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Influence of Oxidative Enzyme on Resistance of Cotton (*Gossypium hirsutum*) against *Helicoverpa armigera*

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ABSTRACT: *A pot experiment was conducted during kharif 2013 to study the effect of insect damage (ID) and mechanical damage followed by insect damage (MDFID) on expression of enzyme activity namely Nitrate Reductase (NRase) and Lipoxygenase (LOX) in Sahana and Laxmi genotypes at different stages of growth. The results revealed that the Nitrate Reductase (NRase) and Lipoxygenase (LOX) activity were significantly higher in insect damage than mechanical damage followed by insect damage.*

Key words: *Enzyme, Nitrate Reductase (NRase) and Lipoxygenase (LOX)*

INTRODUCTION

Cotton is one of the most ancient and important commercial crop next only to food grains and is the principal raw material for a flourishing textile industry. Cotton, although under pressure from synthetic fibers, has made resurgence worldwide and remains as the most improved crop species producing lint plus oil and meal from seed [12].

Cotton is the backbone of our sprawling textile industry contributing 7.00 per cent to our Gross domestic product (GDP), fetching an export earning besides providing employment in the production, promotion, processing and trade. It accounts for 45 per cent of the world fiber and supplies 10 per cent world edible oil [13].

Many factors are responsible for low productivity of cotton but the magnitude of insect pests that damage the crop from sowing to maturity is most important. More than 1326 species of insects have been reported in commercial cotton fields worldwide, but only a small proportion of 162 pests are differing from season to season and between different regions [15]. Of the 30 pests of cultivated cotton, the most important are the caterpillars of *Helicoverpa armigera*, Pink bollworm (*Pectinophora gossypiella) and* spotted bollworm (*Earias vitela)* [5].

The mechanisms of host plant resistance in response to insect infestation consists of a series of biochemical events, including increased production of phenolics, mediated by the activity of such enzymes as phenylalanine ammonia-lyase, tyrosine ammonia-lyase, peroxidase and polyphenoloxidase. The primary metabolites include carbohydrates and proteins, which are exploited by the herbivores for their growth and development. These primary metabolites also function as precursors of secondary substances, which are major elements of resistance in plants. The secondary substances determine the suitability of the substrate for colonization and exploitation by the herbivores and thus govern host preferences and acceptability. Age correlated biochemical profiles of host tissues also significantly influence infestation patterns.

Metabolites play a major role in the adaptation of plants to the changing environment and in overcoming stress constraints. This flows from the large complexity of chemical types and interactions underlying various functions: structure stabilizing, determined by polymerization and condensation of phenols and quinones, or by electrostatic interactions of polyamines with negatively charged loci in cell components; as well as aromatic nuclei and unsaturated aliphatic chains and signal transduction, several plant-biotic and abiotic stress stimuli systems, they evidenced the multiplicity of biochemical mechanisms involved in the protective role by metabolites [6].

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MATERIALS AND METHODS

A pot experiment was conducted on cotton during *kharif 2013*. To study the effect of ID and MDFID on expression of enzymes namely NRase and LOX. The experiment was conducted in pot for maintaining the pest and disease free plants in the glass house condition. One tolerant genotypes *viz*., Sahana and one susceptible genotypes *viz*., Laxmi were used in the study. Seeds of each genotype were sown in 12″ X 8″ pots containing soil and compost mixture in 2:1 proportion and were watered regularly. In each genotype pots were maintained under controlled condition using cages lined with nylen net to prevent from pest and the infection from pests and air borne uredospores to maintain healthy seedlings.

The trifoliate leaves from middle portion of the plants were collected from the control plants of two genotypes for estimation of biochemical constituents which is referred as control and on the day of estimation, the other plants of same genotypes were allowed to ID (*Helicoverpa armigera* L.) by releasing five larvae of 3rd instars per plant and MDFID (releasing five larvae of 3rd instars per plant after mechanical damage using needle) by avoiding inter plant larval movement. After 48hr of releasing larvae, leaves were used for biochemical analysis. The leaf samples collected in ice box containing ice cubes and brought to the laboratory.

Rearing of *Helicoverpa armigera*

Field collected eggs of *H. armigera* were allowed to hatch in multi-cell well plates with semi-synthetic diet at 27±1 ^{0}C and 75% relative humidity.

Sample Preparation for Allelochemical Estimation

Damaged leaves, three to five leaves each, per plant were collected from five plants of each treatment. Leaf samples were also collected from control plants that were not subjected either to mechanical injury or prior herbivory. Samples were prepared from 1 g of fresh leaves homogenized in 10 ml of extraction buffer. The data were statistically analyzed (factorial CRD) and the least significant differences were calculated according to Gomez and Gomez.

RESULT AND DISCUSSION

The enzyme activity like NRase and LOX was recorded significantly higher on the insect damaged plants when compared to the mechanical damage followed by insect damage and control plants. The maximum enzyme activity was noted at 45 DAS in both the genotypes.

Assays were carried out to estimate the NRase activity and LOX enzymes influenced by ID and MDFID differed significantly among genotypes, treatments and their interaction at all three stages of crop growth are represented in Table 1 and Table 2. Amount of NRase activity decreased significantly from 45 to 85 DAS and 85 to 125 DAS in control, ID and MDFID plants in both the genotypes Sahana and Laxmi. LOX enzyme activity was present at negligible concentrations in both the damaged and control plants. However, LOX activity titres were significantly higher in the damaged plant compared to control. In a insect damaged plant, LOX was induced immediately on damage and this phenomenon was same in MDFID. Observations on LOX activity as influenced by ID and MDFID, differed significantly among genotypes, treatments and their interaction at all three stages of crop growth are represented in Table 02. Amount of LOX activity decreased significantly from 45 to 85 DAS and 85 to 125 DAS in control, ID and MDFID treatments in Sahana genotype. In Laxmi genotype lipoxygenase activity decreased from 45 to 85 DAS in ID treatment and lipoxygenase activity at 85 DAS is on par with 125 DAS in control and MDFID treatments.

Induced resistance to herbivores is observed in many crop plants and is being studied intensively in several agricultural systems including soybean, tomato, potato, wheat and more recently, in cotton. The role of induced biochemicals has been extensively studied in plant pathogen interactions in cotton. The area of herbivore induced biochemical changes in cotton is relatively new. It is important to determine if prior herbivory or mechanical damage cause changes in cotton in terms of its susceptibility to insect pests. While the former occurs naturally in field situations, the latter is relatively rare.

Sahana recorded significantly higher NRase activity than Laxmi. Irrespective of genotypes significantly higher NRase activity was observed in ID. Sahana recorded significantly higher NRase activity in ID. Laxmi recorded significantly lower NRase activity in control. High NRase activity was observed in Sahana at 45 DAS in ID and lower NRase activity was observed in Laxmi at 125 DAS in control.

Positive role of nitrate reductase activity in pest tolerance cotton, nitrate reductase (NRase) estimation was done in bollworm tolerant (Sahana) and susceptible (Laxmi) genotypes. Presence of significantly higher amount nitrate reductase (NRase) in bollworm tolerant genotype (Sahana) in all the stages and treatments than Laxmi was observed. The

Table 1

Effect of insect damage and mechanical damage followed by insect damage on Nitrate Reductase activity in cotton leaf at different stages of crop growth

Genotypes	Nitrate Reductase (nmoles of NO2 / g / h)																		
	Control						Insect damage						Mechanical damage followed by insect damage						
	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	Grand mean
Sahana	49.46	47.31	36	-4.35	-27.21	**44.25**	54.66	50.47	40.73	-7.67	-25.48	**48.62**	50.20	48.24	37.13	-3.90	-26.04	**45.19**	**46.02**
Laxmi	38.15	26.76	16.68	-29.86	-56.28	**27.19**	40.58	28.29	19.29	-30.29	-52.46	**29.38**	39.79	28.37	17.98	-28.70	-54.81	**28.08**	**28.22**
Percent increase or decrease over laxmi	-22.87	-43.44	-53.67			-38.55	-25.76	-43.95	-52.64			-39.57	-20.74	-41.19	-51.58			-37.85	-38.68
Grand mean	**43.8**	**37.03**	**26.34**			**35.72**	**47.62**	**39.38**	**30.01**			**39.00**	**45.04**	**38.31**	**27.52**			**36.63**	

	S.Em±	CD at 1%
Factor G	0.61	2.35
Factor T	0.75	2.87
Factor D	0.75	2.87
C x T	1.06	4.07
C x D	1.06	4.07
T x D	1.29	4.98
G x T x D	1.83	7.04

G: Genotype, T: Treatment, D : Days after sowing

Table 2

Effect of insect damage and mechanical damage followed by insect damage on Lipoxygenase (LOX) activity in cotton leaf at different stages of crop growths

Genotypes	Lipoxygenase (units/ min)																		
	Control						Insect damage						Mechanical damage followed by insect damage						
	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	Grand mean
Sahana	5.70	4.30	3.00	-24.56	-47.37	**4.33**	5.80	4.40	3.90	-24.14	-32.76	**4.70**	5.10	3.60	3.30	-29.41	-35.29	**4.00**	**4.34**
Laxmi	4.50	3.40	3.40	-24.44	-24.44	**3.77**	5.20	3.50	3.30	-32.69	-36.54	**4.00**	5.10	3.10	3.10	-39.22	-39.22	**3.77**	**3.84**
Percent increase or decrease over laxmi	-21.05	-20.93	13.33			-13.08	-10.34	-20.45	-15.38			-14.89	0.00	-13.89	-6.06			-5.83	-11.51
Grand mean	**5.10**	**3.85**	**3.20**			**4.05**	**5.50**	**3.95**	**3.60**			**4.35**	**5.10**	**3.35**	**3.20**			**3.88**	

	S.Em±	CD at 1%
Factor G	0.004	0.01
Factor T	0.004	0.02
Factor D	0.004	0.02
G x T	0.006	0.02
G x D	0.006	0.02
T x D	0.008	0.03
G x T x D	0.011	0.04

G: Genotype, T: Treatment, D : Days after sowing

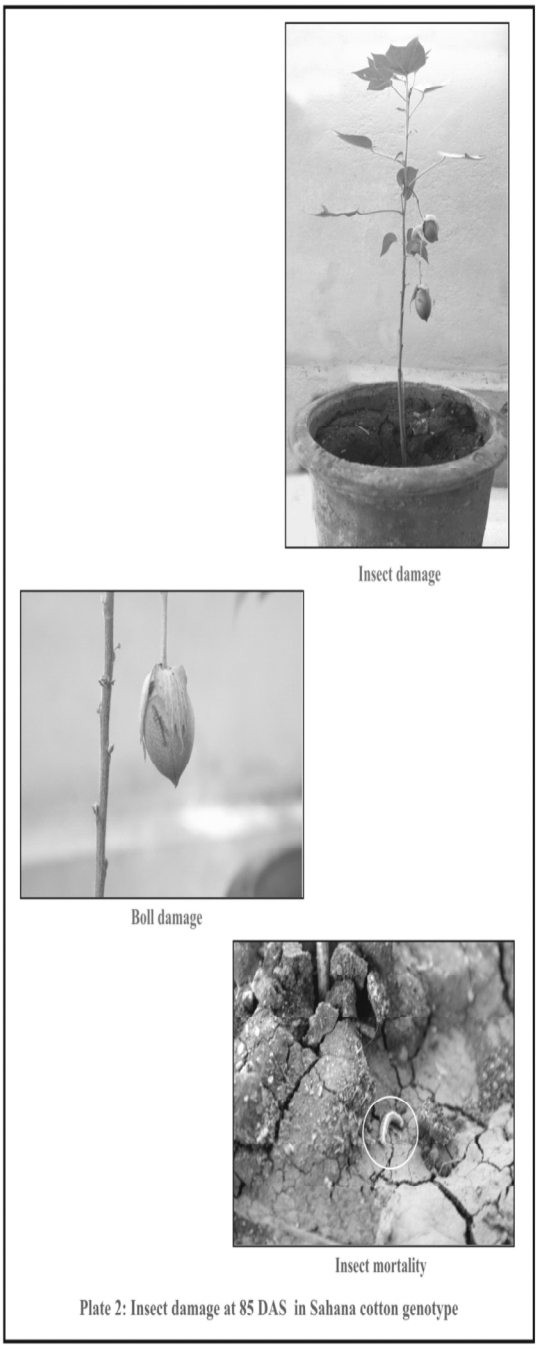

Plate 2: Insect damage at 85 DAS in Sahana cotton genotype

presence of lesser number survived larvae in Sahana than Laxmi indicates its biochemical basis of bollworm tolerance and it has recorded higher nitrate reductase (NRase) activity than Laxmi in both the treatments in this study reports the phenomenon. The phenomenon of increase of nitrate (NRase) reductase activity due to stress has been reported by many workers attributing various reasons. Ananthi and Vijayaraghavan [1] observed Nitrate reductase activity was found to be more in KC2 and AS2 which may be tolerant than the susceptible genotype of cotton. Vamadevaiah [16] reported that the genotypes G.Cot DH-7 and Dhumad possessed higher nitrate reductase activity and carotenoids and exhibited saline tolerance. Sivaramakrishnan *et al.* [14] reported that higher NRase activity was observed at boll development stage for all the genotypes including control. NRase was more in the control than in stressed plants, reduction in enzyme activity either due to the inactivation of the enzyme caused by stress condition.

Higher LOX activity was recorded in Sahana than Laxmi. Irrespective of genotypes, significantly higher LOX activity was observed in ID followed by control and MDFID. Sahana recorded significantly higher LOX activity in ID differed significantly from other two treatments control and MDFID. Laxmi recorded significantly lower LOX activity in both control and MDFID differed significantly from other treatment ID. High amount of LOX activity was observed in Sahana at 45 DAS under ID and lower LOX activity was observed in Laxmi at 85 and 125 DAS under control.

Bollworm tolerant (Sahana) and susceptible (Laxmi) genotypes were used to estimate lipoxygenase (LOX) activity in pest tolerance in Cotton. Presence of significantly higher amount of lipoxygenase (LOX) activity in bollworm tolerant genotype (Sahana) in all the stages and treatments than Laxmi was observed. The presence of lesser number survived larvae in Sahana than Laxmi indicates its biochemical basis bollworm tolerance and it has recorded higher lipoxygenase (LOX) activity than Laxmi in both the treatments in this study reports the phenomenon. The phenomenon of increase of lipoxygenase (LOX) activity due to stress has been reported by many workers attributing various reasons.

Kanofsky and Axelrod [10] reported that there was a significant induction of the LOX enzyme in both the semilooper damaged plants and in mechanically damaged plants, significant detectable induction of LOX has been reported to be more active in the generation of toxic free radicals. Felton *et al.* [7] reported herbivory by the bean leaf beetle, *Ceratoma trifurcata* that causes increase in LOX levels in soybean, while the same was not true of herbivore damaged cotton foliage. While induction occurred soon after mechanical damage, LOX was induced only 48 h after prior herbivory. Bi *et al.* [4] observed insects feeding on plants subjected to prior herbivory or prior damage are more likely to suffer from oxidative and nutritional stress as a result of feeding on foliage that is rich in LOX enzymes, amongst others. Kranthi and

Kranthi, [11] observed that significantly higher lipoxygenase activity in semilooper damage and mechanical damage plant compared to control plants. Gardner, [8] and Vick, [17]. Reported that Lipoxygenase derived fatty acid hydroperoxides can be converted into more stable compounds, including jasmonic acid, which participate in the onset of defense reaction to biotic and abiotic stresses and polyunsaturated fatty acid hydroperoxides resulting from the action of LOX undergoes a variety of reaction including generation of free radicals, which provoke changes in membrane properties, ultimately leading to disfuntioning of the lipid bilayer membrane. Argandona *et al*. [2] *Schizaphis graminum* (R.) herbivory increased hydrogen peroxide content and total soluble peroxidase (POD) activity in barley, with a maximum level of POD activity after 30 min of infestation. Lipoxygenase (LOX) activity was studied by Babitha *et al.* [3] in seedlings of pearl millet genotypes resistant and susceptible to downy mildew pathogen *Sclerospora graminicola*. An increase in LOX activity was observed during the incompatible host-pathogen interaction whereas the activity decreased in compatible ones. Resistant pearl millet seedlings exhibited a 2.4 fold increase in LOX activity after inoculation with the pathogen. The enzyme activity was maximum at 18 h after inoculation. The enzyme activity was maximum in shoot portion of resistant genotype after inoculation. The enzyme activity correlated well with the degree of host-resistance to the pathogen.

In summary, the higher activity of enzymes like NRAase and LOX in Sahana play a defensive role against the insect pest. It is probably due to induction of oxidative enzymes, such as NRAase and LOX.

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