

Original Research Article

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## Evaluation of Antioxidative Responses in Cotton (*Gossypium hirsutum* L.) Genotypes Imparting Resistance to Sucking Pest Attack

Anju Rani, Jayanti Tokas\*, Himani and H. R. Singal

Department of Biochemistry, College of Basic Sciences and Humanities, CCSHAU, Hisar - 125004 (Haryana), India

\*Corresponding author

### ABSTRACT

#### Keywords

Antioxidative enzyme, cotton, sucking pest, resistance, yield

#### Article Info

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Present study was investigated to elucidate the role of antioxidative enzymes in imparting resistance to sucking pest attack. Antioxidative enzymes viz. SOD, CAT, POX, GR and APX were estimated in the leaves (2<sup>nd</sup> leaf & 6<sup>th</sup> leaf) of cotton genotypes infected by sucking pests at 50, 60 and 68 days after sowing (DAS) stage. The antioxidative enzyme activity before infection was maximum in 2<sup>nd</sup> & 6<sup>th</sup> leaves of *G. arboreum* genotypes followed by *G. hirsutum* resistant genotypes and minimum in *G. hirsutum* susceptible genotypes. After infection, antioxidative enzyme activity increased in all the genotypes in both the leaves. The maximum increase in activities of enzymes viz. catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione peroxidase (GR) were observed in 6<sup>th</sup> leaves after pests infection. Maximum increase in antioxidative enzymes was observed in HD418 of *G. arboreum*, H1098 of *G. hirsutum* (R) and H1454 genotype of *G. hirsutum* (S). The results suggested that antioxidative enzymes play an important role in providing resistance to sucking pests infection in cotton genotypes.

### Introduction

Cotton is an important cash crop of India. It belongs to the genus *Gossypium* and family *Malvaceae*. It is grown in India in about 111.55 lakh hectares as against 92.33 lakh hectares witnessed for the same time last year, thereby indicating an increase of close to 21 per cent in the acreage, with annual production of 337.25 lakh bales of 170 kg each. Crop loss due to pest and pathogen attack is a serious problem worldwide. The incidence of insect

pests considerably reduces both the yield and quality of cotton production. In India sucking pest reduces the crop yield to greater extent (Dhawan *et al.*, 1988). Nath *et al.*, (2000) reported that American cotton is more susceptible to the attack of sucking insect pests as well as bollworm complex than indigenous cotton. However, interestingly, the native cotton *Gossypium arboreum* and *Gossypium herbaceum* appears not to be infected with cotton leaf curl disease till the first inception of disease (Akhtar *et al.*, 2010,

2013). Physiological, morphological, and biochemical changes are observed in the plant in response to sucking pest damage (Agrawal *et al.*, 2009). Biotic and abiotic stresses such as drought, salinity, chilling, metal toxicity, and UV-B radiation as well as pathogens attack lead to enhanced generation of ROS in plants due to disruption of cellular homeostasis (Shah *et al.*, 2001; Sharma and Dubey, 2005). Whether ROS will act as damaging or signaling molecule depends on the delicate equilibrium between ROS production and scavenging. Because of the multifunctional roles of ROS, it is necessary for the cells to control the level of ROS tightly to avoid any oxidative injury and not to eliminate them completely. Higher plants have evolved a complex network of antioxidant systems to counteract elevated ROS levels produced in response to pest infestation. This sophisticated machinery encompasses a wide range of lipid and water-soluble antioxidants (e.g., tocopherols,  $\beta$ -carotene, ubiquinone, ascorbate, glutathione) and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST), glutathione peroxidase (GPX), and ascorbate peroxidase (APX) (de Carvalho *et al.*, 2013; Sanchez-Rodriguez *et al.*, 2012). Higher levels of anti-oxidative enzymes such as SOD, CAT, and POX along with polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) were observed in the infested cotton plants. Detailed studies on antioxidant enzymes are important to facilitate our understanding of their role in insect pest resistance. It would, therefore, be the important aim of the cotton breeder to develop cotton genotypes with enhanced protective antioxidative defense system.

## Materials and Methods

The present study was conducted in nine cotton genotypes *viz.* HD418, HD432, HD503, H1439, H1463, H1454, H1464, H1465 and

H1098 during *kharif* season at cotton field of Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. Analysis of antioxidative enzymes was performed at an interval of 50, 60 and 68 days after sowing. Three plants were randomly selected and 2<sup>nd</sup> & 6<sup>th</sup> leaves were taken before and after infection of sucking pests for estimation for biochemical constituents. The enzymes namely superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and glutathione reductase were assayed as per the below mentioned methodology.

### Superoxide dismutase (EC 1.15.1.1)

Superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium, adopting the method of Giannopolities and Ries (1977). The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 7.8), 14 mM L-methionine, 10  $\mu$ M nitroblue tetrazolium, 3  $\mu$ M riboflavin, 0.1 mM EDTA and 0.1 ml of enzyme extract. Riboflavin was added in the end. The tubes were properly shaken and placed 30 cm below light source consisting of two 15 W-fluorescent lamps (Phillips, India). The absorbance was recorded at 560 nm. One enzyme unit was defined as the amount of enzyme which could cause 50 per cent inhibition of the photochemical reaction.

### Catalase (EC 1.11.1.6)

Catalase activity was determined by the procedure of Sinha (1972). The reaction mixture (1.0 ml) consisted of 0.5 ml of phosphate buffer (pH 7.0), 0.4 ml of 0.2 M hydrogen peroxide and 0.1 ml of properly diluted enzyme extract. After incubating at 37°C for 3 min, the reaction was terminated by adding 3 ml mixture of 5% (w/v) potassium dichromate and glacial acetic acid (1:3 v/v) to the reaction mixture. The tubes were heated in

boiling water bath for 10 min. Absorbance of test and control was measured at 570 nm. One unit of enzyme activity is defined as the amount of enzyme which catalyzed the oxidation of 1  $\mu$ mole  $H_2O_2$  per minute under assay conditions.

#### **Peroxidase (EC 1.11.1.7)**

The enzyme activity was estimated by the method of Shannon *et al.*, (1966). The reaction mixture (2.75 ml) contained 2.5 ml of 50 mM phosphate buffer (pH 6.5), 0.1 ml of 0.5% hydrogen peroxide, and 0.1 ml of 0.2% *O*-dianisidine and 0.05 ml of enzyme extract. The reaction was initiated by the addition of 0.1 ml of  $H_2O_2$ . The assay mixture without  $H_2O_2$  served as blank. Change in absorbance was followed at 430 nm for 3 min. One unit of peroxidase was defined as amount of enzyme required to cause change in 0.1 O.D. per minute under assay condition.

#### **Ascorbate peroxidase (EC 1.11.1.11)**

The enzyme activity was determined following the oxidation of ascorbic acid (Nakano and Asada, 1981). The reaction mixture contained 2.5 ml of 100 mM phosphate buffer (pH 7.0), 0.2 ml of 0.5 mM ascorbate, 0.2 ml of 0.1 mM  $H_2O_2$  and 0.1 ml of enzyme extract. The reaction was initiated by the addition of  $H_2O_2$ . The decrease in absorbance at 290 nm was recorded spectrophotometrically which corresponded to oxidation of ascorbic acid. The enzyme activity was calculated using the molar extinction coefficient of 2.8  $mM^{-1} cm^{-1}$  for ascorbic acid. One enzyme unit was defined as amount of enzyme required to oxidize 1 nmole of ascorbic acid per min at 290 nm.

#### **Glutathione reductase (EC 1.6.4.2)**

Method of Halliwell and Foyer (1978) was followed for measuring the enzyme activity.

The reaction mixture consisted of 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of 5 mM oxidized glutathione (GSSH), 0.1 ml of 3.5 mM NADPH and 0.1 ml enzyme extract in final volume of 3 ml. The decrease in absorbance at 340 nm due to oxidation of NADPH was monitored. Non-enzymatic oxidation of NADPH was recorded and subtracted from it. An extinction coefficient of 6.22  $mM^{-1} cm^{-1}$  for NADPH was used to calculate the amount of NADPH oxidized which corresponded to GR activity. One enzyme unit was defined as amount of enzyme required to oxidize 1.0 nmole of NADPH oxidized per min.

### **Results and Discussion**

#### **Superoxide Dismutase (SOD)**

Results depicted in Fig. 1(a) and Fig. 1(b) show the SOD activity in 2<sup>nd</sup> and 6<sup>th</sup> healthy leaves of resistant and susceptible cotton genotypes respectively. The activity of SOD in 2<sup>nd</sup> leaf before infection (50 DAS) was maximum in *G. arboreum* genotypes (41.14-46.66 units  $mg^{-1}$  protein) followed by *G. hirsutum* resistant genotypes (26.58-36.76 units  $mg^{-1}$  protein) and minimum in *G. hirsutum* susceptible genotypes (18.09-20.41 units  $mg^{-1}$  protein). 6<sup>th</sup> leaf had maximum activity in *G. arboreum* genotypes (52.21-56.90 units  $mg^{-1}$  protein) followed by *G. hirsutum* resistant genotypes (29.75-39.18 units  $mg^{-1}$  protein) and minimum in *G. hirsutum* susceptible genotypes (20.85-23.86 units  $mg^{-1}$  protein). SOD activity was higher in resistant genotypes than susceptible genotypes. 6<sup>th</sup> leaf had more activity than 2<sup>nd</sup> leaf in all the genotypes. All the genotypes not differ significantly in SOD activity.

Results depicted in Fig. 1(c) show the effect of pests infection on SOD activity in 2<sup>nd</sup> leaf of resistant and susceptible cotton genotypes and Fig. 1(d) shows the effect of pests infection on

SOD activity in 6<sup>th</sup> leaf of resistant and susceptible cotton genotypes. After infection increase in SOD activity was observed in *G. hirsutum* genotypes. In 2<sup>nd</sup> leaf, at 60 DAS, increase in SOD activity was 30.56- 67.51% in resistant genotypes and 26.34- 43.32% in susceptible genotypes whereas at 68 DAS, more increase in SOD activity was observed and increase was 44.52-83.02% in resistant genotypes and 39.06- 65.27% in susceptible genotypes. In 6<sup>th</sup> leaf increase was 27.44-53.22% in resistant genotypes and 29.09-34.31% in susceptible genotypes at 60 DAS and 68 DAS stage had 41.58- 73.16% increase in resistant genotypes and 39.79-54.45% in susceptible genotypes. Significant increase was observed in all the genotypes.

### **Catalase (CAT)**

Results depicted in Fig. 2(a) and Fig. 2(b) show the CAT activity in 2<sup>nd</sup> and 6<sup>th</sup> healthy leaves of resistant and susceptible cotton genotypes respectively. The activity of catalase followed similar trend as SOD activity in both 2<sup>nd</sup> and 6<sup>th</sup> leaves before infection. Maximum activity of CAT in 2<sup>nd</sup> leaf was in *G. arboreum* genotypes (366.65-422.98 units mg<sup>-1</sup> protein) followed by *G. hirsutum* resistant genotypes (267.65-366.77 units mg<sup>-1</sup> protein) and minimum in *G. hirsutum* susceptible genotypes (226.13-275.29 units mg<sup>-1</sup> protein). In 6<sup>th</sup> leaf, *G. arboreum* genotypes had maximum activity (505.43-535.11 units mg<sup>-1</sup> protein) followed by *G. hirsutum* resistant genotypes (424.99-456.69 units mg<sup>-1</sup> protein) and minimum in *G. hirsutum* susceptible genotypes (258.82-278.60 units mg<sup>-1</sup> protein). 6<sup>th</sup> leaf had more activity than 2<sup>nd</sup> leaf in all the genotypes. All the genotypes differ significantly in CAT activity.

Results depicted in Fig. 2(c) show the effect of pests infection on CAT activity in 2<sup>nd</sup> leaf of resistant and susceptible cotton genotypes and

Fig. 2(d) shows the effect of pests infection on CAT activity in 6<sup>th</sup> leaf of resistant and susceptible cotton genotypes.

After infection increase in CAT activity was observed in *G. hirsutum* genotypes. In 2<sup>nd</sup> leaf, at 60 DAS, increase was 34.78-77.83% in resistant genotypes and 2.92-16.89% in susceptible genotypes whereas at 68 DAS, more increase in CAT activity was observed and increase was 78.04-155.74% in resistant genotypes and 45.84-81.69% in susceptible genotypes. In 6<sup>th</sup> leaf increase was 28.10-39.67% in resistant genotypes and 6.00-15.37% in susceptible genotypes at 60 DAS and at 68 DAS stage increase was 46.73-58.97% in resistant genotypes and 43.87-57.86% in susceptible genotypes. Significant increase was observed in all the genotypes.

### **Peroxidase (POX)**

Results depicted in Fig. 3(a) and Fig. 3(b) show the POX activity in 2<sup>nd</sup> and 6<sup>th</sup> healthy leaves of resistant and susceptible cotton genotypes respectively. In 2<sup>nd</sup> leaf POX activity was maximum in *G. arboreum* genotypes (44.91-47.16 units mg<sup>-1</sup> protein) followed by *G. hirsutum* resistant genotypes (23.34-26.46 units mg<sup>-1</sup> protein) and minimum in *G. hirsutum* susceptible genotypes (12.13-16.96). In 6<sup>th</sup> leaf, *G. arboreum* genotypes had maximum activity (51.82-54.43 units mg<sup>-1</sup> protein) followed by *G. hirsutum* resistant genotypes (22.19-28.31 units mg<sup>-1</sup> protein) and minimum in *G. hirsutum* susceptible genotypes (14.15-17.81 units mg<sup>-1</sup> protein). POX activity was higher in resistant genotypes than susceptible genotypes. 6<sup>th</sup> leaf had more activity than 2<sup>nd</sup> leaf in all the genotypes. All the genotypes not differ significantly in POX activity.

Results depicted in Fig. 3(c) show the effect of pests infection on POX activity in 2<sup>nd</sup> leaf of resistant and susceptible cotton genotypes and



Fig. 3(d) shows the effect of pests infection on POX activity in 6<sup>th</sup> leaf of resistant and susceptible cotton genotypes. After infection increase in POX activity was observed in *G. hirsutum* genotypes. In 2<sup>nd</sup> leaf, at 60 DAS, increase in POX activity was 55.79-139.59% in resistant genotypes and 26.11-43.59% in susceptible genotypes whereas at 68 DAS stage more increase in POX activity was observed and increase was 130.85-140.13% in resistant genotypes and 44.85-74.71% in susceptible genotypes in 2<sup>nd</sup> leaf.

In 6<sup>th</sup> leaf increase was 52-58.82% in resistant genotypes and 19.83-24.60% in susceptible genotypes at 60 DAS and at 68 DAS stage, increase was 156.71-167.54% in resistant genotypes and 55.01-84.64% in susceptible genotypes. Significant increase was observed in all the genotypes.

#### **Ascorbate Peroxidase (APX)**

Results depicted in Fig. 4(a) and Fig. 4(b) show the APX activity in 2<sup>nd</sup> and 6<sup>th</sup> healthy leaves of cotton genotypes respectively. In 2<sup>nd</sup> leaf, APX activity was maximum in *G. arboreum* genotypes (318.60-327.68 units mg<sup>-1</sup> protein) followed by *G. hirsutum* resistant genotypes (201.42-223.60 units mg<sup>-1</sup> protein) and minimum in *G. hirsutum* susceptible genotypes (134.82-147.74 units mg<sup>-1</sup> protein). 6<sup>th</sup> leaf had maximum activity in *G. arboreum* genotypes (377.62-401.42 units mg<sup>-1</sup> protein) followed by *G. hirsutum* resistant genotypes (231.52-275.46 units mg<sup>-1</sup> protein) and minimum in *G. hirsutum* susceptible genotypes (175.28-215.28 units mg<sup>-1</sup> protein). APX activity was higher in resistant genotypes than susceptible genotypes. 6<sup>th</sup> leaf had more activity than 2<sup>nd</sup> leaf in all the genotypes. All the genotypes not differ significantly in APX activity. Results depicted in Fig. 4(c) show the effect of pests infection on APX activity in 2<sup>nd</sup> leaf of resistant and susceptible cotton genotypes and Fig. 4(d) shows the effect of

pests infection on APX activity in 6<sup>th</sup> leaf of resistant and susceptible cotton genotypes. No visible symptoms of infection were observed in *G. arboreum* genotypes. After infection, increase in APX activity was observed *G. hirsutum* genotypes. In 2<sup>nd</sup> leaf, after pests infection at 60 DAS, increase in APX activity was 27.12-45.01% in resistant genotypes and 23.50-38.49% in susceptible genotypes whereas at 68 DAS stage more increase in APX activity was observed and increase was 104.77-134.60% in resistant genotypes and 84.09-95.34% in susceptible genotypes. In 6<sup>th</sup> leaf increase was 73.67-109.31% in resistant genotypes and 32.41-63.48% in susceptible genotypes at 60 DAS and at 68 DAS, increase in APX activity was 106.65-136.43% in resistant genotypes and 96.06-115.05% in susceptible genotypes. Significant increase in APX activity was observed in 2<sup>nd</sup> leaf at 68 DAS, in 6<sup>th</sup> leaf at 60 DAS & 68 DAS stages whereas non-significant increase in APX activity was observed in 2<sup>nd</sup> leaf at 68 DAS.

#### **Glutathione Reductase (GR)**

Results depicted in Fig. 5(a) and Fig. 5(b) show the GR activity in 2<sup>nd</sup> and 6<sup>th</sup> healthy leaves of resistant and susceptible cotton genotypes respectively. In 2<sup>nd</sup> leaf GR activity was maximum in *G. arboreum* genotypes (204.34-214.35 units mg<sup>-1</sup> protein) followed by *G. hirsutum* resistant genotypes (104.56-141.67 units mg<sup>-1</sup> protein) and minimum in *G. hirsutum* susceptible genotypes (68.67-83.04 units mg<sup>-1</sup> protein). In 6<sup>th</sup> leaf, *G. arboreum* genotypes had maximum activity (222.35-230.41 units mg<sup>-1</sup> protein) followed by *G. hirsutum* resistant genotypes (133.68-146.39 units mg<sup>-1</sup> protein) and minimum in *G. hirsutum* susceptible genotypes (86.73-88.77 units mg<sup>-1</sup> protein). GR activity was higher in resistant genotypes than susceptible genotypes. 6<sup>th</sup> leaf had more activity than 2<sup>nd</sup> leaf in all the genotypes. All the genotypes not differ significantly in GR activity.

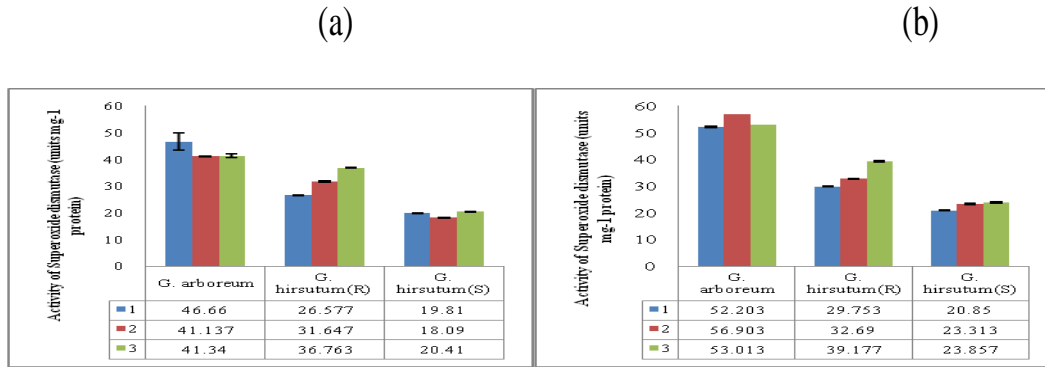


Fig. 1: Superoxide dismutase (units mg<sup>-1</sup> protein) in (a) 2<sup>nd</sup> and (b) 6<sup>th</sup> healthy leaves of resistant and susceptible cotton genotypes

In *G. arboreum* 1=HD 418 2=HD503 3=HD432  
 In *G. hirsutum* (R) 1=H1464 2=H1465 3=H1098  
 In *G. hirsutum* (S) 1=H1463 2=H1454 3=H1439  
 CD at 5%: (a) Genotypes=3.39 (b) Genotypes=0.50

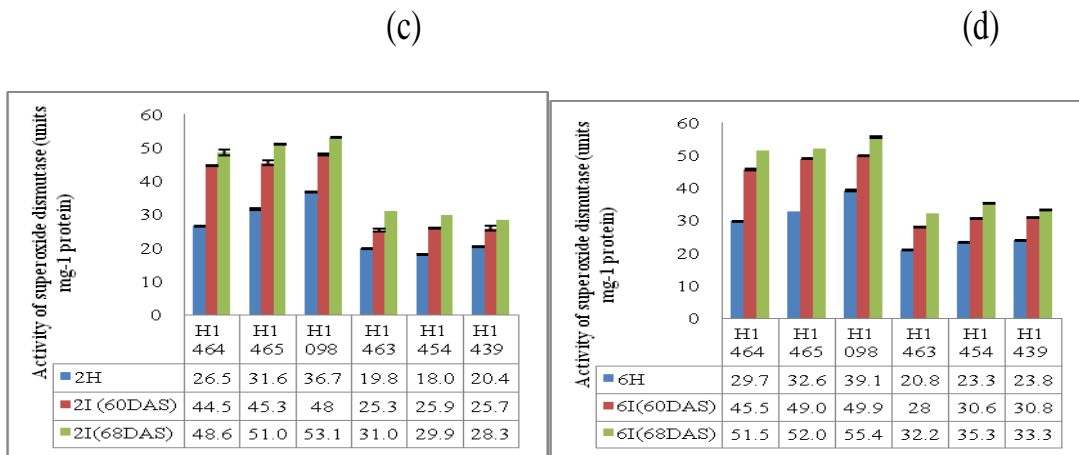
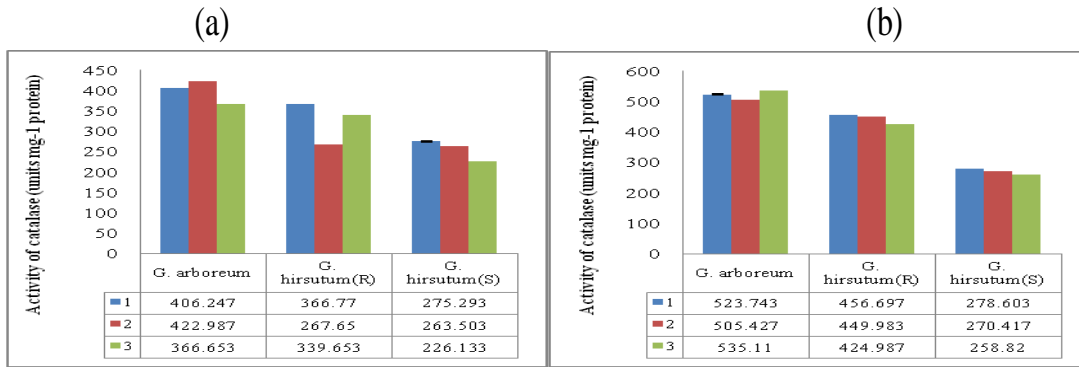


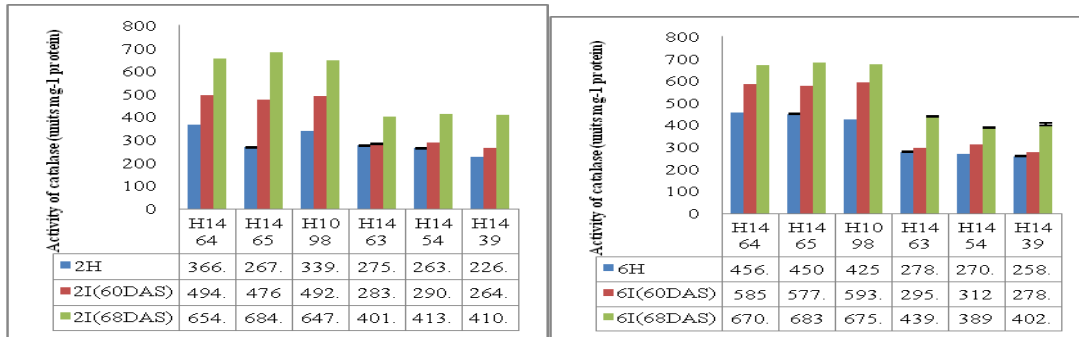
Fig. 1: Effect of pests infection on Superoxide dismutase (units mg<sup>-1</sup> protein) in (c) 2<sup>nd</sup> and (d) 6<sup>th</sup> leaves of resistant and susceptible cotton genotypes

2H= 2<sup>nd</sup> healthy leaf 2I=2<sup>nd</sup> Infected leaf 6H=6<sup>th</sup> Healthy leaf 6I=6<sup>th</sup> Infected leaf  
 (c) H, I (60DAS) H, I (68DAS) (d) H, I (60DAS) H, I (68DAS)  
 Genotypes=0.75 Genotypes=0.61 Genotypes=0.33 Genotypes=0.31  
 Treatment=0.43 Treatment=0.35 Treatment=0.19 Treatment=0.18  
 Genotypes × Treatment=1.06 Genotypes × Treatment=0.86 Genotypes × Treatment=0.46 Genotypes × Treatment=0.44



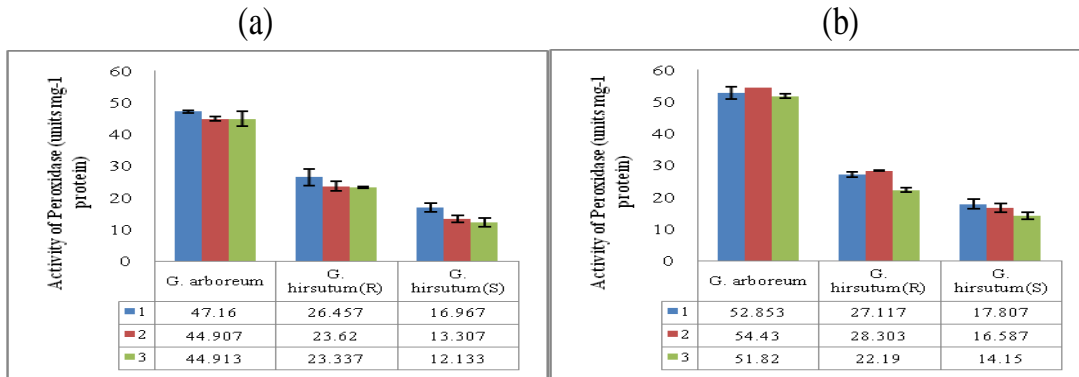
**Fig. 2: Catalase activity (units mg<sup>-1</sup> protein) in (a) 2<sup>nd</sup> and (b) 6<sup>th</sup> healthy leaves of resistant and susceptible cotton genotypes**

In <i>G. arboreum</i>	1=HD 418	2=HD503	3=HD432
In <i>G. hirsutum</i> (R)	1=H1464	2=H1465	3=H1098
In <i>G. hirsutum</i> (S)	1=H1463	2=H1454	3=H1439
CD at 5%: (a) Genotypes=1.25			(b) Genotypes=1.05
	(c)		(d)



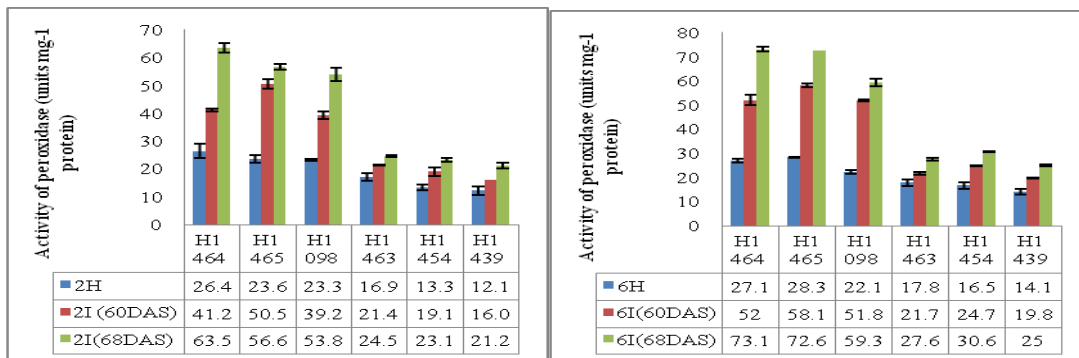
**Fig. 2: Effect of pests infection on Catalase activity (units mg<sup>-1</sup> protein) in (c) 2<sup>nd</sup> and (d) 6<sup>th</sup> leaves of resistant and susceptible cotton genotypes**

2H= 2 <sup>nd</sup> healthy leaf	2I=2 <sup>nd</sup> Infected leaf	6H=6 <sup>th</sup> Healthy leaf	
6I=6 <sup>th</sup> Infected leaf			
(c) H, I (60DAS)	H, I (68DAS)	(d) H, I (60DAS)	H, I
(68DAS)			
Genotypes=1.02	Genotypes=0.71	Genotypes=0.66	Genotypes=3.73
Treatment=0.59	Treatment=0.41	Treatment=0.38	Treatment=2.15
Genotypes × Treatment=1.44 Genotypes × Treatment=1.01 Genotypes × Treatment=0.94 Genotypes × Treatment=5.28			



**Fig. 3: Peroxidase activity (units mg-1 protein) in (a) 2<sup>nd</sup> and (b) 6<sup>th</sup> healthy leaves of resistant and susceptible cotton genotypes**

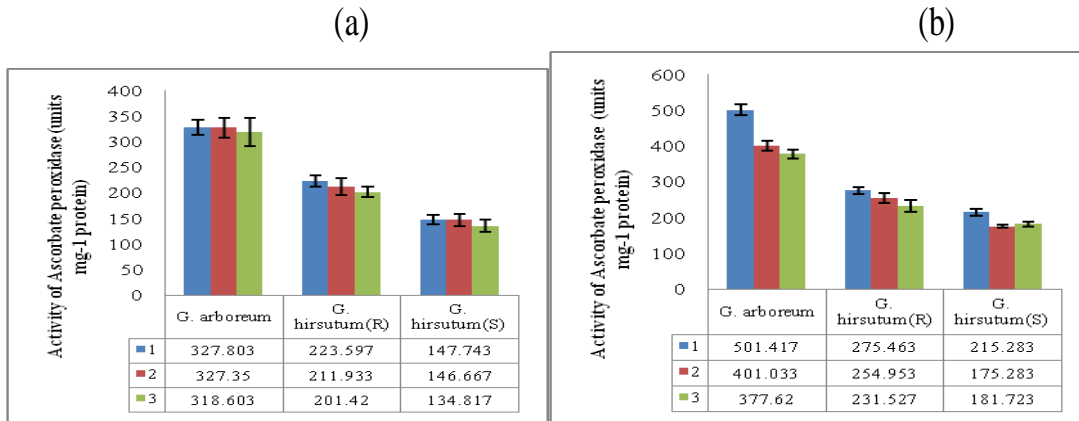
In *G. arboreum* 1=HD 418 2=HD503 3=HD432  
 In *G. hirsutum*(R) 1=H1464 2=H1465 3=H1098  
 In *G. hirsutum*(S) 1=H1463 2=H1454 3=H1439  
 CD at 5%: (a) Genotypes=4.46 (b) Genotypes=3.25  
 (c) (d)



**Fig. 3: Effect of pests infection on Peroxidase activity (units mg<sup>-1</sup> protein) in (c) 2<sup>nd</sup> and (d) 6<sup>th</sup> leaves of resistant and susceptible cotton genotypes**

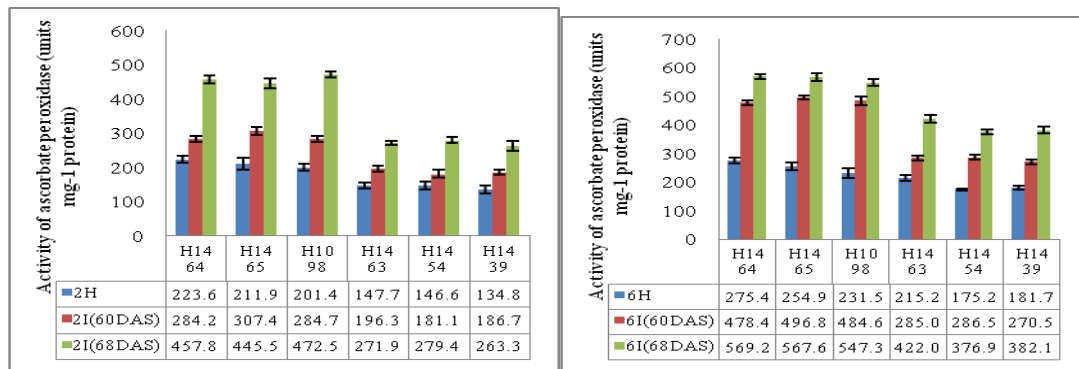
2H= 2<sup>nd</sup> healthy leaf 2I=2<sup>nd</sup> Infected leaf 6H=6<sup>th</sup> Healthy leaf  
 6I=6<sup>th</sup> Infected leaf  
 (c) H, I (60DAS) H, I (68DAS) (d) H, I (60DAS) H, I (68DAS)  
 Genotypes=2.72 Genotypes=3.04 Genotypes=2.09 Genotypes=1.96  
 Treatment=1.57 Treatment=1.76 Treatment=1.21 Treatment=1.13  
 Genotypes × Treatment=3.84 Genotypes × Treatment=4.30 Genotypes × Treatment=2.96 Genotypes × Treatment=2.7





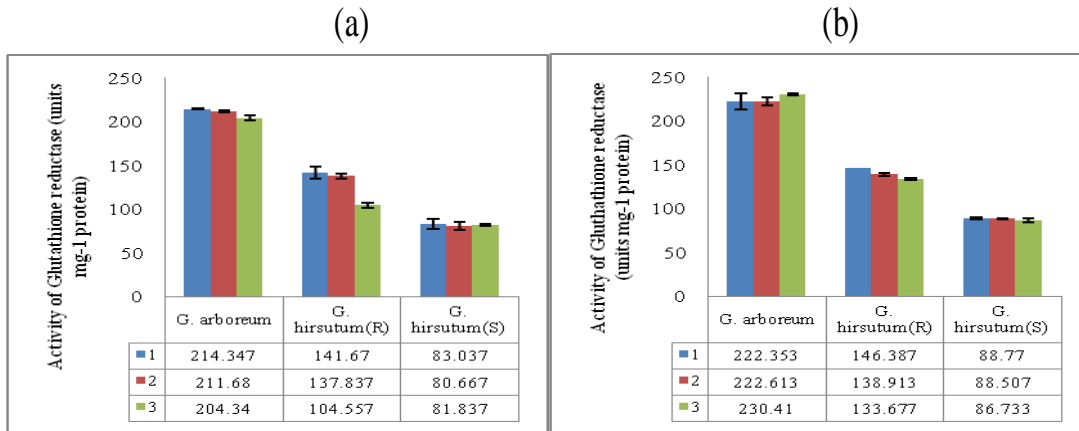
**Fig. 4: Ascorbate peroxidase activity (units mg<sup>-1</sup> protein) in (a) 2<sup>nd</sup> and (b) 6<sup>th</sup> healthy leaves of resistant and susceptible cotton genotypes**

In *G. arboreum*                      1=HD 418                      2=HD503                      3=HD432  
 In *G. hirsutum*(R)                      1=H1464                      2=H1465                      3=H1098  
 In *G. hirsutum*(S)                      1=H1463                      2=H1454                      3=H1439  
 CD at 5%: (a) Genotypes= 15.82                      (b) Genotypes=11.91  
 (c)                      (d)



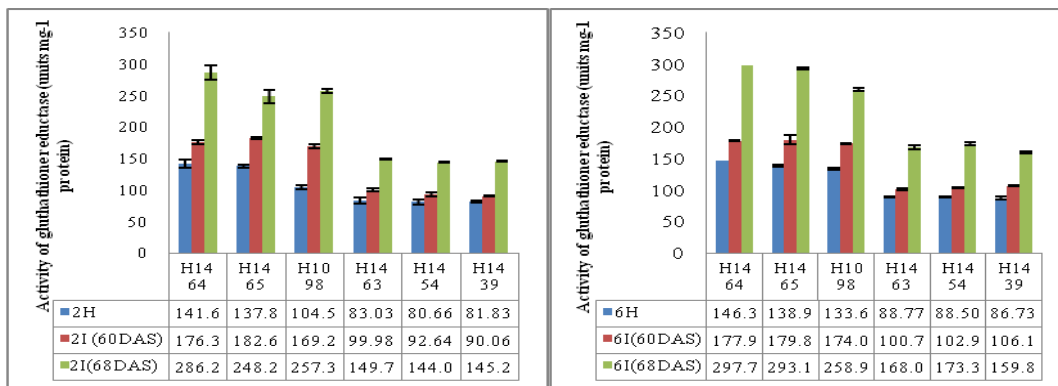
**Fig. 4: Effect of pests infection on Ascorbate peroxidase activity (units mg<sup>-1</sup> protein) in (c) 2<sup>nd</sup> and (d) 6<sup>th</sup> leaves of resistant and susceptible cotton genotypes**

2H= 2<sup>nd</sup> healthy leaf                      2I=2<sup>nd</sup> Infected leaf                      6H=6<sup>th</sup> Healthy leaf                      6I=6<sup>th</sup> Infected leaf  
 (c) H, I (60DAS)                      H, I (68DAS)                      (d) H, I (60DAS)                      H, I (68DAS)  
 Genotypes=22.9                      Genotypes=26.69                      Genotypes=30.77                      Genotypes=22.93  
 Treatment=13.25                      Treatment=15.41                      Treatment=17.77                      Treatment=13.24  
 Genotypes × Treatment= N/A Genotypes × Treatment= 37.76                      Genotypes × Treatment=43.52                      Genotypes × Treatment=32.43



**Fig. 5: Glutathione reductase activity (units mg<sup>-1</sup> protein) in (a) 2<sup>nd</sup> and (b) 6<sup>th</sup> healthy leaves of resistant and susceptible cotton genotypes**

In *G. arboreum*                      1=HD 418                      2=HD503                      3=HD432  
 In *G. hirsutum* (R)                      1=H1464                      2=H1465                      3=H1098  
 In *G. hirsutum* (S)                      1=H1463                      2=H1454                      3=H1439  
 CD at 5%: (a) Genotypes=11.03                      (b) Genotypes=10.49  
 (c)                      (d)



**Fig. 5: Effect of pests infection on Glutathione reductase activity (units mg<sup>-1</sup> protein) in (c) 2<sup>nd</sup> and (d) 6<sup>th</sup> leaves of resistant and susceptible cotton genotypes**

2H= 2<sup>nd</sup> Healthy leaf                      2I=2<sup>nd</sup> Infected leaf                      6H=6<sup>th</sup> Healthy leaf                      6I=6<sup>th</sup> Infected leaf  
 (c) H, I (60DAS)                      H, I (68DAS)                      (d) H, I (60DAS)                      H, I (68DAS)  
 Genotypes=7.42                      Genotypes=14.90                      Genotypes=4.96                      Genotypes=3.75  
 Treatment=4.28                      Treatment=8.60                      Treatment=2.87                      Treatment=2.17  
 Genotypes × Treatment=10.49                      Genotypes × Treatment=21.07                      Genotypes × Treatment=7.02                      Genotypes × Treatment=5.31

Results depicted in Fig. 5(c) show the effect of pests infection on GR activity in 2<sup>nd</sup> leaf of resistant and susceptible cotton genotypes and Fig. 5(d) shows the effect of pests infection on GR activity in 6<sup>th</sup> leaf of resistant and susceptible cotton genotypes. No visible symptoms of infection were observed in *G. arboreum* genotypes. After infection increase in GR activity was observed in *G. hirsutum* genotypes. In 2<sup>nd</sup> leaf, at 60 DAS, increase in GR activity was 24.47-61.84% in resistant genotypes and 10.05-20.41% in susceptible genotypes whereas at 68 DAS stage, more increase in GR activity was observed and increase was 68.11-146.10% in resistant genotypes and 77.50-68.32% in susceptible genotypes. In 6<sup>th</sup> leaf increase was 21.57-30.18.00% in resistant genotypes and 13.49-22.39% in susceptible genotypes at 60 DAS and at 68 DAS stage increase was 93.72-111.03% in resistant genotypes and 84.35-95.82% in susceptible genotypes. Significant increase was observed in all the genotypes.

The activity increased in all the genotypes but the increase was more in resistant genotypes as compared to susceptible genotypes and all the genotypes differ significantly in GR activity.

Among the enzymes involved in antioxidative defense system, superoxide dismutase (SOD) is the first enzyme in ROS detoxifying process. It converts  $O_2^-$  to  $H_2O_2$  and  $H_2O_2$  so produced is scavenged to  $O_2$  and water by the enzymes such as APX, POX and CAT. In present study, SOD activity increased on pests infection and increase was more in resistant genotypes than susceptible genotypes and in all genotypes 6<sup>th</sup> leaf showed enhanced activity than 2<sup>nd</sup> leaf (fig. 1c & 1d). Similarly results were obtained in cotton plants infested by *S. litura* showed induced SOD activity (Usha Rani and Pratyusha, 2013). Similar increase was also observed in the castor and lima bean plants infested by herbivory

(Maffei *et al.*, 2006). The SOD activity was also shown to increase in strawberry leaves infected by *Mycosphaerella fragariae* but the SOD activity for the resistant cultivars was higher than for the susceptible ones (Ehsani-Moghaddam *et al.*, 2006).

Catalase (CAT) activity increased in both 2<sup>nd</sup> & 6<sup>th</sup> leaves after pests infection in both resistant and susceptible genotypes (fig. 2a & 2b). Enhanced CAT activity was observed in resistant genotypes than susceptible genotypes at both 60 DAS and 68 DAS stage in both 2<sup>nd</sup> & 6<sup>th</sup> leaves on pests infection (fig. 2c & 2d). Similarly, a 23 fold increase in CAT activity was observed in maize plants inoculated with *P. indica* as compared to non-inoculated plants (Kumar *et al.*, 2009). Maximum increase in CAT activity after cotton leaf curl burewala virus inoculation was in resistant genotypes as followed by susceptible genotypes as compared to their non-inoculated plants (Siddique *et al.*, 2014). Similar increases in foliar CAT activity were also observed in Algerian-susceptible but not in Algerian-Resistant barley (*Hordeum vulgare* L.) leaves inoculated with *Blumeria graminis* (Vanacker *et al.*, 1998). Cotton plants infested by *S. litura* showed induced the CAT activity (Usha Rani and Pratyusha, 2013).

Peroxidases (POX) are a group of enzymes that detoxify  $H_2O_2$  by utilizing an electron donating substrate for the oxidation of  $H_2O_2$  (Dionisio-sese and Tobita, 1998). POX activity increased in 2<sup>nd</sup> and 6<sup>th</sup> leaves of all the cotton genotypes on pests infection and the increase was higher in resistant genotypes as compared to susceptible genotypes (fig 3c & 3d). Similar to our results, many scientists have reported higher peroxidase activity in resistant cultivars of various crops infected with different types of pathogens. Cotton plants infested by *S. litura* induced the CAT activity (Usha Rani and Pratyusha, 2013).

Infection with plant pathogens led to an induction in POX activity in plant tissues and a greater increase was recorded in resistant plants compared to the susceptible ones (Mydlarz and Harvell, 2006). Similar increase in POX activity has been reported in tomato and bell pepper infected with tobacco mosaic virus and tomato mosaic tobamovirus (Madhusudhan *et al.*, 2009); cucumber mosaic virus and zucchini yellow mosaic virus-infected *Cucumis sativus* and *Cucurbita pepo* plants (Bauer, 2000); tobacco mosaic virus infected tobacco plants (Király *et al.*, 2002); tomato yellow leaf curl virus infected tomato plants (Dieng *et al.*, 2011) and a number of resistant interactions involving several plant patho systems.

Ascorbate peroxidase, a hydrogen peroxide scavenging enzyme is a major enzyme responsible for elimination of hydrogen peroxide. The results of present study showed that APX activity was substantially higher in resistant genotypes as compared to susceptible genotypes in healthy leaves (fig. 4a & 4b).

The APX activity increased in all genotypes infected by pests and increase was higher in resistant genotypes at both 60 DAS and 68 DAS stages (fig. 4c & 4d). Similar observations have been reported for APX activity in soybean and cotton foliage after herbivory attack by *H. zea* (Bi and Felton, 1995; Bi *et al.*, 1997). Lukasik *et al.*, (2012) observed more induction in APX activity in less susceptible cultivars than more susceptible cultivars in triticale after 24 hrs of cereal aphid infestation and there prolonged feeding (after 48 and 72 hrs) caused the strongest induction of APX. Similarly, a rapid increase was observed in more resistant cultivar of chrysanthemum infested by *Macrosiphoniella sanbourni* (Gillete) indicated that the enzyme is involved in early responses to aphid attack (He *et al.*, 2011).

Glutathione reductase (GR) is another specific and important enzyme of ascorbate-glutathione cycle and plays a crucial role in affording protection against oxidative damage in many plants (Foyer *et al.*, 1991) by maintaining endogenous pool of reduced glutathione (GSH). Our results showed that GR activity increased in both 2<sup>nd</sup> and 6<sup>th</sup> leaves of both resistant and susceptible cotton genotypes on pests infection and increase was more pronounced in resistant genotypes than susceptible genotypes (fig. 5c & 5d). Similarly, Hernández *et al.*, (2001) found that the GR activity in the resistant plants was higher than in the susceptible plants of apricot after inoculation with the *Plum pox virus*. Debona *et al.*, (2012) observed that wheat varieties inoculated with *Pyricularia oryzae* for 96 hr at vegetative stage showed increase in GR activity in partially resistant plants (BRS 229) and no significant change in susceptible (BR 18) plants.

To summarize results presented here show that leaves of resistant genotypes had less production of ROS, higher level of ascorbic acid and higher activities of POX, APX, CAT, SOD and GR as compared to susceptible genotypes of cotton. Suggesting that these components of antioxidative defence system play important role in providing pest resistance in cotton genotypes studied here.

The maximum increase in activities of enzymes *viz.* CAT, POX, SOD, APX and GR were observed in 6<sup>th</sup> leaves after pests infection. Maximum increase in antioxidative enzymes was observed in HD418 of *G. arboreum*, H1098 of *G. hirsutum* (R) and H1454 genotype of *G. hirsutum* (S). The results indicated that biochemical parameters studied in the present investigation play important role in providing resistance to sucking pests infection in cotton genotypes studied in the present investigation.

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