Biochemical Defense Responses in Advanced Backcross-Derived Rice Genotypes to *Nilaparvata lugens* (Stal) Infestation

Abstract

BPH,*Nilaparvatalugens* (Delphacidae: Homoptera)infestation on most of the resistant and moderately resistant backcross derived rice genotypes and resistant checks (PTB 33 and BM 71) resulted in an increase in the phenolic content in the infested plants leaf sheath. The percentage increase in phenols in resistant and moderately resistant genotypes ranged from 14.8 % to 67.7 %. Upon infestation of BPH there was a decrease in total reducing sugars in all the resistant and moderately resistant genotypes. The highest quantity of reducing sugars was present in healthy susceptible check TN1 compared to all the resistant and moderately resistant backcross derived rice genotypes. The ascorbic acid content in the resistant and moderately resistant genotypes decreased after BPH infestation and percentage decrease ranged from 16% to 36%. The total N content in the infested resistant and moderately resistant genotypes decreased over healthy genotypes. Decrease in the N content was highest in the susceptible check TN1 (35.2%). In the resistant checks slight decrease in the N content was observed. Potassium and Phosphorous (%) increased in the resistant genotypes and resistant checks, but not in susceptible check TN1. The plants responded defensively upon infestation, resulting in production of higher amount of phenolics, potassium, phosphorous and reduced level of nitrogen, reducing sugars and ascorbic acid.

Key Words:BPH, phenol, ascorbic acid, nitrogen

Introduction

As a major staple crop, *Oryza sativa* (rice) is vital for maintaining global food security (Molla *et al*., 2020).It is grown in 114 countries and acts as the main source of income for over 100 million households in Asia and Africa.Rice, a member of the Gramineae family, possesses a genome size of 430 MB. Globally, it is cultivated across 162.06 million hectares, yielding 500 million metric tons annually, averaging 5.0 tons per hectare (FAO, 2021). India is the second-largest rice-producing country, next only to China.Rice cultivation in India covers 45.5 million hectares, producing 125 million metric tons annually at an average yield of 4.1 metric tons per hectare (USDA, 2023).Nearly, 52% of global rice production is lost due to biotic stresses, with insect pest attacks accounting for 25% of these losses (Bhogadhi*et al*., 2015).Atwal and Dhaliwal (2002) reported that of the over 100 insect species that affect rice, 20 are categorized as major pests. Of all, *Nilaparvata lugens*, is considered a notorious pest in Asia for its damaging impact on rice crops due to its feeding habits on phloem sap (Heong and Hardy, 2009).Nymphs and adults feeding on sap from leaves and leaf sheaths lead to symptoms such as yellowing leaves, reduced tillering, shorter plant height, and increased grain unfilledness.An extensive infestation of BPH leads to a condition termed 'hopper burn' (Vanitha *et al*. 2011).Host plant resistance is a fundamental approach that is an economically viable and advisable tactic in BPH management.Biochemical constituents of the rice plant contributing to resistance has been studied from time immemorial.Here, there are a total of 15 genotypes, including advanced backcross-derived genotypes and resistant and susceptible checks. Siddhi backcross-derived genotypes (12) of F6 generation were used. The genotypes were categorized into resistant, moderately resistant, and susceptible based on our research conducted under both field and glasshouse conditions(Kumari *et al*., 2022).However, the samples for biochemical parameters were taken from glasshouse grown plants. Least square difference (LSD)

test was used to compare the differences in mean. Hence, the current study aimed to identify how certain biochemical parameters impart resistance against BPH.

Materials and Methods

Experimental Area

In *kharif* 2021, this research was conducted at the RARS in Warangal to understand the biochemical defense responses that might be present in the previously screened advanced backcross-derived rice genotypes (Kumari *et al*., 2022).

Estimation of phenols

The phenol content present in 1g of leaf sheath was estimated by Folin-ciocalteau method (Singleton *et al*. 1999).

Reagents: Gallic acid: 100 mg gallic acid was dissolved in 100 ml distilled water for 1000 ppm. Working standard: 1 ml of stock added in 20 ml water for 50 ppm concentration.

Procedure:

A. To 1gm of sample, 10 ml of methanol was added in centrifuge tubes and kept it for maceration for 24hrs.
B. Samples were centrifuged at 6000 rpm for 15 min, taken into 10 ml volumetric flask through filtering and made upto mark with methanol. This extract was used for phenols estimation.
C. After making up the volume to 3.0 ml with distilled water, 1 ml of Folin-Ciocalteau reagent, 2.0 ml of 20% Na2CO3 were added and tubes were placed in a boiling water bath for 1 min. After allowing the content of tubes to cool, the blue solution was diluted to 25 ml with distilled water, the colour developed was measured at 650 nm against a reagent blank in a spectrophotometer.
D. The concentrations of total phenol present in the unknown sample were extrapolated from the standard calibration curve. The amount of total phenol present in the leaf sheath was expressed as mg g^{-1}.

Estimation of reducing sugars

Total Reducing sugars present in rice leaf sheath was estimated by Nelson-Somyogi method (Nelson, 1994). Reagents:

i. Alkaline copper tartarate reagent

Reagent A: 2.5g of anhydrous sodium carbonate, 2 g of sodium bicarbonate, 2.5 g of sodium.

Potassium tartarate, 20 g of sodium sulphate dissolved in 80 ml distilled water and made upto 100 ml.

Reagent B: 15 g of copper sulphate was dissolved in 20 ml distilled water, one drop of concentrated sulphuric acid is added and made upto 100 ml.

Reagent C: 4 ml of B and 96 ml of A solution mixed before use.

ii. Arsenomolybdate reagent

2.5 g of ammonium molybdate was dissolved in 45 ml of distilled water. 2.5 ml of sulphuric acid was added and mixed well. Then 0.3 g of disodium hydrogen arsenate was dissolved in 2.5 ml of water.

iii. 80% ethanol 20 ml of water was added to 80 ml of ethanol.

iv. Glucose standard 100 mg of glucose was dissolved in 100 ml of distilled water for obtaining 1000 ppm.

Procedure:

A. 100 mg of sample was taken into centrifuge tubes and sugars were extracted by adding hot 80% ethanol (twice) 5ml each time.
B. Supernatant was collected into another centrifuge tubes and evaporated it by keeping it on a

water bath at 80°C.

C. Sugars were dissolved by adding 10 ml of water.
D. A set of the test tubes containing 0.2, 0.4, 0.6, 0.8 and 1ml. of standard solution were prepared.
E. 1 ml of aliquot was added into test tubes using pipette. The volume of aliquots in all the test tubes were made up to 2.0 ml with distilled water.
F. Later, 1 ml of alkaline copper tartarate was added to test tubes and were placed in boiling water for 10 min.
G. The test tubes were cooled and 1 ml of arsenomolybdic acid reagent was added.
H. 6 ml of distilled water was added to test tubes and the absorbance (intensity of the colour) was read in a spectrophotometer at 620 nm. The standard graph was drawn from the absorbance values and the amount of sugars was calculated from the standard graph. The amount of sugar present in the sample was expressed as mg g^{-1} of plant tissue.

Estimation of ascorbic acid

Total amount of ascorbic acid present in rice leaf sheath was estimated by Direct Calorimetric determination method described by Sadasivan and Manickam (1996).

Reagents:

a) 2% metaphosphoric acid: 32 g of metaphosphoric acid added to1600 ml of water and made upto 2000 ml. b) Dye solution: 50 mg of 2,6-dichlorophenol indophenol and 42 mg of sodium bicarbonate dissolved in 50 ml hot distilled water. Filtered and 25 ml was diluted to 500 ml with distilled water. c) Standard stock solution: 100 mg ascorbic acid was dissolved in 100 ml of 4% metaphosphoric acid. d) Working standard solution: 5ml of stock was taken into 50 ml volumetric flask and made upto mark.

Procedure:

a. 5 g of sample was taken in centrifuge tubes.
b. 25 ml of 2% metaphosphoric acid was added and homogenized for 10 min.
c. 25 ml of 2% metaphosphoric acid was then made upto 50 ml with metaphosphric acid and centrifuged for 15 min at 4000 rpm.
d. Readings were taken at 518 nm using spectrophotometer.

Estimation of nitrogen

Nitrogen was estimated on whole plant basis using the standard microkjeldhal method (Zuazaga and Ma, 1942) and expressed as percentage.

Procedure: Digestion: 0.2 g of sample was taken and 3 g of catalyst mixture (40 g of potassium sulphate and 8 g of copper sulphate) was added to it. Sample was digested by adding 10 ml of concentrated sulphuric acid at 42°C for 2 hrs 30min. Distillation was done with 4% NaOH, 0.4% Boric acid and titrated with 0.1N sulphuric acid for nitrogen percentage.

Estimation of Phosphorous

Phosphorus content was determined by following Piper, (2019) method. Bortom's reagent :11.5 g of ammonium molybdate was dissolved in 200 ml of water. 0.62 g of ammonium vanadate was dissolved in 300 ml of water. Two solutions were added in 500 ml volumetric flask. 125 ml of nitric acid was added to it and made upto mark with distilled water.

Procedure: 2.5 ml of extract was taken into 25 ml into volumetric flask. 5 ml of Bartom's reagent was added and made upto mark with distilled water. Phosphorous was determined with the help of spectrophotometer at 420 nm after 30 min.

Estimation of Potassium

Potassium content was determined by following Piper, 2019 method. Sample preparation: Plant material was digested by wet digestion method (Piper, 2019) using diacid mixture (nitric and

perchloric acids in 9:4 ratio). 1 g of plant sample was taken in conical flask and 12-15 ml of diacid mixture was added. Later it was kept for digestion at 200°C until clear colour appears on hot plate. After cooling 20 ml of water was added and filtered into 50 ml volumetric flask and kept for analysis. The potassium was determined with the help of ELICO flame photometer.

Statistical analysis: LSD was used to compare all the means among treatments to compare the biochemical parameters.

Results and Discussion

Biochemical basis of resistance was studied for 12 promising resistant and moderately resistant advanced backcross derived genotypes of Siddhi against BPH with regard to phenols, reducing sugars, ascorbic acid, nitrogen, phosphorous and potassium components in comparison with susceptible and resistant checks. The samples were taken before (healthy) and after release (infested) of BPH.

Phenols

Total phenolic content in the leaf sheaths of BPH infested and healthy rice plants was estimated in 12 advanced backcross derived genotypes of Siddhi along with the susceptible check TN1, resistant checks PTB33 and BM71. Total phenols in resistant checks PTB33 and BM71 increased from 0.217 to 0.256 mg g^{-1} tissue (64.02%) and 0.204 to 0.232 mg g^{-1} (62.3%), respectively. Whereas in the susceptible check (TN1) total phenols reduced from 0.186 to 0.133 mg g^{-1} tissue of the leaf sheaths *i.e.*, by 28%. Significantly highest phenolic content (0.295 mg g^{-1} tissue) was observed in infested Siddhi-BC2F6 BPHBL-61. The phenolic content increased in resistantgenotypes after BPH infestation, the highest being 67.7% in Siddhi-BC2F6 BPH BL-64. The phenolic content in the healthy genotypes*viz*., Siddhi-BC2F6 BPH BL-11, Siddhi-BC2F6 BPH BL-19, Siddhi-BC2F6 BPH BL-24, Siddhi-BC2F6 BPH BL-56, Siddhi-BC2F6 BPH BL-57, Siddhi-BC2F6 BPH BL-60 and Siddhi-BC2F6 BPH BL-61 and the infested genotypes*viz*., Siddhi-BC2F6 BPH BL-57 and Siddhi-BC2F6 BPH BL-61 were significantly on par with resistant checks. Slight decrease in the phenolic content was observed in moderately resistant genotype Siddhi-BC2F6 BPH BL-19 and which was on par with susceptible check TN1 (Table 1).

The present results were in accordance to the findings of Reddy *et al*. (2004) who reported that BPH infestation increased the phenolic content of the resistant and moderately resistant cultivars. The present results were similar with the findings of Udayasree*et al*. (2018) who conducted studies on biochemical aspects of resistance against BPH on resistant rice genotypes and stated that the amount of total phenol was observed to be maximum in the leaf sheath of moderately resistant variety. Similarly, Dharshini and Gowda (2014) reported significant increase of phenol content as a result of BPH feeding. Deepa *et al*. (2016) assessed the level of total phenols, crude silica and total sugars in BPH affected rice leaves, resistant varieties showed higher amount of phenols and crude silica as against low quantity of total sugars. Present results were in agreement with Singh (2004) who reported that total phenol contents decreased in all infected plant parts.

Table1.Phenolscontent ofpromisingadvancedbackcrossderivedgenotypes ofSiddhi

Sl. No.	Paddy Genotypes	Phenols(mg g^{-1})		
		Healthy	Infested	% Increase
1	Siddhi-BC_2F_6BPH-BL-30(R)	0.150(0.387)^{f}	0.191(0.437)^{e}	27.8
2	Siddhi-BC_2F_6BPH-BL-43(R)	0.138(0.371)^{f}	0.198(0.444)^{e}	43.2
3	Siddhi-BC_2F_6BPH-BL-64(R)	0.148(0.384)^{f}	0.248(0.497)^{d}	67.7

4	Siddhi-BC_2F_6 BPH-BL11(MR)	0.196(0.443)^{cd}	0.282(0.531)^{cd}	43.7
5	Siddhi-BC_2F_6BPH-BL-12(MR)	0.159(0.399)^{ef}	0.186(0.431)^{ef}	17.0
6	Siddhi-BC_2F_6BPH-BL-19(MR)	0.183(0.428)^{de}	0.156(0.395)^{fg}	-14.7
7	Siddhi-BC_2F_6BPH-BL-24(MR)	0.183(0.425)^{de}	0.260(0.51)^{cd}	42.0
8	Siddhi-BC_2F_6BPH-BL-52(MR)	0.163(0.403)^{ef}	0.195(0.441)^{e}	19.9
9	Siddhi-BC_2F_6BPH-BL-56(MR)	0.201(0.448)^{cd}	0.262(0.512)^{cd}	30.6
10	Siddhi-BC_2F_6BPH-BL-57(MR)	0.254(0.503)^{b}	0.292(0.54)^{bc}	14.8
11	Siddhi-BC_2F_6BPH-BL-60(MR)	0.182(0.427)^{de}	0.269(0.519)^{cd}	47.7
12	Siddhi-BC_2F_6BPH-BL-61(MR)	0.288(0.537)^{a}	0.295(0.543)^{bc}	22.0
13	TN1(S)	0.186(0.431)^{de}	0.133(0.364)^{g}	-28.4
14	PTB33(R)	0.217(0.466)^{cd}	0.356(0.596)^{a}	64.1
15	BM-71(R)	0.204(0.452)^{cd}	0.332(0.574)^{ab}	62.3
	CV	4.559	4.702	
	CD	0.033	0.041	

R=Resistant;MR=Moderatelyresistant;S=Susceptible

Means inacolumnfollowedbysameletterarenotsignificantlydifferentat5%levelbyDMRT

Figuresinparenthesisaresquareroottransformedvalues.

Reducing sugar

BPH infestation had greater influence on reducing sugar content. It was found that reducing sugar content was more in susceptible check than in resistant genotypes of Siddhi. The highest quantity of reducing sugar was present in susceptible check TN1 (1.211 mg g^{-1} tissue) whereas in resistant checks PTB33 and BM71, reducing sugar content was decreased from 0.326 to 0.286 mg g^{-1} tissue (12.4%) and 0.740 to 0.0.610 mg g^{-1} (17.6%), respectively. The percentage reduction of reducing sugars in the resistant genotypes ranged from 18.7% to 36.2%. The reducing sugar content in the healthy genotypes*viz*., Siddhi-BC2F6 BPH BL-11, Siddhi-BC2F6 BPH BL-12, Siddhi-BC2F6 BPH BL-19, Siddhi-BC2F6 BPH BL-52, Siddhi-BC2F6 BPH BL-56 and Siddhi-BC2F6 BPH BL-57 were significantly on par with resistant check BM71. The reducing sugar content in the infested genotypes*viz*., Siddhi-BC2F6 BPH BL-11, Siddhi-BC2F6 BPH BL-12, Siddhi-BC2F6 BPH BL-19, Siddhi-BC2F6 BPH BL-52, Siddhi-BC2F6 BPH BL-56 and Siddhi-BC2F6 BPH BL-57 were significantly on par with resistant check BM71 (Table 2).

The present results were in agreement with the findings of Reddy et al. (2004) who reported that the sugar content was reduced in TN1 after infestation. Similar results were reported by Udayasree*et al*. (2018) who conducted studies on biochemical aspects of resistance to BPH on resistant rice genotype and stated that the amount of total sugars was lowest in RNR 26111 (0.33 mg g^{-1}) and highest in susceptible check TN1 (2.97 mg g^{-1}). The present results were similar to the findings of Jayasimha *et al*. (2015) who reported that the soluble sugar content was reduced in the varieties after BPH damage. Similarly, Vanitha *et al*. (2011) also reported that reducing sugar content was more in susceptible varieties than resistant varieties.

	Paddy Genotypes	**ReducingSugars(mg g^{-1})**

Sl. No.		Healthy	Infested	% Decrease
1	Siddhi-BC_2F_6BPH-BL-30(R)	0.558(0.746)^{def}	0.398(0.624)^{cde}	28.6
2	Siddhi-BC_2F_6BPH-BL-43(R)	0.436(0.659)^{fg}	0.320(0.547)^{def}	26.6
3	Siddhi-BC_2F_6BPH-BL-64(R)	0.286(0.535)^{hi}	0.185(0.424)^{f}	35.4
4	Siddhi-BC_2F_6BPH-BL11(MR)	0.606(0.775)^{cde}	0.488(0.698)^{bcd}	19.5
5	Siddhi-BC_2F_6 BPH-BL12(MR)	0.775(0.877)^{bc}	0.630(0.785)^{b}	18.7
6	Siddhi-BC_2F_6BPH-BL-19(MR)	0.616(0.784)^{cde}	0.423(0.639)^{bcde}	31.3
7	Siddhi-BC_2F_6BPH-BL-24(MR)	0.420(0.644)^{fgh}	0.317(0.557)^{def}	24.6
8	Siddhi-BC_2F_6BPH-BL-52(MR)	0.702(0.833)^{bcde}	0.516(0.717)^{bc}	26.4
9	Siddhi-BC_2F_6BPH-BL-56(MR)	0.836(0.914)^{b}	0.534(0.713)^{bc}	36.2
10	Siddhi-BC_2F_6BPH-BL-57(MR)	0.631(0.792)^{cde}	0.480(0.684)^{bcd}	24.0
11	Siddhi-BC_2F_6BPH-BL-60(MR)	0.549(0.74)^{ef}	0.413(0.636)^{bcde}	24.7
12	Siddhi-BC_2F_6BPH-BL-61(MR)	0.271(0.521)^{i}	0.176(0.416)^{f}	35.0
13	TN1(S)	1.211(1.100)^{a}	0.913(0.955)^{a}	24.6
14	PTB33(R)	0.326(0.567)^{ghi}	0.286(0.528)^{ef}	12.4
15	BM-71(R)	0.740(0.859)^{bcd}	0.61(0.779)^{b}	17.6
	CV	3.006	3.154	
	CD	0.114	0.153	

Table 2. Reducing sugars content of promising advanced backcross derived genotypes of Siddhi

R=Resistant;MR=Moderatelyresistant;S=Susceptible
Means inacolumnfollowedbysameletterarenotsignificantlydifferentat5%levelbyDMRT
Figuresinparenthesisaresquareroottransformedvalues

Ascorbic acid

The studies on ascorbic acid content of leaf sheath of resistant genotypesindicated that the quantity of ascorbic acid in healthy genotypes of Siddhi-BC2F6 BPH BL-64, Siddhi-BC2F6 BPH BL-30 and Siddhi-BC2F6 BPH BL-61 were significantly on par with the resistant checks. The ascorbic acid content in susceptible check TN1 was found as 0.632 mg g^{-1}. Whereas in resistant checks PTB33 and BM71, the percentage decrease in ascorbic acid content was 14.2% and 12%, respectively. The highest quantity of ascorbic acid was present in Siddhi-BC2F6 BPH BL-64 (1.225 mg g^{-1}). The ascorbic content in infested genotypes*viz.*, Siddhi-BC2F6 BPH BL-30 and Siddhi-BC2F6 BPH BL-64 were significantly on par with resistant checks. The highest percentage decrease observed in susceptible check TN1 (59.1%). Percentage reduction of ascorbic acid content in the infested genotypesranged from 16% to 36% (Table 3). The results obtained were similar with the findings of Ramulamma *et al*. (2017) who reported that the BPH infestation caused decrease in ascorbic acid content in infested rice culture**s.**

Table3.Ascorbicacidcontentofpromisingadvancedbackcrossderivedgenotypesof Siddhi

Sl		Ascorbicacid(mg g^{-1})

No	Paddy Genotypes	Healthy	Infested	% Decrease
1	Siddhi-BC_2F_6BPH-BL-30(R)	1.153(1.073)^{ab}	0.899(0.948)^{a}	22.0
2	Siddhi-BC_2F_6BPH-BL-43(R)	0.557(0.744)^{f}	0.356(0.589)^{ef}	36.0
3	Siddhi-BC_2F_6BPH-BL-64(R)	1.225(1.107)^{a}	0.919(0.958)^{a}	25.0
4	Siddhi-BC_2F_6BPH-BL-11(MR)	0.769(0.876)^{cd}	0.646(0.803)^{b}	16.0
5	Siddhi-BC_2F_6BPH-BL-12(MR)	0.563(0.749)^{ef}	0.461(0.676)^{cde}	18.1
6	Siddhi-BC_2F_6BPH-BL-19(MR)	0.757(0.869)^{cd}	0.625(0.79)^{bc}	17.4
7	Siddhi-BC_2F_6BPH-BL-24(MR)	0.820(0.903)^{cd}	0.674(0.82)^{b}	17.8
8	Siddhi-BC_2F_6BPH-BL-52(MR)	0.554(0.743)^{f}	0.416(0.643)^{de}	24.9
9	Siddhi-BC_2F_6BPH-BL-56(MR)	0.760(0.864)^{cde}	0.568(0.73)^{bcd}	25.3
10	Siddhi-BC_2F_6BPH-BL-57(MR)	0.703(0.833)^{def}	0.508(0.711)^{bcd}	27.8
11	Siddhi-BC_2F_6BPH-BL-60(MR)	0.755(0.868)^{cd}	0.617(0.785)^{bc}	18.3
12	Siddhi-BC_2F_6BPH-BL-61(MR)	0.930(0.964)^{ab}	0.665(0.812)^{b}	28.5
13	TN1(S)	0.632(0.794)^{def}	0.258(0.508)^{f}	59.1
14	PTB33(R)	1.118(1.056)^{ab}	0.959(0.979)^{a}	14.2
15	BM-71(R)	1.043(1.021)^{ab}	0.918(0.958)^{a}	12.0
	CV	3.762	3.892	
	CD	0.117	0.116	

R=Resistant;MR=Moderatelyresistant;S=Susceptible

Means inacolumnfollowedbysameletterarenotsignificantlydifferentat5%levelbyDMRT

Figuresinparenthesisaresquareroottransformedvalues

Nitrogen

The nitrogen (N) content in the backcross derived genotypes, resistant check (PTB33) and susceptible check (TN1) varied. The highest percentage of nitrogen (0.91%) was found in the healthy susceptible check TN1 while in the resistant and moderately resistant genotypes, the total N content ranged from 0.73% to 0.89%, thus showed significantly lower N content than susceptible check TN1. The total N content in the genotypes *i.e.*, Siddhi-BC2F6 BPH BL-43 and Siddhi-BC2F6 BPH BL-24 was significantly on par with the resistant checks. In general, the total N content in the infested genotypes decreased over healthy plants in most of the genotypes. The percentage reduction in N content of the genotypes ranged from 6.8% to 25.3%. Percentage decrease in the N content was highest in the susceptible check TN1 (35.2%). Whereas in the resistant checks, PTB33 and BM71 decrease in the N content was 10.1% and 12.5%, respectively (Table 4).

The present results were in accordance with the findings of Dharshini and Gowda (2014) who reported after BPH infestation, the total nitrogen was reduced in all varieties after BPH damage. The present results were similar with the findings of Watanabe and Kitigawa (2000) who reported that BPH feeding on rice plants caused decrease in the contents of N and thus reduced the growth of main shoot and tillers.

Table4.Nitrogencontentof promisingadvancedbackcrossderivedgenotypesofSiddhi

Sl		Nitrogen(%)

No	Paddy Genotypes	Healthy	Infested	% Decrease
1	Siddhi-BC_2F_6BPH-BL-30(R)	0.84(1.16)^{cde}	0.75(1.12)^{bc}	10.7
2	Siddhi-BC_2F_6BPH-BL-43(R)	0.76(1.12)^{ghi}	0.67(1.08)^{efg}	11.8
3	Siddhi-BC_2F_6BPH-BL-64(R)	0.86(1.17)^{cd}	0.79(1.14)^{ab}	8.10
4	Siddhi-BC_2F_6BPH-BL-11(MR)	0.89(1.18)^{ab}	0.80(1.14)^{a}	10.1
5	Siddhi-BC_2F_6BPH-BL-12(MR)	0.73(1.11)^{i}	0.68(1.09)^{ef}	6.80
6	Siddhi-BC_2F_6BPH-BL-19(MR)	0.81(1.14)^{ef}	0.66(1.08)^{efg}	18.5
7	Siddhi-BC_2F_6BPH-BL-24(MR)	0.79(1.13)^{gh}	0.62(1.06)^{hi}	21.5
8	Siddhi-BC_2F_6BPH-BL-52(MR)	0.84(1.16)^{bc}	0.77(1.13)^{bc}	8.30
9	Siddhi-BC_2F_6BPH-BL-56(MR)	0.81(1.14)^{ef}	0.73(1.11)^{cd}	9.90
10	Siddhi-BC_2F_6BPH-BL-57(MR)	0.87(1.17)^{bc}	0.65(1.07)^{gh}	25.3
11	Siddhi-BC_2F_6BPH-BL-60(MR)	0.88(1.17)^{bc}	0.75(1.12)^{bc}	14.8
12	Siddhi-BC_2F_6BPH-BL-61(MR)	0.85(1.16)^{cde}	0.64(1.07)^{gh}	24.7
13	TN1(S)	0.91(1.19)^{a}	0.59(1.04)^{i}	35.2
14	PTB33(R)	0.79(1.14)^{gh}	0.71(1.10)^{cde}	10.1
15	BM-71(R)	0.80(1.14)^{g}	0.70(1.10)^{cde}	12.5
	CV	4.07	4.27	
	CD	0.062	0.058	

R=Resistant;MR=Moderatelyresistant;S=Susceptible

Means inacolumnfollowedbysameletterarenotsignificantlydifferentat5%levelbyDMRT

Figuresinparenthesisaresquareroottransformedvalues

Phosphorus

The percentage increase of phosphorous content in the resistant genotypes ranged from 4.6% to 12.9%. Whereas in resistant checks PTB33 and BM71, the percentage increase in phosphorous content was 11.1% and 15%, respectively. The phosphorous content in healthy genotypes Siddhi-BC2F6 BPH BL-12, Siddhi-BC2F6 BPH BL-19, Siddhi-BC2F6 BPH BL- 56, Siddhi-BC2F6 BPH BL-57 and Siddhi-BC2F6 BPH BL-61 was significantly on par with the resistant checks. However, in Siddhi-BC2F6 BPH BL-11 (2.3%) decrease in the phosphorus content was recorded (Table 5). The results obtained were inconformity with findings of Ramulamma *et al.* (2017) who reported marginal differences in the phosphorus content of the infested cultures compared to uninfested cultures. Similar results were reported by Vanitha *et al.* (2011) who stated that the per cent reduction of P and K was much less upon BPH infestation compared to N content.

Table5.Phosphorouscontentofpromisingadvancedbackcrossderivedgenotypesof Siddhi

Sl. No.	Paddy Genotypes	Phosphorous(%)		
		Healthy	Infested	% Increase
1	Siddhi-BC_2F_6BPH-BL-30(R)	0.519(0.72)^{b}	0.557(0.746)^{bcd}	7.40
2	Siddhi-BC_2F_6BPH-BL-43(R)	0.613(0.783)^{a}	0.675(0.821)^{a}	10.1
3	Siddhi-BC_2F_6BPH-BL-64(R)	0.635(0.797)^{a}	0.664(0.815)^{a}	4.60

4	Siddhi-BC_2F_6BPH-BL-11(MR)	0.617(0.785)^{a}	0.603 (0.776)^{abc}	-2.30
5	Siddhi-BC_2F_6BPH-BL-12(MR)	0.461(0.679)^{c}	0.513(0.716)^{d}	11.2
6	Siddhi-BC_2F_6BPH-BL-19(MR)	0.495(0.703)^{bc}	0.540(0.734)^{cd}	9.10
7	Siddhi-BC_2F_6BPH-BL-24(MR)	0.382(0.618)^{d}	0.505(0.709)^{d}	32.0
8	Siddhi-BC_2F_6BPH-BL-52(MR)	0.590(0.768)^{a}	0.638(0.798)^{ab}	8.10
9	Siddhi-BC_2F_6BPH-BL-56(MR)	0.479(0.692)^{bc}	0.519(0.72)^{cd}	8.40
10	Siddhi-BC_2F_6BPH-BL-57(MR)	0.469(0.685)^{bc}	0.529(0.727)^{cd}	12.9
11	Siddhi-BC_2F_6BPH-BL-60(MR)	0.595(0.771)^{a}	0.652(0.807)^{a}	9.60
12	Siddhi-BC_2F_6BPH-BL-61(MR)	0.368(0.606)^{de}	0.413(0.641)^{e}	12.2
13	TN1	0.481(0.693)^{bc}	0.521(0.721)^{cd}	8.20
14	PTB33	0.325(0.57)^{e}	0.361(0.601)^{e}	11.1
15	BM-71	0.461(0.678)^{c}	0.53(0.726)^{cd}	15.0
	CV	3.344	4.825	
	CD	0.039	0.059	

R=Resistant;MR=Moderatelyresistant;S=Susceptible

Means inacolumnfollowedbysameletterarenotsignificantlydifferentat5%levelbyDMRT

Figuresinparenthesisaresquareroottransformedvalues

Potassium

Percentage increase of potassium content in resistantgenotypes ranged from 7.4% to 33.1%. The potassium content in resistant checks PTB33 and BM71 increased from 16% and 14.1%, respectively. Whereas the resistant and moderately resistant genotypesshowed significant increase in the potassium content after BPH infestation. The potassium content in the healthy resistant genotypes*i.e.*, Siddhi-BC2F6 BPH BL-30, Siddhi-BC2F6 BPH BL-43, Siddhi-BC2F6 BPH BL-11, Siddhi-BC2F6 BPH BL-24, Siddhi-BC2F6 BPH BL-56, Siddhi-BC2F6 BPH BL-57, Siddhi-BC2F6 BPH BL-60 was significantly on par with the resistant checks. Significantly highest potassium content (0.274%) was recorded in infested Siddhi-BC2F6 BPHBL-64 compared to other infested backcross derived genotypes. Potassium content in all the resistant genotypes recorded an increase after BPH infestation, the highest being 33.1% in Siddhi-BC2F6 BPH BL-61 (Table 6).

The present results were in conformity with the findings of Ramulamma *et al.* (2017) who recorded the significant increase in the potassium content in the resistant and moderately resistant cultures of rice after infestation. Similarly, Vanitha *et al.* (2011) reported that K content was slightly increased in the BPH infested plants. Liu *et al*. (2005) also reported that BPH infestation caused a reduction in the K uptake of roots in rice varieties and the influence became more serious with the increase of BPH density and the prolongation of infestation duration. From the present study it can be concluded that the infestation of BPH leads to reduction in nitrogen, ascorbic acid and reducing sugars content but increase in phenols, phosphorus and potassium contents, compared to the susceptible check the increase is greater in resistant and moderately resistant genotypes.

Table6.Potassiumcontentofpromisingadvancedbackcrossderivedgenotypesof Siddhi

Sl. No.	Paddy Genotypes	Potassium(%)		
		Healthy	Infested	% Increase
1	Siddhi-BC_2F_6BPH-BL-30(R)	0.190(0.436)^{cdef}	0.222(0.471)^{e}	16.8

2	Siddhi-BC_2F_6BPH-BL-43(R)	0.232(0.481)^{b}	0.279 (0.528)^{abc}	20.1
3	Siddhi-BC_2F_6BPH-BL-64(R)	0.274(0.524)^{a}	0.297(0.548)^{a}	8.30
4	Siddhi-BC_2F_6BPH-BL-11(MR)	0.224(0.473)^{bc}	0.283(0.542)^{ab}	26.5
5	Siddhi-BC_2F_6BPH-BL-12(MR)	0.159(0.399)^{fg}	0.212(0.473)^{e}	33.1
6	Siddhi-BC_2F_6BPH-BL-19(MR)	0.129(0.359)^{h}	0.164(0.404)^{f}	26.9
7	Siddhi-BC_2F_6BPH-BL-24(MR)	0.208(0.455)^{bcd}	0.233(0.488)^{cde}	12.2
8	Siddhi-BC_2F_6BPH-BL-52(MR)	0.171(0.414)^{efg}	0.158(0.4)^{f}	-8.00
9	Siddhi-BC_2F_6BPH-BL-56(MR)	0.217(0.464)^{bc}	0.233(0.485)^{de}	7.40
10	Siddhi-BC_2F_6BPH-BL-57(MR)	0.198(0.443)^{bcde}	0.217(0.476)^{e}	9.80
11	Siddhi-BC_2F_6BPH-BL-60(MR)	0.216(0.464)^{bc}	0.256(0.519)^{abcd}	18.5
12	Siddhi-BC_2F_6BPH-BL-61(MR)	0.179(0.423)^{def}	0.236(0.486)^{de}	31.8
13	TN1	0.144(0.38)^{gh}	0.153(0.391)^{f}	5.80
14	PTB33	0.225(0.474)^{bc}	0.261(0.506)^{cde}	16.0
15	BM-71	0.224(0.472)^{bc}	0.256(0.502)^{bcde}	14.1
	CV	3.386	4.261	
	CD	0.04	0.042	

R=Resistant;MR=Moderatelyresistant;S=Susceptible

Means inacolumnfollowedbysameletterarenotsignificantlydifferentat5%levelbyDMRT

Figuresinparenthesisaresquareroottransformedvalues

Conclusion

The BPH infestation in resistant genotypes and resistant checks resulted in an increase in the phenolic content in the infested plant, whereas in the susceptible TN1, reduction was observed. Significantly highest phenolic content (0.295 mg g-1 tissue) was observed in infested Siddhi-BC2F6 BPH-BL-61 compared to all genotypes. The total reducing sugar content was significantly highest in the healthy susceptible check TN1 (1.211 mg g-1). There was a decrease in total reducing sugars in all the plants upon BPH infestation. In infested genotypes there was a reduction in the ascorbic acid content in almost all the genotypes including the checks. The lowest quantity of ascorbic acid in healthy resistant and moderately resistant genotypes present in Siddhi-BC2F6 BPH BL-52, Siddhi-BC2F6 BPH BL-43 and Siddhi-BC2F6 BPH BL-12 (0.554 mg g^{-1} , 0.557 mg g^{-1} and 0.563 mg g^{-1}, respectively) which were on par with the susceptible check TN1 (0.632 mg g^{-1}) while the highest ascorbic acid quantity was present in Siddhi-BC2F6 BPH BL-30 and Siddhi-BC2F6 BPH BL-64 (1.153 mg g^{-1} and 1.225 mg g^{-1}). The healthy plants of susceptible TN1 had higher nitrogen content. Significant decrease of nitrogen was recorded in infested plants of susceptible TN1 (35.2%) than the resistant genotypes. The percentage decrease in the nitrogen % of the genotypes ranged from 8.1% to 25.3%. There was increase in phosphorus content in resistant and moderately resistant genotypes before and after infestation, the highest phosphorous % was recorded in infested Siddhi-BC2F6 BPH-BL-43 (0.675%). The highest potassium % was present in infested Siddhi-BC2F6 BPH-BL-64 (0.297%) followed by Siddhi-BC2F6 BPH-BL-11 (0.283%). Increase in potassium content was observed in resistant and moderately resistant genotypes and slight increase was recorded in susceptible check TN1 after BPH infestation.Overall, it can be concluded that upon BPH infestation in resistant and moderately resistant genotypes higher amounts of phenols, potassium, phosphorous and reduced level of nitrogen, reducing sugars and ascorbic

acid were observed.

Future Scope

Understanding the biochemical pathways and defense mechanisms can pave the way for identifying key resistance genes and biochemical markers, facilitating marker-assisted selection in breeding programs.

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1.
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