RESEARCH PAPER

Asian Journal of Bio Science (October, 2010) Vol. 5 Issue 2 : 219-222

Role of detoxifying enzymes in host plant resistance to cotton mealybugs (*Phenococcus solenopsis* Tinsley)

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(Accepted : July, 2010)

A laboratory investigation was conducted to find out role of detoxifying enzymes in defense mechanism of cotton cultivars against mealybugs in Insect Biotechnology Laboratory of Department of Agricultural Entomology,Dr. PDKV, Akola during 2008-09. Quantitative and qualitative studies undertaken for estimation of detoxifying enzymes like Glutathione S-transferases and esterases. GST activity was found higher in resistant variety, PKV Hy-2, followed by AKH-3614-10 (Hirsutum pigmented). The highest esterase activity was found to be present in Bunny-Bt (187.14 nM mg protein^{-1} min^{-1}) than other cotton cultivars. PKV Hy-2 showed very lowest esterase activity (76.97 nM mg protein^{-1} min^{-1}). Glutathione-s-transferase and esterase bands were not detected in the susceptible CAHH-231 (Pigmented hybrid) variety. Three GST isozymes were observed in AKA-8, whereas, CAHH-231 (Pigmented hybrid) did not show any isozyme. The study will be helpful in understanding the biochemical basis of mealy bug resistance in cotton. The outcome of the present investigation will act as stepping stone to develop mealy bug resistant cotton variety.

Key words : Cotton, Detoxifying enzymes, Esterases, GST, Host plant resistance, *Phenococcus solenopsis*

INTRODUCTION

Cotton is an important fibre crop. It is natural gift known for its fibre since time immemorial. It provides 65 per cent raw material to textile industry and contributed one third of total foreign exchange earning of India (Mayee and Rao, 2002).But in recent years mealy bug is becoming one of the major sucking pest of cotton.They suck a large amount of sap from leaves and stems with the help of piercing and sucking mouthparts, depriving plants of essential nutrients. In view of the large infestation of this pest and the huge losses caused by them, this pest must be kept below ETL. Various studies have been done to identify the biochemical basis of sucking pest resistant in host plant.

Though there is huge area under cotton but yield and production is very low. Various factors are responsible for this low productivity in Maharashtra. Among these losses caused by insect pest is major one. In India, 160 species of insect pests have been recorded to damage cotton crop (Agrawal *et al.,* 1979). In Maharashtra, including Vidarbha region about 25 insect pests have been recorded to attack cotton crop regularly (Thakare *et al.*, 1983). Amongst them the sucking pests *i.e.* Aphid (*Aphis gossypii*), Jassid (*Amrasca biguttula biguttula*), thrips (*Thrips tabaci*) and cotton bollworm are major one.

But in recent years mealy bug is becoming one of the major sucking pests of cotton. Mealy bug (*Phenacoccus solenopsis*) belongs to order Hemiptera, sub-order-Homoptera and family–Pseudococcidae, are cottony in appearance, small oval, soft bodied sucking insects. Adult mealy bugs are found on leaves, stems and roots and are covered with white mealy wax which makes them difficult to eradicate. They form colonies on stems and leaves developing into dense, waxy, white masses. They suck a large amount of sap from leaves and stems with the help of piercing and sucking mouthparts, depriving plants of essential nutrients. The excess sap is excreted as honeydew which attracts ants and develops sooty mould inhibiting the plants ability to manufacture food. Serious attack results in retarded growth and late opening of bolls affecting the yield badly. Recently in India the cotton crop in Punjab, Rajasthan, Maharashtra and Gujrat is being seriously infested with mealy bug. During 2005, the sudden appearance of the pest in cotton in Multan, Sanghar, Mirpurkhas and Tando Allahyar of Pakistan destroyed the entire crop within a few days (Tanwar *et al.*, 2007).

MATERIALS AND METHODS

The shoot tips of different cotton cultivars *viz.,* PKV-

Suchita R.Ghule, U.P. Barkhade, M.P. Moharil and T.B. Ugale (2010). Role of detoxifying enzymes in host plant resistance to cotton mealybugs (*Phenococcus solenopsis* Tinsley), *Asian J. Bio. Sci.*, **5** (2) : 219-222

Hy-2,PKV-Rajat,AKH-081,AKA-8,AKH-3614-10 (Hirsutum pigmented),CAHH-231(pigmented hybrid) and Bunny Bt 30 DAE were obtained from the field of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The amount of detoxifying enzymes like GST and esterase was estimated at Insect Biotech Laboratory, Department of Entomology, Dr. P.D.K.V. Akola. Cultivars used in the investigation and their reactions against sucking pests are given in Table 1.

Table 1: Cultivars used in the investigation and their reactions against sucking pests

Sr. No.	Cultivars	Insect reactions
1.	PKV Hy-2	Resistant
2.	PKV Rajat	Tolerant
3.	AKH-081	Tolerant
4.	AKA-8	Susceptible
5.	AKH-3614-10 (Hirsutum pigmented)	Highly susceptible
6.	CAHH-231 (Pigmented hybrid)	Highly susceptible
7.	Bunny Bt	Susceptible

Preparation of sample:

Samples were finally powdered and also crude homogenate was prepared with mortar and pestle and centrifugation method and preserved in refrigerator at 4^0C.

Quantitative estimation of glutathione-S-transferase:

GST quantification was carried out by method describe by Kao *et al.* (1989).

Quantitative estimation of esterase:

An indigenous protocol was developed with modification in the protocols given by Dary *et al.* (1990) and Xu and Bull (1995).

Study of detoxifying and oxidizing enzymes by electrophoresis:

Non denaturing polyacrylamide gel electrophoresis (PAGE) was performed in Hoefer SE 600 slab gel unit (Hoefer, San Fransisco, (A) using Tris-Glycine (pH 8.8) buffer system, 10% running gel and 5% stacking gel were prepared according to manual provided with the Hoefer system. Gel was loaded with the enzyme preparation and electrophoresis was conducted at a constant current of 2 mA per well (Hoefer PS-500 power unit) for 7-8 hrs at 4^0C.

Staining for glutathione-S transferase:

Native 10% PAGE was run at 4^0C for 4-5 hours, until the running front reached to the bottom of the gel. Then gel was transferred into staining solutions.

Staining solution I : 100 ml SPB (0.1 M, SPB 6.5) containing 5.0 mM GSH and 1 mM each of CDNB and NBT.

Staining solution II: 100 ml Tris HCl buffer (pH 9.6) with 4 mM phenozine methosulphate (PMS)

Gel was incubated in freshly prepared staining solution-I in dark at room temperature (at 37^0C) with occasional mild shaking for 20 min. Transferred the gel to the staining solution-II for 5-10 min with intermittent shaking. The gel turned blue because of the formation of insoluble formazan on the gel surface. The areas with glutathione-s-transferase activity remained as colourless bands. Gel was washed twice with DH_2O (6.5 pH), after that gel was transferred into fixing solution (glacial acetic acid: methanol: water as 1:2:7). Gel was stored in 10% glycerol solution and photograph was taken immediately after band observed. Further gel was analyzed for molecular weight and Rf values.

Staining for esterase isozyme:

10% SDS gel was run under semi denaturing condition at 4^0C for 4-5 hrs until the dye reached to the bottom of gel. Then gel was transferred into staining solution immediately. Preincubate the gel into freshly prepared solution 100 ml SPB (0.04 M; 6.5 pH) with 0.02% a-napthyl acetate solution (20 mg a-napthyl acetate in 2 ml acetone) for 20-30 min then gel was transferred into freshly prepared staining solution with 1% fast brilliant blue (BB) salt and 0.02% a-napthyl acetate solution in 100 ml SPB (0.04 M, 6.5 pH) in dark at room temperature. Kept the gel for 30 min with occasional shaking. To reduce non-specific background staining of the gel, 2-4 ml of 4% formaldehyde was added to staining solution prior to staining of gel. Dark blackish bands were observed after staining. Washed the gel twice with dd H_2O (6.5 pH). Gel was transferred into fixing solution as given in GST staining, for one hour. After taking photograph gel was stored in 10% glycerol solution. Further gel was analyzed for molecular weight and Rf values.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below:

Activity of GST from the different cotton cultivars:

The activity of glutathione-s-transferase from AKH-

Table 2: Estimation of detoxifying enzymes from different cotton cultivars

Sr. No.	Cultivars	GST ± SE (nM mg protein^{-1} min^{-1})*	Esterase ±SE (nM mg protein^{-1} min^{-1})*
1.	PKV Hy-2	7.81±0.10	76.97±0.14
2.	PKV Rajat	5.43±0.06	91.61±0.12
3.	AKH-081	7.95±0.03	81.94±0.04
4.	AKA-8	6.67±0.09	160.02±0.05
5.	AKH-3614-10 (Hirsutum pigmented)	7.26±0.08	114.65±0.06
6.	CAHH-231 (Pigmented hybrid)	1.93±0.05	185.04±0.13
7.	Bunny Bt	2.74±0.06	187.14±0.08

* All figures are mean of triplicate ±SE

081 possessed highest GST activity (7.95 nM mg protein^{-1} min^{-1}) followed by PKV-Hy-2 and AKH-3614-10 (Hirsutum pigmented) which recorded 7.81 and 7.26 nM mg protein^{-1} min^{-1}, respectively (Table 2). AKA-8, PKV Rajat and Bunny-Bt contain lower GST activity *i.e.* 6.67, 5.43 and 2.74 nM mg protein^{-1} min^{-1}. Whereas, CAHH-231 (pigmented hybrid) recorded lowest GST activity *i.e.* 1.93 nM mg protein^{-1} min^{-1}.

Activity of esterase from the different cotton cultivars:

Bunny-Bt showed highest esterase activity (187.14 nM mg protein^{-1} min^{-1}) followed by CAHH-231 (Pigmented hybrid) (185.04 nM mg protein^{-1} min^{-1}) AKA-8, (160.02 nM mg protein^{-1} min^{-1}), AKH-3614-10 (Hirsutum pigmented) (114.65 nM mg protein^{-1} min^{-1}), PKV Rajat (91.61 nM mg protein^{-1} min^{-1}) and AKH-081 (81.94 nM mg protein^{-1} min^{-1}), whereas PKV Hy-2 showed lowest esterase activity (76.97 nM mg protein^{-1} min^{-1}) (Table 2).

Data revealed on the esterase activity that susceptible cultivars showed higher esterase activity than tolerant and resistant cultivars. Similar kinds of results were found by Ni *et al.* (2001) in aphid cereal interactions.

Qualitative analysis of detoxifying enzymes:
Isozyme pattern of glutathione-S transferase from the different cotton cultivars:

Banding pattern of glutathione-S -transferase (Table 3) showed 4 isozymes from 2.27 kDa to 13.85 kDa. In CAHH-231 (Pigmented hybrid) any isozyme was not detected. AKA-8 showed three isozymes, AKH-081 and AKH-3614-10 (Hirsutum pigmented) showed two isozymes, whereas PKV Hy-2, PKV Rajat and Bunny-Bt showed single isozyme (Plate 1).

Isozyme pattern of esterase from the different cotton cultivars:

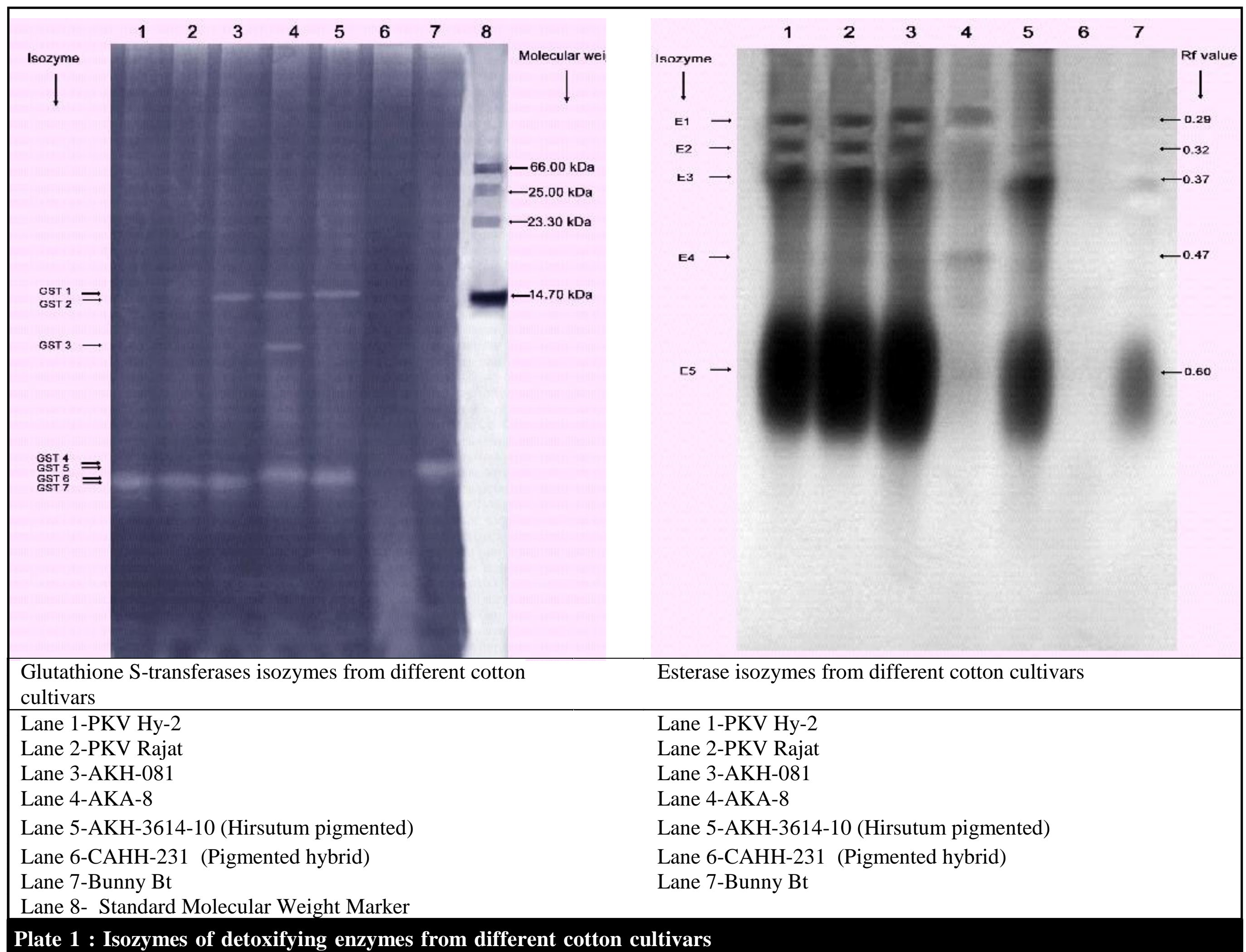
There were about five isozymes (Table 3) in different cotton cultivars. PKV Hy-2, PKV Rajat and AKH-081 showed 4 isozymes, AKH-3614-10 (Hirsutum pigmented) showed 3 isozymes (Plate 1). In CAHH-231 (Pigmented hybrid) no any isozyme was detected. These results are not compared due to want of literature.

This research offers a new perspective on cotton plant resistance against mealybug and provides a model for studying insect plant interactions. Furthermore, the identification of biochemical constituents for mealybug

Table 3: Isozyme pattern of glutathione-s-transferase and esterase from the different cotton cultivars

Cultivars	Glutathione-s-transferase isozymes with molecular weight and Rf values				Total no. of isozymes	Esterase isozymes with molecular weight and Rf value					Total no. of isozymes
Isozyme	GST1	GST2	GST3	GST4		E1	E2	E3	E4	E5	
Molecular wt. (kDa)	13.85	8.22	2.41	2.27		-	-	-	-	-	
Rf values	0.40	0.46	0.59	0.60		0.29	0.32	0.37	0.47	0.60	
PKV Hy-2	-	-	-		1	+, M	+, M	+, D	-	+, D	4
PKV Rajat	-	-	-		1	+, M	+, M	+, D	-	+, D	4
AKH-081	+, M	-	-		2	+, M	+, M	+, D	-	+, D	4
AKA-8	+, M	+, M		-	3	+, L	-	-	+, L	-	2
AKH-3614-10 (Hirsutum pigmented	+, M	-		-	2	-	+, L	+, D	-	+, D	3
CAHH-231 (Pigmented hybrid)	-	-	-	-	N.D.	-	-	-	-	-	N.D.
Bunny Bt	-	-		-	1	-	-	+, L	-	+, L	2

'+' –Presence of band, '-'Absence of band, L-Light intensity band, M- Medium intensity band, D-Dense intensity band, ND- Not detected, Rf-Relative front



Glutathione S-transferases isozymes from different cotton cultivars	Esterase isozymes from different cotton cultivars
Lane 1-PKV Hy-2 Lane 2-PKV Rajat Lane 3-AKH-081 Lane 4-AKA-8 Lane 5-AKH-3614-10 (Hirsutum pigmented) Lane 6-CAHH-231 (Pigmented hybrid) Lane 7-Bunny Bt Lane 8- Standard Molecular Weight Marker	Lane 1-PKV Hy-2 Lane 2-PKV Rajat Lane 3-AKH-081 Lane 4-AKA-8 Lane 5-AKH-3614-10 (Hirsutum pigmented) Lane 6-CAHH-231 (Pigmented hybrid) Lane 7-Bunny Bt

Plate 1 : Isozymes of detoxifying enzymes from different cotton cultivars

resistance provides a novel approach for screening resistant cotton cultivars for further breeding programme or will act as gene source. Ultimately biochemical constituents identified from this research will provide a set of tools for screening cotton cultivars for resistance to mealy bugs.

REFERENCES

Agrawal, R.A., Sharma, G.C., Banerjee, S.K. and Katiyar, Y.N. (1979): A model for integrated control of cotton pest ICCI, Special Issue, No.1.

Dary, O., Georghiuo, G.P., Parsons, E. and Pasture, N. (1990). Microplate adaptation of Gomari's assay for quantitative determination of general esterase activity in single insects. *J. Econ. Ent.,* **83** : 2187-2192.

Kao, C.H., Hung, C.F. and Sun, C.N. (1989). Parathion and methyl parathion resistance in diamond back moth (Lepidoptera : Plutellidae) larvae. *J. Econ. Entomol.,* **82**:1299-1300.

Mayee, C.D. and Rao, M.R.K. (2002). Current cotton production and protection scenarios including G.M. Cotton, *Agrolook*, April-June, pp.14-20.

Ni, X., Quisenberry, S.S., Heng-Moss, J. , Markwell, J., Sarath, G., Klucas, R. and Baxendale, F. (2001). Oxidative responses of resistant and susceptible cereal leaves to symptomatic and non-symptomatic cereal aphid (Hemiptera : Aphididae) feeding. *J. Econ. Entomol.,* **94**:743-751.

Tanwar, R.K., Jeyakumar, P. and Monga, D. (2007). Mealybugs and their management. *Tech. Bull.* 19

Thakare, K.R., Borale, M.N., Ajni, D.S., Raideo, A.K., Gawande, R.B. and Satpute, U.S. (1983). Twenty five years of research on cotton pest management in Maharashtra, Directorate of Research, MPKV, Rahuri, India. pp.1-5.

Xu, G. and Bull, D.L. (1995). Biochemical properties of esterases in pyrethroid resistant and susceptible strains of the hornfly (Diptera : Muscidae). *J. Econ. Entomol.,* **88** (5) : 1186-1191.