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RESEARCH ARTICLE

Phenotypic evaluation of Ptb 33 introgressed rice lines for resistance to brown planthopper, *Nilaparvata lugens* (Stål)

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Abstract

Brown planthopper (BPH), *Nilaparvata lugens* (Stal) continues its dominantce as an important pest in rice and cause considerable yield loss. Ptb 33 is a well-known source of resistance to BPH. Introgressed lines from Ptb 33 were evaluated against BPH using various parameters of resistant mechanisms. Initial screening was done by protray and modified seedbox screening tests. Eight entries illustrate resistance to BPH in mass screening. Further experiments assessed the mechanisms viz., antibiosis, antixenosis and tolerance in selected resistant entries. Antibiosis studies revealed reduced honeydew excretion, lower nymphal survival rates and prolonged developmental periods in resistant lines compared to the susceptible check. Antixenosis was evaluated through nymphal settling preference tests, with resistant entries showing reduced BPH attraction over time. Tolerance parameters, including Functional Plant Loss Index (FPLI) and Plant Dry Weight Loss Index (PDWLI), indicated varying levels of resistance among the introgressed lines. Among the entries identified as resistant through screening, FSR-3 exhibited strong resistance across all three mechanisms, while X21302-145 showed excellent antibiosis but poor tolerance character. Correlation analysis revealed significant interrelation between various resistance parameters. The study identifies promising BPH-resistant rice lines and elucidates their underlying resistance mechanisms, contributing valuable insights for rice breeding programs in developing durable BPH -resistant cultivars.

Keywords

brown planthopper; host plant resistance; introgressed lines; resistance mechanisms; rice

Introduction

Rice (*Oryza sativa* L.) is a vital cereal crop that feeds half of the world's population. The crop is cultivated on approximately 165.25 million hectares globally, with almost 789 million metric tons produced in 2021-22 (1). Rice productivity is often affected by several biotic and abiotic factors and among those, pest attack acts as an important limiting factor in hindering the stability of demand and rice availability (2).

Brown planthopper, *Nilaparvata lugens* (Stål), is an economically significant pest that affects the crop by sucking phloem sap and causing 'hopper burn' condition. Nymphs as well as adult BPH cause damage resulting in wilting, yellowing and ultimately plant death. BPH is an r-strategy pest, the population increases exponentially and results in greater yield loss (3). It also transmits rice wilted stunt virus (RWSV), rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV) leading to more than 60% economic loss (4).

BPH can be effectively controlled by regularly monitoring the field for incidence and by using need-based insecticide application at recommended dosages. But the usage of more insecticides for managing the pest affects the ecological balance and results in the development of pest resurgence and the proliferation of resistant biotypes. Therefore, implementing host plant resistance (HPR) is an eco-friendly method for managing the BPH. Cultivation of resistant varieties is critically important at this time which helps in conserving natural enemies' population and reduces the usage of pesticides (5).

Screening of rice germplasm and breeding for resistance against BPH began with the release of the rice variety 'Mudgo' in 1969. Since then, numerous resistant varieties have been released. Releasing resistant varieties can be a never-ending process, as BPH evolves into new biotypes. Generally, there are three types of mechanisms, viz., antixenosis, antibiosis and tolerance, with unique characteristics contributing to plant resistance against pest attacks. Antixenosis involves traits that deter or disturb insects, leading to reduced colonization or egg-laying. Antibiosis affects insect biology *viz*., survival, growth, or reproduction after consumption of plants. In case of tolerance, plants can still produce a high-quality yield despite being infested by pests (6). Management of pest through host plant resistance begins with phenotypic screening of crop germplasms to ensure the presence of resistance. Phenotypic identification of genotypes is accompanied by evaluating the resistance mechanisms through a series of experiments. Ptb 33 is a well-known resistant variety and has been used as a resistant check in various experiments for comparing any other test genotypes. Since Ptb 33 possesses two resistance genes, viz., b *bph 2* and *Bph 3*, it is taken as the donor parent in introgressing genes for developing high yield BPH-resistant cultivar.

The present study aimed to assess the resistance levels to BPH in a set of introgressed rice lines derived from Ptb 33 as the donor parent and determine the underlying resistance mechanisms.

Materials and Methods

Plant and insect material

Experiments were performed at the Entomology glass house, Department of Rice, Paddy Breeding Station (PBS), Tamil Nadu Agricultural University, Coimbatore. The experimental material consists of a set of 110 F_4 progenies derived from two multiple crosses, viz., Improved Samba Mahsuri / RG 170 // TKM 13 / AD (Bio) 09518) / (CO 52 / PTB 33) and (Improved Samba Mahsuri/ RG 170 // TKM 13 / AD (Bio) 13066) / (CO 52 / PTB 33). Initially, during summer 2020-2021 both the multi-parental crosses were raised and true multi cross hybrid plants were identified based on genotyping the plants using gene-specific markers for BPH. The true hybrids were self-pollinated and developed as multi-parental cross F_2 population. Two F_2 populations (each 1000 plants) of the crosses viz., (Improved Samba Mahsuri / RG 170 // TKM 13 / AD (Bio) 09518) / (CO 52 / PTB 33) and (Improved Samba Mahsuri / RG 170 // TKM 13 / AD (Bio) 13066) / (CO 52 / PTB 33) were raised during wet season of 2021; from these populations about 110 phenotypically superior plants were tagged, self-pollinated and developed next generation without genotyping as F_3 families during 2021-2022 wet season. Single plant selections were made in all the 110 families and developed F_4 generation. In this study, in F_4 generation, phenotyping and genotyping were identified the promising families carrying BPH resistant genes as well as having strong phenotypic resistance mechanism against the pest. Resistant and susceptible checks, Ptb 33 and Taichung Native1 (TN 1) were included in all the phenotypic experiments. BPH insects were mass-cultured in insect-proof cages of glass house. BPH populations were collected from the unsprayed fields initially and maintained in a susceptible rice variety, TN 1 (Fig. 1). The nymphs of subsequent generations were utilized in various experiments.

Fig. 1. Culturing BPH in rice susceptible entry TN 1.

Phenotyping

Along with resistant check (Ptb 33) and susceptible check (TN 1), all the 110 introgressed rice lines were subjected to preliminary screening of Protray Screening Test (PST) (7). Entries were soaked in water for 24 hr and the water was drained and then kept in darkness to enhance sprouting. Pregerminated seeds of entries were sown at the rate of 5-10 seeds in each well of the protray filled with clay soil. It was ensured that the resistant check (Ptb 33) was sown in the middle and susceptible check (TN 1) was sown in two alternate corners of the protray to obtain uniform distribution of the nymphs (Fig. 2). Protray was kept inside the wire mesh cage to protect the seedlings. Seven days after sowing (DAS), seedlings were infested with second and third instar nymphs @ 8-10 nymphs per seedling. Damage scoring was recorded on a 0-9 scale, Standard Evaluation System (SES) for rice, based on the susceptible check or any entries started wilting (8) (Table 1, Fig. 3). Entries that were graded between 0 and 5 were subjected to next level of screening by Modified Seedbox Screening Test method (MSST) (9).

Identification of resistance mechanisms

The entries that were selected as resistant categories based on the damage score in both PST and MSST are further proceeded to phenotyping of resistance mechanisms using various experiments.

Antixenosis mechanism: Antixenosis mechanism was detected by the settling behaviour of nymphs on the selected rice entries. The experiment was carried out in seed box by sowing the seeds @ 10 seeds per entry with three replications. Each row was sown with 3.5 cm distance in the seed box. Susceptible check (TN 1) entry was sown at both corners of the

Fig. 2. Healthy introgressed rice seedlings before infestation.

Fig. 3. Damaged introgressed rice seedlings after infestation.

Table 1. Standard evaluation system for rice (IRRI, 2013)

SYMPTOMS	GRADE
No injury	0
Very slight injury	1
First and second leaves of most plants shown partial yellowing	3
Pronounced yellowing and stunting or about 10 to 25% plants shown wilting Symptom or dead and remaining plants severely stunted	5
More than half of the plants dead	7
All plants dead	9

box and resistant check (Ptb 33) was sown in the middle. At 15 DAS, seedlings were infested with second and third instar nymphs. The number of nymphs settled on the seedlings were recorded periodically at 12, 24, 48 and 72 hr after infestation. The seedlings were disturbed after every count to ensure the reorientation of the nymphs.

Antibiosis parameters: *Nymphal survival and developmental period:* Survival rate of BPH nymphs on selected introgressed lines was observed by releasing of first instar nymphs on 30 day -old seedlings @10 nymphs/seedling with three replications. Seedlings were covered with a mylar sheet cage after release of insects. Seedlings were observed keenly for the emergence of adults. Nymphal survival percentage was worked out (10).

Per cent nymphal survival = (number of adults emerged / number of nymphs released) × 100

Nymphal developmental period was observed by releasing first instar nymphs on 30-day-old seedlings of selected entries @5 nymphs/seedling with three replications. Seedlings were covered with a mylar sheet cage. Nymphs were observed daily for ecdysis. The number of days taken by nymphs to become adults was worked out for each entry.

Growth index of the entries was assessed by dividing the data obtained from nymphal survival and nymphal developmental period (11).

Growth Index = Per cent of nymphs survived / Nymphal developmental period.

***Feeding rate assessment*:** Feeding rate of BPH adult females on selected rice lines was assessed by honeydew excretion analysis using ninhydrin method described by Pathak (1982) (12). Two hr pre-starved freshly emerged female BPH were released into 30-day-old potted plants @ 5 female BPH/seedling with three replications. Circular pieces of Whatman no. 1 filter paper were placed at the base of the seedlings and were removed after 24 hr of infestation. Those filter papers were sprayed with 0.01% (w/v) ninhydrin-acetone solution which resulted in the appearance of purple-coloured spots. The purple spots are developed due to amino acids in the excreted honeydew. These purple spots were traced and were counted over a graph sheet and expressed in mm^2 honey dew area (Fig. 4).

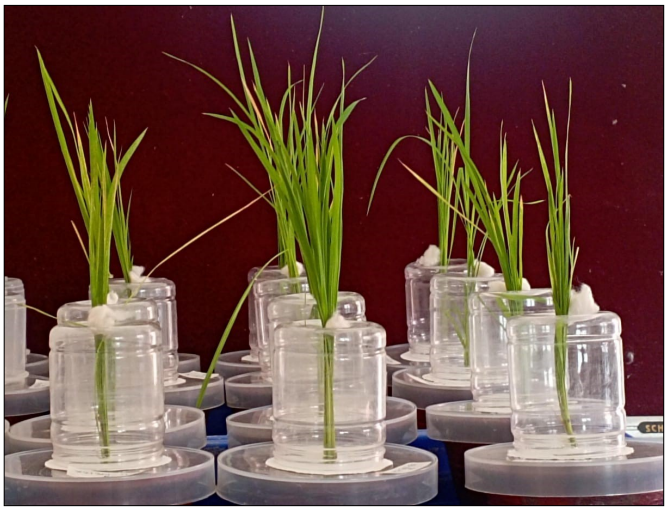

Fig. 4. Feeding rate assay experimental set up.

Tolerance: Tolerance level in the selected lines was estimated by assessing the parameters like functional plant loss index (FPLI), plant dry weight loss index (PDWLI mg) and tolerance index (TI). First instar nymphs were released on 30 days-old seedlings of the selected entries @50 nymphs/seedling with 3 replications and one as a control plant without the release of insects in each rice line. Insects settle and start feeding the seedling., When the entries began to wilt, nymphs were collected and oven dried for 48 h and weighed. Simultaneously, both the infested and uninfested plants were removed along with the roots, washed and air dried for 3 hr and then oven dried at 70℃ for 6 hr and weighed. Tolerance parameters were worked out.

FPLI = [1- (Dry weight of infested plant/Dry weight of uninfested plant)] × 100

PDWLI, mg = (Dry weight of uninfested plant–Dry weight of infested plant)/ Dry weight of BPH progeny on infested plant.

TI = BPH dry weight on test line / BPH dry weight on susceptible check

Statistical analysis

The data from the studies on phenotypic screening with introgressed lines were statistically analysed using analysis of variance (ANOVA) with the help of SPSS software. Standard

Error (SE[d]) and Critical Difference (CD) were computed to evaluate the impact of each parameter and to find significant differences between them respectively. All data were subjected to transformation. Nymphal survival and FPLI data were subjected to arcsine transformation while others were subjected to square root transformation. Correlation studies were analysed using SPSS software to understand the interaction among different resistant parameters.

Results

Phenotyping screening

Out of 110 introgressed entries, none of the entries were categorized as highly resistant in portray screening method. Eight entries viz., X21301-86, X21301-96, X21301-117, X21302-54, X21302-67, X21302-145, X21305-4 and FSR-3 along with the resistant check Ptb 33 were categorized as resistant with the mean damage score between 1 and 3. Eighteen entries showed moderately resistant to brown planthopper (Table 2). Fifty-one entries were recorded with a damage score between 5 and 7; those were categorized as moderate susceptible, and the rest were categorized as susceptible. TN 1, the susceptible check was graded with a damage score of 9.

Entries identified as resistant and moderately resistant in PST were further subjected to MSST and fourteen entries viz., X21301-50, X21301-79, X2130-86, X21301-96, X21301-117, X21302-7, X21302-10, X21302-54, X21302-67, X21302-69, X21302-145, X21305-4, FSR-3 and CB 20166 were categorized as resistant in the modified seedbox screening test. The resistant check (Ptb 33) showed resistance with the mean score of 1.25 and the susceptible check (TN 1) was observed with a damage score of 9 (Table 3).

Antibiosis parameters

Feeding rate: The feeding rate of BPH adults was assessed in selected rice entries by measuring the purple-coloured area marked on the filter paper which directly implies the amount of honeydew excreted. Honeydew area ranged from minimum of 22.18 mm^2 to maximum 961.23 mm^2. The least area of honeydew excretion was found on X21302-145(22.18mm^2) followed by FSR-3 (35.20 mm^2), X21301-79 (54.26 mm^2) and X21301-96 (90.66 mm^2). Honeydew excreted on the entries X21301-86, X21301-50, CB20166, X21301-117, X21302-10 varied between 107 mm^2 and 160 mm^2. Comparatively more honeydew excretion was found on the entry X21302-54 (304.14 mm^2). The resistant (PTB 33) and susceptible checks (TN 1) possessed an area of 108.03 mm^2 and 961.23 mm^2, respectively (Table 4).

Nymphal survival and development period: The mean survival rates of BPH nymphs ranged between 26.55 and 86.98%. The nymphal survival rate was significantly higher on susceptible check TN 1 (86.98%) than all other test entries, including resistant check Ptb 33. Higher survival rates (>50%) were found on X21302-69 (76.80%), X21301-96 and X21302-67 (70.32%), X21305-4 (60.12%) and X21302-54 (53.33%). On the other hand, lower survival rates were found on X21302-145 (29.39%), X21302-10 (36.46%), X21301-79 (39.86%) and X21301-86 (43.18%). Similarly, the nymphal development period varied from 9.7 days to 13.7 days. Among the test entries, the developmental period was quite higher in FSR-3 (13.7 days), X21302-145 and Ptb 33 (13.2 days). While in the susceptible check TN 1, the development period was observed for 9.7 days.

Growth index: The growth Index of the entries ranged between

Table 3. Resistance of rice lines in modified seedbox screening test

S.NO	Entry	Score	Category
1	X21301-11	3.5	MR
2	X21301-13	3.5	MR
3	X21301-30	3.85	MR
4	X21301-50	2.5	R
5	X21301-59	4.2	MR
6	X21301-79	1.5	R
7	X21301-83	3.75	MR
8	X21301-86	1.8	R
9	X21301-96	1.3	R
10	X21301-117	1.5	R
11	X21302-7	2.15	R
12	X21302-10	1.5	R
13	X21302-16	3	MR
14	X21302-36	3.25	MR
15	X21302-54	1.3	R
16	X21302-67	1.28	R
17	X21302-69	2.1	R
18	X21302-87	3.6	MR
19	X21302-89	3	MR
20	X21302-95	3.8	MR
21	X21302-96	3.5	MR
22	X21302-145	1.3	R
23	X21305-1	3	MR
24	X21305-4	1.4	R
25	FSR-3	1.25	R
26	CB20166	2	R
27	TN 1 (Susceptible check)	8.6	S
28	PTB 33 (Resistant check)	1.5	R

Table 2. Resistant levels of introgressed lines of rice in protray screening test

S.no	Entry	No. of Entries	Score	Category
1	X21301-86, X21301-96, X21301-117, X21302-54, X21302-67, X21302-145, X21305-4, FSR-3,	8	1-3	R
2	X21301-11, X21301-13, X21301-30, X21301-50, X21301-59, X21301-79, X21301-83, X21302-7, X21302-10, X21302-16, X21302-36*, X21302-69, X21302-87, X21302-89, X21302-95, X21302-96, X21305-1, CB20166,	18	3-5	MR
3	X21301-9, X21301-24, X21301-26, X21301-28, X21301-35, X21301-37, X21301-51, X21301-68, X21301-71, X21301-75, X21301-80, X21301-85, X21301-109, X21301-116, X21302-2, X21302-4, X21302-6, X21302-9, X21302-12, X21302-31, X21302-32, X21302-35, X21302-36, X21302-37, X21302-45, X21302-46, X21302-58, X21302-60, X21302-62, X21302-64, X21302-65, X21302-66, X21302-70, X21302-71, X21302-75, X21302-76, X21302-77, X21302-78, X21302-80, X21302-82, X21302-83, X21302-90, X21302-91, X21302-95*, X21302-107,X21302-113,X21302-115, X21302-116, X21302-118, X21302-143, FSR-4,	51	5-7	MS
4	X21301-10, X21301-23, X21301-25, X21301-45, X21301-70, X21301-91, X21301-118, X21302-1, X21302-8, X21302-61, X21302-63, X21302-85, X21302-94, X21302-99, X21302-104, X21302-106, , X21302-119, X21302-121,X21302-141, X21310-3, X21310-4, X21310-5, X21310-6, X21310-8, X21310-9, X21310-10, X21310-11, X21310-12, X21313-4, CB 22127,CB 19136, CB 20164, CB 21112	33	7-9	S

Table 4. Evaluation of antibiosis mechanism of resistance in the introgressed lines of rice

Entry	Honey dew (mm²)	Survival (%)	Nymphal development period (days)	Growth Index
X21301-50	109.82^{ef} (10.48)	43.3^{def} (0.72)	10.6^{ef} (3.248)	4.11
X21301-79	54.26^{fg} (7.37)	39.86^{efg} (0.68)	10.3^{fg} (3.214)	3.87
X21301-86	107.12^{ef} (10.35)	43.18^{defg} (0.72)	11.0^{cdef} (3.314)	3.94
X21301-96	90.66^{ef} (9.52)	70.32^{bc} (0.99)	10.8^{def} (3.282)	6.49
X21301-117	144.92^{de} (12.04)	26.55^{g} (0.54)	11.9^{bc} (3.446)	2.24
X21302-7	78.15^{ef} (8.84)	49.99^{de} (0.79)	11.3^{bcde} (3.365)	4.41
X21302-10	156.10^{de} (12.49)	36.46^{efg} (0.65)	12.0^{b} (3.463)	3.06
X21302-54	304.14^{c} (17.44)	53.33^{cde} (0.82)	10.9^{def} (3.299)	4.90
X21302-67	232.27^{cd} (15.24)	70.32^{bc} (0.99)	11.3^{bcde} (3.366)	6.18
X21302-69	298.11^{c} (17.27)	76.80^{ab} (1.07)	10.9^{def} (3.299)	7.04
X21302-145	22.18^{g} (4.71)	29.39^{fg} (0.57)	13.2^{a} (3.636)	2.27
X21305-4	463.83^{b} (21.54)	60.12^{cd} (0.89)	10.5^{ef} (3.248)	5.68
FSR-3	35.20^{g} (5.93)	26.55^{defg} (0.54)	13.7^{a} (3.696)	1.95
CB20166	130.53^{e} (11.43)	49.79^{de} (0.78)	11.7^{bcd} (3.415)	4.33
TN 1	961.23^{a} (31.00)	86.98^{a} (1.20)	9.7^{g} (3.108)	8.96
PTB 33	108.03^{ef} (10.39)	46.63^{def} (0.75)	13.2^{a} (3.635)	3.53
SE(d)	1.474	0.0775	0.2	
CD (p = 0.05)	4.246	0.2232	0.1728	

Figures in parentheses are transformed values given with mean values

Means with same letter are not significantly different at 5% level by DMRT

1.95 and 8.96. The least growth index was observed in FSR-3 (1.95) followed by X21301-117 (2.24), X21302-145 (2.27) and the resistant check Ptb 33 (3.53). The highest growth index was observed in TN 1 (8.96).

***Antixenosis mechanism*:** Settling response of BPH nymphs on selected entries was observed over a period with 12 hr intervals. The preference of nymphs differed significantly among the entries at the release of 12th hr. Among the entries, the least number of nymphs preferred the resistant check, Ptb 33, with the mean population count of 1.9, followed by the entries X21302-7 (2.5), CB 20166 (2.6) and X21302-69 (2.6). Nymphal population on TN 1 was 3.1 at the end of 12th hr release (Fig. 5).

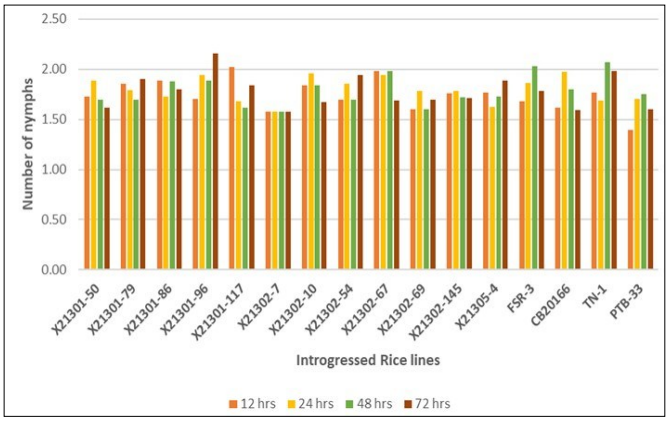

Fig. 5. Settling response of BPH nymphs on rice introgressed lines.

At the release of 24th hr, the nymphal population ranged between 2.5 and 3.9. The entries X21302-7 (2.5) and X21305-4 (2.6) had the least number of nymphs. The entries X21301-86, X21301-117 and the checks possessed an average population of less than 3. The maximum nymphal population was found in the entry CB 20166 with a count of 3.9.

The susceptible check, TN 1, accommodated the maximum population of 4.3 at the end of 48th hr release. The least number of nymphs was found in the entry X21302-7 (2.5), followed by X21302-69 (2.6) and the same population (2.9) existed in three entries, viz., X21301-50, X21301-79 and X21302-54. The resistant check, Ptb 33, possessed a mean population of 3.1.

Likewise, at 72 hr after release, entry X21302-7 was observed to have least nymphal preference (2.5). The maximum number of nymphs got accumulated in the entry X21301-96 (4.7). The nymphal population in the checks, TN 1 and Ptb 33, were 3.9 and 2.6, respectively.

The mean population of nymphs across four-time intervals implies a significant difference in nymphal settlement behaviour among the entries. Among the entries, FSR-3 exhibited the strong resistance indicated by least mean population of 2.30. Ptb 33, the source of introgressed lines was recorded with overall mean population of 2.61 whereas TN 1, in contrast, was noted with a higher population of 3.84, revealing its susceptible nature (Supplementary data table 1).

Tolerance parameters: The functional plant loss index varied significantly among the entries which ranged from 10.48 to 55.70%. FPLI was found least in FSR-3 (10.48%) followed by X21302-145 (11.93%), X21301-86 (17.86%) and the highest in entry X21301-50 (55.70%). It was observed that the resistant check (Ptb 33) had FPLI value of 29.52% and the susceptible check had 41.86%. Also, the plant dry weight loss to BPH dry weight produced differed between 12.27 and 111.1 g/mg. PDWLI was found least in the entry CB20166 (12.27 g/mg), followed by X21302-7 (15.46 g/mg), X21301-50 (17.43 g/mg) and the highest in the entry X21301-86 (Fig. 6). In the case of checks, the PDWLI values of Ptb 33 and TN 1 were computed as 96.38 g/mg and 68.37 g/mg, respectively (Supplementary data table 2).

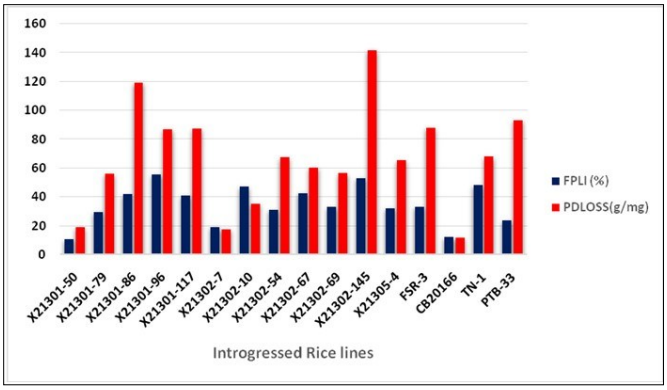

Fig. 6. Quantitative estimation of tolerance parameters.

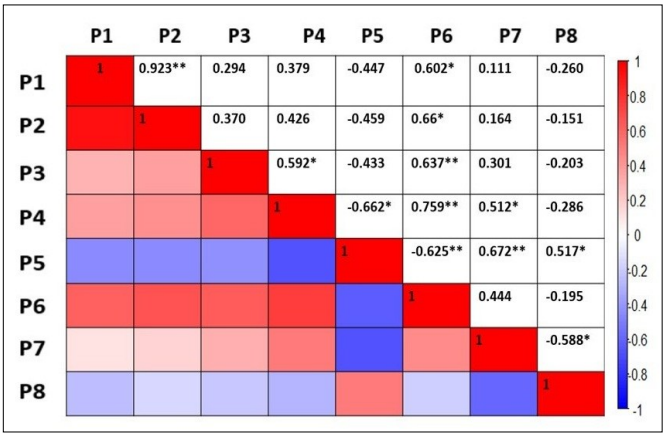

Fig. 7. Interrelation between different resistant parameters against BPH.

Interaction between Damage score and mechanisms of resistance against BPH. Range between -1 to +1 indicates the correlation coefficient between the parameters of the mechanisms. P1- Protray screening score, P2- MSST score, P3- Nymphal preference, P4- Nymphal survival, P5- Nymphal developmental period, P6- Feeding rate, P7- Functional Plant Loss Index, P8- Plant Dry Weight Loss

Interrelation between different resistant parameters: The correlation analysis of phenotypic damage scores along with various resistance parameters in the selected rice entries indicates there was significant interaction within them. The red shade in the heatmap (Fig. 7) indicates the positive correlation, and the blue shade indicates the negative correlation. Stronger correlations are indicated by darker shades of red and blue colors. For instance, the darker red shade shows a strong positive correlation between PST scores and MSST scores (r = 0.923). Also, feeding rate was found to have a better association with both the damage scores (r = 0.602) and nymphal survival (r = 0.759).

Additionally, positive correlations were established between nymphal preference and feeding rate (r = 0.637) and, nymphal survival and nymphal preference (r = 0.592). Similarly, a strong negative correlation was found between the nymphal development period and Functional Plant Loss Index (FPLI) (r = -0.672) and moderate negative correlations between the nymphal developmental period and nymphal survival (r = -0.662) and nymphal development period and feeding rate (r = -0.625).Tolerance parameters, viz., FPLI and PDWLI, were negatively correlated (r = -0.588).

The interrelation studies revealed that on the identified resistant sources, nymphs excreted less amount of honeydew, took more days to become adults and had a smaller number of nymphs settled per plant compared to susceptible check, TN 1.

Discussion

Brown planthopper is the most noxious pest of rice causing drastic yield losses. HPR plays a major role in managing this harmful pest as it targets more specifically without causing any adverse effect on other organisms. Opting for HPR in pest management involves the process of screening, categorization, breeding, and implementation (13). The present research focused on the mechanisms of host plant resistance of introgressed lines from the derivatives of resistant Ptb 33 through various phenotypic measurements.

The mechanisms of resistance against planthoppers were screened through various methods like the standard seedbox method, modified seedbox tests, protray screening method and field tests. Among those, the protray screening method is said to have more advantages, like accommodating 48 genotypes along with checks at a time, making insect movement easier since the seeds are sown in circular fashion and most importantly, being easy to handle (7). From the present study of protray screening test, BPH resistance was identified in seven entries along with Ptb 33 (mean score between 1 and 3) and TN 1 was found to be the most susceptible entry with a score of 9 (Fig. 8). Several studies reported that the PTB 33 has strong resistance and was able to withstand the BPH population, showing no damage or slight damage in the screening test. Also, various reports have revealed the use of PTB 33 as a resistant check in screening tests (14). The rice lines introgressed with brown planthopper (BPH) resistance genes exhibited better performance in comparison to the other genotypes and indicated by better phenotypic responses under BPH infestation (6). In our study, the rice lines introgressed with PTB 33 as a source of BPH resistance, revealed better resistance against brown planthopper. These results were corroborated with the previous work done by several workers (15, 16).

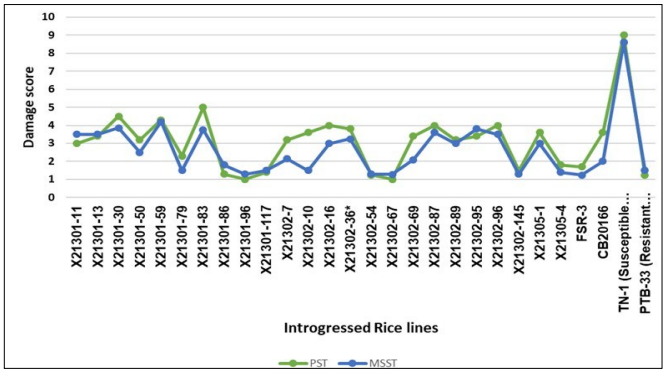

Fig. 8. Damage score in protray and modified seed box screening tests.

Numerous studies suggest that measuring of honeydew excreted by BPH is an effective way to substantiate the scores of seedbox screening. Determining the area of honeydew excretion can help to quantify BPH's feeding since they are directly proportional (17). Resistant cultivars minimize BPH feeding activity due to the presence of various plant metabolites, making them less desirable for feeding, resulting in less honeydew excretion. From the conclusion of a study of certain elite rice genotypes, the resistant genotypes showed significantly less honeydew excretion and susceptible check, the highest honeydew excretion (18). Comparatively in the present study, a minimal area of honeydew excretion was measured in the resistant entries, viz., X21302-145 and FSR-3 when compared to large area of honeydew excretion in the susceptible entries, TN 1.

Predominantly, nymphs settled on resistant genotypes are significantly less when compared to the susceptible genotypes (11, 17). The findings of the current study revealed that the nymphal preference got fluctuated over time and the entry X21302-54 shown relatively high preference across the series of time ranging between 3.9 (12 hr) and 2.9 (72 hr). The entry X21302-7 was observed with constant preference over the time of observation (Fig. 5). Based on the overall mean values, X21305-4 shows the highest mean preference (3.87) and entry FSR-3 shows the least mean preference (2.30), indicating susceptibility and resistance, respectively.

In general, genotypes exhibiting resistance have an adverse effect on the biology of the target insects, while susceptible genotypes support their development and proliferation. Nymphal survival is considered the best method for analyzing the antibiosis component. The resistant genotypes have less nymphal survival and longer developmental periods than others due to the inadequate nutrition in their feed. Accordingly, nymphal survival of the resistant entries in our study was 26%-46% and nymphal development period was in the range of 12-13 days (Fig. 9) (Table 4). Our results were substantiated by the findings of various works (5, 17, 19). Growth index is considered the most reliable parameter for comparing the suitability of the genotypes. The findings of our investigation were like the various studies conducted where the resistant genotypes had lower BPH growth index values and lesser growth index showed the unsuitability of the genotypes for BPH development (19-21).

Resistant rice genotypes were identified using tolerance parameters such as FPLI and PDWLI, which were observed to be lower than susceptible genotypes (11, 17). The present study provides FSR-3 as the resistant entry that is found to have the lowest FPLI and relatively less PDWLI value (Fig. 6).

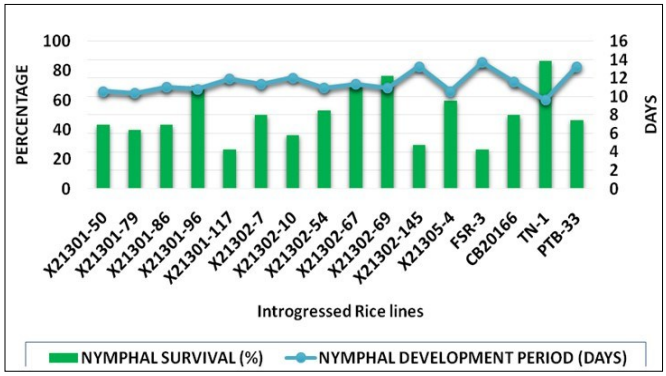

Fig. 9. Survival and developmental period of BPH in introgressed rice lines.

The interrelation of different parameters of resistance revealed complex dynamics between various resistance parameters, highlighting key associations that contribute to BPH resistance. Notably, a strong positive correlation between two screening methods indicates consistent performance of BPH resistance across different seedling stages. A significant positive correlation between PST and the feeding rate of BPH suggests that entries exhibiting good resistance in PST potentially limit BPH feeding, contributing to the overall resistance. Feeding rate assay, honeydew excretion method, is an important tool in assessing antibiosis resistance to identity resistant entries in adult plants. The results of the assay in the study were in line with the seedling stage screening and demonstrated the entries are resistant to BPH at both stages. Additionally, a significant positive linkage between nymphal preference and nymphal survival indicates that nymphs are likely to thrive better on preferred susceptible entries. Similarly, a positive correlation between nymphal preference and feeding rate shows that plants preferred by nymphs tend to have higher feeding rates by adults also, implying that more attractive plants provide a better feeding environment.

The negative correlation between PST and nymphal developmental period suggests that entries with better resistance scores are associated with shorter developmental periods. Furthermore, a strong negative correlation between nymphal survival and nymphal developmental period indicates that entries that reduce nymphal survival tend to slow down nymphal development. Thus, entries with effective resistance mechanisms affect both nymphal survival and developmental period. Among the tolerance parameters, FPLI and PDWLI exhibit a negative relationship, indicating that entries experiencing functional loss may not necessarily show a proportional increase in physical damage.

Conclusion

The time series experiment data revealed that FSR-3 was a resistant entry, which showed high resistance across all three mechanisms, followed by X21302-145, which expressed better antibiosis resistance but poor tolerance.

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Authors' contributions

LSM performed the experiments and wrote the draft of the manuscript. SRP conceptualized the methodology and edited drafting and finalising the manuscript. SV supervised the experiments and provided lab facility. SR made crosses of parental lines and development of introgressed lines. SM helped in the crossing programme and editing the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethical issues: None

References

1. FAOSTAT. Area of Rice production [Internet]. 2022 [cited 2024 Oct 9]. Available from: https://www.fao.org/faostat/en/#data/QCL.
2. Puspito AN, Rozzita N, Nendra Tigara MR, Dinata Putra SL, Purnamasari I, Hartatik S, et al. Molecular screening of local Indonesian rice to identified resistant varieties against brown planthopper (Nilaparvata lugens) attacks. Biodiversitas: Journal of Biological Diversity. 2023;24 (10). https://doi.org/10.13057/biodiv/d241032
3. Bottrell DG, Schoenly, KG. Resurrecting the ghost of green revolutions past: The brown planthopper as a recurring threat to high-yielding rice production in tropical Asia. Journal of Asia-Pacific Entomology. 2012;15(1):122-40. https://doi.org/10.1016/j.aspen.2011.09.004
4. Sharma KR, Raju SVS, Singh SK, Singh R, Meena RS. Rice genotypes and the biochemical basis of resistance against brown planthopper, Nilaparvata lugens (Stål). Entomon. 2024;49(2):215-20. https://doi.org/10.33307/entomon.v49i2.1172
5. Singh I, Sarao PS, Sharma N. Antibiosis components and antioxidant defense of rice as mechanism of resistance to brown planthopper, *Nilaparvata lugens* (Stål). Cereal Research Communications. 2017;45:284-295. https://doi.org/10.1556/0806.45.2017.011
6. Han Y, Wu C, Yang L, Zhang D, Xiao Y. Resistance to *Nilaparvata lugens* in rice lines introgressed with the resistance genes *Bph14Bph14* and Bph15*Bph15* and related resistance types. PLoS One. 2018;13 (6):e0198630. https://doi.org/10.1371/journal.pone.0198630
7. Soundararajan RP, Jeyaprakash P. Rapid screening of rice genotypes for resistance to brown planthopper, Nilaparvata lugens (Stal). Electronic Journal of Plant Breeding. 2019;10(1):76-82. https://doi.org/10.5958/0975-928X.2019.00009.7
8. IRRI. 2013. Standard evaluation system for rice. International Rice Research Institute, Manila, Philippines, 5th Edition. p. 28. Available from https://www.scribd.com/document/333585255/SES-5th-Edition
9. Velusamy R, Heinrichs EA, Medrano FG. Greenhouse techniques to identify field resistance to the brown planthopper,*Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), in rice cultivars. Crop Protection. 1986;5(5):328-33.https://doi.org/10.1016/0261-2194(86)90112-2
10. Heinrichs EA, Medrano FG, Rapusas, HR. 1985. Genetic evaluation for insect resistance in rice. Available from https://www.semanticscholar.org/paper/Genetic-evaluation-for-insect-resistance-in-rice-Heinrichs-Medrano/617bbbe936c1a364ec070fb217c5eab252b4fb0d
11. Alagar M, Suresh S, Samiyappan R, Saravanakumar D. Reaction of resistant and susceptible rice genotypes against brown planthopper (*Nilaparvata lugens*). Phytoparasitica. 2007;35:46-356. https://doi.org/10.1007/BF02980697
12. Pathak PK, Saxena RC, Heinrichs EA. Parafilm sachet for measuring honeydew excretion by *Nilaparvata lugens* on rice. Journal of Economic Entomology. 1982;75(2):194-195. https://doi.org/10.1093/jee/75.2.194
13. Stout MJ. Host-plant resistance in pest management. In: Integrated pest management: Current Concepts and Ecological Perspective. Elsevier Inc. 2014;1-21. https://doi.org/10.1016/B978-0-12-398529-3.00002-6
14. Shilpakala V, Lakshmi VJ, Venkatesarulu NC, Madhav MS, Tamilmurugan R, Devi R. Probing behaviour of brown planthopper Nilaparvata lugens (Stal.) in the Resistant Germplasm Accessions. Journal of Experimental Agriculture International. 2024;46(3):26-34. https://doi.org/10.37992/2023.1403.109
15. Pelhania, Garima, M. Gokulakrishnan, J. Niranjana Devi, S. Yazhini, V. Balasubramani, S. Manonmani, et al. Genotypic and phenotypic analysis of backcross inbred lines for brown plant hopper resistance in rice. Electronic Journal of Plant Breeding. 2023;14(3):976-83. https://doi.org/10.37992/2023.1403.109
16. Lakshmi VI, Sreedhar M, Lakshmi VJ, Gireesh C, Rathod S, Vanisri S. Phenotypic screening and single marker analysis for Brown planthopper resistance in rice (*Oryza sativa* L.). The Journal of Research PJTSA. 2021;49(1&2):1-9. Available from https://epubs.icar.org.in/index.php/TJRP/article/view/118757
17. Roy D, Chakraborty G, Biswas A, Sarkar PK. Antixenosis, tolerance and genetic analysis of some rice landraces for resistance to *Nilaparvata lugens* (Stål.). Journal of Asia-Pacific Entomology. 2021;24(1):448-60. https://doi.org/10.1016/j.aspen.2020.10.012
18. Udayasree M, Rajanikanth P. Non-preference/antixenosis and antibiosis mechanism contributing to BPH resistance in certain identified elite rice genotypes. International Journal of Current Microbiology and Applied Sciences. 2018; 7(06):1908-14. https://doi.org/10.20546/ijcmas.2018.706.226
19. Reddy BN, Lakshmi VJ, Maheswari TU, Ramulamma A, Katti GR. Studies on antibiosis and tolerance mechanism of resistance to brown planthopper, Nilaparvata lugens (Stal) (Hemiptera: Delphacidae) in the selected rice entries. The Ecoscan. 2016;10:269-75.
20. Kumar H, Maurya RP, Tiwari SN. Study on antibiosis mechanism of resistance in rice against brown plant hopper, *Nilaparvata lugens* (Stal.). Annals of Plant Protection Sciences. 2012;20(1):98-101.
21. Tenguri P, Chander S, Ellur RK, Arya PS, Yele Y. Deciphering host plant resistance mechanisms of rice genotypes resistant against Brown Planthopper. Euphytica. 2023;219:8. https://doi.org/10.1007/s10681-022-03136-3