0.500*	-0.487^{NS}	0.390^{NS}	1

observed between damage score and nymphal and adult probing marks while it was positive between damage score and honeydew excretion area by nymphs and adults. In the adults, probing marks is able to explain 4.27 per cent of the variation in damage score and for each unit increase in the probing marks, the damage score is decreased by 0.97 units. In the nymphs, probing marks showed 16.88 per cent of the variation in damage score and for each unit increase in probing marks, the damage score is decreased by 1.34 units. In the adults, total honeydew describes 55.97 percent of the variation in damage score and for each unit increase in the total honeydew, the damage score is increased by 14.92 units. Whereas in case of nymphs, total honeydew is able to explain 4 per cent of the variation in damage score and for each unit increase in the total honeydew, the damage score is increased by 2.99 units.

Table 3: Linear regression among different components of resistance in brown planthopper in N22 mutants.

Variable	No of observations	Regression equation	Standard error	R^2
Probing marks-adults	16	y = -0.9709x+27.543	0.055687117	0.0427
Probing marks-nymphs	16	y = -1.3366x+26.526	0.074897951	0.1688
Honeydew-Adults	16	y = 14.916x+31.207	0.008894529	0.5597
Honeydew-Nymphs	16	y = 2.994x+49.282	0.017484932	0.04

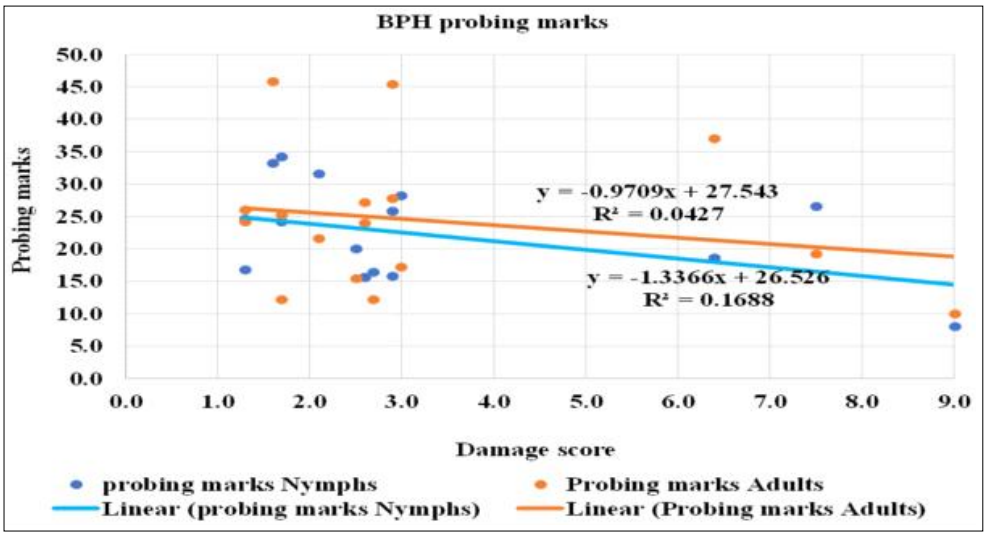


Fig 3: Linear regression between damage score and probing marks in brown planthopper nymphs and adults in N22 mutants.

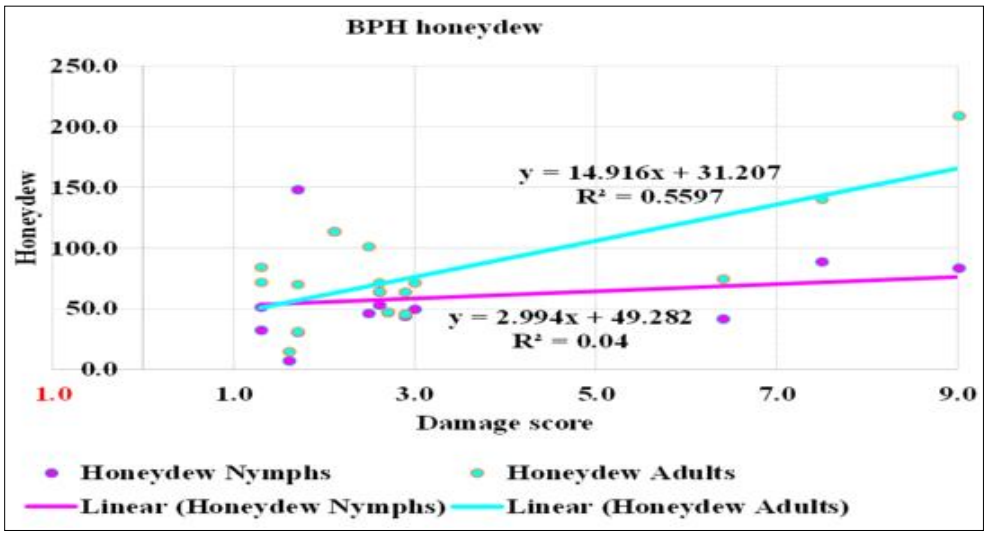


Fig 4: Linear regression between damage score and honeydew excretion area in brown planthopper nymphs and adults in N22 mutants.

Ramesh *et al*. (2014) and Anupama *et al*. (2022) also suggested a positive correlation between damage score and honeydew excretion area and a negative relation between damage score and probing marks made by planthoppers in the mapping population of TN1 X Sinasivappu and rice germplasm accessions.

CONCLUSION

In our study, the EMS N22 mutants resistant to BPH, with more number of probing marks and less honeydew excretion area with least preference for feeding serve as the best donors in the breeding programmes to develop brown planthopper resistant varieties.

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Conflict of interest

The authors declare that they have no conflict of interests.

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