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Biochemical Constituents Imparting Resistance to Sucking Pest Aphid in Cotton (*Gossypium* spp.)

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A B S T R A C T

Present investigation was performed to elucidate the role of selected plant antioxidant enzymes (lipoxygenase and nitrate reductase) and phenolic compounds (total phenol and condensed tannin) in imparting the resistance/susceptibility to aphid infestation in two cotton genotypes (CPD14-1 and CPD14-2). Leaf samples were collected from both aphid infected as well as uninfected plants at 45, 85 and 125DAS (days after sowing) and analysis of the phenolic compounds and enzymatic activities were carried out. Changes in the enzyme activity level were determined at different time interval after infestation. The results indicated that aphid infestation increased the total phenols, condensed tannin, and enzymatic activities. The resistant genotype CPD14-1 recorded higher phenol content (7.07g/100g dry weight), condensed tannin (7.97g/100g dry weight) and enzymatic activities lipoxygenase (3.42U/mg of protein) and nitrate reductase (62.6 nmoles of NO_2 g/h) than susceptible genotype CPD14-2. The results suggested that enhanced activities of enzymes and phenolic compounds may contribute bioprotection of cotton plants against aphid infestation.

Introduction

Since 6000 BC, cotton has been cultivated for lint fiber, which now dominates the natural textile industry worldwide. Cotton fibers sustain one of the world's largest industries, the textile industry, for wearing apparel, home furnishings, and medical supplies. Cottonseed oil is ranked fifth in production and consumption volume among all vegetable oils in the past decades, accounting for 8% of the world's vegetable oil consumption. It provides 65% raw material to textile industry and contributes one third of total foreign exchange earning of India (Mayee and Rao, 2002).

Out of the *Gossypium* species, four are cultivated in agriculture, including two allotetraploids (*G. hirsutum and G. barbadense*) and two diploids (*G. herbaceum and G. arboreum*). *Gossypium hirsutum*, also known as Upland cotton, Long Staple Cotton, or Mexican Cotton, produces over 90% of the world's cotton whereas the other three *Gossypium* species are grown only on comparably small areas. These genotypes were most likely domesticated independently of each other (Fryxell, 1979). Among the several reasons of low yield, the magnitude of insect-pests which damage the cotton crop

from sowing to maturity plays an important role. The insect-pests on an average cause 5-10 percent of losses but under several insect-pests infestations can cause heavy qualitative and quantitative losses varying from 40- 50% (Naqvi, 1976). Cotton insect pest complex is divided into two categories; sucking insect pests and chewing insect pests. Important sucking insect pests are whitefly, *Bemisia tabaci* (Genn.), thrips, *Thrips tabaci* (Lind.) jassid, *Amrasca devastans* (Dist.) and aphid, *Aphis gossypii* (Glov.) which is also designated as key pests causing most of the damage to the cotton crop.

The mechanism of host plant resistance in response to insect infestation consists of a series of biochemical events, including increased production of phenolics, mediated by phenylalanine ammonia-lyase, tyrosine ammonialyase, peroxidase and polyphenol oxidase. 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 namely 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, multiplicity of biochemical mechanisms involved in the protective role by metabolites (Edreva, 2008).

Therefore the aim of present work was, to study the defence mechanism of cotton cultivars against sucking pest aphid, the oxidative responses since they thought to have an important role as antiherbivores.

Materials and Methods

In order to study the factors responsible for resistance to sucking pest aphid infestation, the experiment was conducted during *kharif* 2015-16 at ARS Dharwad farm. Two genotypes were used to study resistant CPD14-1 genotype and susceptible CPD14-2 genotype. In the field one uninfected set was maintained as control by regular spraying and cages lined with nylon mesh to maintain plants free from insect pests and the other set of plants were allowed for naturally aphid infestation and aphid population per plant were recorded and analysis of the biochemical constituents were performed at an interval of 45, 85 and 125 DAS.

Sample preparation

Five plants were randomly selected from each treatment. The top three leaves from the control and infested leaves were chosen for biochemical analysis.

Estimation of total phenol and tannin content

For total phenol estimation 100 mg of oven dried leaf sample was extracted in 10ml of warm 80% ethanol. Then, it was cooled and passed through double layered muslin cloth. The residue of the tissue was ground thoroughly in a morter pestle with hot

alcohol. It was passed through muslin cloth repeated to obtain sufficient amount of extract. The extract was polled and filtered through Whatman NO. 41 filter paper and made up to 10ml volume with alcohol and stored in a refrigerator at 4^{o}C. Total phenols were estimated by Folin-Ciocalteau reagent (FCR) method (Bray and Thorpe, 1954).Tannin was estimated by Folin- Denis reagent (FDR) method (Chan *et al.*, 1978).

Estimation of nitrate reductase activity

Nitrate reductase activity in the leaves was determined by using colorimeter (Hageman and Reed, 1980). A known weight (140 mg) of fresh leaf tissue was cut into pieces and suspended in screw cap vials containing 3.5ml of incubation mixture (20ml of 0.1M phosphate buffer p^{H} 7.5+ 20ml of 5 per cent propanol + 10ml of 0.2 per cent KNO_3). The vials are sealed and kept in dark condition at 30°C for 2hr. Nitrite released into the medium was determined by treating 1ml aliquote with 1ml each of 1 percent sulphanyl amide and 0.02 per cent N-1-napthyl ethylene diamine hydrochloride. After 20 min, solution is diluted to 5 ml with water (final volume should be 5 ml) and absorbance is measured at 540 nm. Standard curve was prepared by using different grade concentrations of nitrite (KNO_2) solution. The nitrate reductase activity is expressed as nmoles of NO_2 formed per gram fresh weight per hour.

Estimation of lipoxygenase activity

Enzyme extraction and assay

1gm of fresh leaf sample was ground using 5ml of 0.1 M Phosphate buffer, pH 6.5 at 4^{0}C, centrifuge at 13000 rpm for 20 min at 4^{0}C, supernatant was collected and used as an enzyme source. Lipoxygenase activity in the leaves was determined colorimetrically (Grayburn *et al.*, 1991). 29.9ml of linoleic acid was taken in 100ml volumetric flask and maintained at 30^{0}C using hot water bath. Aerate gently by gentle steam of air for 2 min and start the reaction by adding 0.1ml of enzyme extract. 1ml of solution was taken from the reaction mixture and transfer to glass tube containing 4ml of 0.1N NaOH at different time interval of 0.5, 1, 1.5, 2 and 2.5 minutes. Simultaneously kept a blank with 1ml substrate and 0.1N NaOH, absorbance was taken at 234 nm.

Statistical Analysis

The data of the experiment was analyzed statistically following the procedure described by Gomez and Gomez (1984). The level of significance used in 'F' and't' test was p = 0.05. The critical difference was calculated wherever the 'F' value was found to be significant by using 3 factorial RCBD (Randomized Complete Block Design).

Results and Discussion

Total phenols

The phenol content of resistant CPD14-1 in control plant at 45, 85 and 125 DAS were (7.35, 6.82 and 6.42 g/100g dry weight) and the susceptible CPD14-2 genotype recorded (6.88, 6.52 and 6.35 g/100g dry weight) respectively as shown in (Fig. 1). However the phenol content was increased under insect damage.

The increase was more in CPD14-1 at 45, 85 and 125 DAS (7.6, 7.28 and 6.96 g/100g dry weight) than CPD14-2 (7.19, 6.79 and 6.62 g/100g dry weight). Results presented in table revealed that the resistant genotype exhibited higher total phenol content compared to susceptible under both control and insect damage and also the total phenol content in both the genotype decreased as the age of the crop advanced.

The results are in line with the findings Kalappanavar and Hiremath (2000) reported higher phenol content in multiple foliar disease resistant sorghum genotypes than susceptible ones. Jain and Yadav, 2003 (2003) who reported total phenol act as antioxidant enzyme substrates. As the high concentrations of phenolic acids are related to resistance to pest (Usha Rani and Jyothsna, 2010), the increased quantity of total phenols might also be attributed to defense mechanism. After infection by a pathogen, plant cells synthesize phenol oxidizing enzymes that oxidise phenols into toxic quinines, which play a crucial role in disease resistance (Jiang *et al.,* 2009). Based on these findings, it could be concluded that rapid accumulations of phenolic compounds occur in incompatible (resistant) host pathogen interaction than the compatible (susceptible) ones.

Condensed tannin

The tannin content of both the genotypes under control and insect damage was measured and percentage decrease was calculated (Table 1). Tannin content of CPD14-1 genotype at 45, 85 and 125 DAS under insect damage was (9.20, 8.78 and 7.53 g/100g dry weight), while under control (8.43, 7.58 and 6.32 g/100g dry weight). On the other hand in insect damage CPD14-2 recorded (8.41, 7.76 and 6.28 g/100g dry weight), respectively, while under control (7.54, 6.87and 5.36 g/100g dry weight).

The results indicated that CPD14-2 susceptible genotype recorded significantly lower tannin content than resistant CPD14-1 with percentage reduction (-25.3-28% 125 over 45DAS). The results of the experiment are in agreement with the findings of Somashekara *et al.*,(2003) the extremity in tannin content of plant was observed among the two groundnut varieties, NAC-6004 was having highest total tannin content as compared to verity CM-300 showed lower tannin content. Khurana *et al.,* (19897) the higher concentration of total tannin had been reported to show antibiotic effect in sorghum against shoot fly and stem borer. However, tannins were associated with repellency or deterrency and jointly contributed to the protection of plant along with other phytochemicals like phenols. Since, tannin present in plant has a role in anti-feedants properties of plant and protects it from being attacked by the pest and diseases.

Lipoxygenase

The leaves of infected resistant genotype showed higher activity of lipoxygenase at 45, 85 and 125 DAS (4.09, 3.83 and 3.13U/mg of protein respectively), compared to infected susceptible genotype (2.43, 2.09 and 1.78 U/mg of protein). Lipoxygenase activity also increased under control resistant genotype (3.73, 3.12 and 2.65U/mg of protein), but it was lower than infected resistant (Table 2). LOX activity was more in insect damage treatment, significantly higher LOX activity was recorded in CPD14-1 genotype with per centage reduction 6.35 per cent in 85DAS over 45DAS and 23.47 per cent in 125 over 85DAS. CPD14-2 genotype recorded significant lower LOX activity with percentage reduction 13.9 per cent in 85DAS over 45DAS and 26.7 per cent in 125 over 85DAS.

The phenomenon of increased lipoxygenase activity due to stress is attributed by various reasons. Mahatma *et al.,* 2011, a higher level of LOX activity was observed in *Fusarium udum* inoculated resistant pigeon pea cultivar than in susceptible cultivar. The increased LOX may generate signal molecules such as jasmonic acid, methyl jasmonic acid or lipid peroxides, which co-ordinately amplify specific responses.

Table.1 Effect of insect damage on accumulation of Tannin content in cotton leaf at different stages of crop growth

Genotype	Tannin(mg/g dry weight)												
	Control						Insect Damage						
	45 DAS	85 DAS	125 DAS	Per cent decrease in 85 DAS over 45 DAS	Per cent decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Per cent decrease in 85 DAS over 45 DAS	Per cent decrease in 125 DAS over 45 DAS	Mean	Grand mean
CPD14-1	8.43	7.58	6.32	-10.08	-25.02	7.44	9.20	8.78	7.53	-4.56	-18.15	8.5	7.97
CPD14-2	7.54	6.87	5.36	-8.88	-28.91	6.59	8.41	7.76	6.28	-7.72	-25.32	7.48	7.03
Per cent increase or decrease over CPD14-2	-10.55	-9.36	-15.8			-11.4	-8.58	-11.6	-16.6			-12	-11.79
Grand mean	7.98	7.22	5.84			7.01	8.8	8.27	6.90			7.99	

	S.Em±	CD@ 1%
Factor G	**0.015**	**0.058**
Factor T	**0.015**	**0.058**
Factor D	**0.019**	**0.071**
G x T	**0.022**	**0.082**
G x D	**0.027**	**0.101**
T x D	**0.027**	**0.101**
G x T x D	**0.038**	**0.143**

Table.2 Effect of insect damage on accumulation of Lipoxygenase activity in cotton leaf at different stages of crop growth

Genotype	Lipoxygenase(u/mg of protein)												
	CONTROL						INSECT DAMAGE						
	45 DAS	85 DAS	125 DAS	Per cent decrease in 85 DAS over 45 DAS	Per cent decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Per cent decrease in 85 DAS over 45 DAS	Per cent decrease in 125 DAS over 45 DAS	Mean	Grand mean
CPD14-1	3.73	3.12	2.65	-16.35	-28.9	3.16	4.09	3.83	3.13	-6.35	-23.47	3.68	3.42
CPD14-2	2.03	1.82	1.20	-10.34	-40.8	1.68	2.43	2.09	1.78	-13.99	-26.7	2.11	1.89
Per cent increase or decrease over CPD14-2	-45.57	-41.66	-54.71			-46.8	-40.5	-45.4	-43.1			-42.6	-44.7
Grand mean	2.88	2.47	1.92			2.42	3.26	2.96	2.45			2.89	

	S.Em±	CD@ 1%
Factor G	0.016	0.062
Factor T	0.016	0.062
Factor D	0.020	0.076
G x T	0.023	0.088
G x D	0.029	0.108
T x D	0.029	0.108
G x T x D	0.040	0.152

Fig.1 Effect of insect damage on accumulation of phenol content in cotton leaf at different stages of crop growth

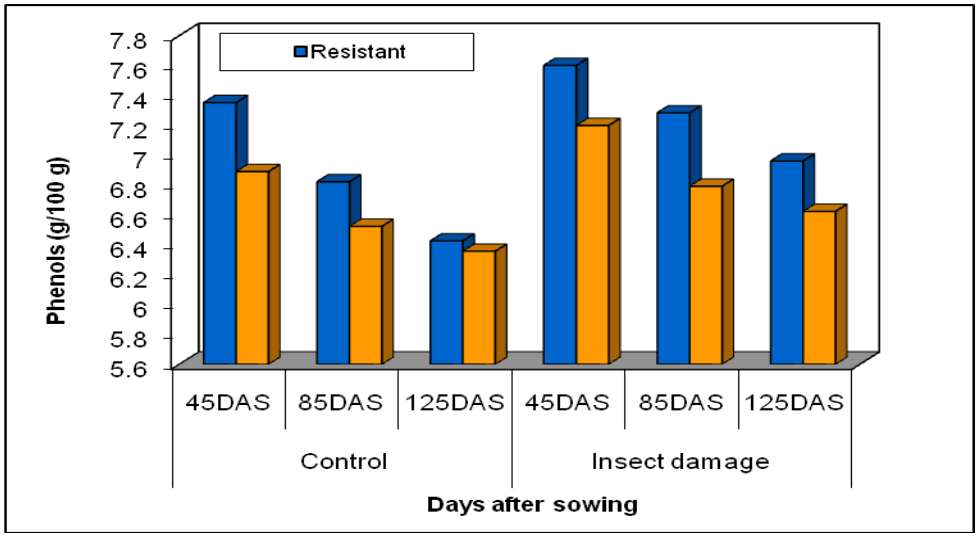
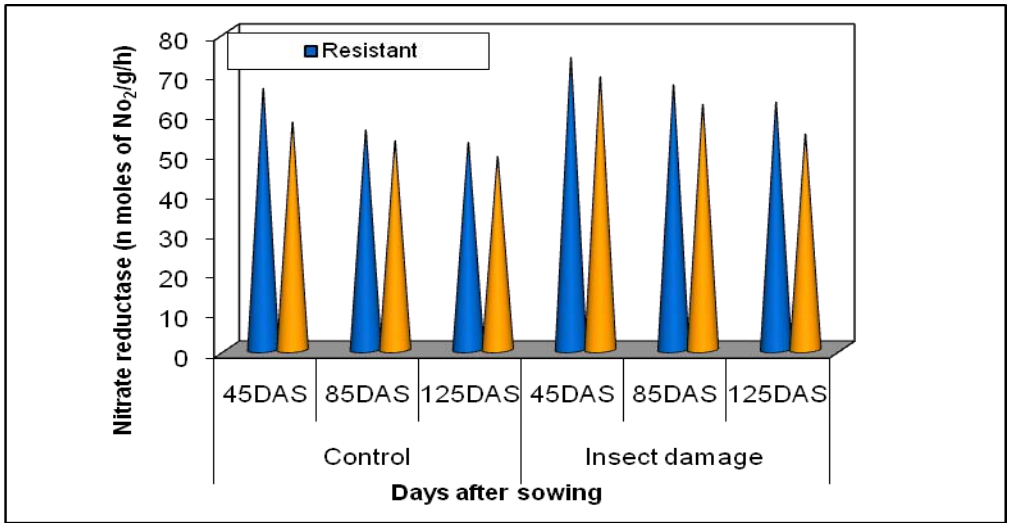


Fig.2 Effect of insect damage on accumulation of Nitrate reductase activity in cotton leaf at different stages of crop growth



Alternatively, LOX catalyzed reactions can result in the production of toxic volatile and non-volatile fatty acid derived secondary metabolites that can directly attack invading pathogens (Croft *et al.,* 1993). Increased LOX activity may also cause irreversible membrane damage, which would lead to the leakage of cellular contents (Mac Carrone *et al.,* 2000).

Nitrate reductase activity

The nitrate reductase activity was decreased as the age of the crop advances as shown in (Fig. 2). CPD14-1 recorded significantly higher nitrate reductase activity at 45, 85 and 125DAS (73.3, 66.7 and 62.3 nmoles of NO_2/g/h) under insect damage than control (65.85, 55.36 and 52.2 nmoles of NO_2/g/h). CPD14-2 genotype recorded lowest nitrate reductase activity in control (57.38, 52.61 and 8.67nmoles of NO_2/g/h) and also insect damage (68.7, 61.7 and 54.3 nmoles of NO_2/g/h) respectively.

The increased NRase activity was attributed to various reasons as follows; the nitrate reductase activity was more in the control than in stressed plants. The NRase, is a substrate inducible enzyme, mediates conversion of nitrate to nitrite. Ananthi and Vijayaraghavan, (2012), observed Nitrate reductase activity was found to be more in KC2 and AS2 which may be tolerant than the susceptible genotype of cotton.. Sivaramakrishnan *et al.,* (1988) studied the midseason drought indicating that there is a sharp decline in NRase activity under water stress situation. NRase activity was found to be more in KC2 and AS2.

This research offers a new perspective on cotton plant resistance against aphid and provides a model for studying insect plant interactions. Furthermore, the identification of biochemical constituents for aphid resistance provides a novel approach for screening resistant cotton cultivars for further breeding programme or as 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 aphids.

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