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Biochemical basis of resistance in rice against Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera : Cecidomyiidae)

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ABSTRACT

A total of 1482 genotypes at Zonal Agricultural Research Station, V. C. Farm, Mandya and 416 genotypes at Agricultural Research Station, Kankanady, Mangalore under AICRP (Rice) were evaluated both in field and greenhouse conditions during Kharif 2006 and 2007. The estimation of biochemical constituents in rice shoot epics (30 day old plants) of selected resistant and susceptible genotypes was done to establish the relationship between various biochemical contents and to compare it with resistance and susceptibility. The studies revealed that the higher level of total phenols and total free amino acids was observed in majority of the resistant genotypes compared to susceptible entries. The amount of total sugars, reducing sugar and crude proteins in all susceptible genotypes was found higher compared to resistant genotypes. However, the amount of total sugars, reducing sugars, crude proteins and amino acids were not related to resistance.

KEY WORDS: *Orseolia oryzae*, boichemicals, host plant resistance, *Oryza sativa* L.

INTRODUCTION

The Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera: Ceceidomyiidae) is a major insect pest of rice in several Asian countries (Bentur *et al.*, 2003). In India, gall midge has been reported from almost all the rice growing states except the Western Uttar Pradesh, Uttaranchal, Punjab, Haryana and Hill states of Himachal Pradesh and Jammu and Kashmir (Bentur *et al.*, 1992). The insect being endoparasitic, use of resistant varieties is the most economical and feasible tool to its suppression (Heinrichs and Pathak; 1981 Khush, 1997; Mathur *et al.*, 1999). But the emergence of new virulent biotypes of gall midge in popular rice varieties is capable of overcoming resistance and this is a cause for concern. So far 6 biotypes of gall midge were identified and characterized in India (Bentur *et al.*, 2003). Widespread cultivation of high yielding varieties made a radical change in the pest status of rice gall midge in coastal Karnataka.

A wide range of allelochemical compounds present in the plants play an important defensive role against insects and other herbivores. Several instances of associations have been reported between phenolics and the resistance of plants to insect damage (Panda and Khush, 1995), Peraiah and Roy (1979) observed higher amount of free amino acid and phenols in resistant varieties Shakti and CR 95-952-1 compared to susceptible variety Ratna. Higher concentrations of phenols in shoot epics of gall midge resistant rice varieties Shakti, Leuang 152, PTB 18, IET 7008, IET 7009 and Siam 29 have been reported without any regard to pest infestation (Vidyachandra *et al.*, 1981; Rajamani, 1982; Joshi and Venugopal, 1984). There were no detailed information is available regarding the association of sugars, total phenols, crude protein and total free amino acids with resistance in rice against homogenous and mixed populations.

MATERIAL AND METHODS

A total of 1482 genotypes at Zonal Agricultural Research Station, V. C. Farm, Mandya 12^0 32'N, 76^0 53'E, and 690 m AMSL) and 416 genotypes at Agricultural Research Station, Kankanady, Mangalore (12^0 54'N, 74^0 51'E, and 30m AMSL) under AICRP (Rice) were evaluated both under field and greenhouse conditions during Kharif 2006 and 2007. The estimation of biochemical constituents *viz.*, total sugars, reducing sugars, total phenols, crude proteins and total free amino acids in shoot epics collected from 30 days old plants of selected resistant and susceptible genotypes were determined.

Extraction of plant tissues in alcohol

Un-infested vegetative shoot epics of 0.5 cm (approx) from 30 days old plants of test entries were collected after stripling of the leaves and leaf-sheaths. The collected plant samples were thoroughly washed with distilled water and dried under shade. One gram of plant sample piece of all the genotypes were taken in separate conical flask and 15 ml of 80 per cent ethanol was added. It was refluxed for 30 minutes on hot water bath. After boiling, the extract was cooled and the pieces of tissues were ground thoroughly in a mortar with pestle in slight ethanol. The supernatant was decanted into another flask and residue was again re-extracted with small quantity of hot ethanol and decanted. The extract was filtered through Whatman No.1 filter paper and made up to a known volume with 80 per cent ethanol. The ethanol part of (alcoholic) extract was stored in refrigerator at 4^0C, and was used for the estimation of total sugars, reducing sugars and phenols.

Total and reducing sugars were estimated following Somogyi (1952). Total phenols in the shoot epics of the plant tissue by following Folin-Ciocalteau method (Bray and Thorpe, 1954), crude proteins by Micro-Kjeldahl method and the amount of total free amino acid by Ninhydrin method developed by Moore and Stein (1948). The data obtained were subjected to Analysis of Variance (ANOVA) (Gomez and Gomez, 1984; Hosmand, 1988) and means were separated by Duncan's multiple range test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

Mandya

During 2006 kharif, total sugars present in susceptible genotypes were 10.13-15.84 mg/g, in resistant genotypes the corresponding values varied from 6.81 to 10.24 mg/g. But in majority of the resistant genotypes lower amounts of sugars were recorded compared to susceptible genotypes. Similarly the amount of reducing sugars in susceptible genotypes varied from 6.22 to 10.21 mg/g. In resistant genotypes it varied between 3.80 to 6.22 mg/g. The total phenols present in resistant genotypes were 0.41 to 0.63 mg/g. In susceptible genotypes the values varied from 0.26 to 0.42 mg/g. Similarly, the amount of crude proteins in resistant genotypes ranged from 2.97 to 4.2 mg/g whereas, in susceptible genotypes it ranged from 5.19 to 6.78 mg/g. The total free amino acids in resistant genotypes varied from 22.56 to 34.82 mg/g. In susceptible genotypes it ranged from 16.45 to 27.63 mg/g. Similar results with respect to total sugars, reducing sugars, total phenols, crude protein and total free amino acids were recorded in 18 test genotypes during 2007 Kharif. In general, a majority of the resistant genotypes showed lower amounts of total sugars, reducing sugars, crude protein and higher amount of total phenols and total free amino acids compared to susceptible genotypes (Table 1).

Mangalore

The biochemical estimation on 20 genotypes during 2006 Kharif showed that the total sugars in resistant genotypes were 6.21 to 12.84 mg/g. In susceptible genotypes it varied from 7.82 to 15.22 mg/g. Similarly, reducing sugars in resistant genotypes varied from 3.86 to 8.24 mg/g. In susceptible genotypes it ranged from 4.71 to 19.84 mg/g. The total phenols in resistant genotypes varied between 0.32 to 0.68 mg/g and in susceptible entries the values ranged from 0.24 to 0.42 mg/g. The crude proteins in susceptible entries were higher with a range of 3.25 to 6.95 mg/g. In resistant genotypes it varied between 2.75 to 4.25 mg/g. Similarly, the total free amino acids in resistant entries were found to be higher with a range of 22.58 to 32.73 mg/g and the corresponding figures in susceptible genotypes varied from 20.21 to 30.28 mg/g. Thus, in general, a majority of the resistant genotypes showed lower total and reducing sugars, crude proteins, higher total phenols and total free amino acids compared to susceptible genotypes. During 2007 Kharif also similar results were obtained. Thus, the studies on biochemical parameters conducted at Mandya and Mangalore during 2006 and 2007 Kharif did not show clear cut evidence regarding association of total sugars, reducing sugars, total phenols, crude proteins and total free amino acids for resistance or susceptibility. But at both the locations, both the years, a majority of resistant genotypes recorded higher amounts of total phenols, total free amino acids and lower amount of total sugars, reducing sugars and crude proteins compared to susceptible genotypes (Table 2). Nevertheless, a correlation between resistance or susceptibility and the amount of above constituents could not be established.

The present results corroborate with that of Peraiah and Roy, (1979) who also reported higher amounts of total free amino acids and total phenols in resistant rice genotypes Shakti and CR-95-95 2-1 compared to susceptible (Rantna). Singh and Salam (1997) reported higher amount of higher amounts of Ortho-dihydroxy, bound and total phenols in all the resistant cultivars compared to susceptible genotypes. They also reported higher amounts of total sugars in susceptible genotypes *viz.*, RP 2346-1323, RPW 9-4-851 and TN1 compared to resistant entries such as ARC 6605, WGL 9181, W1253-1, RPW 9-6-12 and Assam Chudi.

Extensive studies on host plant resistance against insect pests in crop plants have indicated that in most cases the resistance is of biochemical nature (Pathak and Dale, 1983). Poly-phenols are generally associated with plant resistance against insects (Panda, 1979). Phenols were found to act as feeding deterrents to leafhoppers and planthoppers (Sogawa, 1973; Pathak and Khush, 1979) and in general, resistant rice genotypes were found to contain more phenolic compounds than susceptible varieties (Pathak and Khush, 1979). In the present study also indicated higher level of total phenols in resistant genotypes compared to susceptible entries. Das (1976) indicated the possibility high phenol content in Taichung 16 as a factor responsible for stem borer resistance. Vidyachandra *et al.* (1981) and Joshi and Venugopal (1984), reported low soluble sugar content in some gall midge resistant varieties. On the contrary, Peraiah and Roy (1979) found higher sugar content in resistant rice varieties. It is evident from the present investigation in some resistant genotypes (RR 270-56, JGL 11650, NDR 3110 and NDR 2063) that a representative set of resistant varieties had fallen on either side of the susceptible checks for crude proteins and sugars. Risk of generalization based on estimation of biochemical constituents of a selected few resistant varieties, with reference to one or two susceptible checks, is evident from earlier reports. Besides, the physiological state of plant material and portion of the plant selected for estimation undoubtedly influence the parameters concerned. Moreover, plant biochemical defense being a dynamic system, more estimation of constituents and correlation of their concentration with resistance, could only be suggestive, but not decisive.

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Table 1: Biochemical constituents in vegetative stem portion of rice genotypes at Mandya

Genotypes	Biochemical constituents (mg g^{-1})				
	Total sugars	Reducing sugars	Total phenols	Crude proteins	Free amino acids
Wet 2006					
JGL 13595	8.92^{g}	5.34^{f}	0.62^{ab}	3.27^{cde}	34.82^{a}
RP 4518-2-4	9.24^{f}	6.17^{e}	0.48^{e}	3.62^{cde}	29.25^{de}
RP 4615-591	8.78^{g}	4.81^{fgh}	0.58^{c}	3.44^{cde}	26.76^{fg}
RP 4643-1040	7.30^{j}	3.22^{j}	0.52^{d}	4.14^{cd}	32.47^{bc}
WGL 31699-2	6.81^{k}	3.80^{ij}	0.60^{abc}	2.97^{e}	22.56^{hi}
HKR 02-486	8.15^{h}	5.21^{hi}	0.63^{a}	3.37^{cd}	30.15^{cd}
RR 270-56	10.24^{e}	4.92^{fg}	0.59^{bc}	4.13^{cd}	29.32^{de}
NDR 9930096	10.19^{e}	6.22^{e}	0.61^{abc}	3.56^{cde}	24.51^{gh}
IC 114099	8.27^{h}	5.15^{f}	0.49^{e}	4.06^{cde}	26.50^{fg}
IC 115608	8.83^{g}	4.29^{ghi}	0.41^{f}	3.34^{cde}	27.00^{def}
Abhaya	7.70^{i}	3.96^{ij}	0.62^{ab}	2.97^{e}	32.66^{ab}
W 1263	7.28^{j}	4.24^{hi}	0.52^{d}	4.22^{c}	29.18^{de}
Phalguna	8.12^{h}	5.35^{f}	0.61^{abc}	3.17^{de}	32.14^{bc}
RP 4643-829 (S)	10.21^{e}	6.22^{e}	0.47^{e}	5.19^{b}	20.85^{i}
RP 4644-793 (S)	11.37^{d}	7.88^{d}	0.31^{h}	6.34^{a}	27.63^{ef}
RDR 1006 (S)	10.35^{e}	7.15^{d}	0.42^{f}	6.78^{a}	18.47^{j}
NDR 3110 (S)	10.13^{e}	8.44^{c}	0.30^{h}	5.93^{ab}	20.74^{i}
Jaya (S)	14.47^{b}	8.89^{bc}	0.35^{g}	6.15^{a}	24.43^{gh}
IR 20 (S)	13.24^{c}	9.29^{b}	0.37^{g}	6.24^{a}	22.57^{hi}
TN 1 (S)	15.84^{a}	10.21^{a}	0.26^{i}	6.78^{b}	16.45^{j}
Wet 2007					
JGL 11650	10.22^{d}	6.84^{de}	0.49^{ab}	3.45^{e}	27.57^{de}
JGL 13375	9.86^{de}	6.16^{ef}	0.51^{ab}	3.16^{ef}	28.43^{cd}
JGL 13616	9.25^{def}	5.92^{efg}	0.47^{ab}	2.96^{efg}	30.17^{bc}
RP 4643-829	7.45^{fghi}	4.31^{fgh}	0.49^{ab}	3.05^{ef}	24.34^{g}
WGL 44	9.03^{defg}	6.13^{ef}	0.61^{a}	2.84^{efgh}	32.53^{a}
WGL 75	6.53^{hi}	3.77^{h}	0.56^{ab}	2.23^{gh}	30.74^{ab}
RDR 918	6.94^{hi}	3.73^{h}	0.57^{ab}	2.63^{fgh}	31.67^{ab}
Acc No. 2236	8.18^{efgh}	5.27^{efgh}	0.48^{ab}	3.17^{ef}	29.82^{bc}
Acc No. 2270	7.11^{hi}	4.20^{gh}	0.62^{a}	3.52^{e}	28.54^{cd}
Acc No. 2941	7.29^{fghi}	4.07^{h}	0.60^{a}	3.42^{e}	26.39^{ef}
Acc No. 3040	6.12^{i}	3.88^{h}	0.42^{bc}	2.17^{h}	19.50^{h}
JGL 11541 (S)	10.86^{d}	8.13^{cd}	0.31^{cd}	4.89^{cd}	23.33^{g}
RP 4639-110 (S)	8.24^{efgh}	5.95^{efg}	0.32^{cd}	4.23^{d}	24.49^{g}
AS 99035 (S)	9.25^{def}	7.15^{de}	0.30^{cd}	5.21^{bc}	20.11^{h}
PAU 3030-29-2 (S)	13.25^{c}	9.27^{bc}	0.28^{cd}	5.86^{b}	16.23^{i}
MTU 1082 (S)	14.14^{bc}	10.18^{ab}	0.23^{d}	5.92^{b}	25.18^{fg}
Acc No. 2219 (S)	15.54^{ab}	10.83^{ab}	0.22^{d}	5.74^{b}	15.52^{j}
TN 1 (S)	16.84^{a}	11.24^{a}	0.31^{cd}	9.95^{a}	17.76^{i}

Means in a columns followed by common letters are non significant at p=0.05 as per DMRT (Duncan, 1955)

Table 2: Biochemical constituents in vegetative stem portion of rice genotypes at Mangalore

Genotypes	Biochemical constituents (mg g^{-1})				
	Total sugars	**Reducing sugars**	**Total phenols**	**Crude proteins**	**Free amino acids**
Wet 2006					
JGL 13595	8.75^{fgh}	4.91^{ef}	0.58^{bc}	3.74^{h}	22.58^{k}
RDR 987	8.31^{gh}	5.17^{def}	0.32^{ijk}	3.43^{i}	28.71^{ef}
RP 4613-260	7.86^{hi}	4.64^{def}	0.48^{de}	4.05^{fg}	31.35^{abc}
RP 4639-233	6.21^{hi}	4.16^{ef}	0.42^{efg}	2.93^{k}	30.84^{bcd}
RP 4644-1183	6.36^{i}	3.86^{f}	0.46^{def}	3.24^{j}	29.41^{de}
RP 4647-1073	7.10^{hi}	5.13^{def}	0.44^{efg}	3.65^{h}	30.32^{cde}
OR 1914-8	9.74^{efg}	4.68^{ef}	0.52^{cd}	4.25^{e}	27.54^{fg}
OR 2093-4	10.24^{ef}	8.24^{bc}	0.56^{bc}	2.91^{k}	26.22^{gh}
NDR 2063	11.26^{de}	7.65^{c}	0.68^{a}	3.26^{j}	30.09^{cde}
MTU 1075	8.86^{fgh}	5.96^{de}	0.59^{b}	2.75^{l}	25.20^{hi}
NDR 3110	12.84^{bcd}	6.80^{cd}	0.44^{efg}	3.06^{k}	32.73^{a}
Abhaya	8.31^{gh}	4.38^{ef}	0.55^{bc}	2.97^{k}	32.54^{ab}
Phalguna (S)	8.10^{ghi}	4.71^{ef}	0.61^{b}	3.25^{j}	30.28^{cde}
RDR 992 (S)	7.82^{hi}	4.87^{ef}	0.38^{ghi}	6.24^{c}	24.21^{ijk}
JGL 13521 (S)	13.24^{bc}	9.46^{ab}	0.42^{efg}	4.16^{ef}	20.21^{e}
RP 4639-110 (S)	15.22^{a}	10.84^{a}	0.26^{kl}	4.25^{e}	24.26^{ijk}
RP 4644-750 (S)	14.25^{ab}	9.36^{ab}	0.29^{jkl}	3.93^{g}	26.59^{gh}
IR 20 (S)	12.24^{cd}	9.95^{ab}	0.34^{hij}	5.20^{d}	23.33^{jk}
Jaya (S)	14.25^{bc}	8.29^{bc}	0.32^{ijk}	6.42^{b}	25.43^{hi}
TN 1 (S)	13.19^{bc}	10.28^{a}	0.24^{e}	6.95^{a}	23.56^{ijk}
Wet 2007					
JGL 11605	7.22^{de}	4.16^{f}	0.56^{bc}	3.65^{cde}	29.79^{cd}
JGL 11459	7.94^{de}	3.94^{fg}	0.52^{bcd}	3.45^{cde}	30.19^{bc}
JGL 13376	7.12^{de}	4.22^{f}	0.61^{ab}	3.75^{cde}	33.22^{a}
JGL 13418	7.06^{de}	4.13^{f}	0.69^{a}	3.71^{cde}	28.18^{de}
RP 4643-713	7.56^{de}	4.56^{ef}	0.48^{cd}	4.12^{c}	30.33^{bc}
OR 1967-15	8.86^{cd}	5.22^{e}	0.57^{bc}	2.90^{ef}	26.21^{f}
NDR 9930095	6.19^{e}	3.11^{h}	0.62^{ab}	3.16^{cdef}	24.16^{g}
R 1249-1196-2-1	6.54^{e}	3.25^{gh}	0.57^{bc}	2.45^{f}	31.99^{ab}
WGL 157	7.07^{de}	3.84^{fg}	0.61^{ab}	3.06^{def}	32.51^{a}
RDR 918	6.25^{e}	3.22^{gh}	0.57^{bc}	2.93^{def}	29.88^{cd}
JGL 11097 (S)	8.56^{cd}	5.24^{e}	0.28^{e}	3.92^{cd}	19.09^{hj}
JGL 11551 (S)	9.84^{bc}	6.24^{d}	0.42^{d}	6.16^{ab}	20.19^{h}
RP 4639-110 (S)	11.14^{b}	8.16^{c}	0.31^{e}	5.33^{b}	26.69^{ef}
OR 2069-1 (S)	14.24^{a}	10.25^{b}	0.26^{e}	5.74^{ab}	18.25^{i}
TN 1 (S)	15.20^{a}	11.85^{a}	0.23^{e}	6.55^{a}	16.28^{j}

Means in a columns followed by common letters are non significant at p=0.05 as per DMRT (Duncan, 1955)

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