Evaluation of Rice Landraces for Brown Planthopper Resistance Based on Phenotypic Reactions and Biochemical Attributes

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Evaluation of rice landraces for brown planthopper resistance based on phenotypic reactions and biochemical attributes

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Abstract

Brown planthopper (BPH), [*Nilaparvata lugens* (Stål.)] is an economically important pest of rice (*Oryza sativa* L.) throughout Asia, where the damage caused by nymphs and adults, especially during post-tillering to milking stages, significantly reduces grain yield. There is, thus, a pressing need to develop varieties that are resistant to BPH. In this study, the reaction of various rice landraces from Indian origin were assessed (both phenotypically and biochemically) in response to BPH infestation. It was found that the landraces, *viz.* Badshabhog, Gamra, Haldichuri, Janglijata, Kalabhat, Khara, Adanshilpa, Chikonmashuri, Kerala sundari and Lal dudheshwar exhibited resistance to BPH consistently along with the standard check Ptb33, for three consecutive years under both greenhouse and open-field conditions. These phenotypically resistant rice landraces including Ptb33 exhibited lowest feeding rate, least nymphal and adult preference, minimum survival and higher frequency (%) of unhatched eggs when compared with the susceptible check (Swarna). Higher levels ascorbic acid, oxalic acid (OA), crude silica (CS), while lower levels of phenols, reducing sugar and total free amino acid (TFA) were expressed in un-infested resistant and moderately resistant landraces. The resistant plants exposed to herbivory by BPH produced higher levels of phenolic compounds, potassium and TFA than plants of susceptible cultivar Swarna. The feeding rate, settling behaviour and survivability of BPH correlated significantly and negatively with OA and CS, whereas the latter showed a significant and positive correlation with egg hatchability.

Keywords: Plant resistance, *Nilaparvata lugens*, defensive components, antixenosis, correlation, PCA

Declarations

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Key message:

- Phenotypic and biochemical reactions of Indian originated rice landraces were assessed against BPH biotype 4.
- Landraces *viz*. Badshabhog, Gamra, Haldichuri, Janglijata, Kalabhat and Khara exhibited resistance.
- Resistant rice landraces exhibited lowest feeding rate, nymphal and adult preference followed by survival of BPH.
- Crude silica and oxalic acid in resistant landraces could reduce the BPH herbivory.
- Higher quantity of total phenol and free amino acid was observed in BPH infested resistant rice landraces.

Introduction

Rice (*Oryza sativa* L.) is one of the staple food crops in the world and is used by more than one-third of the human population as a primary source of calories (Xu et al. 2015). Of over twenty insects species recognized as economically important pests of this crop, the brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) is one of them (Normile 2008; Heong and Hardy 2009). This phloem sap feeder is known to be one of the most destructive and notorious pests of rice throughout Asia and hold the ability to create as high as 60% yield loss under epidemic conditions (Kumar et al. 2012; Wei et al. 2019). Host-plant resistance to insect injury occurs in plants that use strategies to recover, tolerate or avoid from the attack of pest (Smith 2005); thus, plant characteristics that have negative influences on insect-pest biology, reproduction and development could be used in the screening of plants resistant to insect (Rekha and Singh 2001). The identification of new sources of resistance from landrace, cultivar or germplasms enables the plant breeders to amplify the resistance breeding program through genetic modification (Guo et al. 2019). It has triggered the exploration of resistance sources in rice landraces or traditional folk rice cultivars, which are imperious in different attributes to cultivated rice varieties. The eastern province of India possesses multiple tribal zones, which harbour various rice landraces and also are known to be one of the centres of its origin (Sinha et al. 2015; De 2016). Evaluation of these landraces for true genetic potential, the extent of heterogeneity and biophysical and biochemical differences from commercial varieties is not enrolled elaborately till now. Hence it is essential to gather information on BPH resistance traits in various rice landraces through phenotypic expression and biochemical analysis.

The biophysical factors interfere with feeding, orientation, mating or oviposition mechanisms. In contrast, the biochemical factors are either the primary nutrients or secondary non-nutritional chemicals of plants that affect insect biology. Some of these non-nutritional chemicals are associated with feeding deterrence, repellence or toxicity on insects (Saxena 1986). However, the potential nutritive factors of a plant also play a pivotal role in enhancing its resistance to insects, even in the absence of these chemicals (Mitchell et al. 2016). Thus a strong basis for developing resistant varieties should be aligned towards ascertaining the resistance imparting chemicals and applying those as cues in the breeding program. Host plant shows a varied kind of reactions upon feeding and infesting by insects and alteration of nutritional biochemistry of a plant also takes place in this response (Vanitha et al. 2011). Among the plant chemicals, the presence of increased or decreased amount of nitrogen, potassium, phenolic compounds, reducing sugar, ascorbic acid, crude silica, oxalic acid, free amino acid etc. influence the resistance or susceptibility of rice plants to BPH (Deepa et al. 2016). Keeping these views in backdrop, the present study was undertaken to evaluate different traditional folk rice cultivars of India, commonly known as rice landraces to examine their resistance status to BPH biotype 4 and to quantify the levels of BPH feeding attributing plant defensive traits in selected rice landraces, which will form an integral part of sustainable management of BPH through induced resistance.

Materials and Methods

Plant and insect material

The study materials consisted of 218 rice landraces collected at farmers' field from different districts of eastern India. All landraces were previously registered under West Bengal Biodiversity Board, Department of Environment, Government of West Bengal, Salt Lake, Kolkata – 700106, India. Seeds of resistant check (Ptb33) and susceptible check (Swarna) were collected from National Rice Research Institute, Cuttack, Odisha germplasm unit. The seeds were sown in screening trays and pots, based on the experiment, containing well-puddled soil during rainy season in 2016, 2017 and 2018. The insect BPH biotype 4 was reared on the susceptible variety Swarna (Sai Harini et al. 2020) in the insect-proof glasshouse of Bidhan Chandra Krishi Viswavidyalaya (22.9452º N, 88.5336º E), Nadia, located in West Bengal state of India at 28 ± 2 °C, 75 ± 5% relative humidity and 14:10 h light: dark photoperiod.

Screening for BPH resistance

Free-choice greenhouse screening

The Standard seedbox screening test method of IRRI (2002) with suitable modifications by Jena et al. (2006) was followed to evaluate BPH resistance to 218 selected rice landraces under greenhouse conditions in a complete randomized design and replicated thrice. At the seedling three-leaf stage in the screening trays, 2^{nd} instar nymphs of BPH in the rearing cages were released artificially onto the seedlings by visually ensuring the infestation of each seedling with at least 8-10 nymphs and were monitored at a regular interval for plant damage by BPH. When Swarna plants on one side exhibited intense damage, the entire cage was rotated by 180° for equal reaction on both sides. After complete wilting of more than 90 per cent of plants of susceptible check due to BPH feeding, the tests were terminated and the damage to all seedling rows was computed and converted to a resistant and susceptible score of 0 (Highly resistant), 1 (Resistant), 3 (Moderately resistant), 5 (Moderately susceptible), 7 (Susceptible) and 9 (Highly susceptible) based on the international standard evaluation system (Horgan et al. 2015).

No-choice greenhouse screening

Beside the free-choice test, the no-choice screening was also conducted in the greenhouse by taking 218 numbers of rice landraces to establish their resistance levels against BPH more clearly. The "isolated cage-test method" was followed by taking individual plastic pots (D × H, 8 × 20 cm) for each landrace (Vos and Jander 2008). Twenty freshly germinated seeds of each landrace were individually seeded concentrically in a single pot including Ptb33 and Swarna. At the seedling three-leaf stage in the pots, 2^{nd} instar nymphs of BPH in the rearing cages were released artificially onto the seedlings by visually ensuring the infestation of all seedlings in a single pot with at least 35-40 nymphs and were encircled with a transparent OHP sheet-made hollow cylindrical structure (D × H, 6 cm × 30 cm), roofed with 80-mesh insect-proof net pieces at the top. After complete wilting of more than 90 per cent of plants of Swarna, the tests were terminated and the damage was scored according to Horgan et al. (2015), as mentioned earlier.

Open-field screening

Field screening of all the selected rice landraces was carried out at the farmers' fields, having a long history of BPH occurrence and has sufficient water supply system, in the Burdwan district of West Bengal, India during rainy season of 2016, 2017 and 2018. In 2011, this particular district was designated as a BPH biotype 4 endemic zone in India by IRRI (Krishnaiah and Varma 2012). Nursery of all the rice landraces was prepared in each separate straight row by sowing 200-250 pre-soaked germinated seeds at a specific place beside the main field followed by common agronomic practices. Healthy seedlings were transplanted in the manner of single seedling per hill within the layout of each plot (1 × 1 m) throughout the field in completely randomized block design and replicated thrice (Bhogadhi and Bentur 2015). Transplanting was done deliberately late at the 2^{nd} fortnight of July with a closer spacing (r-r × h-h, 15 × 15 cm) to get a maximum infestation of BPH (Satpathi et al. 2012). Manual weeding operation at 25 days after transplanting (DAT) and 45 DAT was done and a 15 cm water level was maintained for standard BPH multiplication in the field. Scoring based on phenotypic reaction was done when Swarna plots exhibited 'hopper burn' symptoms according to the damage scale 0-9 provided by Sai Harini et al. (2013) from randomly selected 20 plants per replicated plot. Numbers of BPH nymphs and adults per three plants, selected at random from each plot, were also counted simultaneously. At the same time, per cent chaffy grain was enumerated by counting total numbers of spikelets from randomly selected three panicles in each plot at the harvesting stage according to Timmanagouda and Maheswaran (2017).

Phenotyping

Phenotypic tests were conducted in a set of three replications with 40 rice landraces, selected from the results of three years screening (2016-2018) of 218 landraces, in 2019.

Feeding rate by honeydew excretion test

The honeydew excretion test of BPH was carried out with 30 day-old potted seedlings by the method recounted by Pathak et al. (1982). Five numbers of each 2nd instar nymphs and one-day-old adult females were introduced separately to the bottom portion of the seedling with an orange coloured bromocresol green treated filter paper around the base and an inverted and basal perforated transparent plastic cup (80 ml volume) on the filter paper incarcerating the insects to the stem portion of about 9 cm long. The hole of the cup was closed with a ball of non-absorbent cotton to prevent the escape of insects. The honeydew droplets excreted by BPH were turned into blue spots when they came in contact with the filter paper after 48 h of insect imprisonment. The area marked with blue colour was measured on millimetre squared (mm^2) graph paper sheet as the extent of feeding and also interpreted statistically.

Settling behaviour of BPH nymphs

This experiment was conducted following the method of Sarao and Bentur (2016) by taking 40 selected landraces seeded at random rows (10 seeds per row), 3.0 cm apart in a seedbox. The susceptible check Swarna was sown in two frontier rows, while a single row of Ptb33 was in the centre of the box. The 15 day-old seedlings were infested with 2nd – 3rd instar BPH nymphs with at least 12 – 15 individuals per seedling and the tray was immediately covered with insect-proof cage to prevent the escape of nymphs. The number of nymphs settled on each seedling was counted at 1, 3 and 5 days after infestation from randomly selected five plants in each row. The seedlings were manually disturbed after each observation for proper reorientation of the BPH nymphs.

Settling behaviour of BPH adults

The tested landraces were grown in a tray previously filled with fertilizers enriched puddled soil. Around 800 pairs of adults were released onto the 30 day-old seedlings with the help of a giant aspirator under free-choice test, and the tray was again covered with insect-proof cage (Sarao and Bentur 2016). Numbers of adult male and female alighting on various landraces were visually counted at 6, 12, 24, 48, 72 and 96 h after release. Like nymphal settling test, seedlings were manually disturbed after each count for proper reorientation of the BPH adults.

Nymphal survival

The experiment on nymphal survivability was carried out by caging one-day-old freshly hatched 1st instar BPH nymphs on 15 day-old seedlings (20 nymphs per plant and replicated thrice) of all the landraces separately along with standard check varieties (Jena et al. 2015). The seedlings were monitored at regular interval for consecutive

18 days, and the numbers of adults were counted whenever they emerged and carefully removed from the seedlings. The per cent nymphal survival was calculated using the formula of Heinrichs et al. (1985).

$$\% \text{ nymphal survival} = \frac{\text{number of adults emerged}}{\text{number of nymphs released}} \times 100 \text{.........................} (1)$$

Ovicidal test

Like the nymphal survivability test, one pair of three-day-old BPH adult was confined on 30 day-old seedlings of the tested landraces and check varieties separately, each in three replications. The adults were removed on the 7th day of release and all the seedlings were observed for nymphal hatching from the day onward. The number of hatched nymphs was counted and removed from the plant through an aspirator. After 15-18 days when nymphs stopped coming out, seedlings were collected and dissected under a stereoscopic zoom binocular microscope (40x magnifications) to examine the number of egg masses and the number of unhatched eggs. A total number of eggs were assumed to be the sum of the number of nymphs counted and the number of unhatched eggs. The per cent unhatched eggs were enumerated by using the formula of Khan and Saxena (1985).

$$\% \text{ unhatched eggs} = \frac{\text{number of unhatched eggs}}{(\text{number of nymphs emerged} + \text{number of unhatched eggs})} \times 100 \text{.........................} (2)$$

Biochemical study

Bio-chemical analysis of healthy and BPH infested rice plants was carried out for the comparative estimation of total phenol (TP), reducing sugar (RS), ascorbic acid (AS), oxalic acid (OA), crude silica (CS) and total free amino acid (TFA) along with the estimation of nutrient composition *viz.* nitrogen (N), phosphorus (P) and potassium (K) with 40 selected rice landraces in 2018-2019. This set of experiment was conducted under well-equipped laboratory condition at Department of Biological Sciences, Indian Institute of Science Education and Research (IISER) Kolkata, West Bengal, India. Seeds were sown separately in two plastic containers for each landrace with no additional nutrient. One set of 30 day-old seedlings were infested with 2^{nd} – 3^{rd} instar BPH nymphs for a week. The green leaf sheaths of both healthy and infested plants were used for the biochemical analysis of TP, RS, AS, OA, CS and TFA, while N, P and K were estimated from oven-dried (60 °C for 72 hours) and ground materials. The detail estimation procedures and the required reagents have been narrated in the supplemental material 1.

Total phenol

The quantity of TP present in 1 g of leaf sheath was estimated using a spectrophotometer (Shimadzu, UV-1900) based on the calorimetric assay described by Sadasivam and Manickam (2008).

Reducing sugar

Estimation and comparison of RS in rice leaf sheath between the healthy and infested plants were made by Dinitrosalicylic Acid Reagent (DNS) method described by Sadasivam and Manickam (2008).

$$10 \text{ ml contain} = \frac{x \times 10 \text{ mg of glucose}}{0.1} = \% \text{ of reducing sugar} \text{ .... } (3)$$

Absorbance corresponding to 0.1 ml of test sample = x mg of glucose.

Ascorbic acid

The AS content in healthy and infested rice leaf sheath of different landraces was estimated by the volumetric method described by Sadasivam and Manickam (2008).

$$\text{Quantity of ascorbic acid (mg per 100 g sample)} = \frac{0.5 \text{ mg} \times V2 \times 100 \text{ ml} \times 100}{V1 \text{ ml} \times 5 \text{ ml} \times \text{Wt.of the sample}} \text{ .... } (4)$$

Where V1 = known volume and V2 = titrated volume

Oxalic acid

Quantitative estimation of OA in healthy and infested plants of rice landraces was done by a direct calorimetric method with Indole reagent by following the method of Bergerman and Elliot (1955).

Crude silica

The CS content in both healthy an infested rice plants was estimated through spectrophotometer (Shimadzu, UV-1900) according to the method suggested by Wei-min et al. (2005).

Total free amino acid

The TFA content in both healthy an infested rice plants was estimated using a spectrophotometer (Shimadzu, UV-1900) by following the method described by Moore and Stein (1948).

Nitrogen

One gram each of oven dried plant sample was taken from both healthy and infested plants and N was estimated on whole plant basis by using the standard micro Kjeldahl method by following Piper (1966) and data was expressed as percentage.

Phosphorus and potassium

Two-hundred and fifty mg of plant material was digested by wet digestion method according to Piper (1966) using a tri-acid mixture (nitric, sulphuric and perchloric acids in 9:2:1 ratio). The P and K were then estimated with the help of a Systonics Digital Flame Photometer (Model S-931) and were expressed as percentage.

Statistical analysis

The data obtained from different experiments related to mass screening, phenotyping and bio-chemical parameters were analyzed using analysis of variance (ANOVA) with the help of IRRISTAT 4.0 software (Sarao and Bentur 2016) developed by the Biometrics Unit of International Rice Research Institute, the Philippines.

Data which lacked normality were transformed using arcsine and square root transformations before subjected to statistical analysis. Cluster analysis of 218 rice landraces including Ptb33 and Swarna was done based on the similarity in resistance reactions under free-choice, no-choice and field screening and other quantitative parameters like number of BPH and per cent chaffy grains.The similarity matrix was generated through the simple Euclidean distance across all parameters of different landraces, and this matrix was used in a hierarchical clustering technique of Ward's minimum variance method using R software, version 4.0.2 (https://www.R-project.org). A relationship was established among different bio-physical and bio-chemical parameters of tested rice landraces using pairwise correlation coefficients of their mean values by Pearson correlation with the help of XL-Stats 2020 software (https://www.xlstat.com/en/).

Principle Component Analysis (PCA) is one of the most frequently used methods of multivariate data analysis. It was used as a method that transforms an original set of variables into a smaller set of uncorrelated linear variables by retaining most of the information in the former (Ray et al. 2014) and was executed using the XLSTAT 2020 software. The independent factors in the total data set those mostly contributed to the infestation by BPH on rice were selected for PCA. The total variance is simply the sum of variances of these variables. As they have been standardized to have a variance of one, each observed variable contributes one unit of variance to the total variance in the data set where total nine independent BPH infestation attributing traits were selected for this purpose. The array of communality, the amount of variance of a variable accounted by the common factors together was estimated by the highest correlation coefficient in each array according to Seiller and Stafford (1985). Factor loadings after varimax rotation along with Kaiser Normalization (Kaiser 1974) were estimated for determining the correlation of a variable with a factor. The highest value of the factor loading (squared cosine is the largest) of a particular variable in a particular factor among the extracted factors plays the important role to churn out the factor. After performing PCA, both observations (selected rice landraces) and variables (BPH infestation attributing traits) represented graphically in the factor space through distance biplot analysis (Legendre and Legendre 1998) using XLSTAT 2020 software. The biplot was used to interpret the distances between the observations as these are an approximation of their Euclidean distance in the p-dimensional variable space. The position of two observations projected onto a variable vector was used to determine their relative level for this variable.

Results

Mass screening

The results of 218 rice landraces that were initially screened in the glasshouse as well as in the field during three years period for their reactions to brown planthopper and scored on 0-9 scale. The level of resistance was noticed among 218 landraces ranged from 1.2-9.0 (glasshouse) and 1.1-9.0 (field), indicated a wide variation. The 5 landraces *viz*. Badshabhog, Haldichuri, Janglijata, Kalabhat and Khara were observed as resistant against BPH by showing their damage score (DS) in the range of 1.2-2.0, 1.5-2.8 and 1.1-1.9 under free-choice, no-choice and field screening, respectively. The 218 rice landraces, along with Ptb33 and Swarna, could be easily classified into four major clusters at 8 unit distance by the scale of similarity (Figure 1). Most of the resistant and moderately resistant landraces were grouped under the major cluster I and II, respectively. Cluster III comprised of 46 landraces closest to Swarna in similarity matrix, where most of the landraces showed highly susceptible and susceptible features. However, the majority of moderately susceptible landraces constituted two sub-clusters under the major cluster IV.

Phenotyping

Honeydew excretion

The amount of honeydew excretion is directly proportional to the quantity of food intake by BPH. The quantity of honeydew excreted by BPH nymphs varied significantly among the tested landraces (Table 1). The lowest feeding rate was recorded in Janglijata (27.9 mm^2) Badshabhog (30.3 mm^2), Kalabhat (30.7 mm^2) and Haldichuri (33.3 mm^2), respectively, equivalent to Ptb33. Similar trend of honeydew excretion was also observed for one-day-old adult BPH females.

Nymphal settling

Settling behaviour of BPH nymphs differed significantly among the tested landraces, where the least number of nymphs settled on Kalabhat, followed by Ptb33 and Khara (Table 1). All most identical behaviour of nymphal settling was noticed on all the observation days. Overall, the number of nymphs settled 80.00% less on Kalabhat, 78.12% on Ptb33 and 73.75% on Badshabhog concerning the susceptible check Swarna.

Settling behaviour of BPH adults

The significantly lower number of adult males settled on Kalabhat, Ptb33 and Hanumanjata, while Ptb33 and Khara registered a significant lower number of adult females of BPH (Table 1). The observations for both adult males and females were also found to be supplementary to the screening result of the landraces.

Nymphal survival

Mean per cent survival rate of BPH nymphs on phenotypically resistant landraces was lower than on the susceptible check (Table 1). The landraces such as Badshabhog (25.6%), Janglijata (25.6%) and Raghushal

(27.1%) had the lowest survival rates, equivalent to Ptb33 (26.6%), which were significantly different from Swarna (96.1%).

Hatching of eggs

Among the landraces tested to assess the per cent unhatched eggs of BPH, it was observed that Ptb33 (89.2%), Haldichuri (83.4%), Kalabhat (81.2%) and Badshabhog (78.8%) had the higher per cent of unhatched eggs, and that in Swarna lowest per cent (24.8%) of eggs remained unhatched (Table 1).

Biochemical components

Total phenol

The TP content in the leaf sheaths of the BPH infested and healthy rice plants was estimated and differed significantly among the selected rice landraces (Figure 2). In the healthy plants, TP content was found to be 0.28 mg g^{-1} tissue in Ptb33, whereas Swarna exhibited 0.48 mg g^{-1} tissue. After the BPH infestation, per cent increase in TP content was observed in most of the resistant and moderately resistant rice landraces in the range of 22.22 to 51.28%.

Nitrogen

The per cent N content was not varied significantly among the selected rice landraces, including susceptible checks in case of the healthy plants, while the BPH infested plants showed a significant variation (Figure 3). Higher per cent of N content was noticed in the moderately susceptible rice landraces (1.25 to 1.61%) with the highest in Swarna (1.72%), but the significant lower range of N accumulation was found in the resistant landraces (1.12 to 1.31%).

Phosphorus

Very marginal difference of P content was observed among the BPH infested rice landraces, which was clear from the value registered by Ptb33 (0.50%) and Swarna (0.41%) depicted in Figure 4. Most of the resistant and moderately resistant landraces exhibited an increase in the per cent P content except Bahurupi (-22.73%), Kabirajshal (-37.50%), Lilabati (-31.71%) and Raghushal (-5.77%).

Potassium

Unlike N and P, significant variation in both the healthy and BPH infested plants was observed in the case of K (Figure 5). Here also, per cent increase in K was observed in most of the rice landraces, whereas a negative value was encountered in some resistant and moderately resistant landraces. However, Lilabati exhibited a consistent behaviour before and after the BPH feeding in total K content.

Reducing sugar

Reducing sugar, another biochemical component present in rice leaf sheath influences the infestation of BPH, varied significantly among all the rice landraces both in healthy and BPH infested plants (Figure 6). Higher quantity of RS was observed in moderately susceptible landraces with the highest in susceptible check Swarna (1.20 mg g^{-1} glucose equivalent), compared to Ptb33 (0.35 mg g^{-1} glucose equivalent). After BPH feeding, per cent decrease in RS took place in the range of 1.32 to 65.71%, irrespective of all the rice landraces including standard check varieties.

Ascorbic acid

Figure 7 revealed that, AS varied significantly among the healthy and BPH infested rice landraces with the reduction of quantity after feeding. Healthy leaf sheaths of Ptb33 (1.15 mg g^{-1} tissue) followed by Haldichuri (1.06 mg g^{-1} tissue) registered the highest amount of ascorbic acid content compared to Swarna (0.65 mg g^{-1} tissue), whereas 23.48%, 14.15% and 13.85% reduction were observed after the infestation by BPH, respectively.

Oxalic acid

Oxalic acid also varied significantly among the selected rice landraces both in healthy and after the infestation by BPH (Figure 8). In the healthy plants, higher range of OA content was noticed in resistant and moderately resistant rice landraces (0.27-0.46 mg g^{-1} tissue) and was statistically at par with Ptb33 (0.40 mg g^{-1} tissue), compared to Swarna (0.18 mg g^{-1} tissue). Per cent reduction in OA was observed irrespective of all the rice landraces including the standard checks after feeding of BPH on them.

Crude silica

Crude silica content was observed to be significantly higher in resistant and moderately resistant rice landraces (Figure 9). Adanshilpa (17.52%) followed by Laldudheshwar (16.60%) exhibited higher CS content and were found to be statistically at par with Gamra (15.85%), Kalabhat (15.80%) and Ptb33 (14.53%). Swarna registered significantly lower CS content and was found to be equivalent with the moderately susceptible landraces. Though the per cent decrease in CS content among all the landraces was observed after the BPH infestation, and higher per cent reduction was shown by the moderately susceptible landraces than those of the resistant landraces.

Total free amino acids

Total free amino acid content has differed significantly amongst the tested landraces, and the relative quantity was also varied after the infestation of BPH (Figure 10). The highest quantity of TFA was observed in susceptible check Swarna (2148.2 µg g^{-1} of glutamic acid equivalent), followed by a moderately susceptible landrace Maltu (2041.7 μg g^{-1} of glutamic acid equivalent). Resistant landraces registered significantly lower amount of TFA in the range of 1125.8-1575.2 μg g^{-1} of glutamic acid equivalent and were statistically at par with Ptb33 (1356.6 μg g^{-1} of glutamic acid equivalent). However, BPH feeding resulted in the increasing of the quantity of TFA among all the landraces except Maltu.

Correlation studies

Pairwise correlation among the biochemical parameters of rice plants tested in various rice landraces and has been depicted in Table 2. The plant nutrient N was non-significantly correlated with all the biochemical factors except K (negatively) and free amino acid (positively), whereas a significant and positive correlation was observed between OA, CS and K and negative among K, RS and TFA. TP significant but negatively correlated with K, OA and CS while, correlated positively with RS.

Table 3 revealed that, N content in plants exhibited a significant and positive correlation with honeydew excretion and nymphal survival. P, on the other hand, was significant but negatively correlated with nymphal survival, while both TP and K showed significant positive and negative correlation with per cent un-hatched eggs and settling of BPH nymphs and adult females, respectively. Both OA and CS correlated significant but negatively with honeydew excretion, settling of nymphs and adult females and nymphal survival, while CS posed a significant and positive impact on per cent un-hatched eggs. In contrast, honeydew excretion, settling of three BPH morphs and nymphal survival correlated significantly and positively with free amino acid.

Principal component and diversity analysis

Data presented in the tables (Table 4 and 5) revealed that the first, second and third principal components explained about 48.35%, 14.08% and 11.43% for healthy and 48.89%, 13.47% and 11.59% for infested plants of the total sample variance respectively. The first three components containing the Eigen values greater than 1 have been retained for the study; hence, the first three components explain the variance of the sample reasonably. Scree-plot test, which is based on the decreasing curve of Eigen values, also provided a transparent visual aid for justification of retaining three components effectively. Table 6 and 7 showed the correlation of variables to the different principal components in the form of the corresponding factor loadings after varimax rotation for healthy and infested plants, respectively. In case of healthy rice plants, the 1st factor consists of N, TP, RS, OS, CS and TFA, while 2nd, 3rd and 4th factors consist only K, AS and P, respectively. Similarly, N, RS, AS, CS and TFA consisted in 1st factor for BPH infested rice plants, while OA and P shifted into the 4th factor. Here, it has been seen that both RS and AS registered highest squared cosine values (0.828) followed by CS (0.756) in the first factor with maximum load.

The scattered plot matrix score clustered the different biochemical components related to BPH feeding into groups sowing superiority with a mass of selected rice landraces (Figure 11 and 12). It was clear from the biplot that for healthy plants, resistant and moderately resistant landraces including Ptb33 were closely associated with P, CS, AS, OA and K, while with the higher values TP, TFA, N and RS were closely associated with Swarna. Besides, biplot of BPH infested plants exhibited that TFA appeared in close association with resistant landraces and in contrast, AS shifted towards the susceptible check.

Discussion

The resistance of rice to BPH has been studied, documented and reported by several workers and traditional varieties have been identified as most of the resistant donors (Kalode and Krishna 1979; Jena et al. 2006; Jena et al. 2015). Identification of resistant donors is made through the mass screening technique due to its direct relationship with the feeding of the pest. In the present study, the landraces with score '1', neither preferred both nymphs and adults to feed and settle significantly on them as shreds of evidence from the honeydew and settling tests nor they were allowed for surviving and egg-laying (nymphal survivability and ovicidal test). These findings might be linked to the less ingestion of food and its improper usage impaired the development and survival of BPH on resistant varieties (Alagar et al. 2007). Rate of feeding varied from landrace to landrace, and it determined the food intake by BPH. Feeding can only be determined preciously through computing the area of honeydew excretion and several studies recognized this method as the best for complementing the phenotypic screening (He et al. 2013; Jena et al. 2015). Various plant metabolites present within resistant rice cultivars inhibit the feeding activities of BPH due to the less preference and that was reflected in low honeydew excretion (He et al. 2013). Besides resistant cultivars, significantly lower amounts of honeydew excreted by BPH, when feeding on moderately resistant landraces, confirmed the accuracy of phenotypic screening (Singh et al. 2017) and the possible trend of resistance among the respective rice landraces (Ritu and Ravi 2006). Soundarajan et al. (2002) also conceded that the enumeration of the feeding rate of BPH is a potential indicator to differentiate the resistant and susceptible genotypes of rice.

Results of the present study showed that comparatively lower per cent nymphal population of BPH survived on phenotypically resistant rice landraces than Swarna. These results were corroborated by the findings of Vanita et al. (2011) and Kumar et al. (2012), who confirmed the reduced survival and longevity of BPH nymphs and adults on resistant and moderately resistant genotypes. Reduced and poor survival of BPH might be due to the lower feeding rate on resistant landraces, which may be attributable to the lack of phagostimulant or presence of antifeedants (Seo et al. 2009; He et al. 2013; Sable et al. 2015). However, it may also be possible that, these rice landraces lack essential nutrients which are solely required for the survival of BPH. Alternatively, Bing et al. (2007) and Syobu et al. (2011) acknowledged regarding some mechanisms or other factors, responsible for preventing ingestion of the required quantity of nutrients from a particular plant, imparted by the resistant landraces. Early embryonic development implied by the onset of eye pigmentation process normally, but hatching of eggs was affected probably due to the failure of developing nymphs to split chorion (Ramulamma 2014). Zheng et al. (2017) substantiated the fact that the lower nymphal survival in a rice variety SD15 possibly due to the lower rate of egg hatching and thus tend to be resistant with varying host adaptabilities also supported the present investigation. However, available literatures revealed that, antibiotic resistance levels in some resistant rice accessions were positively associated with the quantity of BPH feeding (Hao et al. 2008; Yang et al. 2017; Han et al. 2018). Therefore, variable resistant traits among different rice landraces could be attributable for the antibiotic reactions against BPH (Darshini and Sidde Gowda 2015). Similar observations were also documented by Kumar et al. (2013), where it has been noticed that resistant rice landraces were statistically at par with Ptb33 in terms of lower per cent egg hatchability, than TN1.

Nitrogen (N) content is regarded as an indicator of plant quality which was reported to induce a barrier against the resistance of BPH in rice (Lu and Heong, 2009; Salim 2002). Higher quantity of honeydew excretion by BPH was obtained in susceptible cultivars, and N was significant and positively correlated with this behaviour. The synergistic relationship between N in rice leaf and higher feeding rate of BPH possibly due to the ready-made succulence in leaf sheath for higher N content, which may not affect the insect biology directly, but changes the host biochemistry and plays a significant role in the reduction of plant resistance (Rashid et al. 2016). Ramulamma (2014) also indicated that N was negatively correlated with the resistance of rice against BPH. Moreover, Watanabe and Kitigawa (2000) also documented the effect of BPH feeding on rice plants resulted into the reduction of total N content and photosynthetic products in the leaf sheath and drastically hampered the plant growth. Results of the present study on the role and impact of N against BPH are also in parity with the elaborative findings of Lu et al. (2004); Lu et al. (2005) and Horgan et al. (2018). Besides N, P and K are also required by the herbivores for ATP and nucleic acid synthesis along with several physiological activities. Per cent reduction of P in BPH infested rice plants was conceded by Vanitha et al. (2011), while K showed a positive influence with the resistance parameters of rice against BPH (Lu et al. 2005; Amtmann et al. 2008). It was very clear from the result that K had a significant and negative impact on feeding along with BPH settling, survival and reproduction, may be attributable to the distribution of primary metabolites in plant tissues, which in turn could affect the attractiveness of the plant for insects as well as their subsequent growth and development on it (Rashid et al. 2017a). However, some workers found that higher level of K was associated with a lower population of BPH possibly due to the reduced level of RS and TFA in K rich rice cultivars (Vanitha et al. 2011). Correlation and PCA strongly boosted these obtained results and the possible mechanisms were also supported by Rashid et al. (2017b) and Yin et al. (2005). The phenolic compounds were found to be the feeding deterrents to BPH in rice and generally have a positive correlation with host plant resistance (Singh 2004). In the present study, quite lower level of TP was observed in Ptb33 and some resistant landraces compared to susceptible check, where higher per cent increase took place in the resistant landraces. Implication of phloem chemistry of rice, comprises of silicic acid, oxalic acid and phenolic compounds, provokes resistance to BPH (Ghaffar et al. 2011), but the latter usually possess a negative impact over the formers (Ciulu et al. 2018). Grayer et al. (1994) reported that higher silicon content in rice leaf sheath of a resistant variety can reduce the TP content at a lower level without disrupting the phenotypic resistance of the concerned rice variety to BPH. However, Mishra and Misra (1991) found a significantly lower quantity of TP in the resistant varieties Pundia and Handisarakanthi than TN1 and corroborates the findings of the present investigation. Plant vitamins like AS at a higher concentration inhibits the feeding rate of BPH (Sakai and Sogawa 1976) and the statement fully supports the present results in terms of resistance reactions of rice landraces. Both OA and CS were already recognized as the sucking inhibitor against BPH in rice, and in the present experiment a significant and positive correlation was also observed between them. For BPH, reduced performance with impaired feeding behaviours and poor population growth on rice were recorded in higher silicon content cultivars (He et al. 2015; Reynolds et al. 2016; Yang et al. 2017) and positively boosted our findings. The possible mechanisms of plant resistance related to higher silicon content may be the increased rigidity and reduced digestibility of plant tissues due to a physical barrier formed from higher deposition of silica in epidermal cells of rice plants (Massey et al. 2006; Massey and Hartley 2009; Han et al. 2015). Moreover, this physical barrier has a potentiality to reduce the food quality of herbivores and thus impairs their feeding capability followed by the reduction of growth rate (Han et al. 2015; Calandra et al. 2016). In addition, several workers indicated OA as another sucking inhibitor beside silicon (Yoshihara et al. 1979), and Nagata and Hayakawa (1998) found some antifeeding activity of OA against BPH. The TFA also played a significant role in BPH infestation on rice where most of the resistant landraces, including Ptb33 registered lower TFA content. This might be attributable that resistant cultivars against sap suckers usually possess a lower quantity of TFA by limiting the nutritive value of plant tissues for the herbivores Golan et al. (2017). Biplot of PCA suggests that TFA, RS and N content were in a close association in the healthy rice plants, while the distance between the former and two later was largest after BPH feeding. It was evident that the level of TFA content in leaf sheath increased after the BPH infestation and corroborated the findings of Sempruch et al. (2011). Although, it is still not clear by the researchers regarding the mechanisms of resource allocation when attacked by herbivores, but it can be hypothesized that higher cell damage would make the plant resource sequestration a possible preferred strategy (Orians et al. 2011). Moreover, Rashid et al. (2017b) linked higher K level with a lower level of TFA in the rice plants and observed the increment of both the compounds after BPH feeding.

In conclusion, it may be suggested that the activity of various nutrients and some biochemical components like OA, CS, and TFA in resistant landraces could reduce the feeding rate, nymphal and adult preference, survival and egg hatching of BPH which may in turn be useful in developing IPM strategy of BPH in rice. Understanding these biochemical mechanisms underlying resistance in rice landraces will also contribute to the effective management of BPH and facilitate resistance breeding program more efficiently.

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Figure legends

Fig. 1

Circular cluster dendrogram based on similarity matrix enumerated from 218 rice landraces (RL) and 2 standard checks (Ptb33 and Swarna) varieties. The dendrogram was created using Ward.D2 package.

Fig. 2

Total phenol (TP) content in the healthy and BPH infested rice landraces.

Fig. 3

Nitrogen (N) content in the healthy and BPH infested rice landraces.

Fig. 4

Phosphorus (P) content in the healthy and BPH infested rice landraces.

Fig. 5

Potassium (K) content in the healthy and BPH infested rice landraces.

Fig. 6

Reducing sugar (RS) content in the healthy and BPH infested rice landraces.

Fig. 7

Ascorbic acid (AS) content in the healthy and BPH infested rice landraces.

Fig. 8

Oxalic acid (OA) content in the healthy and BPH infested rice landraces.

Fig. 9

Crude silica (CS) content in the healthy and BPH infested rice landraces.

Fig. 10

Total free amino acid (TFA) content in the healthy and BPH infested rice landraces.

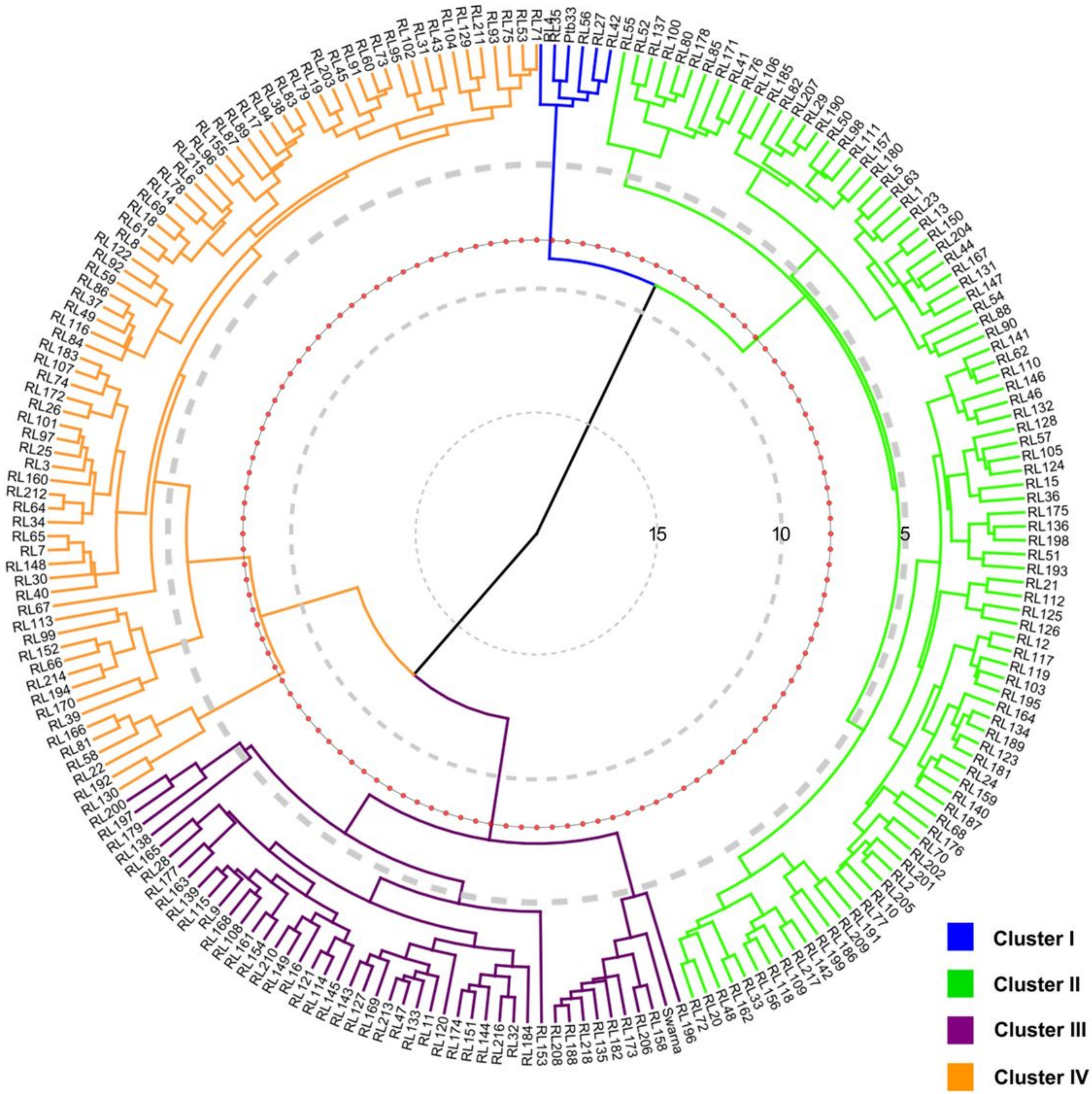
Fig. 11

Scattered plot matrix score of healthy (H) rice landraces and biochemical components.

Fig. 12

Scattered plot matrix score of infested (I) rice landraces and biochemical components.

Figures



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Circular cluster dendrogram based on similarity matrix enumerated from 218 rice landraces (RL) and 2 standard checks (Ptb33 and Swarna) varieties. The dendrogram was created using Ward.D2 package.

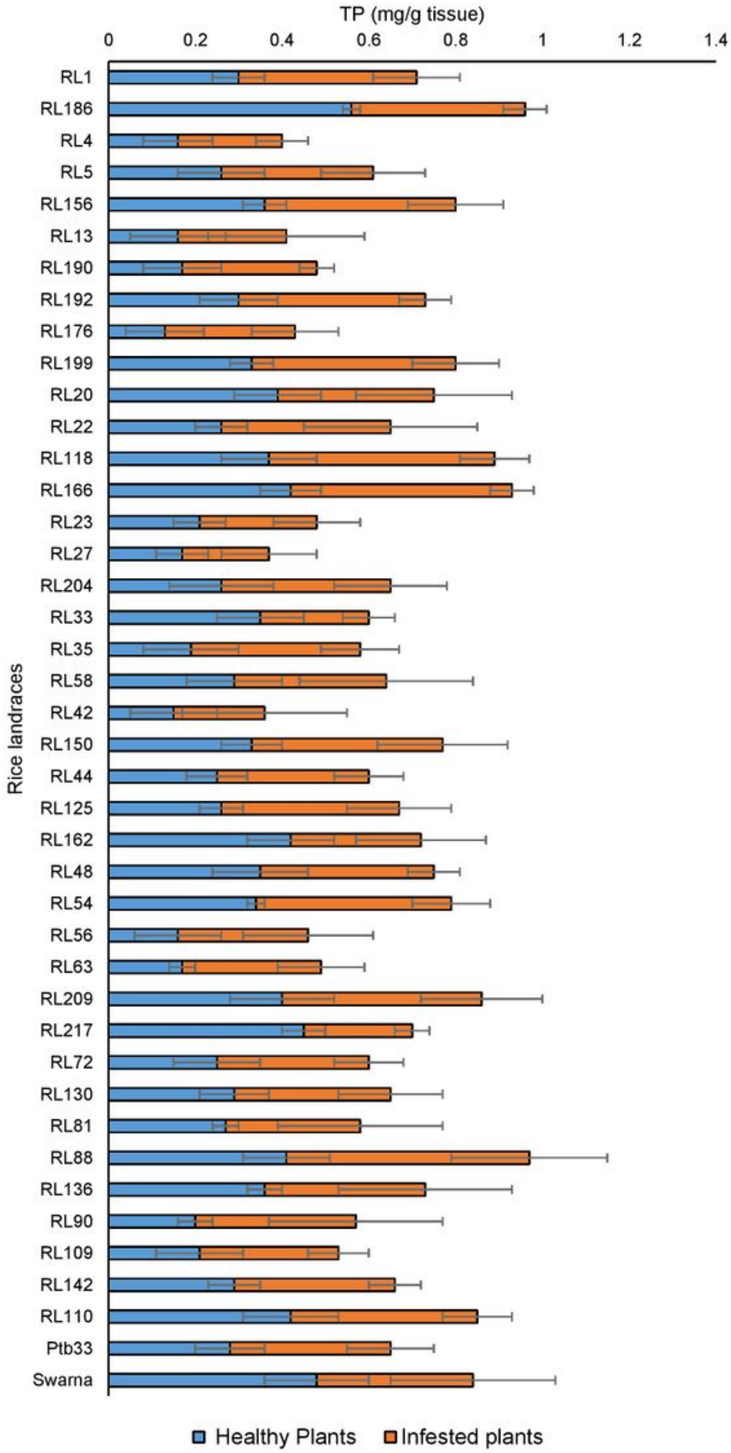


Figure 2

Total phenol (TP) content in the healthy and BPH infested rice landraces.

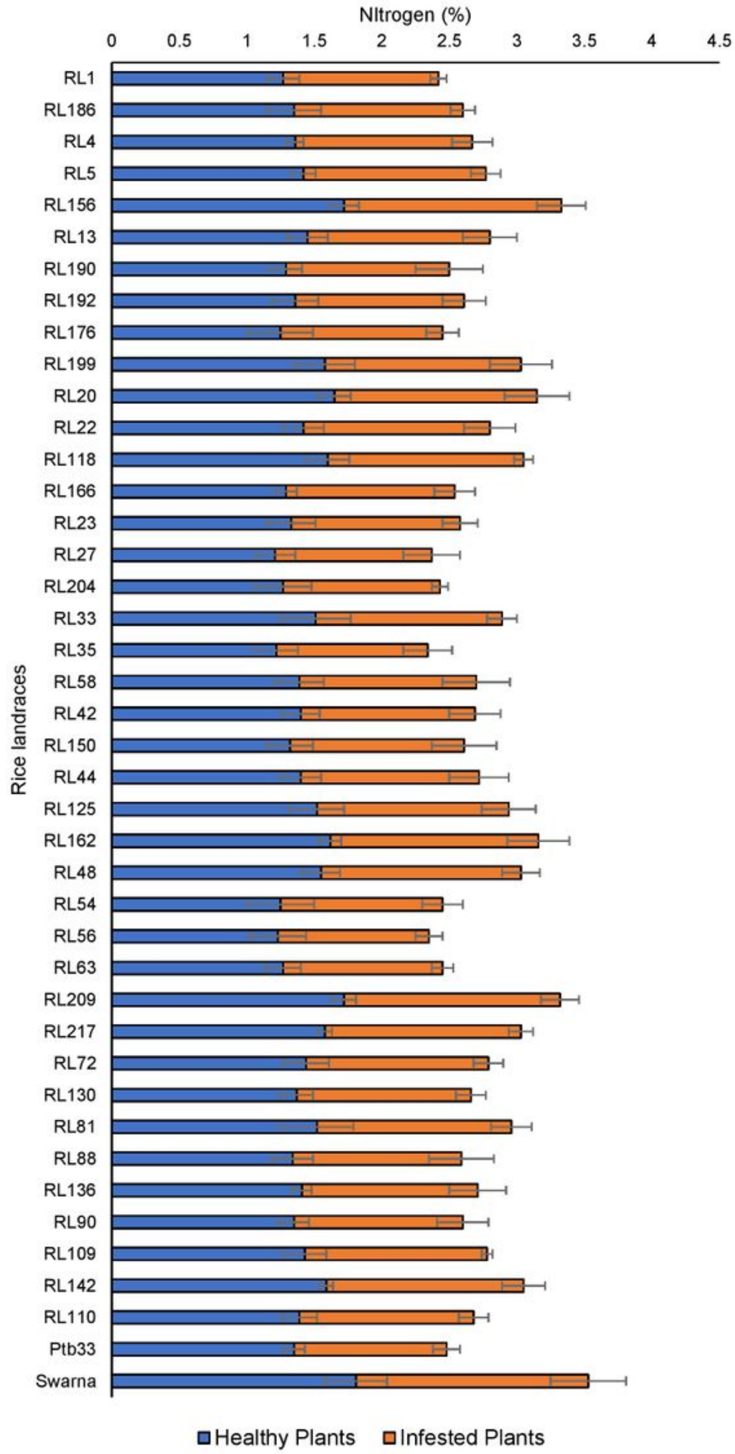
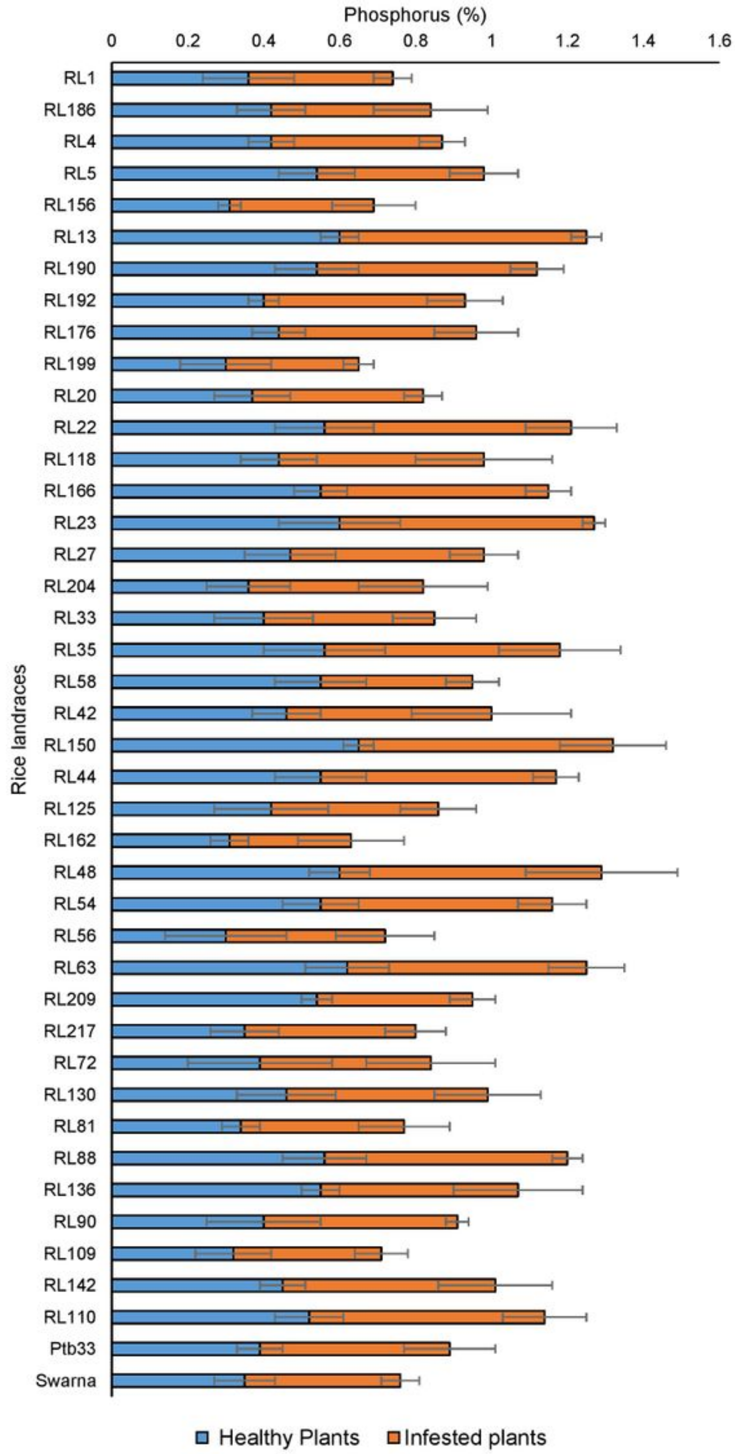


Figure 3

Nitrogen (N) content in the healthy and BPH infested rice landraces.



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Phosphorus (P) content in the healthy and BPH infested rice landraces.

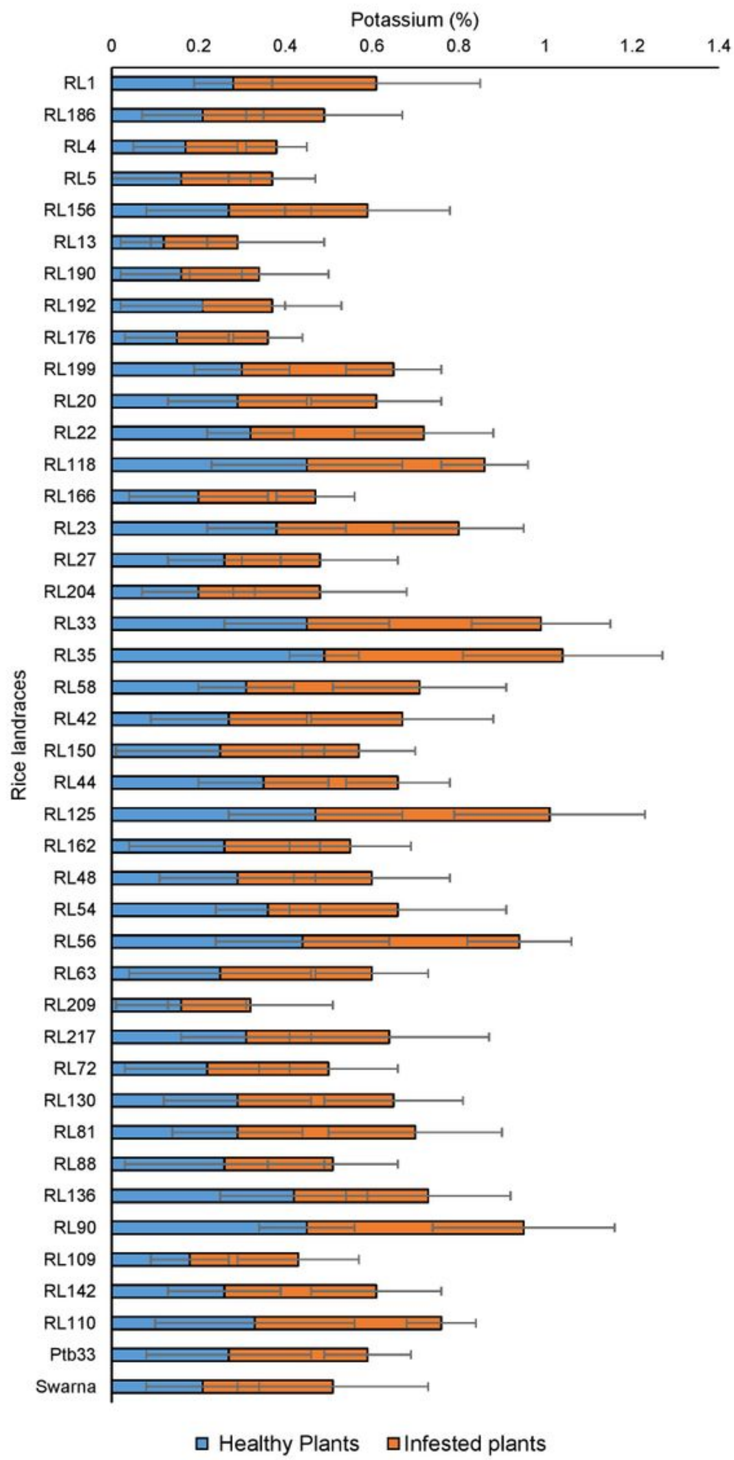

Figure 5

Potassium (K) content in the healthy and BPH infested rice landraces.

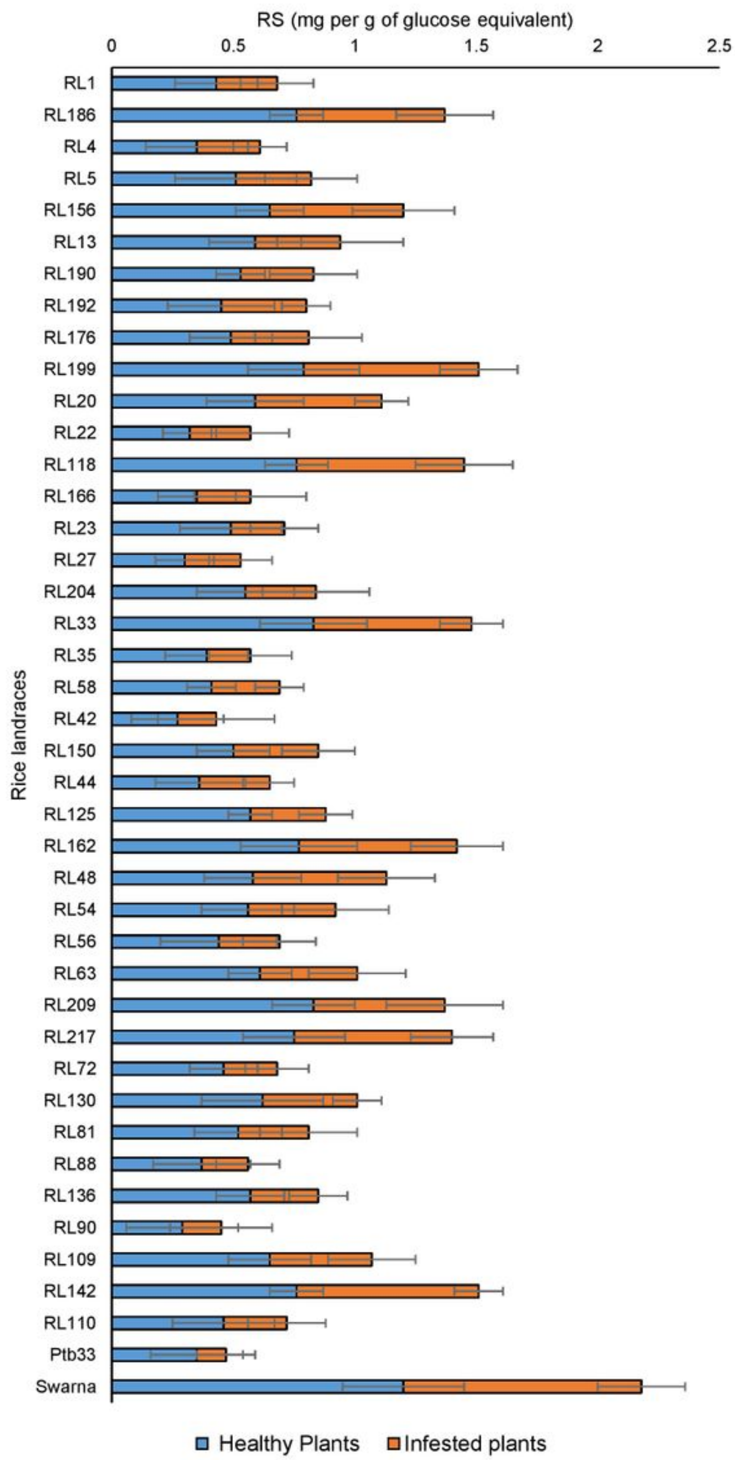

Figure 6

Reducing sugar (RS) content in the healthy and BPH infested rice landraces.

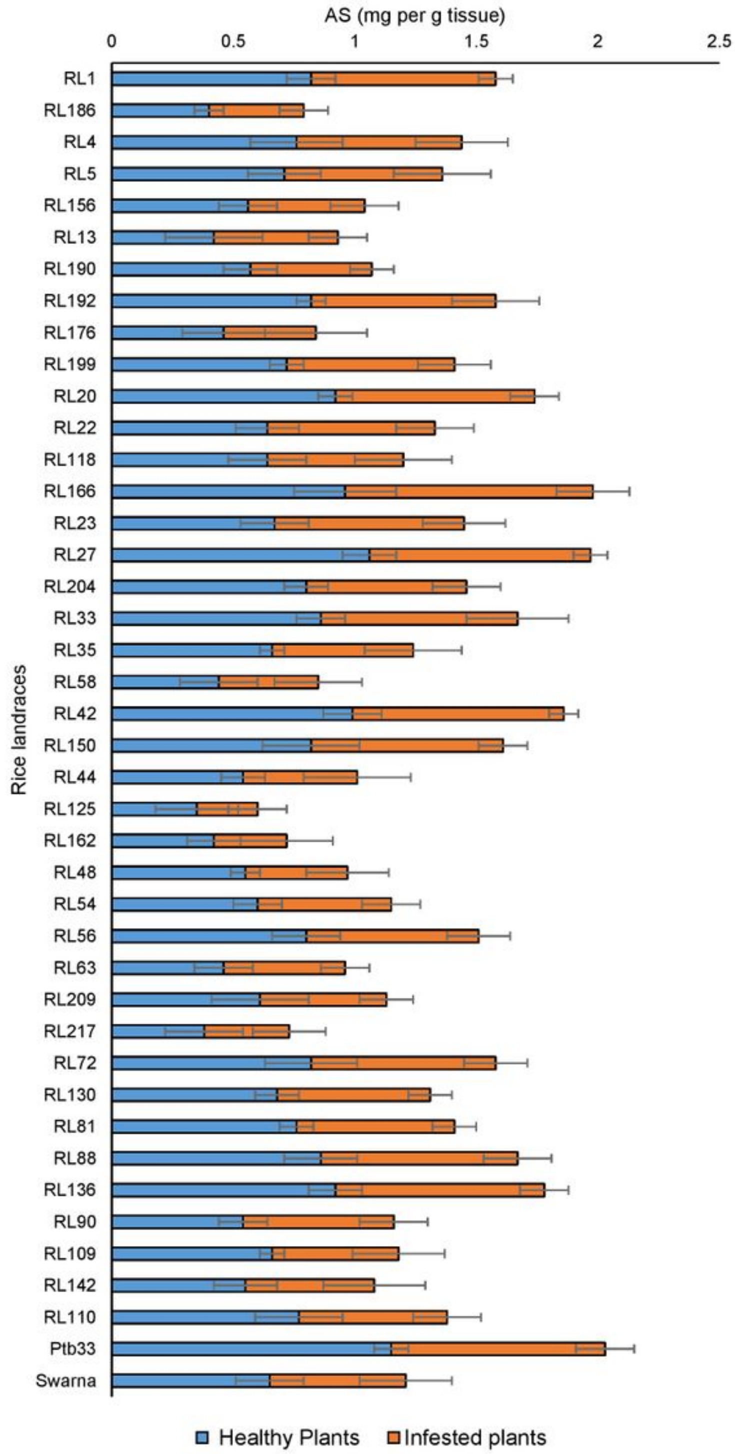

Figure 7

Ascorbic acid (AS) content in the healthy and BPH infested rice landraces.

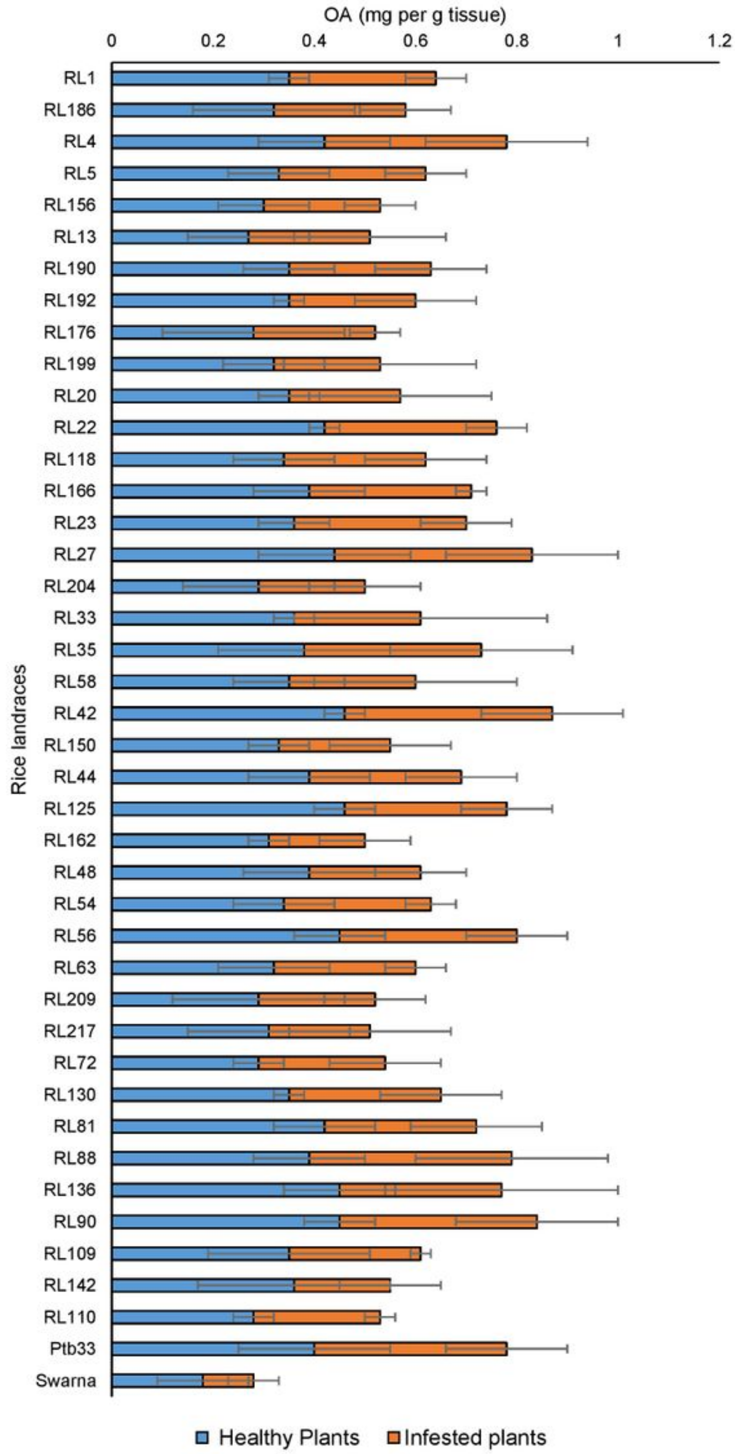

Figure 8

Oxalic acid (OA) content in the healthy and BPH infested rice landraces.

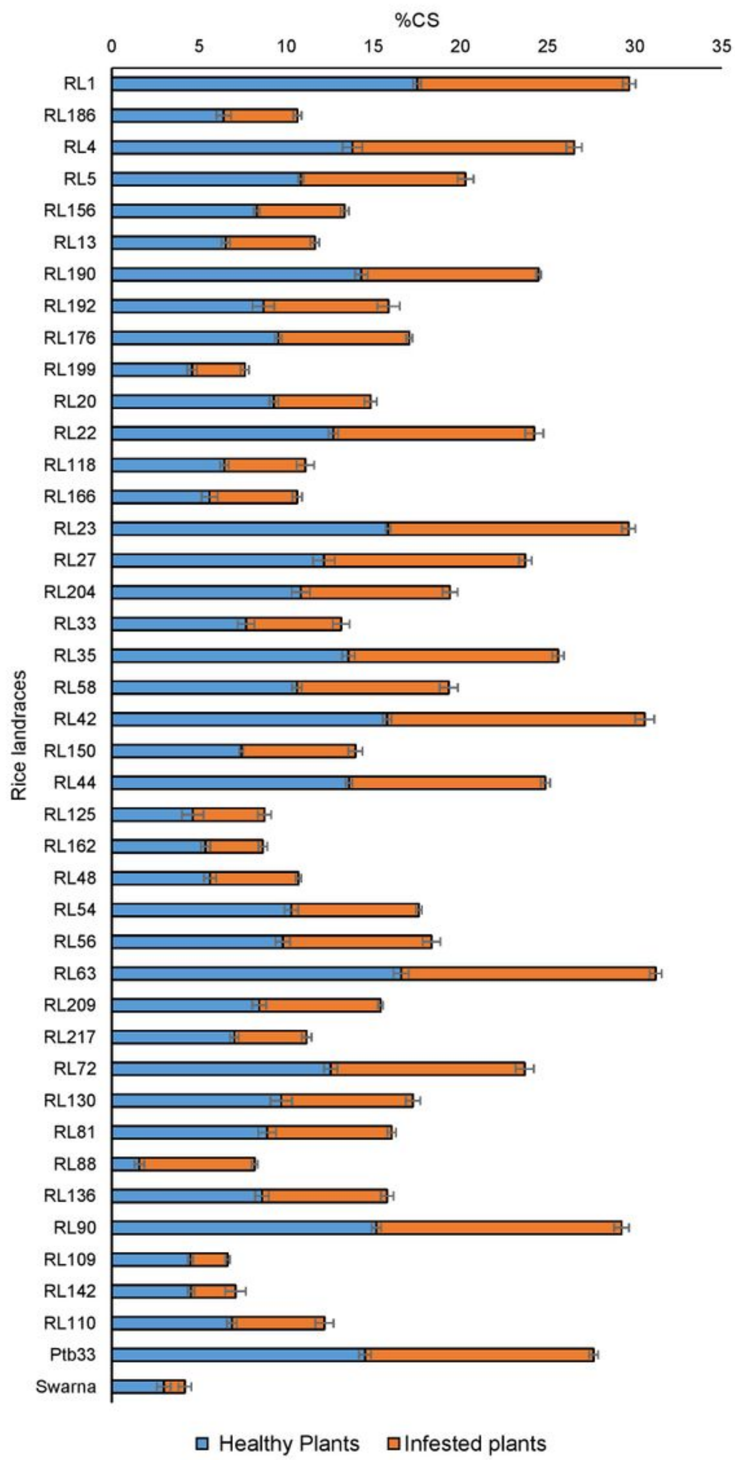

Figure 9

Crude silica (CS) content in the healthy and BPH infested rice landraces.

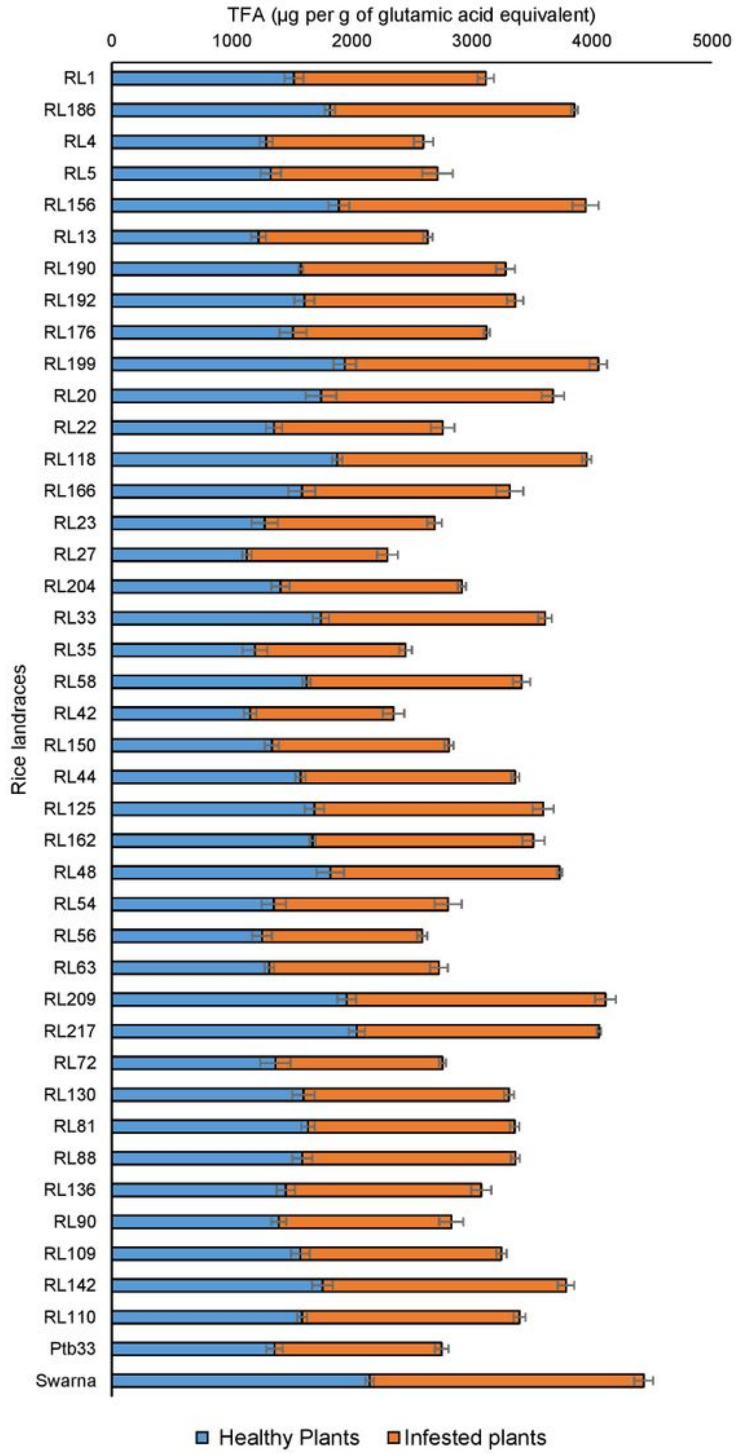

Figure 10

Total free amino acid (TFA) content in the healthy and BPH infested rice landraces.

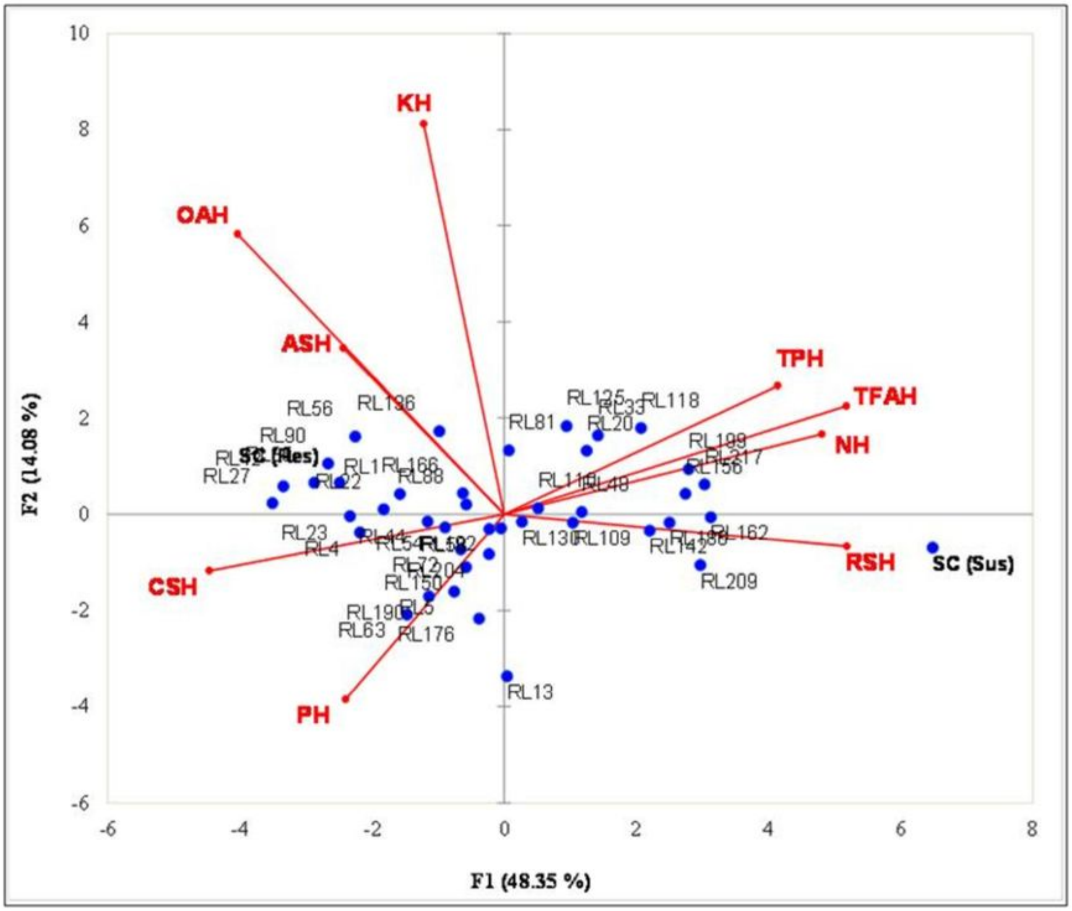

Figure 11

Scattered plot matrix score of healthy (H) rice landraces and biochemical components.

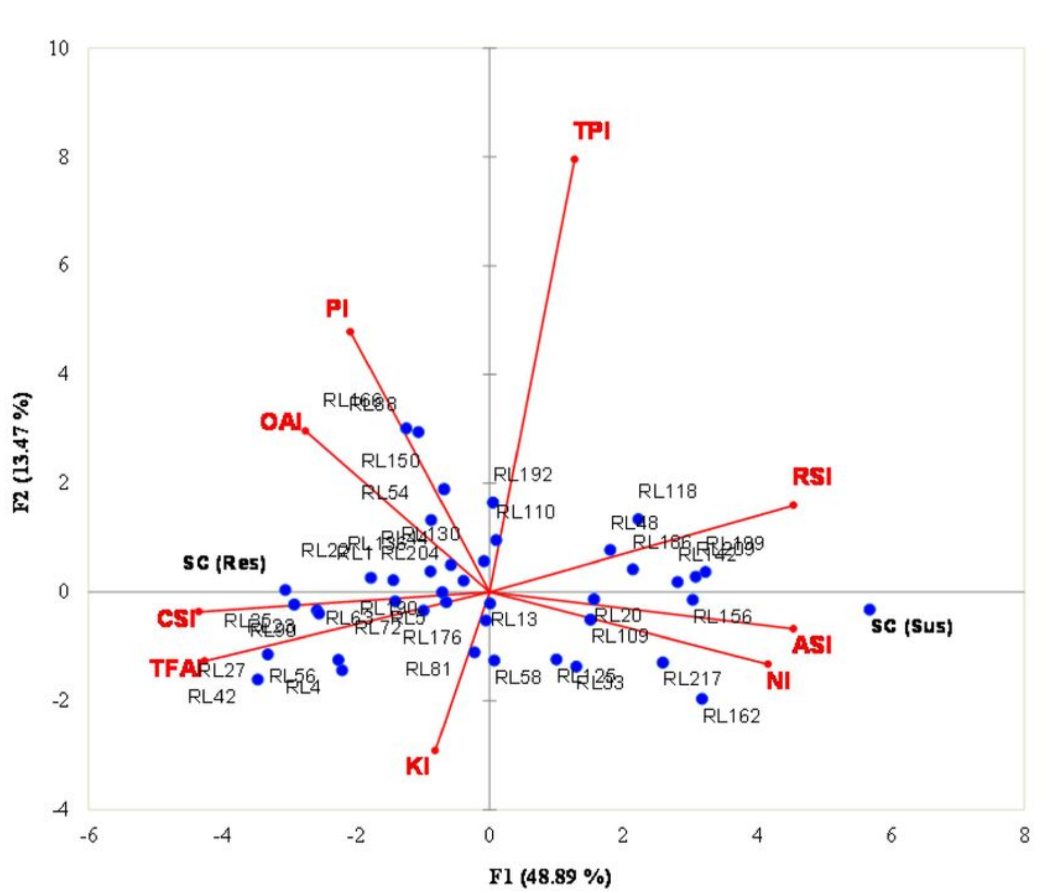

Figure 12

Scattered plot matrix score of infested (I) rice landraces and biochemical components.

Supplementary Files

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- Supplementalmaterial1.pdf