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Studies on Non-preference Mechanism and Biochemical Aspects of Resistance to BPH *Nilaparvata lugens* (Stal.) on Resistant Rice Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To conduct studies on non-preference mechanism and biochemical aspects of resistance to BPH on resistant rice genotype.

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 2017 and May 2018.

Methodology: The selected rice entries were selected including PTB33 (resistant check) and TN1 (susceptible check), this was replicated thrice. After 30 days, about hundred first instar nymphs were released in the pots. The number of nymphs settled on each entry was counted from randomly selected 10 hills at 24, 48, 72, 96 and 120 hours after release. Number of probing marks made by a day old single female insect during 24 hours of its feeding was recorded on all the selected entries along with resistant and susceptible rice cultures. Estimation of total phenols, total sugars and proteins was done for selected rice genotypes.

Results: Among all the test cultures, KNM 2305, KNM 2307, JGL 24423 and Sabita recorded lowest number of nymphal settlement. Biochemical aspects of resistance like total phenols, total

sugars and protein content in selected rice genotypes was done. The amount of total phenol was observed to be maximum in the leaf sheath of moderately resistant JGL 24423 (2.70 mg/g). The amount of total sugars was lowest in RNR 26111 (0.33 mg/ g), RNR 21571(0.86 mg/g) followed by JGL 24423 (1.40 mg/g) and highest in susceptible check TN-1 (2.97 mg/g). Least amount of protein was observed in JGL 24423 (0.76 mg/g) followed by IET 23993 (1.43 mg/g).

Keywords: Rice; Nilaparvata lugens; brown planthopper; non-preference; probing test.

1. INTRODUCTION

Approximately 52% of the global production of rice is lost annually owing to the damage caused by biotic stress factors, of which 25% is attributed to the attack of insect pests [1]. Rice is infested by more than hundred species of insects and about twenty of them are considered serious pests as they cause significant damage to rice crop. Among them brown plant hopper (BPH), Nilaparvata lugens (Homoptera: Delphacidae) is one of the most destructive insect pests causing significant yield loss in most of the rice cultivars of Asia [1]. It is a phloem-sapsucking insect pests of tropical and temperate rice in Asia feeds on the rice phloem sap using its piercing-sucking mouthparts, which affects the growth of rice plants and results in "hopperburn" [2]. BPH is also a vector, transmitting viral diseases such as Grassy stunt, Rugged stunt and associated diseases.

Host plant resistance is a major economic and desirable practice for the management of Brown Plant Hopper (BPH) [3]. Understanding the mechanism of resistance is important before evolving resistant varieties. Analyses of biochemical constituents revealed low content of total sugars as against higher quantities of total phenols, Ortho-dihydroxy phenols and silica in all the moderately resistant varieties. Understanding the mechanism of resistance is important before evolving resistant varieties. The objective of the study is to study mechanisms of resistance and to quantify the biochemical basis of resistance in the elite rice lines to BPH.

2. MATERIALS AND METHODS

2.1 Studies on Preference/Nonpreference by BPH Nymphs

The studies were conducted in polyhouse, Rice Research Centre, ARI, Hyderabad. The selected rice entries were sown in a big tray (60×45×20 cm) along with PTB33 (resistant check) and TN1 (susceptible check), this treatments were replicated thrice. After 30 days, about hundred first instar nymphs were released in the pots. The pots were covered with mylar cage to prevent escape of insects. The number of nymphs settled on each entry was counted from randomly selected 10 hills at 24, 48, 72, 96 and 120 hours after release. Based on the data, the mean no. of nymphs settled/hill were computed and statistically analyzed by completely randomized design.

2.2 Probing Test

Number of probing marks made by a day old single female insect during 24 hours of its feeding was recorded on all the selected entries along with resistant and susceptible rice cultures. One day old adult female insect was released on a seven day old test entry seedling placed in a test tube and allowed to feed for 24 hours. Each entry was replicated six times. After 24 hours, the insect was removed and the test plant was stained by dipping in one per cent aqueous Erythrosin-B solution for one hour to distinguish the feeding marks on the test entries (Naito, 1964). The feeding marks were counted using magnifying hand lens.

2.3 Biochemical Aspects of Resistance

2.3.1 Estimation of total phenols

Sample extraction: Ten mg of oven-dried powdered sample was extracted in 20 ml of warm 80 per cent ethanol and the extract was centrifuged at 6000 rpm for 30 minutes. The supernatant was evaporated to dryness on a water bath and the residue was dissolved in 5 ml of water. The alcohol free extract was used for estimation of total phenols.

Estimation

An aliquot sample of 1 ml was diluted to 5 ml with water and 0.5 ml of phenol reagent was added and mixed. Exactly after 5 minutes, 1.5 ml of 20 per cent sodium carbonate solution was added

and kept for incubation for two hours. After cooling under running tap water, the absorbance was read at 750 nm, against the reagent blank in a spectrophotometer.

Estimation of Total sugars: Total sugar content of the selected rice accessions were determined by Nelson- Somogyi method (Nelson, 1944; Somogyi, 1952).

One gram of the sample was made to 50 ml by adding distilled water then the sample was hydrolyzed by keeping it in a water bath for 20 minutes at 100°C. Five ml of lead acetate was added, after 30 minutes three small spatulas of potassium oxalate was added. The sample was made to 50 ml and then it was filtered. From the filterate, 10 ml of solution was taken and 1 ml of Hydrochloric acid was added. The solution was boiled for 5 min then 5 drops of sodium hydroxide and 5 drops of phenol solution was added. The solution was made up to 10 ml by adding distilled water.

Aliquot of 0.1 ml was prepared and 1 ml of copper reagent was added and it is boiled for 30 minutes. After cooling, 1 ml of arsenomolybdate was added. The sample was made up to 25 ml by addition of distilled water. Aliquot of 0.1 ml with 1 ml of copper reagent is taken as blank. The absorbance was read at 750 nm, against the blank in a spectrophotometer.

Estimation of total protein content: The total protein content of rice plant samples were determined by the using kjeldhal method (AOAC).

Estimation

The sample size used in the Kjeldahl procedure was around 1.00 gm. Samples were weighed and transferred into Kjeldahl digestion tube containing 3 gms of digestion mixture in each tube (prepared by mixing 50 gms of potassium sulphate and 10 gms of Copper sulphate) and 10 ml of concentrated sulphuric acid. After 3 hrs of digestion in a unit with electrical heat and fume removal and cooling to room temperature was done and 10 ml of distilled water was added into each tube. By distillation, sodium hydroxide (40%) was trapped in boric acid solution (500 mg in 1000 ml of water). Bromocresol green (13 ml) and methyl red indicator (15 ml) was added into boric acid solution. Total protein was determined by titration with 0.1 N hydrochloricacid.

3. RESULTS AND DISCUSSION

3.1 Nymphal Settlement

Number of nymphs settled per seedling on moderately resistant cultures along with susceptible check (TN1) and resistant check (PTB 33) was recorded at 1, 2, 3, 4 and 5 days after release for determining BPH nymphal feeding preference. Among the tested entries, differences were evident in the nymphal counts during the period of observation with BPH nymphs showing tendency to move away from the moderately resistant entries compared to susceptible entries.

The number of nymphs settled on moderately resistant entries one day after release varied across the genotypes. Sinnasivappu had lowest number of nymphs (3.8) per seedling followed by KNM 2305 (4.2 nymphs) and RNR 25993/2 (4.4 nymphs) which were on par with resistant check, PTB 33 that has recorded 4.3 nymphs per seedling. Among the remaining test entries KNM 2307 (7.5 nymphs), MTU 1010 (7.1 nymphs), MTU 1001 (9.2 nymphs) and susceptible check TN1 (9.4 nymphs) showed highest nymphal settlement (Table 1).

The nymphal preference / settlement slightly varied two days after release with test entries, KNM 2305 showing lowest number of nymphs (4.3) per seedling followed by Sinnasivappu (4.5 nymphs), RNR 21571 (4.6 nymphs) while the resistant check, Ptb 33 continued to show lowest number of nymphs per seedling (3.5) on second day also with reduced number of nymphs compared to 1st day. Similarly the susceptible check (TN1) has recorded highest number of nymphs (9.6).

Preference by nymphs became more evident with more time allowed for settling of nymphs for three days. Among all the entries, RNR 25993/2 (3.7 nymphs), Sinnasivappu (3.8 nymphs) and resistant check, PTB 33 (2.5 nymphs) have recorded less number of nymphs per seedling. This was followed by RNR 21571 (4.2 nymphs), KNM 2305 (4.4 nymphs), Sabita and IET 23993 (4.5 nymphs). While MTU 1001, MTU 1010, RNR 23079 and RNR 25838 that have recorded higher number of nymphs (ranging from 5.5 to 5.9 nymphs) which remained more or less equal to those recorded at two days after release.

The data on nymphal settlement four days after release showed least number of nymphs settled on RNR 21571 (3.4 nymphs), Sinnasivappu (3.5

nymphs), JGL 24423 (3.6 nymphs) and resistant check, PTB 33 (1.2 nymphs) while higher number of nymphs were found on test entry, RNR 23079 (5.1 nymphs). The susceptible check, TN1 registered highest number of nymphs per seedling (10.5).

The test entry KNM 2307 (3.0 nymphs) recorded lowest number of nymphs per seedling five days after release, followed by IET 23993 (3.1 nymphs), while the resistant check PTB 33 recorded significantly lowest number of nymphs (1.3) per seedling. The test RNR 21571 recorded highest nymphal settlement (4.0) per seedling while the susceptible check, TN1 recorded significantly highest nymphs per seedling (10.3).

Bhanu et al. [4] studied non preference mechanism in selected rice entries to brown plant hopper (*Nilaparvata lugens*) and reported MTU IJ 206-7-4-1 as highly resistant with significantly low nymphal settlement per seedling (3.22 nymphs) and susceptible check, TN1 with more number of nymphs (12.01) per seedling. Similar results were obtained in the present study, where most of the selected moderately resistant test entries showed non-preference for nymphal settlement, while highest nymphal settlement was noticed on TN1.

3.2 Probing Test

To study the feeding behaviour of brown planthopper, feeding marks or probing marks

made by one day old female insect were recorded on seven day old test entry plant by allowing to feed for 24 hours in a test tube. Results indicated that there was significant difference among the entries with regard to probing marks (Table 2). Among the screened entries, KNM 2305 (22.6) and KNM 2307 (20.0) received maximum number of feeding punctures, which were on par with resistant check, PTB 33 (25.6 feeding punctures). Though the test entries RNR 21571 (19.6) and JGL 24423 (17.3) also received significantly more number of feeding punctures, they differed significantly with the resistant check PTB 33.

The test entries RNR 25993/2 (11.3) and MTU 1010 (13.0) recorded least number of probing marks and were on par with susceptible check TN 1 (10.3). More number of feeding punctures on test entry signifies the resistant behavior of that entry towards BPH. This might be due to reason that, these entries did not sustain prolonged feeding by BPH due to presence of certain feeding deterrents or toxic chemicals or absence of feeding stimulants or some essential nutrients. Hence, the insect had to probe more and more on the resistant genotypes to locate feeding sites (Sogawa, 1982). The present study clearly suggests the differences in the level of resistance offered by test entries to BPH with test entries like KNM 2305 and KNM 2307 offering higher resistance by making it difficult for BPH to feed on it.

| S. no. | Rice genotype | 1DAR | 2DAR | 3DAR | 4DAR | 5DAR |
|--------|---------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 1. | MTU 1001 | 9.2 ^g | 7.7 ⁹ | 5.9 ^{tg} | 4.6 ^{cd} | 3.9 ^c |
| 2. | MTU 1010 | 7.1 ^f | 6.3 ^{fg} | 5.8 ^f | 4.7 ^{cd} | 3.8 ^{bc} |
| 3. | RNR 23079 | 6.6 ^e | 5.6 ^{ef} | 5.7 ^{ef} | 5.1 ^d | 3.4 ^{bc} |
| 4. | IET 23993 | 5.7 ^{cd} | 6.3 ^{fg} | 4.5 ^{cd} | 3.7 ^{bc} | 3.1 ^{bc} |
| 5. | JGL 24423 | 6.9 ^{ef} | 5.2 ^{de} | 4.8 ^d | 3.6 ^{bc} | 3.2 ^{bc} |
| 6. | SABITA | 6.3 ^{de} | 5.1 ^{de} | 4.5 ^{cd} | 3.7 ^{bc} | 3.2 ^{bc} |
| 7. | KNM 2307 | 7.5 ^{fg} | 6.2 ^{fg} | 5.4 ^e | 3.8 ^{bc} | 3.0 ^b |
| 8. | RNR 21571 | 6.0 ^d | 4.6 ^{cd} | 4.2 ^c | 3.4 ^b | 4.0 ^{cd} |
| 9. | SINNA SIVAPPU | 3.8 ^a | 4.5 ^c | 3.8 ^{bc} | 3.5 ^{bc} | 3.4 ^{bc} |
| 10. | RNR 25838 | 6.2 ^{de} | 5.4 ^e | 5.5 ^{ef} | 4.4 ^c | 3.7 ^{bc} |
| 11. | RNR 25993/2 | 4.4 ^{bc} | 5.0 ^d | 3.7 ^b | 3.8 ^{bc} | 3.7 ^{bc} |
| 12. | RNR 26111 | 5.5 ^c | 6.0 ^f | 5.1 ^{de} | 4.4 ^c | 3.6 ^{bc} |
| 13. | KNM 2305 | 4.2 ^{ab} | 4.3 ^b | 4.4 ^{cd} | 4.6 ^{cd} | 3.2 ^{bc} |
| 14. | PTB 33 | 4.3 ^b | 3.5 ^ª | 2.5 ^ª | 1.2 ^a | 1.3 ^a |
| 15. | TN1 | 9.4 ^{gh} | 9.6 ^h | 11.6 ⁿ | 10.5 ^e | 10.3 ^d |
| | C.D. | 0.52 | 0.46 | 0.54 | 0.53 | 0.99 |
| | SE(m) | 0.19 | 0.16 | 0.18 | 0.18 | 0.34 |
| | SE(d) | 0.27 | 0.22 | 0.26 | 0.26 | 0.48 |
| | C.V. | 5.38 | 5.01 | 6.25 | 7.22 | 15.27 |

Table 1. Number of nymphs settled per seedling of selected rice entries

(DAR – Days after Release)

Similar studies conducted by Sable (2010) also proved resistance and moderately resistant rice genotypes recorded probing frequency ranging from 21.40 to 38.80 which in accordance with the results obtained from the present study. Udayababu et al. (2011) studied probing behaviour of BPH on six selected highly resistant advanced rice breeding lines along with a resistant check (PTB 33) and susceptible check (TN 1) and reported that highly resistant lines exhibited highest probing marks (30.4 to 42.9 per female) than the susceptible check (TN 1).

Table 2. Number of probing marks of adultBPH on selected rice genotypes

| S. no. | Rice genotype | Probing frequency |
|--------|---------------|--------------------|
| 1. | MTU 1001 | 16.6 ^e |
| 2. | MTU 1010 | 13.0 ^{fg} |
| 3. | RNR 23079 | 13.6 ^f |
| 4. | IET 23993 | 14.6 ^{ef} |
| 5. | JGL 24423 | 17.3 ^d |
| 6. | SABITA | 15.0 ^{ef} |
| 7. | KNM 2307 | 20.0 ^c |
| 8. | RNR 21571 | 19.6 ^{cd} |
| 9. | SINNA SIVAPPU | 13.6 ^f |
| 10. | RNR 25838 | 15.0 ^{ef} |
| 11. | RNR 25993/2 | 11.3 ⁹ |
| 12. | RNR 26111 | 15.6 ^{ef} |
| 13. | KNM 2305 | 22.6 ^b |
| 14. | PTB 33 | 25.6 ^a |
| 15. | TN1 | 10.3 ^{gh} |
| | C.D. | 2.27 |
| | SE(m) | 0.78 |
| | SE(d) | 1.10 |
| | C.V. | 8.33 |

3.3 Biochemical Aspects of Resistance

Total Phenols: Total phenolic content in the leaf samples of eight selected rice cultures along with susceptible check TN1 and resistant check Ptb 33 (Table 3) were analysed. The amount of total phenol was observed to be maximum in the leaf sheath of KNM -2307 (3.03 mg/g) which was higher the resistant check, PTB 33 (2.97 mg/g) but was on par with PTB -33, JGL 24423 (2.70 mg/g) and RNR 21571 (2.66 mg/g). The rice cultures, KNM 2305 (2.53 mg/g), RNR 23079 (2.20 mg/g), IET 23993 (2.13 mg/g) and RNR 26111 (2.00 mg/g) showed moderate total phenolic content. The least amount of total phenol was observed in rice culture, RNR 25993/2 (1.80 mg/g) which was on par with the susceptible check TN1 (1.46 mg/g). In general the total phenolic content was two times higher than TN1 in moderately resistant accessions.

Grayer et al. [5] found higher levels of phenols in the resistant rice varieties compared to susceptible varieties and suggested their involvement in offering resistance to BPH. The phenolic compounds were found to be feeding deterrents to leaf and planthoppers and in general, resistant varieties were found to have more phenolic compounds than susceptible varieties [6]. Sujatha et al. [7] stated that phenols were positively correlated with resistance against BPH. Akshaya [8] reported that BPH infestation on rice resulted in an increased phenolic content in resistant varieties. The results of the present study are also in accordance with the studies of the earlier researchers wherein the cultures with high total phenolic content have shown lower damage score and were also found to show higher antixenosis and antibiosis effect on BPH.

Total Sugars: The total sugar content of leaf sheath of eight rice genotypes along with susceptible check TN1 and resistant check PTB 33 are presented in Table 3. The results of the experiment showed that all the test cultures were not only significantly different from each other, but also were significantly different from the susceptible check TN1 and resistant check PTB 33. Lowest quantity of total sugars was recorded in test culture RNR 26111 (0.33 mg/g) which was significantly lower than the resistant check PTB-33 (0.51 mg/g). This was followed by the test cultures RNR 21571 with 0.86 mg/g of total sugars. The cultures JGL 24423 (1.40 mg/g) and KNM 2307 (1.87 mg/g) were found to record moderately high quantity of total sugars.

The cultures RNR 25993/2 (2.13 mg/g), IET 23993 (2.20 mg/g), RNR 23079 (2.29 mg/g) and KNM 2305 (2.42 mg/g) recorded highest total quantity of sugars however, they were significantly less than the susceptible check TN1 which recorded highest total sugar content of 2.97 mg/g.

Sugars play a significant role in pest incidence has been implicated in host- plant resistance studies in number of instances [9]. Many researchers suggested that sugar content in rice varieties acts as a potent sucking stimulant for BPH. Sogawa [10] reported preference of BPH for various sugars like sucrose, glucose and dextrose under laboratory conditions and hence low sugar has been always considered as one of the desirable qualities of a resistant plant [11,7], mentioned that higher quantity of total sugars was recorded in BPH susceptible Tellahamsa and Jaya varieties. Total Protein content: The content of total protein in the leaves of eight rice genotypes along with susceptible and resistant checks were analysed and presented in Table 3. It was observed that the susceptible check TN1 has significantly highest quantity of total soluble protein (6.26 mg/g) compared to test varieties. In contrast all the test entries and resistant check PTB 33 recorded lesser quantity of total soluble protein ranging from 3.76 to 0.76 mg/g of leaf. Significantly least quantity of protein was observed in JGL 24423 (0.76 mg/g) followed by RNR 25993/2 (1.43 mg/g). The test entries IET 23993, RNR 23079, KNM 2305, RNR 21571 with a total protein content of (1.93 mg/g), (2.13 mg/g), (2.53 mg/g) and (2.90 mg/g) respectively were found to contain moderate quantity of proteins and were on par with each other. Among the test entries KNM 2307 recorded a protein content of 3.80 mg/g which was slightly above resistant check PTB-33 but was on part with it.

Protein as an important nutrient plays a vital role in plant metabolism, the decrease in protein content may induce several changes in plants which may effect the plant yield and guality of the produce. Sujatha et al. [7] indicated that protein negatively correlated content was with resistance. Similar inference was also reported by Sogawa [12,13]. Vanitha et al. [14] reported that protein content in the basal stem of rice was higher in susceptible plants compared to resistant plants and the per cent reduction in protein content was higher in susceptible variety when compared to resistant variety.

Though less quantity of protein makes plant less palatable for insects and also retard their

developmental physiology considering the negative effects it causes on plant metabolism yield and quality it is attributed that moderate content of protein is highly desirable. As high content promotes susceptibility of plant to insect attack and less content effects the plant physiology, though it is not exactly proved beyond doubt that a threshold protein level at which it actually promotes susceptibility to insect attack or a level at which it effects plant physiology. It can be concluded from this part of the study that genotype (PTB-33) possessing moderate quantity of protein offered high resistance to BPH and genotype (TN1) possessing very high quantity of protein induced susceptibility to BPH, which suggests that proteins play a key role in inducing susceptibility at levels far above certain critical levels while at normal levels and very low levels it doesn't promote resistance (JGL 24423 and RNR 25993/2).

In the present study wherein many of the test entries recorded moderate content of protein and it was on par with the highly resistant check PTB-33. Though JGL 24423 and RNR 25993/2 recorded less protein content the results from the antixenosis and antibiosis do not suggest these entries as resistant. However, as discussed the moderate content is highly desirable compared to less or higher content of protein which is evident from the results of the present study that PTB-33 with moderate content of protein is designated as the resistant check for BPH and TN1 with very high content of protein is designated as susceptible check for BPH studies.

| S. no. | Rice genotype | Total phenol (mg/g) | Total sugars (mg/g) | Protein content (mg/g) |
|--------|---------------|------------------------|------------------------|---------------------------|
| 1. | KNM 2305 | 2.53 ^b | 2.42 | 2.53 ^{cd} |
| 2. | RNR 21571 | 2.66 ^{ab} | 0.86 ^c | 2.90 ^{cd} |
| 3. | RNR 23079 | 2.20 ^{bc} | 2.29 ^h | 2.13 ^{bc} |
| 4. | KNM 2307 | 3.03 ^a | 1.87 ^e | 3.80 ^{de} |
| 5. | JGL 24423 | 2.70 ^{ab} | 1.40 ^d | 0.76 ^a |
| 6. | IET 23993 | 2.13 ^{bc} | 2.20 ^g | 1.93 ^{bc} |
| 7. | RNR 26111 | 2.00 ^{bc} | 0.33 ^a | 2.46 ^c |
| 8. | RNR 25993/2 | 1.80 ^c | 2.13 ^f | 1.43 ^b |
| 9. | PTB 33 | 2.96 ^{ab} | 0.51 ^b | 3.76 ^d |
| 10. | TN 1 | 1.46 ^{cd} | 2.97 ^j | 6.26 ^e |
| | C.D. | 0.589 | 0.151 | 0.907 |
| | SE(m) | 0.198 | 0.051 | 0.305 |
| | SE(d) | 0.280 | 0.072 | 0.432 |
| | C.V. | 14.617 | 5.192 | 18.887 |

Table 3. Total phenol, total sugar and protein content of selected rice germplasm accessions

4. CONCLUSION

Among the selected rice genotypes KNM 2305, RNR 21571 and JGL 24423 exhibiting resistance mechanisms and possessing biochemical constituents in proportions that could offer resistance to BPH and hence can be used as best source of donors for breeding BPH resistant varieties with acceptable quality traits.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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