Indices of oxidative stress in cotton (Gossypium hirsutum L.) genotypes after infestation of sucking pest

Anju Rani, Jayanti Tokas, Anamika and HR Singal

Abstract
Cotton is a crop of great economic importance in India. In the present study, H₂O₂ content, MDA content and LOX activity were estimated in the leaves (2nd & 6th) of cotton genotypes infected by sucking pests at 50, 60 and 68 days after sowing (DAS). H₂O₂ content, MDA content and LOX activity was found to be maximum in 2nd & 6th leaves of G. hirsutum susceptible genotypes followed by G. hirsutum resistant genotypes and minimum in G. arboreum genotypes. The leaves of resistant genotypes had less production of H₂O₂, MDA and LOX as compared to susceptible cotton genotypes.

Keywords: Cotton, lipoxygenase, malondialdehyde, oxidative stress, pest, resistance

Introduction
Cotton is a crop of great economic importance in India. It is a natural fibre of vegetable origin, like linen, jute or hemp and composed of cellulose. Out of about 50 species of cotton plants in the world, only four have been domestically cultivated for cotton fibres namely Gossypium hirsutum, Gossyium barbedense, Gossypium arboreum and Gossypium herbaceum. Gossypium hirsutum and Gossypium barbedense are the most commonly cultivated species of cotton in the world. Gossypium hirsutum variety is the most important agricultural cotton, accounting for more than 90 per cent of world fibre production. As per CAB report (July, 2014) during 2013-14, cotton was grown in India in about 117.27 lakh hectares with a total production of 390 lakh bales and in Haryana, it occupied an area of about 5.66 lakh hectares with annual production of 23 lakh bales (Cotton corporation of India, 2014) [3]. World wide area under cotton production is estimated at around 33.1 million hectares (Johnson et al. 2014) [8]. India, China, United State, Pakistan and Brazil are main producers of cotton in the world (Johnson et al. 2014) [8] and account for almost 80% of global production. India ranks first in area of cultivation and second in production after China. The sustainability of cotton production worldwide has been affected due to piercing, sucking insect pests and bollworms which are a serious threat to the cotton crop. The cotton ecosystem includes a wide variety of arthropods throughout the world. More than 1326 species of insects have been reported attacking cotton in the world. In India, 162 species have been recorded among which only 15 species consider potential threat to the crop. When plants are subjected to biotic or abiotic stresses, reactive oxygen species (ROS) such as \( \cdot \mathrm{O}_2, \cdot \mathrm{OH} \) and \( \mathrm{H}_2 \mathrm{O}_2 \) are generated in response to stress conditions which are considered to be indicators of oxidative damage (Dat et al., 2000) [1]. ROS can cause oxidative damage to many cellular components, including membrane lipids, proteins, nucleic acids, and chlorophyll. The balance between ROS production and activities of antioxidative enzymes determines whether oxidative signalling and/or damage will occur. Lipoxygenases (LOXs) constitutes another group of anti-oxidative enzymes involved in plant defense against many stresses through octadecanoid pathway (Bruinsma et al., 2009). They catalyze hydroperoxidation of polyunsaturated fatty acids resulting in formation of fatty acid hydroperoxides. The latter are enzymatically and/or chemically degraded to unstable and highly reactive aldehydes, \( \gamma \)-ketois, epoxides, (Bruinsma et al., 2009) and ROS such as hydroxyl radicals, singlet oxygen, superoxide ion and peroxyl, acyl and carbon-centered radicals (Maffei et al., 2007). Therefore, studies on indices of oxidative stress are important to facilitate our understanding of their relationships between the oxidative burst and plant defense responses against insect pest attack.
Material and Methods
The present investigation was carried out at the fields of Cotton Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during the year 2013-14 and 2014-15. Leaves (2nd & 6th) of nine genotypes viz. HD418, HD432, HD503, H1464, H1465, H1098, H1463, H1454 and H1439 were collected before and after the attack of sucking pests. HD418, HD432, HD503 (G. arboreum) and H1464, H1465, H1098 (G. hirsutum) were the resistant genotypes and H1463, H 1454, H1439 (G. hirsutum) were the susceptible cotton genotypes. Sampling was done at 50, 60 and 68 days after sowing (DAS). The leaf samples were collected in ice box containing ice cubes and brought to the laboratory.

Malondialdehyde: Malondialdehyde content was estimated according to the method of Heath and Packer (1968) [7]. To 0.5 ml of supernatant, 2.5 ml of 20% (w/v) trichloro acetic acid (TCA) containing 0.5% thiobarbituric acid was added. The mixture was heated in a water bath at 95°C for 30 min and quickly cooled in ice bath. Then the absorbance was recorded at 532 nm and the value of non-specific absorption at 600 nm was subtracted from it. The concentration of malondialdehyde was calculated using the extinction coefficient of 155 mM⁻¹ cm⁻¹.

Hydrogen peroxide: H₂O₂ was estimated by the method of Sinha (1972) [12]. To 0.4 ml extract, 0.6 ml of 0.1 M phosphate buffer (pH 7.0) and 3 ml mixture of 5% (w/v) potassium dichromate and glacial acetic acid (1:3, v/v) was added. The mixture was heated for 10 min in a boiling water bath. Colour of solution changed to green due to the formation of chromic acetate. After cooling, absorbance was recorded at 570 nm against the reagent blank without sample extract. The quantity of H₂O₂ was determined from the standard curve 10-160 μmoles of H₂O₂.

Lipoygenase estimation: The enzyme activity was determined spectrophotometrically at 234 nm by the method of Catherine et al. (1998) [2]. The reaction was started by the addition of enzyme. Increase in absorbance was measured at room temperature for 2 min. Lox activity was measured by monitoring the formation of conjugated dienes from linoleic acid. One enzyme unit was defined as the amount of enzyme causing 0.1 unit change in O.D. at 234 nm⁻¹.

Results and Discussion
Malondialdehyde (MDA): Results depicted in Fig. 1(a) and Fig. 1(b) shows the MDA content in 2nd and 6th healthy leaves of resistant and susceptible cotton genotypes respectively. The content of MDA was maximum in G. hirsutum susceptible genotypes (11.12-12.78 μ moles g⁻¹ dry wt.) followed by G. hirsutum resistant genotypes (6.73-9.25 μ moles g⁻¹ dry wt.) and minimum in G. arboreum genotypes (5.18-6.00 μ moles g⁻¹ dry wt.). MDA content was higher in susceptible genotypes than resistant genotypes in both 2nd and 6th leaves. In 6th leaf MDA content was 12.94- 14.13 μ moles g⁻¹ dry wt. in G. hirsutum susceptible genotypes followed by 9.89-12.21 μ moles g⁻¹ dry wt. in G. hirsutum resistant genotypes and minimum 6.56-8.60 μ moles g⁻¹ dry wt. in G. arboreum genotypes. All the genotypes differed significantly in MDA content.

Results depicted in Fig. 1(c) shows the effect of pest infection on MDA content in 2nd leaf of resistant and susceptible cotton genotypes and Fig. 1(d) shows the effect of pests infection on MDA content in 6th leaf of resistant and susceptible cotton genotypes. After infection, increase in MDA content was observed in G. hirsutum genotypes. In 2nd leaf at 60 DAS, increase in MDA content was 17.29-37.59% in resistant genotypes and 43.37-58.65% in susceptible genotypes. At 68 DAS, however more increase in MDA content was observed and increase was 62.03-101.83% in resistant genotypes and 96.50-117.99% in susceptible genotypes. In 6th leaf increase was 19.01-26.05% in resistant genotypes and 54.38-65.83% in susceptible genotype at 60 DAS stage. At 68 DAS, more increase in MDA content was observed and increase was 37.48-55.22% in resistant genotypes and 97.42-106.56% in susceptible genotypes. Significant increase was observed in all the genotypes. The content increased in all the genotypes but the increase was more in susceptible genotypes as compared to resistant genotypes and all the genotypes differed significantly in MDA content.

A striking early response is the transient accumulation of ROS at the plant surface against pathogenic infection. Prolonged overproduction of ROS can lead to peroxidation of membrane lipids and pigments, denaturation of proteins, damage to DNA and fragmentation of polysaccharides (Rady and Osman, 2012). Lipid peroxidation is measured as the amount of Thiobarbituric acid reactive substances or malondialdehyde (MDA) produced when polyunsaturated fatty acids in the membrane undergo oxidation by accumulation of free oxygen radicals. As lipids peroxidation is the symptom mostly ascribed to oxidative damage, it is often used as an indicator of increased membrane damage (Moller et al. 2007). In the present results, higher MDA content was observed in susceptible genotypes as compared to resistant genotypes as shown in fig. 3a & 3b and on pests infection there was increase in MDA content in both 2nd and 6th leaves (fig.1c & 1d) at both 60 DAS and 68 DAS. MDA content was higher in more infected leaves (68 DAS) as compared to less infected leaves (fig. 1c & 1d). Similarly, higher H₂O₂ and MDA concentrations were recorded in bean yellow mosaic virus infected fava bean leaves as compared to corresponding controls (Radwan et al., 2010) [10]. Debona et al. (2012) [5] observed similar results in wheat varieties inoculated with Pyricularia oryzae at vegetative stage showed increase in MDA content in susceptible plants (BR18) and no significant change in partially resistant plants (BRS 229). Whereas, Siddique et al. (2014) observed increased level of MDA in resistant genotype (Ravi) of cotton infected with cotton leaf curl Burewala virus.

Hydrogen Peroxide (H₂O₂): Results depicted in Fig. 2(a) and Fig. 2(b) show the H₂O₂ content in 2nd and 6th healthy leaves of resistant and susceptible cotton genotypes respectively. The content of H₂O₂ followed similar trend as MDA content in both 2nd and 6th leaves before infection. In 2nd leaf maximum H₂O₂ content was observed in G. hirsutum susceptible genotypes (4.38-4.74 m moles g⁻¹ dry wt.) followed by G. hirsutum resistant genotypes (3.44-3.52 m moles g⁻¹ dry wt.) and minimum was in G. arboreum genotypes (2.22-3.16 m moles g⁻¹ dry wt.). Similar trend was observed in 6th leaf where maximum content 5.67-5.88 m moles g⁻¹ dry wt. was in G. hirsutum susceptible genotypes followed by G. hirsutum resistant genotypes (3.65-3.93 m moles g⁻¹ dry wt.) and minimum was in G. arboreum genotypes (2.34-3.24 m moles g⁻¹ dry wt.). H₂O₂ content was higher in susceptible genotypes than resistant genotypes. All the genotypes did not differ significantly in H₂O₂ content. Results depicted in Fig. 2(c) show the effect of pests infection...
on H\textsubscript{2}O\textsubscript{2} content in 2\textsuperscript{nd} leaf of resistant and susceptible cotton genotypes and Fig. 2(d) shows the effect of pest infection on H\textsubscript{2}O\textsubscript{2} content in 6\textsuperscript{th} leaf of resistant and susceptible cotton genotypes. Significant increase was observed in all the genotypes.

After infection, increase in H\textsubscript{2}O\textsubscript{2} content was observed in G. hirsutum genotypes. In 2\textsuperscript{nd} leaf, at 60 DAS stage, increase in H\textsubscript{2}O\textsubscript{2} content was 47.93-52.40\% in resistant genotypes and 79.78-100.39\% in susceptible genotypes whereas 68 DAS stage had more increase in H\textsubscript{2}O\textsubscript{2} content and increase was 56.05-143.11\% in resistant genotypes and 124.90-146.74\% in susceptible genotypes. In 6\textsuperscript{th} leaf increase was 46.34-49.68\% in resistant genotypes and 48.63-59.20\% in susceptible genotypes at 60 DAS. In 68 DAS, in H\textsubscript{2}O\textsubscript{2} content was 139.77-155-17\% in resistant genotypes and 162.34-226.92\% in susceptible genotypes. H\textsubscript{2}O\textsubscript{2} content increased in all the genotypes but the increase was more in susceptible genotypes as compared to resistant genotypes and all the genotypes differed significantly in H\textsubscript{2}O\textsubscript{2} content.

Hydrogen peroxide, a natural toxic plant metabolite is known to increase membrane permeability by attacking membrane lipids. In the present study, H\textsubscript{2}O\textsubscript{2} was higher in sensitive genotypes than tolerant genotypes in healthy leaves (fig. 2a & 2b) and pest infection resulted in the increased production of H\textsubscript{2}O\textsubscript{2} at both 60 DAS and 68 DAS stages but increase was more in susceptible genotypes than G. hirsutum resistant genotypes (fig. 2c & 2d). Similarly, colonization of pea aphids (Acyrthosiphon pisum) in pea led to significant insect density and time dependent enhancement in the rate of H\textsubscript{2}O\textsubscript{2} and H\textsubscript{2}O\textsubscript{2} production (Mai et al. 2013). Similar results were also observed in Potato (Solanum tuberosum) leaves, attacked by green peach aphids (Myzus persicae), have nearly twice the H\textsubscript{2}O\textsubscript{2} content than uninfested leaves (Kerchev et al., 2012) [9]. Debona et al. (2012) [5] observed that wheat varieties inoculated with Puccinia oryzae at vegetative stage resulted into increased H\textsubscript{2}O\textsubscript{2} content in susceptible plants (BR18) and no significant change in partially resistant plants (BRS 229). El-Beltagi et al. (2012) [6] observed increased level of H\textsubscript{2}O\textsubscript{2} and MDA in tomato plants infected with Meloidogyne incognita as compared to uninfected leaves and this increased content of MDA and H\textsubscript{2}O\textsubscript{2} accounted for the defense mechanism against the invasion by this root-knot nematode.

**Lipoxigenase (LOX):** Results depicted in Fig. 3(a) and Fig. 3(b) shows the LOX activity in 2\textsuperscript{nd} and 6\textsuperscript{th} healthy leaves of resistant and susceptible cotton genotypes respectively. The activity of LOX in 2\textsuperscript{nd} leaf before infection was maximum in G. hirsutum susceptible genotypes (147.74-151.89 units mg\textsuperscript{-1} protein) followed by G. hirsutum resistant genotypes (131.96-143.37 units mg\textsuperscript{-1} protein) and minimum in G. arboreum genotypes (124.03-131.12 units mg\textsuperscript{-1} protein). In 6\textsuperscript{th} leaf maximum activity (156.81-162.13 units mg\textsuperscript{-1} protein) was in G. hirsutum susceptible genotype followed by G. hirsutum resistant genotypes (143.32-156.81 units mg\textsuperscript{-1} protein) and minimum in G. arboreum genotypes (136.43-142.93 units mg\textsuperscript{-1} protein). LOX activity was higher in susceptible genotypes than resistant genotypes. All the genotypes did not differ significantly in LOX activity.

Results depicted in Fig. 3(c) show the effect of pest infection on LOX activity in 2\textsuperscript{nd} leaf of resistant and susceptible cotton genotypes and Fig. 3(d) shows the effect of pests infection on LOX activity in 6\textsuperscript{th} leaf of resistant and susceptible cotton genotypes. After infection increase in LOX activity was observed in G. hirsutum genotypes. In 2\textsuperscript{nd} leaf, at 60 DAS, increase in LOX activity was 7.53-16.19\% in resistant genotypes and 10.86-26.21\% in susceptible genotypes whereas at 68 DAS, more increase in LOX activity was observed and increase was 17.32-31.88\% in resistant genotypes and 23.58-40.47\% in susceptible genotypes. In 6\textsuperscript{th} leaf increase was 10.90-13.49\% in resistant genotypes and 13.36-20.72\% in susceptible genotypes at 60 DAS and 68 DAS stage had 22.60-27.87\% increase in resistant genotypes and 30.74-36.57\% in susceptible genotypes. Significant increase was observed in all the genotypes. The activity increased in all the genotypes but the increase was more in susceptible genotypes as compared to resistant genotypes and all the genotypes differed significantly in LOX activity.

Lipoxygenases (LOXs) are non-heme-iron-containing enzymes, which catalyze the oxidation of polyunsaturated fatty acids containing a cis, cis-1, 4-pentadiene site. LOXs are another group of anti-oxidative enzymes involved in plant defense against many stresses through octadecanoid pathway. LOX activity was higher in sensitive genotypes in healthy leaves (50 DAS) as compared to tolerant genotypes (fig. 3a & 3b). Increase in activity was observed in all the genotypes on pest infection in both 2\textsuperscript{nd} & 6\textsuperscript{th} leaves at both 60 DAS and 68 DAS stages (fig. 3c & 3d). Similar results were observed by Bi et al. (1994) [1] in soyabeen when infected by herbivore corn earworm H. zea. Zhao et al. (2009) [13] observed increase in LOX activity in wheat when infected by Sitobion avenae (F.). They catalyze hydroperoxidation of polyunsaturated fatty acids resulting in formation of fatty acid hydroperoxides.

The leaves of resistant genotypes had less production of H\textsubscript{2}O\textsubscript{2}, MDA and LOX as compared to susceptible cotton genotypes suggesting that these components play important role as indices of oxidative stress on sucking pests infection in cotton genotypes studied in the present investigation.

![Fig 1(a, b): Malondialdehyde content (µ moles g\textsuperscript{-1} dry wt.) in (a) 2\textsuperscript{nd} and (b) 6\textsuperscript{th} leaves healthy leaf of resistant and susceptible cotton genotypes](image-url)
Fig 1(c, d): Effect of pests infection on Malondialdehyde content ($\mu$ moles g\(^{-1}\) dry wt.) in (c)\(2^{nd}\) and (d)\(6^{th}\) leaves of resistant and susceptible cotton genotypes

Fig 2(a, b): Hydrogen Peroxide content (m moles g\(^{-1}\) dry wt.) in (a)\(2^{nd}\) and (b)\(6^{th}\) healthy leaves of resistant and susceptible cotton genotypes

Fig 2(c, d): Effect of pests infection on Hydrogen Peroxide content (m moles g\(^{-1}\) dry wt.) in (c)\(2^{nd}\) and (d)\(6^{th}\) leaves of resistant and susceptible cotton genotypes
1 Unit = One unit of LOX is the amount of enzyme required to cause change in 0.1 O.D. min⁻¹.
In G. Arboresum
  1=HD 418
  2=HD 503
  3=HD 432
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=3.68 (b) Genotypes=2.43

Fig 3(a, b): Lipoxygenase activity (Units mg⁻¹ protein) in (a) 2nd and (b) 6th healthy leaves of resistant and susceptible cotton genotypes

1 Unit = One unit of LOX is the amount of enzyme required to cause change in 0.1 O.D. min⁻¹.
2H = 2nd healthy leaf
2I = 2nd Infected leaf
6H = 6th Healthy leaf
6I = 6th Infected leaf
(c) H, I (60DAS)
  Genotypes = 8.45
  Treatment = 4.88
(d) H, I (68DAS)
  Genotypes = 6.13
  Treatment = 3.54
Genotypes × Treatment = 11.94
Genotypes × Treatment = 6.67
Genotypes × Treatment = 6.77
Genotypes × Treatment = 6.19

Fig 3(c, d): Effect of pests infection on Lipoxygenase activity (units mg⁻¹ protein) in (c) 2nd and (d) 6th leaves of resistant and susceptible cotton genotypes

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References

