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Non-Preference Mechanism of Resistance in Rice Germplasm Accessions to Whitebacked Planthopper *Sogatella furcifera* (Horvath)

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***Abstract*:** A total of 1010 germplasm accessions collected from different parts of India were mass screened for their reaction to Whitebacked planthopper, *Sogatella furcifera* (Horvath) by standard seedbox screening technique during 2016-2018 at ICAR-Indian Institute of Rice Research, Hyderabad. 43 accessions exhibited a damage score (DS) ranging from 1.39 to 5.0 and were designated as resistant and moderately resistant to WBPH, and 967 accessions were susceptible (DS 5.1 to 9.0). Two germplasm accessions *viz.,* IC 75864 (DS-1.39), IC 215295 (DS-2.65) were resistant and 41 accessions were moderately resistant (DS 3.1 to 5.0). The selected resistant accessions were assessed for their feeding preference by WBPH by measuring feeding/probing marks and honeydew excretion area. The resistant accessions exhibited more number of probing marks ranging from 4.6 to 24.0/seedling indicating the non-suitability of the accessions for feeding by WBPH. Resistant check MO1 recorded 31.6, PTB33 recorded 17.8 probing marks and susceptible check TN1 recorded 8.2 probing marks/seedling. WBPH fed less and excreted less honeydew on the resistant germplasm accessions indicating their non-preference. Negative correlation existed between resistance and probing marks whereas it is positively correlated with honeydew excretion. The identified resistant germplasm accessions can be used in the breeding programmes to develop WBPH resistant varieties.

Keywords: Germplasm accessions, Honeydew excretion, Host plant resistance, Mass screening, Probing marks, *Sogatella furcifera*, Whitebacked planthopper

Rice is one of the world's most important staple food crops. There are many constrains in rice production among which insect pests remain a constant problem in all rice growing areas. One of the most economically important insects is the Whitebacked planthopper, *Sogatella furcifera* (Horvath) (Hemiptera: Delphacidae) which is a migratory insect which can cause huge damage where both the nymphs and adults suck the plant sap directly and results in loss of water and nutrients (Park et al 2008) and indirectly transmits viral diseases such as black-streaked dwarf virus (Pie et al 2016). The control of WBPH with chemical insecticides results in insecticide resistance development, detrimental impact on natural enemies and environmental pollution (Jhansi Lakshmi et al 2010a,b,c, Dhawan et al 2013, Kamala 2020, Vijay Kumar Yadav et al 2021). Host plant resistance is the most important measure to keep the insect pests under control. 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. The necessity to identify suitable new resistant donors for whitebacked planthopper from different sources is important in order to combat the pest and develop resistant varieties. 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. Measurement of honeydew excretion and number of feeding marks made by WBPH are used as tools to assess the resistance and susceptibility of a genotype. Keeping this in view, present investigation was planned to evaluate the germplasm accessions for their resistance to Whitebacked planthopper and to study the antixenosis mechanism of resistance for feeding.

MATERIAL AND METHODS

Mass rearing of whitebacked planthopper: WBPH was mass reared on the susceptible rice variety TN1 (Jhansi Lakshmi et al 2010c). WBPH 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 70±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. Adult gravid female hoppers were collected and 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. Using this technique, a continuous pure culture of WBPH was maintained during the period of study.

Mass screening of germplasm accessions: In order to identify the sources of resistance to WBPH, 1010 germplasm accessions were mass screened under controlled greenhouse conditions as per the technique described by (Nagendra Reddy et al 2016). The entries were pre-germinated in petridish and sown individually in screening trays 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 (MO1) in the middle and two rows of susceptible check (TN1) in the border. When the seedlings are 12 days old, first and second instar nymphs of WBPH were released on the seedlings 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° 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 (IRRI 2013) (Table 1). All the 1010 germplasm entries were screened in two replications and the identified resistant accessions were further screened in 5-7 replications.

Feeding behaviour of whitebacked planthopper based on probing marks: The 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 adults and third instar nymphs of whitebacked planthopper expressed in terms of feeding marks or probing marks on the leaves and stems of the rice entries (Ponnada et al 2011). For this purpose, a single one-day old adult female and third instar nymph were separately confined 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 analyzed statistically in Completely Randomized Design (CRD) and the means were separated using DMRT.

Table 1. Criteria for WBPH 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

Honeydew excretion: This parameter was used to know about non-preference mechanism for feeding. The amount of honeydew excreted by the insects was measured which was an indication of the feeding preference and efficiency of WBPH to feed on a rice variety. Whatman No.-1 filter paper was dipped in ethanol solution dissolved with 0.02% Bromocresol green powder, allowed to dry for 1 hour and dipped again till the filter paper turned yellowish orange (Nanthakumar et al 2012). The treated filter paper was placed at the bottom of 30 days-old plants, planted in small plastic pots. A small plastic cup with a hole was placed over the filter paper and five pre-starved adult insects/3rd instar nymphs were released into the cup. The hole was plugged with cotton to prevent the escape of the insects. The adults were allowed to feed for 24 h at the base of the stem of the plant. The honeydew droplets excreted by the adults/nymphs turn into blue spots when come in contact with the filter paper. The relative area of the spots produced by honeydew excreted on bromocresol green treated filter paper were traced on tracing paper and placed on millimeter square graph paper and the squares were counted and expressed in mm^2. The germplasm accessions were statistically compared on the basis of mean value obtained from 3 replications each.

Correlation and regression analysis: Pearson correlation analysis and linear regression analysis among the damage score, probing marks and honeydew excretion was done using OP Stat software to understand their relationship and interdependence.

RESULTS AND DISCUSSION

Germplasm accessions resistant to WBPH: Out of these 1010 germplasm accessions, 43 accessions exhibited a damage score (DS) ranging from 1.39 to 5.0 and were designated as resistant and moderately resistant to WBPH, and the remaining 967 accessions were susceptible with a damage score of 5.1 to 9.0 (Table 2). Out of 43 germplasm accessions, two accessions *viz.,* IC 75864 (DS-1.39), IC 215298 (DS-2.65) were resistant and rest 41 accessions were moderately resistant (DS-3.1 to 5.0) (Fig. 1a and 1b). The frequency distribution graph (Fig. 2) shows that in the remaining 967 germplasm accessions, 204 accessions were

Table 2. Damage score, probing marks and honeydew excretion of WBPH in the germplasm accessions

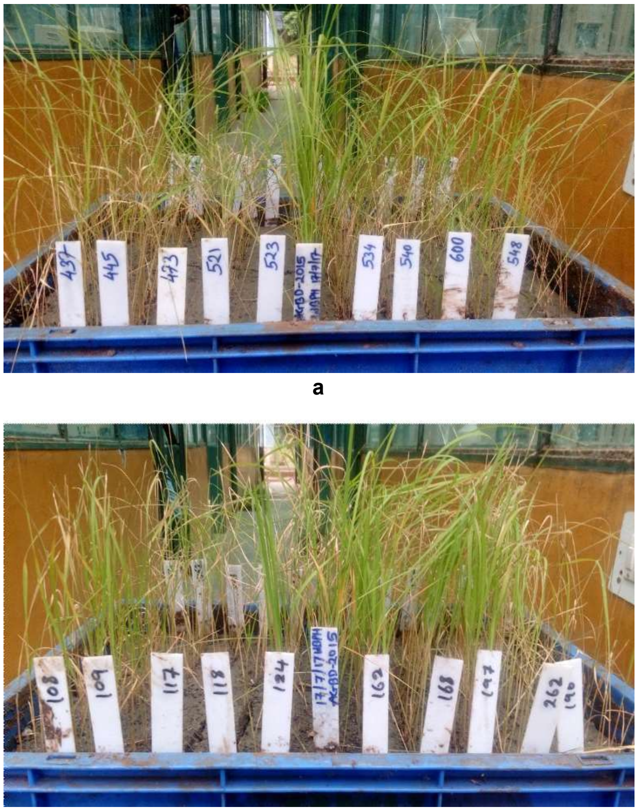
Germplasm accessions	Damage score	Probing marks		Honeydew excretion (mm^2)	
		Adults	Nymphs	Adults	Nymphs
IC75864	1.4 (R)	16.6 (4.07)$^{c-f}$	12.6 (3.52)$^{c-e}$	23.6^{o}	6^{o}
IC215298	2.7 (R)	18.8 (4.28)^{cd}	10.4 (3.22)$^{d-g}$	63.3 $^{j-n}$	18.7 $^{i-m}$
IC216620	3.2 (MR)	13.8 (3.69)$^{e-i}$	13.8 (3.69)^{c}	72.6^{g-n}	31^{e-i}
IC75877	3.5(MR)	12.4 (3.49)$^{g-k}$	10.8 (3.28)$^{c-f}$	121.6^{b-f}	28^{g-k}
IC216897	3.9 (MR)	15.0 (3.80)$^{d-i}$	7.0 (2.62)$^{j-m}$	-	-
IC216632	4.0 (MR)	14.2 (3.73)$^{e-i}$	13.2 (3.58)^{cd}	68.3^{h-n}	32.3^{e-i}
IC216901	4.0 (MR)	16.6 (4.07)$^{c-f}$	10.2 (3.11)$^{e-h}$	76.3^{f-n}	44^{b-f}
IC210765	4.1 (MR)	12.0 (3.43)$^{g-k}$	7.0 (2.64)$^{i-m}$	109.3^{b-h}	15.7^{k-n}
IC216856	4.1 (MR)	15.2 (3.87)$^{d-h}$	7.0 (2.64)$^{i-m}$	-	-
IC216563	4.2 (MR)	13.4 (3.65)$^{f-j}$	4.2 (2.04)$^{o-s}$	66.3^{i-n}	17.3^{j-n}
IC216710	4.3 (MR)	11.4 (3.37)$^{h-l}$	5.4 (2.30)$^{l-p}$	98^{c-k}	15.7^{k-n}
IC216612	4.3 (MR)	11.4 (3.37)$^{h-l}$	2.2 (1.47)^{uv}	129.6^{b-d}	48.7^{b-e}
IC540676	4.3 (MR)	12.4 (3.49)$^{g-k}$	9.2 (3.03)$^{f-j}$	74.6^{g-n}	36^{d-h}
IC216944	4.3 (MR)	24.5 (4.91)^{b}	10.0 (3.09)$^{e-i}$	84.3^{e-l}	11.3^{m-o}
IC540644	4.4 (MR)	20.2 (4.42)^{bc}	2.6 (1.60)$^{r-v}$	64.3^{j-n}	15.7^{k-n}
IC216628	4.4 (MR)	11.6 (3.39)$^{g-k}$	5.0 (2.2)$^{l-p}$	101^{b-j}	25^{h-l}
IC211233	4.4 (MR)	15.6 (3.90)$^{c-g}$	2.4 (1.52)^{uv}	85^{e-m}	54.7^{bc}
IC75735	4.5 (MR)	18.2 (4.21)$^{c-e}$	7.8 (2.78)$^{g-k}$	53.3^{l-n}	23.7^{h-l}
IC216678	4.5 (MR)	13.8 (3.69)$^{e-i}$	4.2 (2.04)$^{o-s}$	45.3^{no}	16.3^{j-n}
IC75747	4.5 (MR)	14.4 (3.78)$^{d-i}$	2.0 (1.37)^{v}	-	-
IC216640	4.6 (MR)	7.4 (2.71)$^{n-p}$	4.8 (2.18)$^{m-q}$	61^{k-n}	26.7^{g-k}
IC215276	4.6 (MR)	7.2 (2.65)$^{n-q}$	2.0 (1.37)^{v}	49^{n}	13^{l-o}
IC216874	4.7 (MR)	6.6 (2.56)$^{o-q}$	3.8 (1.94)$^{p-u}$	-	-
IC216566	4.8 (MR)	14.0 (3.70)$^{e-i}$	4.0 (1.98)$^{p-t}$	105.6^{b-i}	42.7^{b-g}
IC216479	4.8 (MR)	7.2 (2.67)$^{n-p}$	1.8 (1.31)^{v}	50.3^{n}	8.7^{no}
IC216525	4.9 (MR)	11.0 (3.31)$^{i-m}$	6.8 (2.59)$^{k-m}$	88^{d-l}	31.3^{e-i}
IC17045X	4.9 (MR)	10.0 (3.13)$^{j-n}$	3.0 (1.72)$^{q-v}$	-	-
IC216596	4.9 (MR)	6.4 (2.52)^{pq}	2.4 (1.54)$^{t-v}$	76^{g-n}	41.7^{d-g}
IC17037X	4.9 (MR)	9.4 (3.06)$^{k-o}$	2.6 (1.60)$^{r-v}$	-	-
IC75955	4.9 (MR)	-	-	90.3^{c-k}	15.7^{k-n}
IC216693	5.0 (MR)	8.4 (2.89)$^{l-p}$	5.0 (2.22)$^{l-p}$	136^{a-c}	29.3^{f-j}
IC458401X	5.0(MR)	12.0 (3.45)$^{g-k}$	6.6 (2.54)$^{k-m}$	78.3^{e-n}	51.3^{b-d}
IC75958	5.0 (MR)	6.8 (2.60)$^{o-q}$	6.8 (2.59)$^{k-m}$	76.6^{g-n}	55.3^{bc}
IC75748	5.0 (MR)	4.6 (2.14)^{q}	6.4 (2.52)$^{k-n}$	123.3^{b-e}	55^{bc}
IC75800	5.1 (MS)	6.2 (2.46)^{pq}	7.4 (2.66)$^{h-l}$	104.6^{b-i}	43.3^{b-f}
IC540720	5.1 (MS)	7.0 (2.64)$^{n-q}$	6.2 (2.47)$^{k-o}$	110.3^{b-g}	32.7^{e-h}
IC216609	5.1 (MS)	7.8 (2.78)$^{m-p}$	5.0 (2.22)$^{l-p}$	144.3^{ab}	62^{ab}
IC75737	5.2 (MS)	9.6 (3.06)$^{k-o}$	6.8 (2.59)$^{j-m}$	-	-
MO1	1.4 (R)	31.6 (5.55)^{a}	31.0 (5.51)^{a}	28.7^{g-k}	21.6^{o}
PTB 33	3.4 (MR)	17.8 (4.20)$^{c-e}$	19.4 (4.37)^{b}	25.3^{o}	10^{m-o}
TN1	9.0 (HS)	8.2 (2.83)$^{m-p}$	4.4 (2.06)$^{n-r}$	185^{a}	82^{a}
CD		0.4665	0.6157	46.985	28.42
CV(%)		10.94	37.11	32.99	5.7123

Note: Means in a column followed by same letter are not significantly different from each other by LSD (P=0.05). Figures in parenthesis are square root transformed values moderately susceptible with a damage score of 5.1 to 7.0, 329 accessions were susceptible with a damage score of 7.1 to 8.9 and the remaining 434 accessions were highly susceptible with a damage score of 9.0. The resistant check MO-1 recorded a damage score of 1.4, PTB 33 recorded a damage score of 3.4 and the susceptible check TN1 recorded a damage score of 9.0. Host plant resistance is the most economical and desirable method for the management of crop pests (Horgan et al 2015). Screening for resistance to whitebacked planthopper is a continuous process to identify new sources of resistance. In India, host plant resistance to WBPH is being exploited in several research centres and very important sources of resistance have been identified. Beant Singh and Shukla (2007) screened 1224 rice accessions out of which 57 accessions were resistant, 370 were moderately resistant to WBPH. Three lines, viz., RIC 06-0305, RP 4334-TSH-41-8-1-1-2-6 and MO1 were resistant and 28 were moderately resistant out of fifty-eight rice lines (Sarao and Mahal 2007). Sarao and Mahal 2012 screened 66 rice germplasm lines out of which two lines, *viz*., IR 59 547-235-3-3 and SPR 85 163-5-1-2-4 were resistant, 20 lines were moderately resistant and the remaining lines were susceptible to WBPH. Zhu_et al 2016 evaluated 218 common wild rice materials out of which one was highly resistant and twenty-one were moderately resistant to WBPH. Out of seventy-four rice landraces, eight landraces *viz*., Kudai Vazhai, Karthi Samba, Vadivel, Ponmani Samba, Kallimadayan, Panamara Samba, Kodaivilayan and Kalyani were resistant and 18 landraces were moderately resistant to WBPH (Venkatesh et al 2019). Four varieties viz., Pathara, Pratap, Tejaswini and Santpheal were resistant and fifteen were moderately resistant out of ninety-four released varieties (Meher et al 2020). The results are in conformity with the findings of Sarao and Mahal 2007, Kumar et al 2018 and Meher et al 2020, where TN1 was highly susceptible, MO1 was resistant and PTB33 was moderately resistant to WBPH.

Probing marks: A total number of 37 resistant, moderately resistant and susceptible germplasm accessions along with susceptible check TN1 and resistant checks *viz*., MO1 and PTB33 were selected to find out the feeding behaviour of third instar nymphs and one-day old adult WBPH expressed in terms of feeding marks or probing marks on the stems of rice plants (Table 2).

WBPH Adults: There was a significant difference among the germplasm accessions with regard to probing marks. The resistant accession IC 216944 had maximum number of probing marks (24.5) while susceptible check TN1 recorded lowest number of marks (8.2) by adult whitebacked 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 216944 (24.5) followed by IC 540644 (20.2), IC215298 (18.8), IC75735 (18.2), IC 75864 (16.6), and IC 216693 (16.6). The resistant check MO1 had more number of probing marks (31.6). The susceptible entries were probed less number of times (average 7.9 probing marks/seedling) compared to resistant germplasm accessions (13.2 marks/seedling) (Table 2).

WBPH nymphs: WBPH nymphs probed more number of times on the resistant germplasm accessions compared to susceptible accessions. The resistant germplasm accession IC216620 was probed more number (13.8) of times followed by IC216632 (13.2), IC75864 (12.6) and IC75877 (10.8). The resistant check MO1 received maximum number of probing marks (31.0) and PTB33 was probed 19.4 times. However, the susceptible germplasm accessions recorded less number of probing marks (5.0-7.4). The susceptible check TN1 had 4.4 probing marks. The susceptible germplasm accessions were probed less number of times (average 5.4 probing marks/seedling) compared to resistant accessions (7.2 marks/seedling). The nymphs probed less number of times (7.1) than the adults (12.5). The present study on



a

b

Fig. 1a and 1b. Mass screening of the germplasm accessions probing marks indicated that the presence of non-preference mechanism for feeding in the case of resistant checks and resistant germplasm accessions. 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" (Ponnada et al 2011). The feeding mark is a spherical protrusion, 15-17 p.m. in height, with a circular flange. It is possible to ascertain the probing frequency in a given period on different plant materials by counting the number of feeding marks.

Honeydew excretion: The honeydew excretion in one-day old WBPH female adult in the germplasm accessions ranged from 23.6 mm^2 (IC75864) to 185.0 mm^2 (TN1) (Table 2). The feeding and honeydew excretion in the resistant germplasm accessions was less (38.5mm^2) compared to moderately resistant germplasm accessions (82.7mm^2) and in the susceptible germplasm accessions the honeydew excretion was high (136 mm^2). In the case of WBPH nymphs, the honeydew excretion ranged from 6 mm^2 (IC75864) to 82 mm^2 (TN1). The honeydew excretion in the susceptible accessions including check TN1 was highest and ranged from 32.7 to 82.0 mm^2 with a mean of 55.0 mm^2. In the resistant entries the honeydew excretion was less (15.4mm^2) compared to moderately resistant accessions (28.9 mm^2). In general, the adults fed more and excreted more honeydew (83.4mm^2) compared to nymphs (30.7mm^2). This revealed that difference in the amount of honeydew excretion is mainly attributed to the difference in the relative amount of sap intake. Less intake of sap on resistant varieties, despite successful stylet penetration into the vascular bundles indicates the occurrence of certain undesirable gustatory factors that governed sustained sucking by the insect. It is further considered that this gustatory reaction is responsible for the abnormally high frequency of stylet probing, lack of satisfactory intake of nutrients, and subsequent reduction in fecundity or non-preference response. Therefore, the amount of honeydew excreted by the insect in unit time when fed on different rice cultures could be considered as an index for its feeding preference. WBPH has copiously excreted honeydew on the susceptible TN1.

Correlation and regression between damage score, probing marks and honeydew excretion: Correlation analysis between damage score and probing marks of adults (R^2 = -0.605) and nymphs (R^2 = - 0.624) indicated negative correlation which is significant. More number of probing marks were observed on the germplasm accessions which are resistant and vice versa (Table 3 and Fig. 3). There is a significant and positive correlation between the damage score and honeydew excretion of the whitebacked planthopper (adults R^2 =0.684 and nymphs R^2 =0.631). The honeydew excretion was less in the resistant germplasm accessions and vice versa. There was a significant and negative correlation between probing marks and honeydew excretion by the adults (-0.483) and nymphs (-0.410) (Table 3 and Fig. 4). The negative relation between probing marks and feeding/honeydew excretion tells that WBPH was a phloem feeder, probed readily and fed longer on the susceptible accessions. In contrast, the insect made brief and repeated probes on the resistant germplasm accessions, consequently reducing the effective ingestion period. Ramesh et al (2014) also suggested a positive correlation between damage score and honeydew excretion area in whitebacked planthopper in the mapping population

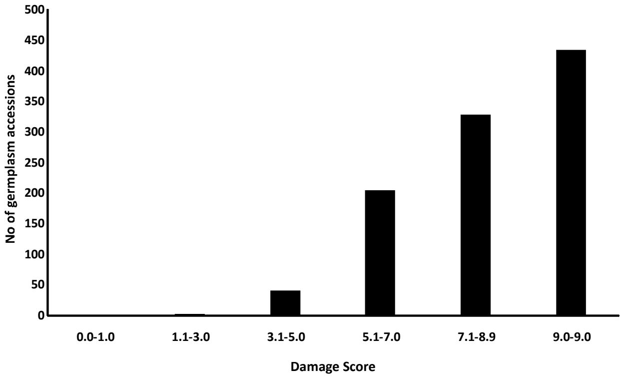


Fig. 2. Frequency distribution of damage score in germplasm accessions

Table 3. Correlation matrix among damage score, probing marks and honeydew excretion of WBPH

Components of resistance	Damage score	Probing marks (Adults)	Probing marks (Nymphs)	Honeydew excretion (Adult)	Honeydew excretion (Nymphs)
Damage score					
Probing marks- adults	-0.605^{**}				
Probing marks- nymphs	-0.624^{**}	0.670^{**}			
Honeydew excretion- adult	0.684^{**}	-0.483^{**}	-0.412^{*}		
Honeydew excretion- nymphs	0.631^{**}	-0.410^{*}	-0.238^{NS}	0.686^{**}	1

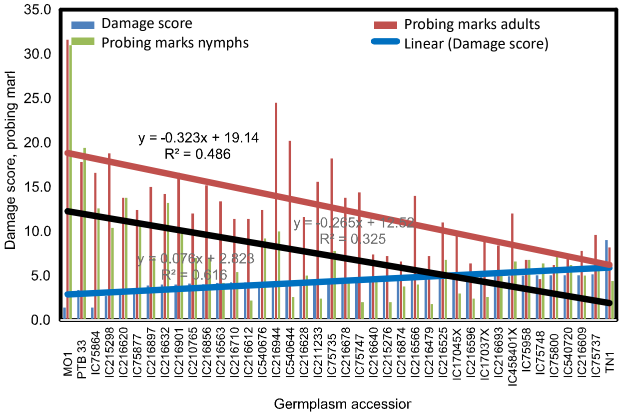


Fig. 3. Damage score, probing marks in the WBPH adults and nymphs in the germplasm accessions

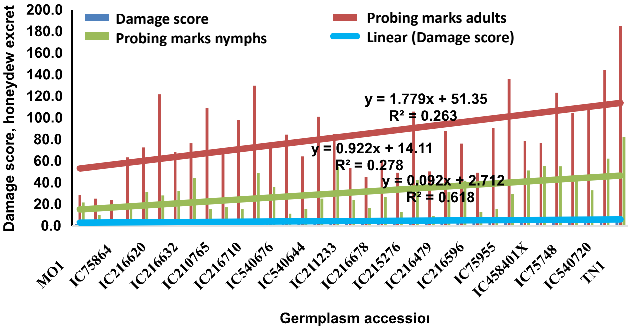


Fig. 4. Damage score, honeydew excretion in the WBPH adults and nymphs in germplasm accessions

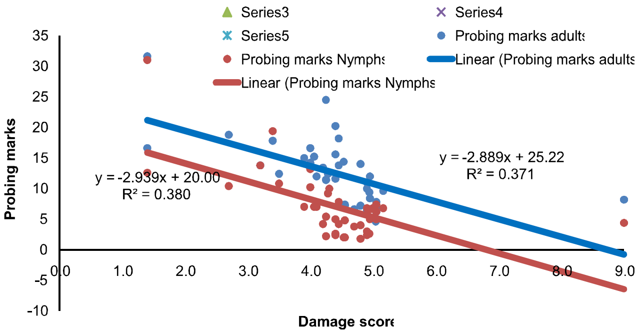


Fig. 5. Regression analysis between damage score and probing marks

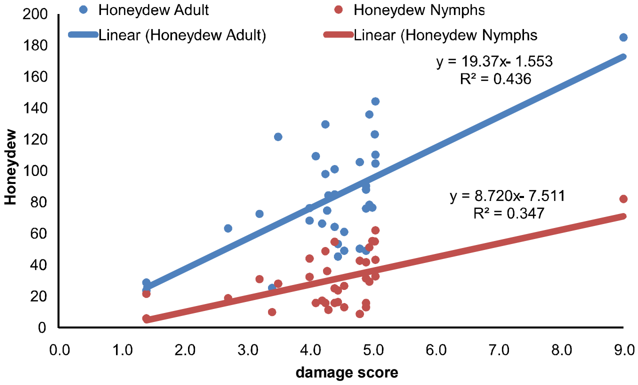


Fig. 6. Regression between damage score and honeydew excretion

Table 4. Linear regression analysis among different components of resistance

Component	No of observations	Regression equation	Standard error	R^2
Probing marks- adults	40	y = -2.8896x + 25.222	4.3526	0.3714
Probing marks- nymphs	40	y = -2.9396x + 20.004	4.344366	0.3807
Honeydew excretion-adults	35	y = 19.37x - 1.5534	0.922515	0.4363
Honeydew excretion-nymphs	35	y = 8.7202x - 7.511	0.9928	0.3472

of TN1 X Sinasivappu. There was a negative relation between damage score and probing marks and the probing marks could explain 75% variation in the damage score and for each unit increase in the probing marks, the damage score is decreased by 5.8 units (Table 4 and Fig. 5). A positive relation was observed between damage score and honeydew excreted (feeding) by both nymphs and adults. The honeydew excretion (feeding) by the nymphs and adults together explains 78.4% variation in the damage score and for each unit increase in the honeydew excretion/feeding, the damage score is increased by 28.1 units (Table 4 and Fig. 6).

CONCLUSIONS

Two germplasm accessions *viz.,* IC 75864 (DS-1.39), IC 215295 (DS-2.65) and MO1 were resistant to whitebacked planthopper and the insect exhibited non-preference for feeding with more number of probing (feeding) marks and less feeding and honeydew excretion on resistant accessions. The identified resistant germplasm accessions with known resistant mechanisms can be used as donors in the breeding programmes to develop WBPH resistant varieties.

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