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Evaluation of antioxidative metabolites in cotton (*Gossypium hirsutum* L.) genotypes in response to sucking pest attack

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Abstract
Nine cotton genotypes *viz*. HD418, HD432, HD503, H1439, H1463, H1454, H1464, H1465 and H1098 were evaluated under field conditions to compare their resistance levels to sucking pest attack. In the present study, ascorbate and β-carotene content of cotton genotypes infected by sucking pests were estimated in the leaves (2^{nd} & 6^{th}) at 50, 60 and 68 days after sowing (DAS). The ascorbate and β carotene content before infection was maximum in 2^{nd} & 6^{th} leaves of *G. arboreum* genotypes followed by *G. hirsutum* resistant genotypes and minimum in *G. hirsutum* susceptible genotypes. After infection the ascorbate and β carotene content increased in all the genotypes in both the leaves. The maximum increase in content of ascorbate and β-carotene were observed in 6^{th} leaves after pests' infection and more increase was in resistant genotypes than susceptible genotypes.

Keywords: ascorbate, β-carotene, cotton, gossypium hirsutum, metabolite, sucking pest

Introduction
Cotton belongs to the genus Gossypium and family Malvaceae. It is the principal commercial and cash crop of India since time immemorial. India has a pride place in the global scenario with the cultivation of all four species of cotton *viz.*, *G. hirsutum* L., *G. barbadense* L., *G. arboreum* and *G. herbaceum.* Textile industry is a number one enterprise in the country which consumes nearly 70 per cent of total fibre produced (Itnal, 2004) [11]. India, China, United State, Pakistan and Brazil are main producers of cotton in the world (Johnson *et al.*, 2014) [12] and account for almost 80% of global production. India ranks first in area of cultivation and second in production after China.

Cotton crop is infested by several pests right from germination to harvest and the pest spectrum of cotton is quite complex. As many as 1326 species of insect and mite pests have been reported to attack cotton at various stages of crop growth, across the globe (Hargreaves, 1948) [9]. However, in India the number is limited to 130 species (Agarwal *et al.*, 1984) [1]. Among them the boll worms *viz.*, American boll worm, *Helicoverpa armigera* (Hubner), spotted boll worm, *Earias vittella* (Fabricius), spiny boll worm, *Earias insulana* (Boisduval) and pink boll worm, *Pectinophora gossypiella* (Saunders) pose greater threat to cotton production. Besides, a complex of sucking pests *viz.*, Green leaf hopper, *Amrasca biguttula biguttula* (Ishida), thrips, *Thrips tabaci* (Linnman), aphid, *Aphis gossypii* (Glover) and whitefly, *Bemisia tabaci* (Gennadius) are known to have occupied major pest status (Gosh, 2001) [7] and account for the yield loss of 22.85 per cent (Satpute *et al.*, 1990) [16].

A lot of attention is being paid towards improvement of tolerance of crops to pest attack. Antioxidative defense system also plays an important role in resistance mechanism. When plants are subjected to biotic or abiotic stresses, reactive oxygen species (ROS) such as. O_2^-, OH^- and H_2O_2 are generated in response to stress condition (Dat *et al.*, 2000) [6] which are considered to be indicators of oxidative damage (Wise and Naylor, 1987). To control the level of ROS and to protect the cells, plants possess a number of low-molecular weight antioxidants *viz.* ascorbate, glutathione, phenolic compounds, tocopherols and enzymes *viz.* SOD, CAT, APX, and GR which cause scavenging of ROS and regeneration of the active forms of antioxidants. Detailed studies on antioxidant enzymes are important to facilitate our understanding of their role in insect pest resistance. It is, therefore, the important aim of the cotton breeder to develop cotton genotypes with enhanced protective antioxidative defense system.

Material and Methods

The present study was carried on nine genotypes HD418, HD432, HD503, H1439, H1463, H1454, H1464, H1465 and H1098 of cotton, samples of leaves (2nd and 6th) were taken before and after infection of sucking pests from field of Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. These genotypes differ, in their resistance to sucking pests. HD418, HD432, HD503 were resistant genotypes of *G. arboreum*, H1464, H1465 and H1098 were resistant genotypes of *G. hirsutum and* H1439, H1463 and H1454 were susceptible genotypes of *G. hirsutum*.

β Carotene content

β carotene content was determined using the method of AOAC, 2000 [2]. Absorbance was measured at 440 nm in spectrometer using saturated n-butanol as a blank and amount of β carotene was calculated using equation.

β-Carotene (ppm) = 0.0105+23.5366×A

Ascorbate

Ascorbic acid content was estimated by the method of Mukherjee and Choudhuri, 1983 [14]. The concentration of ascorbic acid was calculated from a standard curve plotted with known concentration of ascorbic acid by measuring absorbance at 530nm.

Results and Discussion

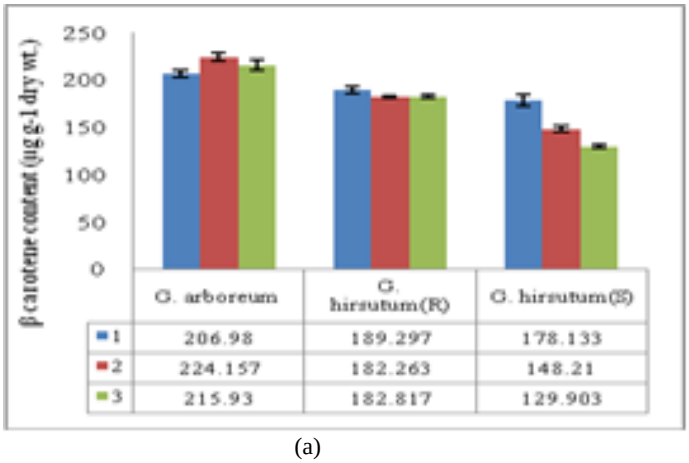
β carotene- Carotenoids are C_{40} isoprenoids that play critical role not only in plant defense, but also in plant growth and development. In addition to their many economic and health benefits (Carvajal-Lérida *et al.,* 2012) [4], carotenoids are critical components of the photosynthetic machinery, and play a role in protecting the plant from photooxidative damage (Howitt and Pogson, 2006) [10]. Results depicted in Fig. 1(a) and Fig. 1(b) show the β carotene content in 2nd and 6th healthy leaves of resistant and susceptible cotton genotypes respectively.

β carotene content before infection was maximum in 2nd leaf of *G. arboreum* genotypes (206.98-224.16 µg g^{-1} dry wt.) followed by *G. hirsutum* resistant genotypes (182.26-189.29 µg g^{-1} dry wt.) and minimum in *G. hirsutum* susceptible genotypes (129.90-178.13 µg g^{-1} dry wt.). 6th leaf of *G. arboreum* genotypes had 217.77-237.85 µg g^{-1} dry wt β carotene content followed by *G. hirsutum* resistant genotypes (203.03-211.40 µg g^{-1} dry wt.) and minimum in *G. hirsutum* susceptible genotypes (195.82-203.87 µg g^{-1} dry wt.). β carotene content was higher in resistant genotypes than susceptible genotypes in both 2nd and 6th healthy leaf and 6th leaf had more β carotene content than 2nd leaf in all the genotypes. All the genotypes did not have significant difference in β carotene content in 2nd and 6th leaves.

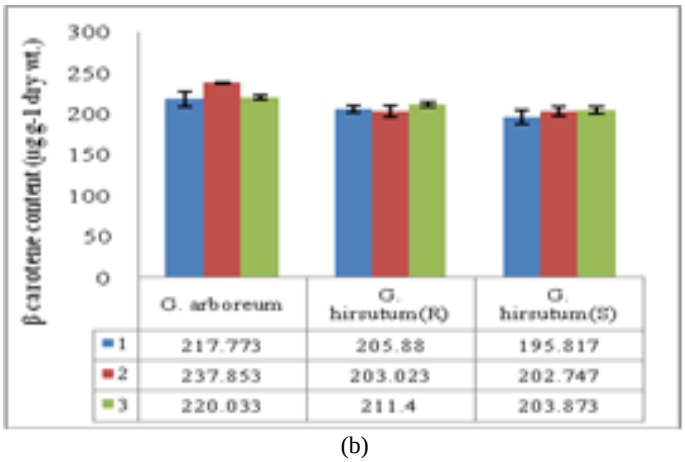
Results depicted in Fig. 1(c) show the effect of pests infection on β carotene content in 2nd leaf of resistant and susceptible cotton genotypes and Fig. 1(d) shows the effect of pests infection on β carotene content in 6th leaf of resistant and susceptible cotton genotypes. After infection, there was increase in β carotene content. In 2nd leaf at 60 DAS, increase in β carotene content was 43.17-55.98% in resistant genotypes and 22.34-41.12% in susceptible genotypes whereas at 68 DAS the increase was 81.90-103.07% in resistant genotypes and 60.63-60.37% in susceptible genotypes in case of *G. hirsutum*.

In 6th leaf increase was 29.59-32.84% in resistant genotypes and 19.97-23.95% in susceptible genotypes at 60 DAS and at 68 DAS, increase was 131.12-142.31% in resistant genotypes and 74.58-102.56% in susceptible genotypes. Significant increase in β carotene content was observed in 2nd leaf at 60 DAS & 68 DAS and in 6th at 68 DAS whereas in 6th leaf at 60 DAS increase was not as significant.

Tolerant genotypes had more β-carotene content than sensitive genotypes in both 2nd & 6th leaves (fig. 1a & 1b) and there was increase in β-carotene content on pest infection in all the genotypes but increase was more in resistant genotypes than susceptible genotypes in both 2nd & 6th leaves (fig. 1c & 1d). Increased carotene content could be associated with the higher production of singlet oxygen species along with other oxyradicals (Mishra *et al.,* 1993) [13].

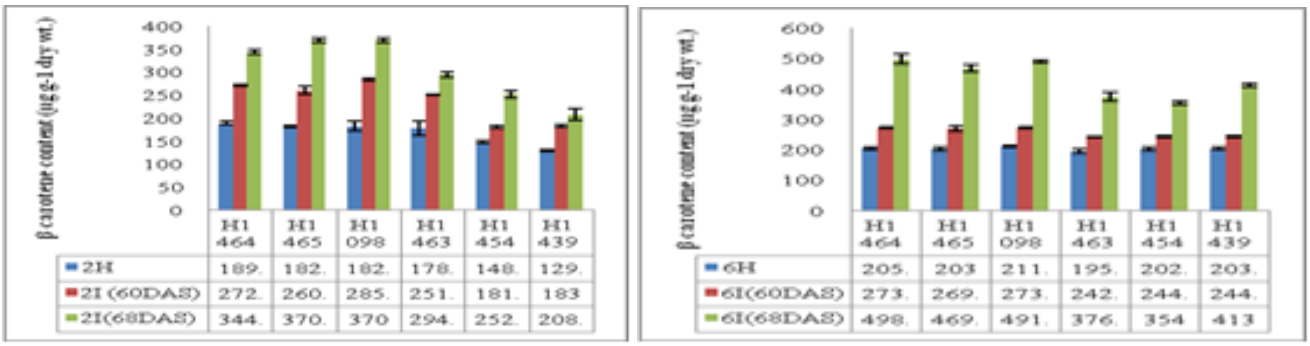


(a)



(b)

Fig. 1: β carotene content (µg g^{-1} dry wt.) in (a) 2nd and (b) 6th healthy leaves of resistant and susceptible cotton genotypes.
CD at 5%: (a) Genotypes=21.32 (b) Genotypes= N/A



(c) (d)

Fig 1: Effect of pests infection on β carotene content (µg g^{-1} dry wt.) in (c) 2nd and (d) 6th leaves of resistant and susceptible cotton genotypes.

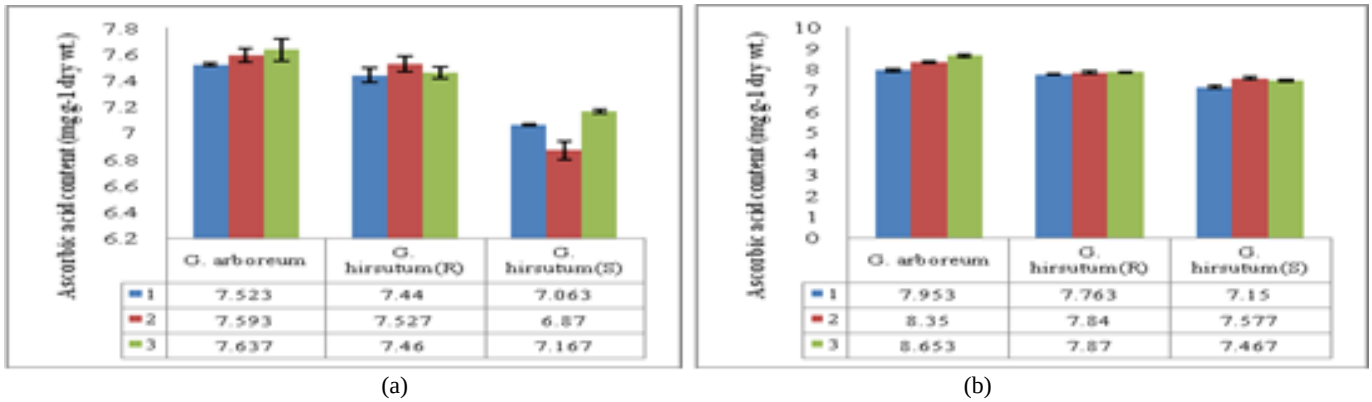
(c) H, I (60DAS)	H, I (68DAS)	(d) H, I (60DAS)	H, I (68DAS)
A=15.04	A=16.06	A= N/A	A=23.19
B=8.69	B=9.26	B=10.65	B=13.39
A × B=21.28	A × B=22.61	A ×B=N/A	A×B=32.79

4.1.2 Ascorbate: Ascorbate plays an important role in affording protection against active oxygen species, as it acts as electron donor for APX. Ascorbate is main non-enzymatic antioxidants which play a key role in protecting plants against ROS-mediated oxidative damage (Smirnoff, 2005) [7]. It is involved in maintenance of redox state of cells. Moreover, ascorbate provides membrane protection by directly scavenging. O_2^- and H_2O_2 (Dalton, 1995) [5].

Results depicted in Fig. 2(a) and Fig. 2(b) show the ascorbate content in 2nd and 6th healthy leaves of resistant and susceptible cotton genotypes respectively. Ascorbate content followed similar trend as β carotene content in both 2nd & 6th leaves before infection. Ascorbate content was higher in *G. arboreum* genotypes followed by *G. hirsutum* resistant genotypes and minimum in *G. hirsutum* susceptible genotypes in both 2nd and 6th leaves. Ascorbate content was higher in resistant genotypes than susceptible genotypes in both 2nd and 6th leaves. 6th leaf had more ascorbate content than 2nd leaf in all the genotypes. All the genotypes not differ significantly in ascorbate content.

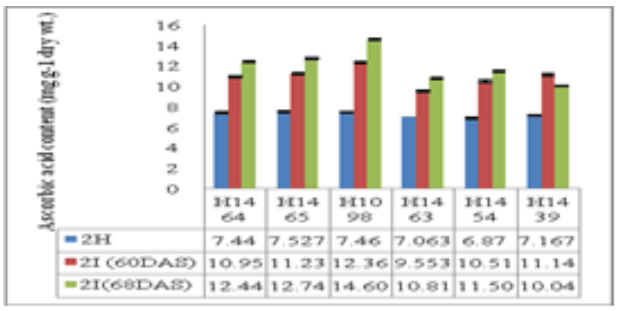
Results depicted in Fig. 2(c) show the effect of pests infection on ascorbate content in 2nd leaf of resistant and susceptible cotton genotypes and Fig. 2(d) shows the effect of pests infection on ascorbate content in 6th leaf of resistant and susceptible cotton genotypes. After infection, increase in ascorbate content was observed in *G. hirsutum* genotypes. In 2nd leaf, at 60 DAS, the increase in ascorbate content was 47.18-65.78% in resistant genotypes and 35.25-55.53% in susceptible genotypes whereas at 68 DAS the increase was 67.20-95.68% in resistant genotypes and 40.13-53.05% in susceptible genotypes.

In the present study resistant genotypes showed pronounced increase in ascorbate content than susceptible genotypes in both 2nd & 6th leaves (fig. 2a & 2b). The present results indicated that there was increase in ascorbate content on pests infection at both 60 DAS and 68 DAS stages (fig. 2c & 2d). Similar reports suggest that wounded jasmonate influenced ascorbate accumulation in various plants (Suza *et al.*, 2010) [18]. The results of present study are in agreement with those obtained by Gossett *et al.* (1994) [8] in cotton. Noctor and Foyer (1998) [15] observed increased ascorbate level increase in the susceptible varieties of barley infected with powdery mildew disease and proposed that ascorbic acid is present in high concentration in plant tissues and possesses a prominent antioxidative role in barley leaves as the most important reducing agent. Whereas, Arrigoni (1979) [3] reported that the amount of ascorbic acid in susceptible plant was unaltered by nematode attacks, but in resistant plants, ascorbic acid synthesis was always stimulated.

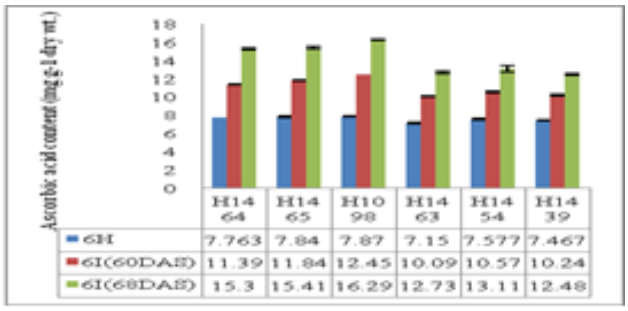


(a) (b)

Fig. 2: Ascorbic acid content (mg g^{-1} dry wt.) in (a) 2nd and (b) 6th healthy leaves of resistant and susceptible cotton genotypes.
CD at 5%: (a) Genotypes=0.15 (b) Genotypes=0.16



(c)



(d)

Fig 2: Effect of pests infection on Ascorbic acid content (mg g^{-1} dry wt.) in (c) 2nd and (d) 6th leaves of resistant and susceptible cotton genotypes.

(c) H, I (60DAS)	H, I (68DAS)	(d) H, I (60DAS)	H, I (68DAS)
A=0.14	A=0.12	A=0.13	A=0.27
B=0.08	B=0.07	B=0.07	B=0.16
A× B=0.19	A × B=0.17	A × B=0.38	A × B=0.18

The present study revealed maximum increase in content of ascorbate and β-carotene in 6th leaves after pests infection and more increase was in resistant genotypes than susceptible genotypes. Thus, these biochemical parameters studied in the present investigation play important role in providing resistance to sucking pests infection in cotton genotypes.

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