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Biochemical Analysis of Resistance in Paddy to BPH, Nilaparvata Lugens

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Abstract The present studies were carried out in the glasshouse condition at DRR, Rajendranagar, Hyderabad, to study the biochemical basis of resistance in the identified sources of resistance. BPH fed rice plants were analyzed spectrophotometrically for quantitative changes in biochemical profile that occurs as plant's defensive responses. The plant responded defensively upon infestation, resulting in low level of protein synthesis, production of higher amounts of phenolic compounds and reduced level of reducing sugars and ascorbic acid. The per cent reduction of P was less and K was much less upon BPH infestation compared to N content.

Keywords Mechanisms of resistance, Screening, Germplasm accessions, Honeydew, Antixenosis.

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Introduction

Rice (*Oryza sativa*) is an important cereal crop and a source of calories for one-third of world population. Rice has been reported to be infested by a wide array of pests and among them, the brown planthopper (BPH) *Nilaparvata lugens* (Stal.) (Homoptera: Delphacidae), is one of the major pest causing frequent outbreaks [1] and severe yield reductions. Host plant resistance is one of the most economical, effective and most practical methods of pest management. The effect of resistant variety on the pest population is specific, cumulative and persistent. A resistant plant variety that reduces the insect population by 50% in each generation is sufficient to eliminate an insect of economic importance within a few generations [2].

The necessity to identify suitable new donors resistant to BPH from different sources is of utmost importance in order to combat the pest and develop material resistant to different biotypes. It is also necessary to understand the factors which are responsible for manifesting the resistance into the selected cultures with desirable characters so that these can be utilized effectively in the breeding program. The germplasm accessions that were found to be resistant and moderately resistant [3] were analysed for buiochemical bases of resistance.

Materials and Methods

BPH was mass reared on the susceptible rice variety Taichung Native 1 (TNI) to produce enough nymphs

Table 1. Total phenols, reducing sugars and ascorbic acid in the healthy and BPH infested plants of selected rice germplasm accessions. R=Resistant; MR=Moderately resistant; S=Susceptible. Means with same letter are not significantly different at 5% level by DMRT.

		Total phenols mg/g tissue			Total reducing sugars mg/g tissue			Total ascorbic acid mg/g tissue		
Sl. No.	IC Nos.	Healthy plants	Infested plants	% increase	Healthy plants	Infested plants	% decrease	Healthy plant	Infested plants	% decrease
1	578151 (R)	0.142^{g}	0.242^{bcd}	70.42	0.306^{hg}	0.153^{g}	49.98	1.171^{b}	1.054^{a}	9.99
2	464186 (R)	0.147^{fg}	0.233^{cd}	58.50	0.185^{i}	0.162^{fg}	12.43	1.405^{a}	0937^{b}	33.30
3	463837 (R)	0.157^{fg}	0.189^{def}	20.38	0.682^{d}	0.531^{d}	22.14	1.171^{b}	0.820^{c}	29.98
4	577478 (MR)	0.175^{efg}	0.245^{bcd}	40.28	0.478^{e}	0.203^{fg}	57.52	0.937^{d}	0.626^{e}	33.19
5	578145 (MR)	0.240^{bcd}	0.198^{def}	-17.49	0.682^{d}	0.223^{f}	52.63	0.820^{e}	0.937^{b}	-14.26
6	578665 (MR)	0.243^{bcd}	0.172^{ef}	-29.21	0.392^{f}	0.165^{fg}	57.90	0.468^{g}	0.351^{e}	25.00
7	463851 (MR)	0.297^{ab}	0.199^{def}	-33.51	0.303^{g}	0.190^{fg}	37.28	0.820^{e}	0.585^{f}	28.64
8	577741 (MR)	0.206^{cdef}	0.151^{f}	-26.58	0.242^{h}	0.184^{fg}	23.98	0.937^{d}	0.820^{de}	12.47
9	578144 (MR)	0.160^{fg}	0.148^{f}	-7.36	0.643^{d}	0.303^{e}	52.87	0.820^{e}	0.699^{d}	14.74
10	578443 (MR)	0.248^{bc}	0.298^{ab}	20.25	0.971^{b}	0.696^{c}	28.32	0.468^{g}	0.351^{f}	25.00
11	464128 (MR)	0.187^{defg}	0.205^{def}	9.59	0.494^{e}	0.203^{fg}	58.90	1.171^{b}	0.937^{b}	19.98
12	578413 (MR)	0.162^{fg}	0.172^{ef}	6.17	0.468^{e}	0.168^{fg}	64.10	0.468^{g}	0.351^{f}	25.00
13	578017 (MR)	0.203^{cdef}	0.283^{abc}	39.4	0.762^{c}	0.776^{b}	-1.83	0.820^{e}	0.585^{e}	28.64
14	TN1 (S)	0.318^{a}	0.214^{de}	-32.82	1.206^{a}	0.921^{a}	23.63	0.585^{f}	0.234^{g}	60.00
15	Ptb33 (R)	0.231^{cde}	0.335^{a}	45.02	0.282^{gh}	0.180^{fg}	36.17	1.054^{c}	0.937^{b}	11.10
SEm±		0.022	0.025		0.02	0.019		0.021	0.034	
CD (p=0.05		0.065	0.074		0.059	0.056		0.054	0.1	

for infestation. BPH population was initially collected from the rice fields and pure culture was maintained in the glasshouse at a temperature of 30^{o}C±5^{o}C with a relative humidity of 60±5% on 40–50 day old potted plants of NT1. Mass rearing was done in cages. Second and third instars were collected and used for experiments.

Fourteen germplasm accessions that were found to be resistant and moderately resistant to BPH along with susceptible check TN1 and resistant check Ptb 33 were sown separately in pots. Later, fifteen-day-old seedlings were transplanted in 10-cm diameter clay pots. The potted plants were covered with cylindrical Mylar cages (13×90 cm). After 30-days of transplanting, second or third-instar BPH nymphs were released at the rate of 10/plant. Biochemical analyses were carried out at seven days after infestation. The plants maintained without any BPH infestation served as control for each analysis. Three replications were maintained for each test. One gram of oven dried plant sample taken from both control and infested plants in each variety was analyzed for nitrogen on whole plant basis by using the standard microkjeldahl method [4] and were expressed as percentage. Potassium and phosphorus were determined by wet digestion method [4] using triacid mixture (nitric, sulfuric and perchloric acids in 9:2:1 ratio with the help of ELICO flame photometer (Model Cl-22A) and spectronic 20, respectively and data were expressed as percentage. Quantity of total phenols present in leaf sheath was estimated by calorimetric assay, reducing sugars by DNS method and total ascorbic acid by volumetric method as described by Sadasivan and Manickam [5] and expressed as mg g-l dry weight. The data obtained from the biochemical experiments were statistically analyzed in a completely randomized block design and different parameters observed in the experiments were subjected to LSD by using the DMRT package which was used for comparing the means.

Results and Discussion

Nutrients may play an important role in resistant mechanism in rice against BPH. The growth and development of rice plants are mainly affected by the function of nutrient uptake particularly N, P and K in

Table 2. Total nitrogen and protein % in the healthy and BPH infested plants of selected rice germplasm accessions. R=Resistant; MR=Moderately resistant; S=Susceptible. Means with same letter are not significantly different at 5% level by DMRT.

Sl. No.	IC Nos.	% Nitrogen Healthy plants	% Nitrogen Infested plants	% Nitrogen % decrease	% Protein Healthy plants	% Protein Infested plants	% Protein % decrease
1	578151 (R)	1.28^{hi}	1.260^{e}	1.27	7.98^{gh}	7.88^{f}	1.27
2	463837 (R)	1.25^{f}	1.24^{e}	0.77	7.79^{h}	7.73^{f}	0.77
3	577478 (MR)	1.48^{g}	1.47^{d}	1.25	9.28^{f}	9.16^{e}	1.25
4	578145 (MR)	1.49^{g}	1.47^{e}	1.24	9.34^{f}	9.23^{de}	1.24
5	463851 (MR)	1.52^{g}	1.54^{c}	-0.9	9.54^{f}	9.63^{c}	-0.09
6	578665 (MR)	1.65^{f}	1.53^{c}	7.32	10.33^{de}	9.57^{cd}	7.32
7	577741 (MR)	1.64^{f}	1.58^{c}	3.82	10.24^{e}	9.85^{c}	3.82
8	465106 (MR)	1.72^{de}	1.65^{b}	4.43	10.76^{c}	10.29^{b}	4.43
9	577663 (MR)	1.69^{def}	1.53^{c}	9.48	10.59^{cd}	9.59^{cd}	9.48
10	578144 (MR)	1.67^{ef}	1.45^{d}	13.25	10.44^{de}	9.05^{e}	13.25
11	578443 (MR)	1.79^{bc}	1.74^{a}	3.08	11.20^{b}	10.86^{a}	3.08
12	464128 (MR)	1.82^{ab}	1.71^{a}	6.15	11.38^{ab}	10.68^{a}	6.15
13	578017 (MR)	1.74^{cd}	1.55^{c}	10.44	10.85^{c}	9.72^{c}	10.44
14	578413 (MR)	1,80^{ab}	1.75^{a}	2.95	11.29^{ab}	10.96^{a}	2.95
15	TN1 (S)	1.85^{a}	1.53^{c}	17.28	11.56^{a}	9.57^{cd}	17.28
16	Ptb 33 (R)	1.32^{h}	1.218^{e}	7.39	8.22^{g}	7.61^{f}	7.39
SEm±		0.015	0.019		0.183	0.117	
CD (p=0.05)		0.044	0.055		0.529	0.338	

root systems and so pests feeding on them. The biochemical bases of resistance with regard to phenols, reducing sugars, ascorbic acid and NPK among the resistant and moderately varieties in comparison with susceptible and resistant checks are presented hereunder:

Phenols

The total phenolic content in the leaf sheaths was significantly different between the susceptible check (TN1) and the uninfested (healthy) resistant and moderately resistant cultures (Table 1). Significantly highest phenolic content (0.297 mg g^{-1} tissue) was observed in uninfested 463851 compared to all test cultures including resistant check Ptb 33 except susceptible check TN1. The BPH infestation influenced phenolic production in most of the resistant and moderate resistant cultures whereas in the BPH susceptible TN1 the total phenols reduced from 0.318 to 0.214 mg/g tissue of the leaf sheaths i.e. by 32.82%. 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 Pathak and Khush [6]. Grayer et al. [7] found higher levels of phenolics in the resistant rice varieties than in susceptible ones studied, suggesting their involvement in the resistance of rice to BPH. In contrast, Mishra and Misra [8] reported that the resistant varieties such as Pundia and Handi Sarakanthi possessed lower concentration of phenols than susceptible check TN1. Singh [9] reported that total phenol contents decreased in all infected plant parts. Similar results were reported by Kushwaha and Narain [10]. In our findings, slight decrease in the phenolic content was observed in moderately resistant cultures 578145, 578665, 463851, 577741 and 578144 which were either on par or significantly lower than in TN1. Sujatha et al. [11] stated that phenols were positivity correlated with resistance against BPH.

Reducing sugar

BPH infestation had greater influence o reducing sugar content. It was observed that reducing sugar content was more in susceptible varieties than in resistant varieties. The highest quantity of reducing sugar was present in susceptible variety TN1 (1.206 mg/g tissue) (Table 1). Resistant and moderately resistant cultures had less quantity of reducing sugars than susceptible variety which ranged between 0.185 to 0.971 mg/g tissue. Percentage of decrease in reducing sugars over healthy cultures were observed in 578413 (64.10%) and 464128 (58.90%). This finding is in line with the earlier findings which stated that reducing sugar content was high in susceptible varieties than in resistant varieties Vanitha et al. [12]. The quantity of reducing sugars decreased in the infested cultures than healthy ones (12.43% to 64.10%) except in 578017 wherein a slight increase was observed (1.83%). Jain and Yadav [13] reported higher total phenol content in resistant genotypes and high reducing sugars in susceptible genotypes of finger millet.

Ascorbic acid

The studies on ascorbic acid content (Table 1) of leaf sheaths indicated that less quantity of ascorbic acid was present in healthy cultures of 578665, 578443 and 578413 (0.468 mg/g each) which were on par with the susceptible check TN1 (0.585 mg/g). The highest quantity of ascorbic acid was present in 578151, 463837 and 464128 (1.171 mg/g) followed by 464186 (1.405 mg/g) and the remaining six moderately resistant accessions. The highest % decrease observed in susceptible check TN1 (60.00%). In the infested paddy cultures, there was a reduction in the ascorbic acid content in almost all the resistant and moderately resistant cultures including the checks (9.99%) to 60%) except in 578145 where a marginal increase (14.26%) was recorded. Studies on the effect of nutrient compounds on sucking response of BPH Sakai and Sogawa [14] showed that vitamins such as ascorbic acid inhibited feeding at higher concentrations than at lower concentrations.

The studies on biochemical aspects of resistance to BPH indicated that mechanism of resistance in case of the cultures tested is complex. It showed that the plant responds defensively upon infestation, resulting in low level of protein synthesis; production of higher amounts of phenolic compounds and reduced levels of reducing sugars and ascorbic acid. Probably these factors alone or in combination might have a role in antibiosis and non preference. In contrast, lower availability of phenolic compounds as toxic principle, depletion of reducing sugars and ascorbic acid might have increased the susceptibility of TN1 plants to the BPH.

Nitrogen

The nitrogen (N) content in the selected healthy rice germplasm accessions, resistant check (Ptb 33) and susceptible check (TN1) varied with the culture. The data showed highest per cent (1.85%) N is the susceptible check TN1 (Table 2) while in the resistant and moderately resistant cultures, the total N content ranged from 1.25 (463837) to 1.82% (464128), thus, showed significantly lower N content than susceptible check TN1 (1.85%). The total N content in the resistant cultures 463837 and 578151 was 1.25 and 1.28%, respectively. These were on par with the resistant check Ptb 33 (1.32%). In general the total N content in the infested cultures decresed over healthy plants in most of the rice cultures and it varied with the culture. Decrease in the N content was highest in the susceptible variety TN1 (17.28%). Sujatha et al. [11] also indicated that nitrogen was negatively correlated with resistance. Hu et al. [15] reported that the yield loss in mid season hybrid rice due to WBPH and BPH injury was positively correlated with the level of nitrogen fertilizer applied. BPH feeding on rice plants caused decrease in the contents of N and photosynthetic products in the leaves and thus reduced the growth of main shoot and tillers [16]. Heinrichs and Medrano [17] reported that weight, feeding rate, and population growth of BPH increased with nitrogen application on IR 26, Utri Rajapan and Triveni. The population growth increased 3- fold with added nitrogen on IR 26 and Utri Rajapan and - fold in the moderately resistant Triveni.

Protein content

The total protein content in plant in selected germplasm accessions in healthy and uninfested plants is presented in Table 2. Similar to nitrogen, total protein content varied with the healthy rice cultures. Total protein was highest in the susceptible TN1 (11.56%) and lowest in the resistant culture 578151 (7.98%) and 463837 (7.79%) and these were on par with each other and the resistant check Ptb 33. The total protein content decreased in the infested

Table 3. Total phosphorus and potassium % in the healthy and BPH infested plants of selected rice germplasm accessions. R=Resistant; MR=Moderately resistant; S=Susceptible. Means with same letter are not significantly different at 5% level by DMRT.

Sl. No.	IC Nos.	% Phosphorus Healthy plants	% Phosphorus Infested plants	% Phosphorus % increase	% Potassium Healthy plants	% Potassium Infested plants	% Potassium % increase
1	578151 (R)	0.348^{g}	0.359^{h}	3.25	0.226^{bcd}	0.304^{b}	34.51
2	463837 (R)	0.515^{d}	0.5^{ef}	-2.91	0.218^{abcd}	0.394^{a}	80.73
3	577478 (MR)	0.646^{b}	0.681^{b}	6.07	0.181^{def}	0.215^{de}	18.78
4	578145 (MR)	0.367^{fg}	0.584^{cd}	58.98	0.272^{b}	0.258^{bcde}	-5.14
5	463851 (MR)	0.468^{de}	0.489^{f}	4.55	0.244^{bc}	0.361^{a}	47.95
6	578665 (MR)	0.454^{e}	0.457^{fg}	0.66	0.124^{f}	0.158^{f}	27.41
7	577741 (MR)	0.826^{a}	0.852^{a}	3.14	0.261^{b}	0.286^{bc}	9.57
8	465106 (MR)	0.615^{bc}	0.679^{b}	10.40	0.248^{bc}	0.283^{bc}	14.11
9	577663 (MR)	0.668^{b}	0.847^{a}	26.79	0.258^{b}	0.236^{cde}	-8.52
10	578144 (MR)	0.636^{bc}	0.552^{de}	-13.20	0.158^{ef}	0.267^{bcd}	68.63
11	578443 (MR)	0.615^{bc}	0.662^{b}	7.69	0.327^{a}	0.363^{a}	11.00
12	464128 (MR)	0.671^{b}	0.675^{b}	0.59	0.271^{b}	0.284^{bc}	4.79
13	578017 (MR)	0.579^{c}	0.609^{c}	5.12	0.231^{bcd}	0.249^{bcde}	7.79
14	578413 (MR)	0.447^{e}	0.472^{fg}	5.59	0.178^{def}	0.268^{bcd}	50.00
15	TN1 (S)	0.385^{fg}	0.424^{g}	10.03	0.193^{cde}	0.202^{ef}	4.663
16	Ptb 33 (R)	0.414^{ef}	0.448^{fg}	8.21	0.214^{bcde}	0.236^{cde}	10.28
SEm±		0.006	0.033		0.029	0.017	
CD (p=0.05)		0.017	0.095		0.085	0.049	

cultures compared to healthy cultures. Liu et al. [18] and Wang et al. [19] reported that BPH feeding caused only slight reduction in protein contents in resistant varieties and also grew normally. Sujatha et al. [11] indicated that protein content was negatively correlated with resistance. This decrease in nutrient content may induce several changes in plants, which make plant less palatable for insects and also their development Usharani and Jyothsna [20]. Vanitha et al. [12] 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.

Phosphorus content

Phosphorus content in the uninfested resistant and moderately resistant cultures ranged between 0.414 to 0.826% and variations appeared to be marginal even though the differences were significant (Table 3). Marginal differences were observed in phosphorus content of the infested cultures compared to healthy cultures. In many cultures marginal increase in the phosphorus content was observed in the infested compared to healthy ranging from 0.59 to 26.79, except 578145 where the increase was 58.96%. However, in 463837 (2.91%) and 578144 (13.20%) marginal % decrease in the phosphorus content was observed. Sujatha et al. [11] proved that phosphorus positively correlated with BPH resistance. The per cent reduction of P was less and K was much less upon BPH infestation compared to N content Vanitha et al. [12].

Potassium content

Potassium content in resistant, moderately resistant cultures varied between 0.124 (578665) to 0.327 (578443) % in the healthy cultures. The variations in the potassium content in resistant and moderately resistant cultures were marginal (Table 3). Interestingly, in the resistant and moderately resistant cultures significant increase in the potassium content was recorded after infestation. The increase was highest in the resistant culture 463837 and ranged from 0.217 in the healthy to 0.394% in the infested culture i.e. 80.73% increase. In the susceptible and resistant checks, the increase in potassium content in the infested compared to healthy, was marginal. Even though the potassium content was more or less similar in resistant and susceptible cultivars, some increase in K content was observed in resistant cultures while it remained almost same in susceptible TN1 after BPH infestation. Sujatha et al. [11] positively correlated potassium content with BPH resistance. Vanitha et al. [12] reported that K content was slightly increased in the BPH infestation plants. From the present studies it can be referred that the infestation of BPH leads to reduction in nitrogen and protein content but increase in phosphorus and potassium contents. Compared to the susceptible plant the increase is greater in resistant and moderately resistant plants.

Conclusion

The plants responded defensively upon infestation, resulting in low level of protein synthesis; production of higher amounts of phenolic compounds and reduced level of reducing sugars and ascorbic acid. The percent reduction of P was less and K was much less upon BPH infestation compared to N content.

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