Supplemental File 1. Materials and methods

Plant materials and insect culture

Five wild rice accessions along with one susceptible and one resistant check were evaluated under screen house conditions at the Rice Research Laboratories (RRL), Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India during wet seasons of 2017 and 2018. Seeds of wild rice accessions namely, *Oryza nivara* (IRGC104646, CR100204), *O. punctata* (IRGC99577), *O. australiensis* (IRGC105270, IRGC105275), susceptible check TN1 and resistant check Pt33 were sown in earthen pots. After germination, individual plant of each accession was transferred in plastic pots (10 cm diameter) containing well puddled nutrient rich soil except for modified seed box screening. Thirty-day-old plants were studied with BPH biotype 4 population, collected from rice fields and reared on TN1 plants in rectangular cages (68 cm × 50 cm × 50 cm) under screen house conditions at the RRL positioned at 30°54′N and 75°48′E at (28 ± 2) °C, 75% ± 5% relative humidity and 14 h light/10 h dark photoperiod (Heinrichs et al, 1985).

Antibiosis resistance parameters

**Modified seed box screening**

The pre-germinated seeds of wild rice accessions were sown in rows at spacing of 3.5 cm randomly in a seed box (45 cm × 35 cm × 10 cm) and after 30 d of sowing, 2nd – 3rd instar BPH nymphs @ 6-8 nymphs per plant were released. The damage score was recorded on 0-9 scale when 90%-100% of TN1 plants get wilted following Standard Evaluation System (SES) of rice (IRRI, 2014). Each accession was scored on an individual plant basis as 0 = no visible damage, 1 = partial yellowing of first leaf, 3 = first and second leaves of most plants partially yellowing, 5 = pronounced yellowing and stunting or about half of the plants wilting or dead, 7 = more than half the plants wilting or dead and remaining plants severely stunted, and 9 = all plants wilted and dead. Each accession with mean rating of 0-3.49, 3.50–5.49, and 5.50–9.00 was designated as resistant, moderately resistant and susceptible, respectively (Heinrichs et al, 1985). The experiment was replicated thrice in one crop season.

**Honeydew excretion**

Feeding preference of BPH on wild rice accessions was measured by honeydew excretion test (Heinrichs et al, 1985). The pre-germinated seeds of rice accessions were sown in pots as mentioned above and 30-day-old plants were used for measuring area of honeydew excretion. Each accession was replicated five times. Individual plants of each accession were enclosed with a plastic cup (having a hole at the top). The Whatman filter paper no. 1 discs dyed with 0.1% bromocresol green solution were placed at the base of the plant inside the cup. Five one-day-old BPH females starved for 2 h were released in each cup for 24 h. The honeydew drops turned to blue coloured spots on the treated filter paper. Firstly, the area of each spot was measured by graph paper and then these spots were cut and weighted to represent the feeding activity of BPH in mm² and mg, respectively.

**Ovicidal response**

Ovicidal response of accessions towards BPH eggs was evaluated followed by Yang et al (2014). This response of plants results in production of watery lesions (WLs) or necrotic lesions at oviposition site and the intensity of these lesions was observed and rated from 0 based on following categories: 0 = no visible necrotic symptoms, 1 = brownish oviposition damage but no watery lesions, 2 = discontinuous watery lesions, 3 = conspicuous, vertically elongated watery lesions. An average score below 1.0 was considered as non-ovicidal response and above 1.0 was considered as an ovicidal response of the accessions (Yang et al, 2014). This experiment was replicated five times.

**Biochemical constituents**

The wild rice accessions were assayed thrice in each season for their biochemical constituents at constitutive and induced levels so as to evaluate the role of enzymatic and/or non-enzymatic factors of plants in imparting resistance against BPH. On 30-day-old plants, 2nd – 3rd instar BPH nymphs were released @ 10 nymphs per plant and after 72 h of infestation, leaf blade and leaf sheath tissues were sampled from both infested and uninfested plants for biochemical analysis.

**Enzymatic constituents: Phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) enzymes**

For the extraction of PAL and PPO, samples of leaf blade and leaf sheath (250 mg) were homogenized with 5 mL of ice cold 0.1 mol/L Tris-HCl buffer (pH 7.5), containing 5 mmol/L β-mercaptoethanol using pre-chilled pestle and mortar. The homogenate was centrifuged at 10 000 × g at 4 °C for 25 min and the supernatant was used for enzyme estimation. PAL and PPO was assayed following the method described by Burrell and Rees (1974) and Bastin and Unhler (1972), respectively.

**Phenolic constituents: Total phenols and flavonols**

Dried and grounded samples of leaf blade and leaf sheath (40 mg) were refluxed with 80% aqueous methanol for 1 h at 60-80 °C. The refluxed material was then filtered through Whatman filter paper No. 1 and the volume was made to 10 mL with 80% methanol. The extract was used for the estimation of total phenol and flavonol contents. Total phenol content was
estimated as per method described by Swain and Hillis (1959) using Folin-Ciocalteau phenol reagent. The determination of flavonol content was done by the method of Balabaa et al (1974) using 0.1 mol/L methanolic solution of aluminium chloride.

**Free amino acids**

For the extraction of free amino acids, tissues of leaf blade and leaf sheath (1 g) were refluxed with 80% and 70% ethanol for 15 min. The supernatants were pooled and filtered through Whatman filter paper No. 1. The filtrate was made free from ethanol by evaporation on a flash evaporator. The extract was diluted to 25 mL and used for estimation of free amino acids according to Lee and Takahashi (1966).

**Statistical analysis**

The data were subjected to one-way analysis of variance using statistical software SPSS v 20.0 and analysis was done using completely randomized design (CRD). Mean ± SE were calculated at 5% level of significance (Gomez and Gomez, 1984). Correlation analysis was done to find relationship between antibiosis parameters and biochemical constituents and regression models were developed using stepwise regression analysis to study the dependence of antibiosis parameters on biochemical constituents in rice accessions. Post-hoc test was performed using Duncan’s multiple range test (DMRT).

**References**


**Supplemental Fig. 1. Antibiosis resistance parameters.**

A. Modified seed box screening (MSBS) of wild rice accessions.

B. Honeydew excretion by five adult females of *Nilaparvata lugens* on filter paper.
Supplemental Table 1. Total phenol, flavonol and total free amino acid contents in leaf blade and leaf sheath of rice accessions at constitutive and induced levels against *N. lugens*.

<table>
<thead>
<tr>
<th>Accession</th>
<th>Leaf blade</th>
<th>Leaf sheath</th>
<th>Leaf blade</th>
<th>Leaf sheath</th>
<th>Leaf blade</th>
<th>Leaf sheath</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constitutive level</td>
<td>Induced level</td>
<td>Constitutive level</td>
<td>Induced level</td>
<td>Constitutive level</td>
<td>Induced level</td>
</tr>
<tr>
<td>IRGC99577</td>
<td>6.18 ± 0.06 d</td>
<td>6.36 ± 0.05 d</td>
<td>2.63 ± 0.08 d</td>
<td>2.87 ± 0.03 c</td>
<td>2.49 ± 0.07 e</td>
<td>2.97 ± 0.06 e</td>
</tr>
<tr>
<td>IRGC105270</td>
<td>5.58 ± 0.09 c</td>
<td>5.94 ± 0.05 c</td>
<td>2.16 ± 0.06 c</td>
<td>2.72 ± 0.04 c</td>
<td>1.85 ± 0.13 cd</td>
<td>2.61 ± 0.07 d</td>
</tr>
<tr>
<td>IRGC105275</td>
<td>5.06 ± 0.06 b</td>
<td>5.30 ± 0.04 b</td>
<td>1.87 ± 0.04 b</td>
<td>2.25 ± 0.07 b</td>
<td>1.68 ± 0.07 bc</td>
<td>1.99 ± 0.06 b</td>
</tr>
<tr>
<td>IRGC104646</td>
<td>5.97 ± 0.04 d</td>
<td>6.21 ± 0.06 d</td>
<td>2.18 ± 0.06 c</td>
<td>2.78 ± 0.03 c</td>
<td>2.02 ± 0.04 d</td>
<td>2.34 ± 0.05 c</td>
</tr>
<tr>
<td>CR100204</td>
<td>4.61 ± 0.07 a</td>
<td>5.16 ± 0.07 b</td>
<td>1.79 ± 0.07 b</td>
<td>2.09 ± 0.07 b</td>
<td>1.49 ± 0.14 b</td>
<td>1.87 ± 0.13 b</td>
</tr>
<tr>
<td>Ptb33</td>
<td>7.62 ± 0.11 e</td>
<td>7.79 ± 0.13 e</td>
<td>2.72 ± 0.07 d</td>
<td>3.77 ± 0.05 d</td>
<td>2.75 ± 0.06 e</td>
<td>3.07 ± 0.06 e</td>
</tr>
<tr>
<td>TN1</td>
<td>4.35 ± 0.10 a</td>
<td>4.90 ± 0.03 a</td>
<td>1.42 ± 0.09 a</td>
<td>1.69 ± 0.06 a</td>
<td>1.15 ± 0.08 a</td>
<td>1.52 ± 0.07 a</td>
</tr>
</tbody>
</table>

**LSD (P = 0.05)**
- Accession: 0.08
- Infestation: 0.16

Values are Mean ± SE (n = 3). Means within a column followed by the same letter are not significantly different at P ≤ 0.05 according to the Duncan’s multiple range test.

Supplemental Table 2. Linear regression analysis between antibiosis parameters and biochemical constituents.

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>Coefficient of determination ($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_1 = -51.05 - 17.39X_1 + 208.48X_2 - 24.87X_3 + 105.65X_4 + 37.08X_5$</td>
<td>0.99</td>
</tr>
<tr>
<td>$Y_2 = -215.32 + 193.31X_1 + 267.66X_2 + 1667.12X_3$</td>
<td>0.81</td>
</tr>
<tr>
<td>$Y_3 = 19.06 - 18.75X_1 + 1.14X_2 - 3.29X_3 - 12.32X_4$</td>
<td>0.92</td>
</tr>
</tbody>
</table>

$Y_1$ = Damage score, $Y_2$ = Honeydew excretion, $Y_3$ = Ovicidal response, $X_1$ = Phenylalanine ammonia lyase, $X_2$ = Polyphenol oxidase, $X_3$ = Total phenols, $X_4$ = Flavonols, $X_5$ = Free amino acids.