

Phenotypic Screening of Elite Rice Germplasms against Brown Planthopper [*Nilaparvata lugens* (Stal.)] for Identification of Resistant Donor Lines

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Authors' contributions

This work was carried out in collaboration among all authors. Author MNR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PRK and NRGV managed the analyses of the study. Author MB managed the literature searches. All authors read and approved the final manuscript

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ABSTRACT

Aims: To identify Brown Planthopper (BPH) resistant rice genotypes and categorize all the test entries based on their level of resistance against BPH.

Study Design: Completely Randomized Design.

Place and Duration of Study: Poly-house, Department of Entomology, Rice Research Centre, Agriculture Research Institute (ARI), Rajendranagar, Hyderabad, India, between June 2016 and July 2017.

Methodology: A total of 61 elite rice genotypes selected including resistant (PTB33) and susceptible check (TN1). All these test entries were screened against Brown Planthopper (BPH) using Standard Seedbox Screening Technique (SSST) inside poly-house conditions. Based on the

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Damage Score (DS) achieved during study, all entries were categorized into resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS).

Results: Among 61 cultures, the resistant check PTB33 and BM71 exhibited R reaction to BPH with a DS of 3.0, while twelve cultures viz. Milyang 63, IET 23993, HHZ 5 DT-1 DT-1, HHZ 25 SAL DT-1 DT-1, Bobhu Kongbu, BPT 2671, BPT 2611, MTU 1121, MTU 1001, MTU 1010, RNR 23079 and GSR 234 exhibited MR reaction to BPH with a DS ranging between 3.1 to 5.0. The rest of the cultures showed MS and S reactions while the susceptible check, TN1 along with other 12 cultures exhibited HS reaction to BPH with a DS of 9.0.

Conclusion: Resistant and moderately resistant rice genotypes have been identified for development of BPH resistant lines. Further detailed studies are required to understand the underlying mechanisms of resistance among the R and MR genotypes.

Keywords: Rice; *Nilaparvata lugens*; brown planthopper; Standard Seedbox Screening Test (SSST); damage score; host plant resistance.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a diploid ($2n = 24$), short day, self-pollinated crop widely grown in tropical agro-climatic zone around the world. Rice being cultivated in warm and humid tropical conditions is prone to high insect pest attack. It is estimated that approximately 52 per cent of global rice production is lost annually by biotic stresses (viz., insects, diseases, weeds, etc.), of which one-fourth is due to insect pests [1]. Rice is attacked by more than hundred species of insects, of which around 20 cause significant economic damage and one among them is brown planthopper (BPH), *Nilaparvata lugens* (Stal.) [2]. Brown planthopper is a very dangerous pest; under favourable conditions its population can increase rapidly and result in plant death in large areas known as "hopper burn" [3]. It is also reported to cause huge yield losses every year in East and South Asian countries [4]. Before 1970s BPH was just a minor pest in the Asian countries, except Japan, but during the 1970s, it suddenly rose to major pest status and started to cause heavy infestation leading to high yield losses in rice fields. It caused havoc among rice-growing farmers and researchers with its unpredictability of infestation and dramatically higher level of damage [5]. The major reason behind this sudden outbreak of BPH in South-East Asia during 1970s was due to the disrupted ecological balance between BPH and natural enemies by insecticides, which were accepted as an important component to ensure high output from high yielding varieties [6]. Meanwhile the BPH outbreak in India was primarily due to disrupted co-evolution between BPH and local BPH resistant rice cultivars by sudden replacement of BPH resistant traditional rice cultivars with susceptible exotic high yielding varieties during

the promotion of High Yielding Varieties Program in the late 1960s [6]. These circumstances lead to better thrust in the field of research for BPH resistant varieties as early as 1966 at IRRI, Philippines, where thousands of rice accessions were screened against BPH [7] and the process of identification of newer and better donor lines still continues in almost all rice growing nations because with time these varieties exhibit breakdown of resistance in the field, by formation of BPH biotypes [6], biotypes which can feed, survive and damage these previously known resistant varieties. Until recent years BPH management strategy was focused mainly on the use of synthetic insecticides, which led to environmental pollution as well as development of resistance in BPH against the commonly used insecticide groups [8]. Under these circumstances utilisation of Host Plant Resistance (HPR) for the development of resistant or tolerant varieties against BPH, and then integrating it with other feasible integrated pest management components will be most economic and effective approach for mitigating the BPH problem [9,10]. In the present study, an effort was made following Standard Seedbox Screening Test (SSST) under poly house conditions to identify the presence of resistance, if any, towards BPH in some of the elite rice germplasms possessing desirable yield and quality traits.

2. MATERIALS AND METHODS

2.1 Mass Rearing of BPH

Mass rearing of BPH was done on BPH susceptible rice variety Taichung Native 1 (TN1). Pre-germinated seeds of TN1 were sown in three litre plastic pots (3-4 hills per pot) filled with

fertilizer enriched soil, and watered regularly in poly-house till plants reached 60 days of age. These plants were then transferred to insect proof cages (5-6 pots per cage) and inoculated with 12-15 gravid females per cage and watered regularly. Once the BPH population developed and first and second instar nymphs started to emerge, they were used in the screening studies.

2.2 Screening

A total of 61 rice germplasms including a susceptible check (TN1) and a resistant check (PTB33) were screened against BPH (Table 1) using the Standard Seedbox Screening Technique (SSST) developed by the International Rice Research Institute (IRRI) [11]. The seeds of selected genotypes were kept in separate petri plates and poured water till all seeds gets submerged in water. After 24 hours the excess water was drained and seeds were kept for germination for one more day. After 48 hours, the pre-germinated seeds were sown in plastic trays (42 x 32 x 15 cm) and labelled accordingly. The seeds were sown in the plastic trays in a specific layout (Fig. 1) in which different treatments were planted randomly with help of a random number table, Resistant check (PTB33)

was planted in the middle row and the susceptible check (TN1) was planted around the perimeter of the rectangular tray, each entry SSST tray was replicated three times.

Seedlings were watered regularly and allowed to grow till three leaf stage in a healthy manner inside protected conditions to avoid insect pest incidence. On reaching three leaf stage, seedlings were infested with first instar nymphs of BPH. It was verified that every test seedling had 6-7 nymphs. BPH infected seedlings were kept in insect proof cages and water level was maintained uniform throughout the tray. The tray was also rotated 180° at regular intervals to get a uniform reaction to the seedlings by the released insects. Once 90 per cent mortality was observed in seedlings of susceptible check (TN1), the rice germplasm entry seedlings were then scored based on a 0-9 scale using the Standard Evaluation System (SES) [12] as described in Table 2. After scoring as per SES, means of three replications were calculated and a damage score (DS) calculated. All the SSST entries were then categorized as resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) based on damage score, the categorization chart is provided in Table 3 [13].

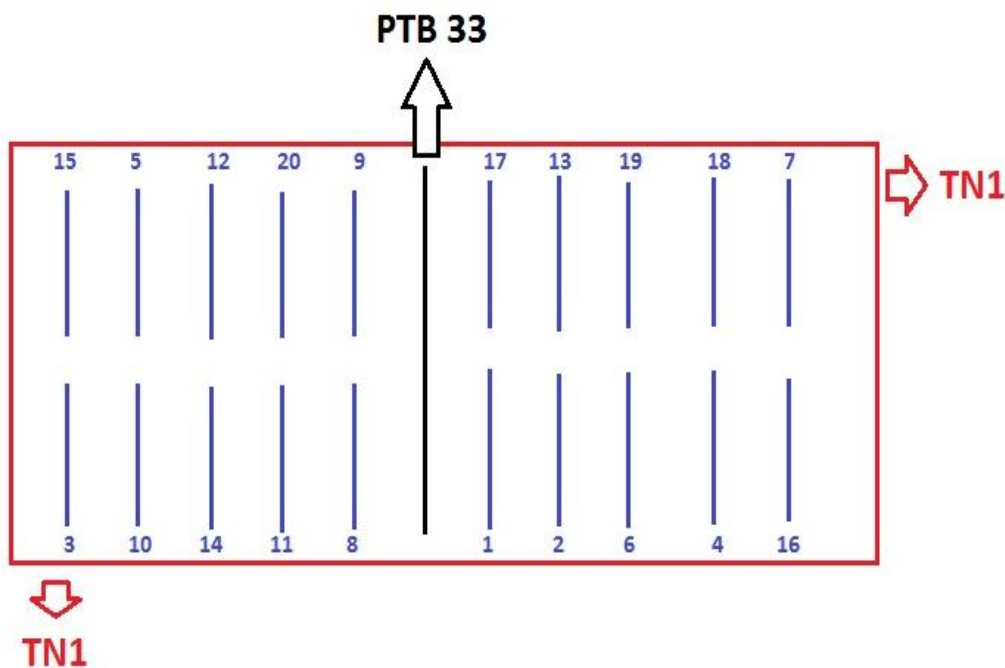


Fig. 1. Layout of Standard Seedbox Screening Test (SSST)
 PTB33 - Resistant check, TN1 - Susceptible check, 1 to 20 - Treatment lines

Table 1. Selected rice genotypes for mass screening against BPH using SSST

| Sl. no. | Rice genotype | Parentage / Cross | Sl. no. | Rice genotype | Parentage / Cross |
|---------|-------------------------------|--|---------|---------------|------------------------------|
| 1 | PTB 33 | Pure line selection from land race from RARS, Pattambi | 32 | BPT 2601 | MTU 1001 x N 22 |
| 2 | BM71 | Vajram/ Darrington | 33 | KNM 1723 | JGL15185 x HPR2443 |
| 3 | Milyang 63 | * | 34 | RNR 8860-1 | MTU1071 x BPT5204 |
| 4 | IET 23993 | IR64/ Ady. Selection from IIRR | 35 | RNR 8860-5-1 | MTU1071 x BPT5204 |
| 5 | HHZ 5 DT-1 DT-1 | Huang – Hua – Zhan*2/OM 1723 | 36 | BPT 2659 | MTU 2077/NBR 16/MTU 2077 |
| 6 | HHZ 25 SAL DT-1 DT-1 | - | 37 | RNR 8860-7 | MTU1071 x BPT5204 |
| 7 | Bobhu Kongbu | - | 38 | RNR 23109-3 | PUSA1121 x BASMATI370 |
| 8 | BPT 2671 | MTU 2067 x AJAY | 39 | JGL 24527 | JGL11727 x VD82 |
| 9 | BPT 2611 | IR 64 x LALNAKAND | 40 | BPT 2742 | MTU 2716 x MTU 7029 |
| 10 | MTU 1121 | BPT5204 x MTU DP 13 | 41 | IR 77384 | |
| 11 | MTU 1001 | Vajram x MTU 7014 | 42 | KNM 1638 | JGL3844 x BPT5204 |
| 12 | MTU 1010 | Krishnaveni x IR 64 | 43 | BPT 2782 | NLR 145 x MTU 2077 |
| 13 | RNR 23079 | CR 1009 x NLR145 | 44 | RNR 21278 | RNR2465 x NLR34449 |
| 14 | GSR 234 (HHZ 5-Y3-SAL 2-SUB 1 | Huang – Hua – Zhan*2/OM 1723 | 45 | RNR 23109 | PUSA1121 x BASMATI370 |
| 15 | MTU 1244 | - | 46 | KNM 736 | KRISHNA HAMSA x JGL3844 |
| 16 | BPT 2613 | MTU 7029 x MOROBERKAN | 47 | BPT 2780 | BPT 2270/NLR 145 |
| 17 | MTU 1240 | MTU3626 x GEDANZIBETON | 48 | KNM 626 | MTU1010 x JGL11470 |
| 18 | BPT 2688 | PLA 1100 x CR 683-164-1 | 49 | JGL 25153 | JGL17653 x RP2421 |
| 19 | IET 23081 | BPT5204 x BPT4358 | 50 | RNR 8860-5 | MTU1071 x BPT5204 |
| 20 | BPT 2743 | MTU 7029 x NCR 34449 | 51 | RNR 8860-11 | MTU1071 x BPT5204 |
| 21 | JGL 26951 | JGL18047 x BADRAKALI | 52 | RNR 8860-15 | MTU1071 x BPT5204 |
| 22 | MUT NS1 | Mutant derived from Nizersail # | 53 | RNR 23564 | RNR 2458 x BM 71 |
| 23 | IET 24146 | NK16520 (selection) | 54 | KNM 1616 | JGL11470 x BPT5204 |
| 24 | MTU 1241 | MTU3626 x GEDANZIBETON | 55 | BPT 2600 | BPT 5204 x WGL 357 |
| 25 | RNR 11718 | MTU1010 x NLR34449 | 56 | BPT 2618 | BPT 5204 x AZNCENA |
| 26 | KNM 2213 | MTU 1001x JGL 11470 | 57 | BPT 2861 | MTU 2077 x NLR 34449 |
| 27 | MTU 1243 | MTU1081 x MTU1064 | 58 | MTU 1184 | PLA 1100 x BM71 |
| 28 | JGL 26959 | JGL18047 x BADRAKALI | 59 | MTU 1194 | MTU1081 x MTU1064 |
| 29 | RNR 23599 | PUSA1121 x BM71 | 60 | MTU 1242 | MTU1081 x MTU1064 |
| 30 | RNR 8860-4 | MTU1071 x BPT5204 | 61 | TN 1 | Dee-Geo-Wu-Gen/Tsai-yuan-chu |
| 31 | KNM 604 | MTU1010 x JGL11727 | | | |

*Culture from Republic of Korea, # culture from Bangladesh, - information unavailable with author, RARS- Regional Agricultural Research Station, IIRR - Indian Institute of Rice Research

Table 2. Damage score based on standard evaluation system [12]

| Plant state | Damage score |
|---|--------------|
| No damage | 0 |
| Very slight damage | 1 |
| Lower leaf wilted with two green upper leaves 3 | 3 |
| Two lower leaves wilted with one green upper leaf | 5 |
| All three leaves wilted but stem still green | 7 |
| Plant is dead | 9 |

Table 3. Categorization of levels of resistance based on damage score [13]

| Sl. no. | Reaction | Damage score |
|---------|-----------------------------|--------------|
| 1 | Resistant (R) | 1.0 - 3.0 |
| 2 | Moderately resistant (MR) | 3.1 - 5.0 |
| 3 | Moderately susceptible (MS) | 5.1 - 7.0 |
| 4 | Susceptible (S) | 7.1 - 8.9 |
| 5 | Highly susceptible (HS) | 9.0 |

Table 4. Reaction of different rice genotypes screened against BPH in SSST

| Sl. no. | Rice genotype | Mean damage score | Reaction | Sl. no. | Rice genotype | Mean damage score | Reaction |
|---------|----------------------|-------------------|----------|---------|---------------|-------------------|----------|
| 1 | PTB 33 | 3.0 | R | 32 | BPT 2601 | 8.2 | S |
| 2 | BM71 | 3.0 | R | 33 | KNM 1723 | 8.3 | S |
| 3 | Milyang 63 | 3.2 | MR | 34 | RNR 8860-1 | 8.3 | S |
| 4 | IET 23993 | 3.2 | MR | 35 | RNR 8860-5-1 | 8.3 | S |
| 5 | HHZ 5 DT-1 DT-1 | 3.3 | MR | 36 | BPT 2659 | 8.4 | S |
| 6 | HHZ 25 SAL DT-1 DT-1 | 3.5 | MR | 37 | RNR 8860-7 | 8.5 | S |
| 7 | BOBHU KONGBU | 3.5 | MR | 38 | RNR 23109-3 | 8.5 | S |
| 8 | BPT 2671 | 3.7 | MR | 39 | JGL 24527 | 8.5 | S |
| 9 | BPT 2611 | 3.9 | MR | 40 | BPT 2742 | 8.7 | S |
| 10 | MTU 1121 | 4.0 | MR | 41 | IR 77384 | 8.7 | S |
| 11 | MTU 1001 | 4.2 | MR | 42 | KNM 1638 | 8.7 | S |
| 12 | MTU 1010 | 4.3 | MR | 43 | BPT 2782 | 8.7 | S |
| 13 | RNR 23079 | 4.3 | MR | 44 | RNR 21278 | 8.8 | S |
| 14 | GSR 234 | 4.9 | MR | 45 | RNR 23109 | 8.8 | S |
| 15 | MTU 1244 | 5.1 | MS | 46 | KNM 736 | 8.8 | S |
| 16 | BPT 2613 | 5.3 | MS | 47 | BPT 2780 | 8.8 | S |
| 17 | MTU 1240 | 5.6 | MS | 48 | KNM 626 | 8.9 | S |
| 18 | BPT 2688 | 5.7 | MS | 49 | JGL 25153 | 9.0 | HS |
| 19 | IET 23081 | 6.3 | MS | 50 | RNR 8860-5 | 9.0 | HS |
| 20 | BPT 2743 | 6.4 | MS | 51 | RNR 8860-11 | 9.0 | HS |
| 21 | JGL 26951 | 6.6 | MS | 52 | RNR 8850-15 | 9.0 | HS |
| 22 | MUT NS1 | 6.6 | MS | 53 | RNR 23564 | 9.0 | HS |
| 23 | IET 24146 | 6.7 | MS | 54 | KNM 1616 | 9.0 | HS |
| 24 | MTU 1241 | 6.8 | MS | 55 | BPT 2600 | 9.0 | HS |
| 25 | RNR 11718 | 6.9 | MS | 56 | BPT 2618 | 9.0 | HS |
| 26 | KNM 2213 | 7.5 | S | 57 | BPT 2861 | 9.0 | HS |
| 27 | MTU 1243 | 7.5 | S | 58 | MTU 1184 | 9.0 | HS |
| 28 | JGL 26959 | 7.7 | S | 59 | MTU 1194 | 9.0 | HS |
| 29 | RNR 23599 | 7.9 | S | 60 | MTU 1242 | 9.0 | HS |
| 30 | RNR 8860-4 | 8.0 | S | 61 | TN 1 | 9.0 | HS |
| 31 | KNM 604 | 8.2 | S | | | | |

R- Resistant, MR- Moderately Resistant, MS- Moderately Susceptible, S- Susceptible, HS- Highly Susceptible

3. RESULTS AND DISCUSSION

The results of screening are presented in Table 4. Out of the 61 entries screened for BPH

resistance by SSST, the damage score ranged from 3.0 to 9.0 and 14 entries have shown high to moderate resistance (DS <5). The remaining entries recorded damage score more than five

and were found susceptible to BPH. Among the 14 resistant to moderately resistant cultivars (DS <5) two check lines PTB 33 (DS 3.0) and BM 71 (DS 3.0) have shown highest resistance with a damage score of 3.0 while the remaining twelve cultivars registered a damage score between 3.1-5.0 and were categorised as moderately resistant (MR) to BPH. These twelve entries include ; Milyang 63 (DS 3.1), IET 23993 (DS 3.2), HHZ 5 DT-1 DT-1 (DS 3.3), HHZ 25 SAL DT-1 DT-1 (DS 3.5), Bobhu Kongbu (DS 3.5), BPT 2671 (DS 3.7), BPT 2611 (DS 3.9), MTU 1121 (DS 4.0), MTU 1001 (DS 4.2), MTU 1010 (DS 4.3), RNR 23079 (DS 4.3) and GSR 234 (DS 4.9). The remaining 45 lines along with susceptible check TN1 (DS 9.0) recorded a damage score above five and hence were categorised as susceptible to BPH. Among these 11 lines have shown moderate susceptibility (MS) with a damage score ranging from 5.1 to 7.0, and remaining 36 entries, including susceptible check TN1 recorded damage score of 7.1 and above, and were categorised as susceptible (DS 7.1 to 8.9) and highly susceptible (DS 9.0) to BPH respectively.

In accordance with previous studies [13,14], PTB 33 gave a Resistant (R) reaction with a damage score of 3.0 and TN1, gave a Highly Susceptible (HS) reaction with a damage score of 9.0, justifying their use as resistant and susceptible checks in this study. Bhogadhi et al. (2015) reported that in Standard Seedbox Screening Test (SSST) BM71 and MTU 1001 have shown resistant reaction against BPH, with a mean damage score of 3.0 and 4.0, respectively [15]. Thus from the results obtained it is evident that the present work is in accordance with several previous works [13,14,15]. Further, a detailed investigation of their mechanisms of host plant resistance is required to elucidate the information regarding the type of resistance viz. antixenosis, antibiosis, and tolerance, in each genotype. Proper and scientific use of these data will lead to the development of resistant varieties which can resist and overpower the BPH menace for longer duration in the field conditions with least pesticide interventions.

4. CONCLUSION

Among all the entries in SSST only two genotypes (resistance check PTB33 and BM71) exhibited resistant reaction to BPH with a damage score of 3.0, while twelve genotypes viz., Milyang 63, IET 23993, HHZ 5 DT-1 DT-1, HHZ 25 SAL DT-1 DT-1, Bobhu Kongbu, BPT

2671, BPT 2611, MTU 1121, MTU 1001, MTU 1010, RNR 23079 and GSR 234 exhibited moderate resistance reaction to BPH with a damage score ranging between 3.1 to 5.0. The rest of the genotypes showed moderately susceptible, susceptible and highly susceptible reactions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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