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TITLE

Genetic and Biochemical Basis of Gall Midge Resistance in some Cultivars of Indica Rice

FINAL REPORT FOR THE PERIOD

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GENETIC AND BIOCHEMICAL BASIS OF GALL MIDGE RESISTANCE IN SOME CULTIVARS OF *INDICA* RICE

FINAL REPORT

I.A.E.A. PROJECT

(RESEARCH CONTRACT NO.2475/R4/RB)

OCTOBER 1, 1980 - NOVEMBER 30, 1986

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I.A.E.A. PROJECT

FINAL REPORT

Research Contract No.	:	2475/R4/RB
Title of the project	:	"Genetic and biochemical basis of Gall Midge Resistance in some cultivars of Indica rice".
Date of commencement	:	OCTOBER 1, 1980
Date of Expiry	:	NOVEMBER 30, 1986
Institute where research is being carried out.	:	Department of Genetics Osmania University HYDERABAD 500 007 A.P., INDIA.
Chief Scientific Investigator	:	Dr. G.M. Reddy Professor of Plant Genetics Department of Genetics Osmania University HYDERABAD-500007 A.P., INDIA.
Time period covered	:	(OCTOBER 1, 1980 - NOVEMBER 30, 1986)

Rice, the staple food of more than half the World's population occupies about 1/5th of the total World's acrage under cereals. India is the largest rice growing Country with approximately 39.7 million hectares under rice cultivation. The stability of high productivity of modern rice varieties is greatly affected by insect pests. Rice gall midge is a serious insect pest of rice that is prevalent in several south eastern asian Countries. In India, the pest is endemic to parts of Karnataka, Maharashtra, Andhra Pradesh, Orissa and Bihar.

Gall midge resistance has been mainly attributed to antibiosis. No progress has sofar been made in identifying the exact biochemical nature of resistance. In Indica sub-species the understanding of chemical nature of disease would be helpful in the control of the disease and also in breeding programme aimed at developing resistance varieties.

Studies were undertaken to establish the biochemical basis of resistance. Biochemical characterisation of resistant and susceptible varieties were carried out. The parameters considered were:

(i) Total sugar and Reducing sugar content
(ii) Total phenol content
(iii) Amino acid profile
(iv) Past infectional changes in sugar & phenol
(v) Isozyme studies.

Materials and Methods:

Eight resistant vars. Ptb 21, Ptb 33, JBS 346, RP-4-14, Surekha, Kakatiya, WGL 26358, IET 6290 and 4 susceptible vars. IR8, IR20, Jaya and TN_1 were used in the biochemical analysis.

(1) Sugar:

8 resistant and 4 susceptible varieties were studied for their total sugar and reducing sugar content. Total sugar was estimated by Anthrone method and reducing sugar by Nelson's method.

Results are summarised in Table-1.

(ii) Total phenols:

8 resistant and 4 susceptible varieties were studied for their total phenols. Results are summarised in Table-II.

(iii) Amino Acid Analysis:

The Amino Acid composition of calli and seedlings of a resistant (Kakatiya) and susceptible (TN_1) variety were studied. For free amino acid analysis, ten day old seedlings and 30 day old calli of TN_1 & Kakatiya (2 gm/each) were homogenised in 70% ethyl alcohol. Free amino acids were extracted and dried.

Chlorophyll, organic acids, cations and sugars were removed by eluting the dried extracts through Dowex-50X4 (50-100 mesh) on 40X1 cm column with 3N NH_4OH. Ammonia was completely removed and the dried samples were dissolved in sodium citrate (pH 2.2) diluent buffer. For total amino acids, 5 mg each of 10 day seedlings and calli of TN_1 & Kakatiya were hydrolysed with 6N Hcl for 24 hours at 105°C. The samples were evaporated and taken in sodium citrate diluent buffer. Amino acid analysis were carried out on JOEL automatic amino acid Analyser. Data of the acidic, neutral and basic amino acid pools were presented in Table-III.

(iv) Changes in sugar & phenol contents after infection:

The changes in phenol and sugar contents were noted in susceptible plant (TN_1) after 15 days of gall midge infection and after silver shoot formation.

The results of the experiment are summarised in Table-IV.

Study of total sugar and reducing sugar.

In resistant varieties the range of total sugar content was 19.10 mg/g f.wt (JBS 446) to 30.4 mg/g f.wt. (Kakatiya). In susceptible varieties it ranged from 26.75 mg/g f.wt. (IR 8) to 30.35 mg/g f.wt. (IR 20). No significant differences between resistant and susceptible varieties in regard to levels of total sugar could be observed.

Reducing sugar for resistant variety ranged from 7.45 mg/g f.wt. (Kakatiya) to 10.02 mg/g f.wt. (JBS 446) and for susceptible variety 5.05 (IR 20) to 7.25 TN_1. Pre-infectional reducing sugar level in resistant variety is comparatively more than susceptible variety.

Phenols:

Study of total phenols revealed that it ranged from 0.973 to 2.400 mg/g f.wt. in resistant vars. which is higher than susceptible varieties i.e. 0.475 mg/g f.wt. in Jaya to 0.754 mg/g f.wt. in IR 20.

Study of Amino Acid Profile:

In susceptible variety TN_1 the amino acid proline was absent both in seedling and in calli but it was present in the

resistant variety Kakatiya both in the seedling and in the calli. The amino acid valine and methionine was not present both in the seedlings and calli of both the varieties. Leucine, tyrosine, phenyl alanine was absent in calli of both the varieties. Seedlings showed a higher concentration of amino acids except for alanine for both TN_1 and Kakatiya and cysteine and isoleucine in case of TN_1.

Post infectional changes for total phenols, total sugars and reducing sugar.

Post infectional increase in total phenols was observed in TN_1 plants. Healthy plants exhibited a total phenol content of 0.36 mg/g f.wt. which increased to 0.6 mg/g f.wt. after 15 days and 0.58 mg/g f.wt. after silver shoot formation. A reduction in level of total sugar was observed. Healthy plants exhibited 4.24 mg/g f.wt. which decreased to 2.72 mg/g f.wt. after 15 days after infestation and 2.14 mg/g f.wt. after silver shoot formation.

Increased levels of reducing sugars were observed after infection. Healthy plants showed 0.34 mg/1 f.wt. of reducing sugars which increased to 0.64 mg/g f.wt. after 15 days post infection and 1.02 mg/g f.wt. after silver shoot formation.

In all the resistant varieties studied, the total phenolic content was more compared to the susceptible ones. It is quite possible that endogenous high levels of phenols render resistance against the disease.

The total sugar content did not reveal significant differences but reducing sugar was comparatively higher in resistant varieties.

Analysis of 10 day old seedlings and 30 day old calli of both susceptible (TN_1) and resistant (Kakatiya) varieties contained aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cysteine, isoleucine, lysine, histidine, and arginir arginine, as free amino acids. Valine and methionine were absent in seedlings and calli of both the varieties. However, proline was found exclusively in Kakatiya (resistant). The neutral amino acids i.e. leucine, tyrosine and phenylalamine were absent in calli of both the resistant and susceptible types, whereas these were found in seedlings. Generally, the pooled amino acid concentration is higher in seedlings than in calli. However, 2 free amino acids glutamic acid and alanine were found more in calli as compared to seedlings.

It is known in some cases that proline metabolism is severely affected in disease affected material. The key enzymes in proline metabolism are glutamic semi aldehyde dehydrogenase and proline oxidase. Since it has been observed that proline was present only in resistant variety it may be assumed that either glutamic semialdehyde dehydrogenase was more active in resistant varieties or proline oxidase was less active in resistant varieties leading to an accumulation of proline.

It was observed that phenols and reducing sugars increased after infestation while a decrease was observed for total sugars.

Conclusion:

Endogenous high quantity of phenols and amino acid proline were seen in resistant varieties which were probably responsible for resistance against gall midge. The role of sugar in biochemical resistance was insignificant.

Isozyme studies: In the present investigation eight resistant and four susceptible varieties were selected to study the isozymes of peroxidase and esterase to know the association of these isozymes with gall midge resistance.

Isozyme studies with peroxidase and esterase have shown that all the resistant varieties contained additional band, (Table 5 and 6) which was absent in susceptible varieties, suggesting that this band may be associated with resistance (fig.1 and 2).

The F1's of (Resistant x resistant) PTB21XWG2 2245; PTBxWG2 28259; PTBxIET 6293; (Resistant x susceptible) PTB1XIR8 PTB21XJaya and (Susceptible x Susceptible) Jaya XIR8 and JayaXIR20; crosses were used for biochemical basis confering resistance. Results showed that the amount of peroxidase was markedly high in lines showing resistance to gall midge. In resistance Xresistance and resistanceXsusceptible lines, the analysis for enzymes participating in resperatory metabolism were assayed for malate dehydrogenase (MDH) and cytochrome 'C' oxidase which show that at the flowering stage, the activity was high relatively. The reasoning may be attributed to high respiration levels probably to maintain resistance to the pest invasion.

Genetic Studies:

Genetic studies have been carried by selecting resistant and susceptible varieties for gall midge resistance and the following crosses were made.

1) Resistance X Resistant:

a) PTB 21 x PTB 33
X Surekha
X IET 6290
X IET 6291
X IET 6292
X IET 6293
X WGL 22245
X WGL 28259

b) WGL 28259 X PTB 33
X Surekha
X IET 6290
X IET 6291
X IET 6292
X IET 6293
X WGL 22245

2) Resistant X Susceptible:

a) PTB 21 X IR 8
X IR 20
X Jaya
X TN_1

b) PTB 33 X IR 8
X IR 20
X Jaya
X TN_1

c) WGL 22245 X IR 8
X IR 20
X Jaya
X TN_1

d) IET 6290 X IR 8
X IR 20
X Jaya
X TN_1

3) Susceptible X Susceptible:

a) Jaya X IR 8
X IR 20
X TN_1

b) TN_1 X IR 8
X IR 20

The F2 generation was grown at Research Station, Warangal, Natural infested location which is 150 km away from this University and the studies are in progress. To confirm the results obtained on genetic analysis, one more year's data is required without any additional grant.

Papers published on work done under the contract:

Reddy, G.M. 1982. Genetic and some biochemical studies of gall midge resistance in rice. Paper presented at joint FAO/IAEA Research Co-ordination meeting on 'Use of isotopes in pest management with emphasis on rice insects'. Jakarta, Indonesia 1-5 February, 1982.

SUGARS & REDUCINGSSUGARS

(mg/g. fresh wt.)

IN SOME GALL MIDGE RESISTANT & SUSCEPTIBLE VARIETIES (10 DAY OLD SEEDLINGS)

Sl. No.	Variety	Resistant/ Susceptible	Total sugar	Reducing Sugar
1.	Ptb 21	R	21.36	7.50
2.	Ptb 33	R	20.68	8.35
3.	JBS 446	R	19.10	10.02
4.	RP-4-14	R	21.45	7.50
5.	Surekha	R	22.90	7.55
6.	Kakatiya	R	30.40	7.45
7.	WGL 26358	R	26.70	7.56
8.	IET 6290	R	25.62	7.60
9.	IR 8	S	26.75	5.85
10.	IR 20	S	30.35	5.05
11.	Jaya	S	28.60	5.40
12.	TN 1	S	30.25	7.25

TABLE-II

TOTAL PHENOLS

(mg/g. fresh wt.)

IN SOME GALL MIDGE RESISTANT & SUSCEPTIBLE VARIETIES

(10 DAY OLD SEEDLINGS)

Sl.No.	Variety	Resistant/ Susceptible	Total phenols
1.	Ptb-21	R	0.973
2.	Ptb-33	R	1.440
3.	JBS-446	R	1.850
4.	RP-4-14	R	1.820
5.	Surekha	R	2.400
6.	Kakatiya	R	0.992
7.	WGL 26358	R	1.451
8.	IET 6290	R	0.996
9.	IR 8	S	0.668
10.	IR 20	S	0.754
11.	Jaya	S	0.475
12.	TN 1	S	0.482

TABLE-III

AMINO ACID (FREE AMINO ACID POOL) COMPOSITION OF SEEDLINGS (10 DAYS) AND CALLUS (30 DAYS) OF SUSCEPTIBLE (TN_1) AND RESISTANT (KAKATIYA) RICE VARIETIES

AMINO ACIDS	Concentration, u moles/100gm.fresh wt.			
	TN_1 (susceptible)		Kakatiya (Resistant)	
	Seedling	Callus	Seedling	Callus
1. Acidic Amino Acids:				
Aspartic acid	9.37	4.10	8.60	6.20
Threonine	4.68	3.80	5.20	2.50
Serine	7.81	2.00	7.00	3.10
Glutamic acid	6.25	8.04	5.80	6.00
Proline	Nil	Nil	2.40	1.50
Glycine	4.20	2.80	3.20	2.50
Alanine	9.37	12.20	10.50	11.50
Cysteine	1.56	2.00	2.10	1.50
Valine	Nil	Nil	Nil	Nil
Ninhydrin positive material with high 570 mm & 440 mm absorption	50	50	50	50
2. Neutral amino acids:				
Methionine	Nil	Nil	Nil	Nil
Isoleucine	2.34	2.50	3.60	2.80
Leucine	2.80	Nil	2.50	Nil
Tyrosine	1.56	Nil	2.80	Nil
Phenyl alanine	1.80	Nil	1.20	Nil
3. Basic amino acids:				
Lysine	8.50	6.20	10.00	7.20
Histidine	4.20	1.50	3.80	1.80
Arginine	2.50	2.00	3.20	1.50

TABLE-IV

TOTAL PHENOLS, SUGARS AND REDUCING SUGARS

(mg/g fresh wt.)

IN HEALTHY AND GALL MIDGE INFESTED TN_1 PLANTS

Sl. No.	Stage	Total Phenols	Total sugars	Reducing sugars
1.	Healthy	0.36	4.24	0.34
2.	15 days after infection	0.60	2.72	0.64
3.	After silver shoot formation	0.58	2.14	1.02

TABLE-V

PEROXIDASE ISOZYME PATTERN IN RESISTANT AND SUSCEPTIBLE VARIETIES

VARIETY	Rf VALUES							
	0.06	0.11	0.52	0.70	0.78	0.79	0.84	0.93
1. Ptb 21 (R)	+	+	+	+	-	+	+	+
2. Ptb 33 (R)	+	+	+	+	-	+	+	+
3. JBS 446 (R)	+	+	+	+	-	+	+	+
4. RP-4-14 (R)	+	+	+	+	-	+	+	+
5. Surekha (R)	+	+	+	+	+	-	+	+
6. Kakatiya (R)	+	+	+	+	+	-	+	+
7. WGL 26358 (R)	+	+	+	+	+	-	+	+
8. IET 6290 (R)	+	+	+	+	+	-	+	+
9. IR 8 (S)	+	+	+	+	-	-	+	+
10. IR 20 (S)	+	+	+	+	-	-	+	+
11. Jaya (S)	+	+	+	+	-	-	+	+
12. TN_1 (S)	+	+	+	+	-	-	+	+

RESULT

Resistant cultivars showed an extra band (Rf 0.78 or Rf 0.79)

TABLE-VI

ESTERASE ISOZYME PATTERN IN RESISTANT AND SUSCEPTIBLE VARIETIES

VARIETY	Rf VALUES								
	0.04	0.12	0.25	0.49	0.53	0.628	0.636	0.643	0.78
1. Ptb 21 (R)	+	+	+	+	+	-	-	+	+
2. Ptb 33 (R)	+	+	+	+	+	-	-	+	+
3. JBS 446 (R)	+	+	+	+	+	+	-	-	+
4. RP-4-14 (R)	+	+	+	+	+	+	-	-	+
5. Surekha (R)	+	+	+	+	+	-	-	+	+
6. Kakatiya (R)	+	+	+	+	+	-	-	+	+
7. WGL 26358 (R)	+	+	+	+	+	-	+	-	+
8. IET 6290 (R)	+	+	+	+	+	-	+	-	+
9. IR 8 (S)	+	+	+	+	+	-	-	-	+
10. IR 20 (S)	+	+	+	+	+	-	-	-	+
11. Jaya (S)	+	+	+	+	+	-	-	-	+
12. TN_1 (S)	+	+	+	+	+	-	-	-	+

RESULT: The resistant varieties had a extra band

IR8, IR20, Jaya and TN_1 were used in the biochemical analysis.

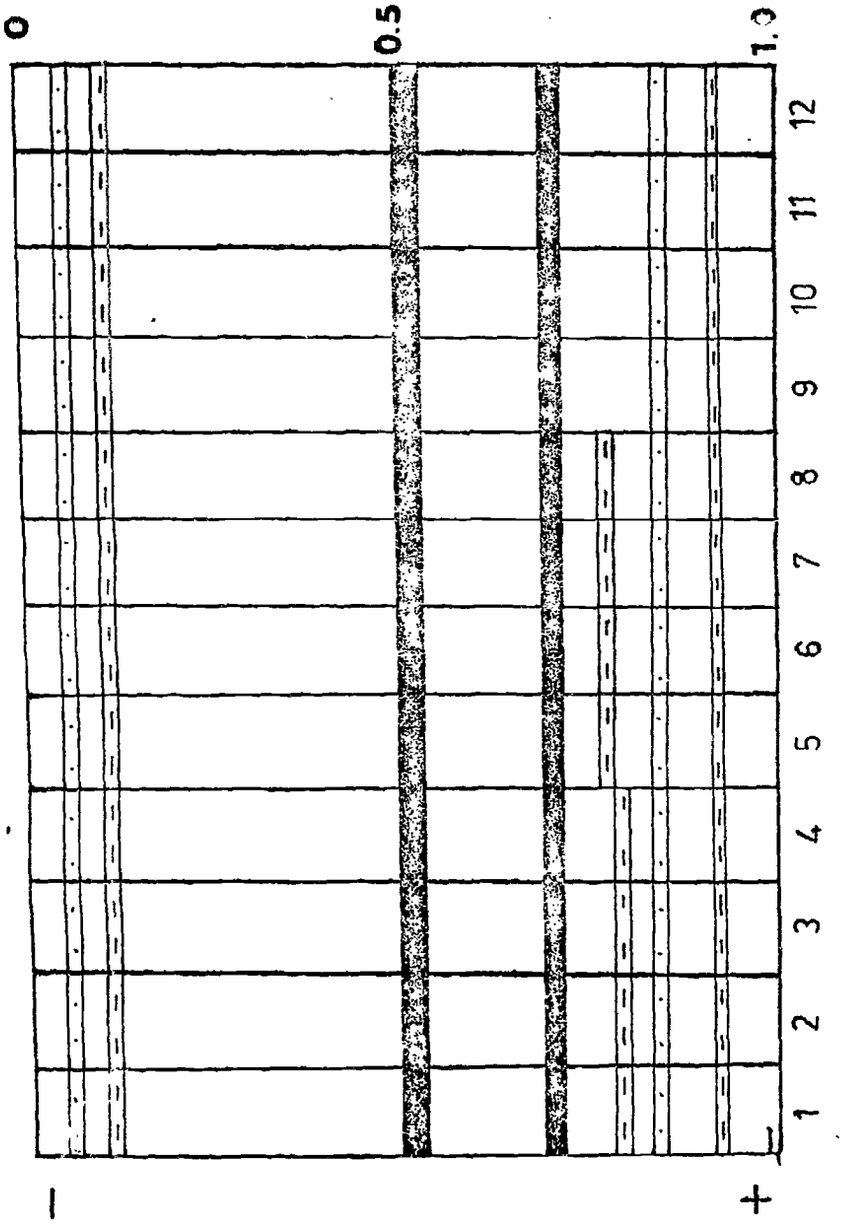

Fig I. PEROXIDASES

Fig 2 - ESTERASES