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Evaluation of biochemical defense response to *Spodoptera litura* infestation in cotton (*Gossypium hirsutum* L.) genotypes

Arslan Hafeez (arslanhafeezuaf@gmail.com)
University of the Free State - Bloemfontein Campus: University of the Free State https://orcid.org/0000-0003-0053-091X

Samina Jam Nazeer Ahmad
University of Agriculture Faisalabad

Jam Nazeer Ahmad
University of Agriculture Faisalabad

Muhammad Imran Tipu
Nuclear Institute for Agriculture and Biology

Tanwir Ahmad Malik
University of Agriculture Faisalabad

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Abstract

Background: The cotton armyworm (*Spodoptera litura) is one of* the most devastating pests of many economically important crops including cotton which cause substantial yield losses due to its feeding pattern on leaves and other plant parts. Plants respond to herbivore damage through an array of defense responses to ensure their survival. This study was aimed to appraise biochemical defense responses of cotton genotypes to *S. litura* infestation.

Methods: Two Bt cotton cultivars namely Bt-886 and CIM-622 and one non-Bt PB-896 cultivar were used in the study. The experiment was conducted in greenhouse conditions. Leaf samples for biochemical analysis were collected after 24 hrs of infestation by third instar larvae.

Results: Data revealed that infestation caused significant reduction in chlorophyll pigments of all cultivars. Infestation caused a marked increase in hydrogen peroxide and malondialdehyde concentrations as well as activities of various antioxidant enzymes such as superoxide dismutase, peroxidase and catalase. The levels of other secondary metabolites such as phenolics, proline and glycine betaine were also found to be higher after infestation.

Conclusion: Among the cotton cultivars, cv. PB-896 was found to be considerably resistant to pest attack due to an efficient antioxidant system, lower chlorophyll degradation, and lesser accumulation of hydrogen peroxide and malondialdehyde that manifested minimal oxidative injury.

Introduction:

Plants being sessile face many abiotic and biotic stresses from the natural environment. Biotic stresses coming from herbivores and pathogens are significant constraints that adversely affect plant growth and yield (Bruce, 2010). Plants have developed an intricate morphological, physiochemical and molecular defense mechanism to cope with herbivore attack (LIU et al., 2020; Palial et al., 2018; Tian et al., 2018). However, the capacity of defense response varies from plant species to species (Rathinam et al., 2019).

Infestation led to the generation of *in planta* reactive oxygen species (ROS) such as superoxide radicle (O2•-), hydrogen peroxide (H_2O_2) and hydroxyl radicles (OH•-) which cause oxidative stress in cellular environment (Moloi and van der Westhuizen 2006; O'Brien et al. 2012). Production of ROS is an early response to pest damage and is believed to provide a signal for plant-insect interaction (Saed-Moucheshi et al., 2014a; Scheler et al., 2013). Accumulation of ROS in response to infestation damage activates NADPH (Nicotinamide Adenine Dinucleotide Phosphate) oxidases located on plasma membrane which in turn reduces oxygen through NADPH as an electron donor (Apel and Hirt, 2004; Saed-Moucheshi et al., 2014b) Also, excessive production of ROS above threshold level leads to DNA damage, lipid peroxidation, denaturation of proteins and pigment oxidation leading to substantial cellular damage (Ashraf et al., 2015).

To survive ROS-mediated oxidative injury, plant uses defensive mechanisms that include production of enzymatic or non-enzymatic antioxidants. Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are major enzymatic antioxidants (Mahawar and Shekhawat, 2019; Rasheed et al., 2020). For instance, *S. litura* feeding causes considerable inflation in SOD, POD and CAT activities in infested cotton plants (Usha Rani and Pratyusha, 2013). In this context, the defensive responses of plant against insect attack have been reported in various studies (Abbate et al., 2018; Li et al., 2018; Mitra et al., 2019; Timbó et al., 2014; Zhang et al., 2019). Induction of these enzymes plays a significant role in homeostasis and detoxification of lethal ROS, enabling signal transduction and starting a cascade of defenses against herbivore attack through mediating the plant's secondary metabolites (Gulsen et al., 2010; Mai et al., 2013). Plants also accumulate organic osmolytes such as proline and glycine betaine under environmental stresses, which play role in osmotic adjustment as well as cyto-protection through enhanced antioxidant system (Mittler, 2002). Kaur et al. (2015) reported increased level of proline in pigeon pea plants as a response of induced defense response to the *Helicoverpa armigera* infestation. The role of glycine betaine as an osmo-protectant under abiotic stress is extensively reported in the literature, however, its role in biotic stress tolerance is not well understood yet.

Cotton (*Gossypium hirsutum* L.) is a source of natural plant fiber for the textile industry and plays a pivotal role in the country's economy with a significant contribution to GDP (10%) and foreign exchange earnings (55%) of Pakistan (Afridi et al., 2016; Rehman et al., 2019, 2015). It is usually referred to as white gold in Pakistan. Substantial yield losses in cotton production have been recorded in the tropical and subtropical regions due to biotic and abiotic stress, including pest attack. The cotton armyworm, *S. litura* is among major polyphagous pests causing significant yield loss in crops (Saleem et al., 2016). This insect feeds on a broad range of valuable crops including cotton (Ashfaq et al., 2011) causing severe leaf damage to the plant. *S. litura* induced biochemical changes have been reported earlier in various plants (Usha Rani and Pratyusha, 2013; Vijaya and Rani, 2017). Therefore, this study was performed to appraise the biochemical mechanisms of resistance manifested by cotton during *S. litura* feeding. Such information may be useful for breeding programs to produce tolerant plants.

Materials And Methods

Plants

Three cotton cultivars namely Bt-886, CIM-622 and PB-896 were used for this experiment. Seeds of all three cotton cultivars were immersed in water for 10 h and incubated at 28°C for a day. The germinated seeds were planted in plastic pots (23.5 x 29 cm) each containing 10 kg of soil in a greenhouse (35 ± 10° C) and watered every two days. This was followed by weekly fertilizer treatment (N: P: K = 20: 20: 20). The trial was carried out under greenhouse at Postgraduate agriculture research station (PARS), Department of Botany, University of Agriculture, Faisalabad, Pakistan.

Insects and pest feeding

The eggs of *S. litura* was procured from the fields of Faisalabad and reared in the laboratory on agar-based artificial diet. This was done in a climate-controlled insectary (25 ± 2 ◦C, 75 ± 5% R.H. and 16:8 (L:D) light: dark photoperiod) in plastic box (10 L capacity) with proper ventilation in the IGCDB laboratory at the Entomology department, University of Agriculture Faisalabad, Pakistan. During early hours of the day, top fully expanded leaves of 45 days old cotton plants were allowed for infestation with pre-starved 3rd instar larvae for 24 hrs confined by enclosing with a muslin bag. Infested leaves were detached and immediately frozen in liquid nitrogen and stored at -20°C for further biochemical analysis. Leaves from un-infested plants were used as the controls.

Chlorophyll content

Measurement of chlorophyll *a*, *b* pigments and total chlorophyll contents were done by using the Arnon (1949) protocol. The leaf sample (0.1 g) was homogenized in 80 % pure acetone (marked the final volume up to 5 mL). Supernatant absorbance was monitored at 645, 663 and 480 nm spectrophotometrically (Hitachi, U-2900, Japan).

Proline estimation

Free proline estimation was done following Bates et al. (1973) protocol. Leaf material (0.1 g) was crushed in 5 mL of 3% aqueous sulpho-salicylic acid. Filtrate (1 mL) was transferred to a test tube and diluted with 1 mL of acid ninhydrin followed by 1 mL of glacial acetic acid. Mixture was maintained in a water bath at 100 °C for 10 minutes and cooled on ice. Toluene (4 mL) was added to the mixture and shaken vigorously for 20s and absorbance was taken at 520 nm by using a spectrophotometer (Hitachi U-2900 Japan). Standard curve was used to calculate free proline concentration as µmol g−1 fresh weight.

Hydrogen peroxide (H_2O_2) concentration

Velikova et al. (2000) protocol was used for the estimation of endogenous H_2O_2 concentration. Leaf tissue (0.1 g) was homogenized in 1 mL of 0.1 % trichloroacetic acid (TCA) (w/v) at 4ºC and centrifuged at 10,000 x *g* for 10 min. The supernatant (0.5 mL) taken and was added with 0.5 mL potassium phosphate buffer (10 mM; pH 7.0) and 1 mL potassium iodide (1M) and gently vortexed. The absorbance was taken at 390 nm using spectrophotometer (Hitachi U-2900 Japan). H_2O_2 content was calculated from the H_2O_2 standard curve and expressed as µmol g-1 fresh weight.

Determination of Malondialdehyde (MDA)

Concentration of MDA in leaf tissue was measured using Cakmak and Horst (1991) protocol. Plant leaf sample (0.5 g) was ground in 10 mL of 6% trichloroacetic acid (TCA, w/v) and centrifuged at 11,000 x *g* for 20 min. Supernatant taken was equally mixed with 4ml of 0.5 % thiobarbituric acid (TBA). The reaction sample was maintained in a water bath at 95°C for 30 min and allowed to cool at room

temperature. After cooling, reaction mixture was centrifuged at 8000 x *g* for 5 min and absorbance was read at 532 and 600 nm.

Antioxidant enzymes activities

Leaf sample (0.5 g) was grinded in liquid nitrogen and dissolved in 10ml of 100 mM chilled potassium phosphate (K-P) buffer (pH 7.5). The mixture was centrifuged at 10,000 x *g* at 4 °C for 15 minutes and the supernatant collected was referred to as enzyme extract.

Superoxide dismutase (SOD) activity

Giannopolitis and Ries (1977) protocol was used to observe SOD activity. The reaction solution constitutes nitro-blue tetrazolium (NBT; 50 µM), riboflavin (1.3 µM), methionine (13 mM), potassium phosphate buffer (100 mM) with pH 7.5 and enzyme extract (0.1 ml). The reaction solution was irradiated at 77 µmol m^{-2} s^{-1} for 20 min using a fluorescent lamp. Later, the mixture absorbance was recorded at 560 nm through spectrophotometer (Hitachi U-2900, Japan). The enzyme activity was expressed in U mg^{-1}protein.

Catalase (CAT) and Peroxidase (POD) activity

Activities of CAT and POD were evaluated following protocol by Chance and Maehly (1955). The reaction solution for CAT activity constitutes potassium phosphate buffer (100 mM; 7.5 pH), enzyme extract (0.1 mL) and H_2O_2 (5.9 mM). The absorbance of reaction solution was observed at 240 nm for every 20 sec intervals. The POD reaction mixture (0.1 mL) contained potassium phosphate buffer (50 mM; pH 7.5), guaiacol (20 mM) and H_2O_2 (40 mM). The change in absorbance was measured spectrophotometrically at 470 nm for every 20 sec. The enzymatic activities were expressed in U mg^{-1}protein.

Phenolics

Julkunen-Tiitto (1985) protocol was used for the determination of phenolic content. Plant leaf tissue (0.5 g) was grinded in 5 mL of 80 % acetone and centrifuged at 10,000 x *g* for 10 min. Supernatant (0.1 mL) taken was homogenized with milli-Q water (2 mL) and Follin-ciocalteu phenol reagent (1 mL) and gently mixed. Subsequently, 5 mL sodium carbonate (Na_2CO_3; 20 %) was added and the final volume was raised to 10 mL by adding distilled H_2O. This was followed by vigorous vortexing (5-10 s) and incubation for 20 minutes at room temperature. Absorbance was measured spectrophotometrically (Hitachi U-2900, Japan) at 720 nm.

Estimation of total soluble protein (TSP)

Leaf material was grinded in 10 mL of cooled K-phosphate buffer (100mM; pH 7.5) and centrifuged at 10,000 x g for 15 min at 4°C. After centrifugation, supernatant was collected for the analysis. Total soluble protein concentration was estimated by following Bradford (1976) method.

Glycine Betaine (GB) content

Leaf glycine betaine contents were estimated by using protocol given by Grieve and Grattan (1983). Leaf material (0.5 g) was homogenized in de-ionized water (20 mL) and mechanically shaken for 24 h at 25°C. Homogenate (1 mL) was filtered and diluted with 2N H_2SO_4 (1 mL). Of this, 0.5 mL was added to a centrifuge tube, cooled on ice for 1 h. The mixture was added up with per iodide solution (0.20 mL) and vortexed gently. The tubes were maintained at 4 °C for 16 h. Later, tubes were centrifuged for 15 minutes at 10,000 x *g*, carefully aspirated the supernatant. To dissolve per-iodide crystals, 9 ml of 1,2-dichloromethane was added. The reaction was kept at ambient temperature for 2 h. The absorbance of reaction mixture was read spectrophotometrically at 365 nm.

Statistical analysis

All data from the completely randomized experiment (three-independent replications) regarding above-mentioned parameters were statistically analyzed using COSTAT 6.303 window software (Cohort Software, Monterey, CA, USA). Analysis of Variance (ANOVA) technique at $P \leq 0.05$ was applied to analyze all data gathered. Graphical representation of the correlation plot was carried out using R studio software.

Results

Photosynthetic pigments:

The infestation by *S. litura* caused a notable reduction ($P \leq 0.001$) in the chlorophyll *a* content in all cotton cultivars. The cv. PB-896 had relatively higher chlorophyll *a* after infestation (Fig. 1A). Although cultivars were not significantly different for chlorophyll *b*, yet infestation led to a noticeable reduction ($P \leq 0.001$) in chlorophyll *b* contents for all cotton cultivars (Fig. 1B). In parallel to Chlorophyll *a* and Chlorophyll *b*, infestation led to a substantial reduction ($P \leq 0.001$) in total chlorophyll content with minimum reduction observed in cv. PB-896 as compared to the other two cultivars (Fig. 1C).

Oxidative stress indicators (H_2O_2 and MDA):

Consequent upon the Infestation by *S. litura*, there was a considerable increase ($P \leq 0.001$) in the endogenous level of H_2O_2 for all three cultivars. The cultivars varied significantly ($P \leq 0.001$) for this parameter. The endogenous level of H_2O_2 was higher in the cv. Bt-886 while the minimal increase was observed in cv. PB-896 under infestation (Fig. 2A). Insect feeding had a significant effect ($P \leq 0.001$) in the accumulation of MDA content in all infested cotton plants. The MDA contents were higher in cv. Bt-886 as compared to CIM-622 and PB-896. The minimal values for MDA contents were observed in cv. PB-896 infested plants (Fig. 2B).

Antioxidant enzymes activities:

Infested plants showed significantly higher ($P \leq 0.001$) SOD activity for all three cotton cultivars. Insect damaged plants of cv. PB-896 had markedly higher SOD activity over CIM-622 and Bt-886. Cv. Bt-886 had

higher values for this attribute (Fig. 3A). Plants of three cultivars exposed to *S. litura* infestation manifested a remarkable increase (P≤0.001) in POD activity. Among the three cultivars tested, cv. PB-896 showed considerably higher POD activity in infested plants than that of Bt-886 and cv. CIM-622 (Fig. 3B). *S. litura* infested cotton plants exhibited significantly enhanced (P≤0.001) CAT activity for all cultivars. There existed notable differences (P≤0.001) among cultivars for this variable. Cv. PB-896 displayed maximal CAT activity over cv. Bt-886 and CIM-622 in infested plants (Fig. 3C).

Proline

The accumulation of proline among the three cotton cultivars was significantly affected (P≤0.001) by *S. litura* infestation as compared with controls. All cultivars performed significantly different (P≤0.001) for this variable. In this regard, more proline accumulation was noted in the infested plants of cv. PB-896, while the lowest was in the infested plants of cv. CIM-622 (Fig. 4A).

Leaf phenolics and GB contents

Infestation leads to the significant (P≤0.001) accumulation of phenolic contents in all three cultivars. There existed considerable (P≤0.001) differences between cotton cultivars for this parameter. Significantly higher (P≤0.001) phenolic contents were observed in cv. PB-896 infested plants compared with the other two cultivars (Bt-886 and CIM-622) (Fig. 4B). Infestation caused distinguishable enhancement (P≤0.001) in the accumulation of GB contents in damaged plants of all cultivars. However, cotton cultivars did not vary significantly for this attribute. Maximal GB contents were recorded in cv. PB-896 while minimal in cv. CIM-622 (Fig. 4C).

Total soluble proteins (TSP)

As a result of *S. litura* infestation, TSP increased considerably (P≤0.001) in plants exposed to infestation by *S. litura*. All three cultivars had a significant difference (P≤0.001) for TSP contents. For instance, the maximal level of TSP contents was observed in cv. PB-896 while minimal in cv. Bt-886 (Fig. 4D).

Correlation analysis

The Pearson correlation analysis was conducted to evaluate the relationship between various studied attributes in cotton plants under *S. litura* infestation (Fig. 5). Oxidative stress indicators (H_2O_2 and MDA) are negatively correlated with photosynthetic pigments. However, antioxidant enzymes (SOD, POD, CAT), TSP, Proline and GB are positively correlated with each other and with oxidative stress indicators but negatively correlated with photosynthetic pigments. This correlation exhibited a close connection between chlorophyll pigments, oxidative stress indicators and antioxidant enzymes in different cotton cultivars.

Discussion

The present results showed that infestation by *S. litura* has a significantly negative impact on individual (chl a and chl b) and total chlorophyll content in all cotton cultivars. Similar findings were reported by Nagrare et al. (2017) that chl a, chl b and total chlorophyll content were reduced to 21.2%, 19.1% and 23.7% respectively in the cotton plants infested by mealybug. Our results are corroborated with Heng-moss et al. (2003) that *Diuraphis noxia* (Russian wheat aphid) feeding caused a marked decrease in Chl. a, b and carotenoid contents of susceptible wheat isolines as compared to resistant lines. Huang et al. (2013) also observed significant decline in chlorophyll contents of tomato leaves due to mealybug infestation. This loss of chlorophyll contents may be attributed towards the supressed pigment biosynthesis due to deficiency of Mg (a major constituent of chlorophyll), herbivory induced damaged chloroplasts in palisade tissues, loss of assimilates due to insect feeding or ROS damage to the pigments (Khattab, 2007). In addition, Goławska et al. (2010) reported significantly less chlorophyll synthesis in stressed plants as compared to unstressed plants as a mechanism of defense response towards pest.

S. litura feeding markedly influenced the levels of the antioxidant enzymes in infested cotton plants. A considerable rise in SOD activity was recorded in all cotton cultivars in response to *S. litura* feeding. SOD play role as the first line defense system involved in the detoxification and conversion of superoxide radicles into oxygen and hydrogen peroxide (Cavalcanti et al., 2007; Raychaudhuri and Deng, 2000) generated either by Mehler's reaction or photorespiration under various stresses (Khattab and Khattab 2005; Rani and Jyothsna 2010). Similar to our findings, War et al. (2013) noticed a considerably increased activity of SOD in groundnut plants challenged with both *Helicoverpa armigera* and *Aphis craccivora* herbivory. Elevated POD activity has been regarded as an immediate response of plants to insect attack through cell wall strengthening as it is considered as a critical enzyme involved in lignin biosynthesis (Duan et al., 2014; He et al., 2011; Mehdy, 1994; Moloi and van der Westhuizen, 2006). In addition, POD together with phenols act as toxin to the pest and discourage its feeding and health (Duffey and Stout, 1996; Ni et al., 2009; Tan et al., 2011; Zhang et al., 2008). Moreover, POD is reported to cause immediate toxicity in the gut of herbivore (Zhu-Salzman et al., 2008). Similarly, in the present investigation, POD activity was significantly enhanced in the infested cotton plants, which is corroborated to an earlier investigation of Ni et al. (2001) in which they found significantly higher POD activity in resistant wheat cultivars as compared to susceptible cultivars infested with RWA (Russian wheat aphid). Catalase (CAT) is one of the important components of ROS scavenging system that converts H_2O_2 into water and oxygen (Bittner et al., 2017). Also, an increased CAT level is known to play role in cell wall strengthening and act as a signal transducer to induce defense genes (Chen et al., 1993). In this study, higher activity of CAT was observed in *S. litura* infested cotton plants. A similar observation was reported by Bi and Felton (1995) in soybean plants damaged by *Helicoverpa zea* and in rice plants infested by yellow stem borer and leaf roller (Rani and Jyothsna, 2010). Overall, in the present experiment, increased activities of SOD, POD and CAT after *S. litura* infestation in cotton confers resistant to *S. litura* feeding.

An efficient antioxidant system readily scavenges ROS (H_2O_2) and protects membranes which is evident in terms of minimal MDA accumulation. H_2O_2 is produced after herbivore feeding (Mithöfer et al., 2004). In the present experiment, an increased level of H_2O_2 was observed in infested cotton plants. These

results are corroborated with other authors (Argandoña et al., 2001; Maffei et al., 2006; Walling, 2000; War et al., 2012a, 2011). Higher levels of H_2O_2 in plants considered to be damaging for insect gut through oxidative damage in infested plants (Orozco-Cardenas and Ryan, 1999). Kaur et al. (2014) reported a negative correlation (r = -0.73) regarding CAT activity and H_2O_2 contents in infested leaves, indicating that the higher CAT activity decreases H_2O_2 accumulation as CAT transforms H_2O_2 into H_2O and O_2.

Increased level of proteins is a typical response occurring in plants under biotic and abiotic stress (Broz et al., 2010). Our results exhibited significantly increased levels of total soluble proteins (TSP) in leaves of infested cotton plants as compared with non-infested plants. Similarly, War et al. (2012b) reported an increased level of protein content in insect-infested plants of three groundnut genotypes as compared to non-infested plants. Also, Usha Rani and Pratyusha (2013) found higher expression of TSP in infested cotton plants as compared to healthy control plants. An increased protein concentration in response to insect infestation is attributed to the generation of defense-related enzymes and proteins under stressful conditions (Chen et al., 2009; Helmi and Mohamed, 2016; Lawrence and Koundal, 2002).

Plants tend to accumulate phenolic compounds in response to herbivory (Rani and Jyothsna, 2010; Sharma et al., 2009). In our study, it was observed that *S. litura* influenced a marked increase in the accumulation of phenolic compounds in plants exposed to herbivory. Kaur et al. (2014) observed similar findings in infested pigeon pea genotypes. Phenolic compounds with antioxidant potential reduce highly reactive oxygen radicles (Ashraf, 2009) and are toxic to insects (Bhonwong et al., 2009; Walling, 2000).

Glycine betaine is a quaternary organic compound that protects plants under various stresses via stabilizing proteins, maintaining the integrity of membranes (Habib et al., 2012) as well as modulation of several physiochemical mechanisms in plants (Kurepin et al., 2017). However, its role in biotic stress tolerance is not well understood. In the present study, higher contents of GB were observed in infested cotton plants as compared to control. Kaur et al. (2017) reported an average mean of GB in chickpea resistant and susceptible genotypes challenged by *Helicoverpa armigera* infestation as 2149.34 ug/g and 1760.5 ug/g respectively. They also observed 27.07% higher GB in the pod wall of resistant chickpea plants as compared with susceptible plants.

Free proline play role as an osmo-protectant as well as contributes to stabilization of proteins and membranes by attenuating ROS adverse activity, probably playing a role in plant adaptation to unfavorable conditions (Ashraf and Foolad, 2007; Kmieć et al., 2014). In this experiment, Increased proline content was observed in cotton plants as a result of pest damage. *Phenacoccus peruvianus* feeding on *Bougainvillea glabra* induced higher proline content than the un-infested plants (Abbate et al., 2018). Our experimental results manifested significantly higher content of proline in all infested cotton cultivars, however, PB-896 still had higher proline.

Conclusions

In conclusion, the antioxidant enzymes such as SOD, POD and CAT are involved in *S. litura* resistance response. The accumulation of total soluble proteins, phenolics, proline and glycine betaine also formed an integral part of cotton resistance response towards pest damage. Cv. PB-896 showed prominently higher accumulation of defense related biochemicals and thus could be considered to have better defense response over cv. Bt-886 and cv. CIM-622. Our results may help improve the understanding of the underlying biochemical mechanisms of *S. litura* resistance in cotton, which can be helpful in crop protection and integrated pest management.

Declarations

Ethics approval and consent to participate

Not applicable

Competing interest

The authors declare that they have no competing interests.

Consent for publication

Not applicable

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Authors contribution

Hafeez A conducted the experiment and drafted the manuscript. Ahmad SJN designed and supervised the experiment and revised the manuscript. Ahmad JN provided lab facility and assisted in biochemical analysis. Tipu MI helped with methodology. Malik TA provided seeds of the cotton cultivars. All authors read and approved the final manuscript.

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Figures

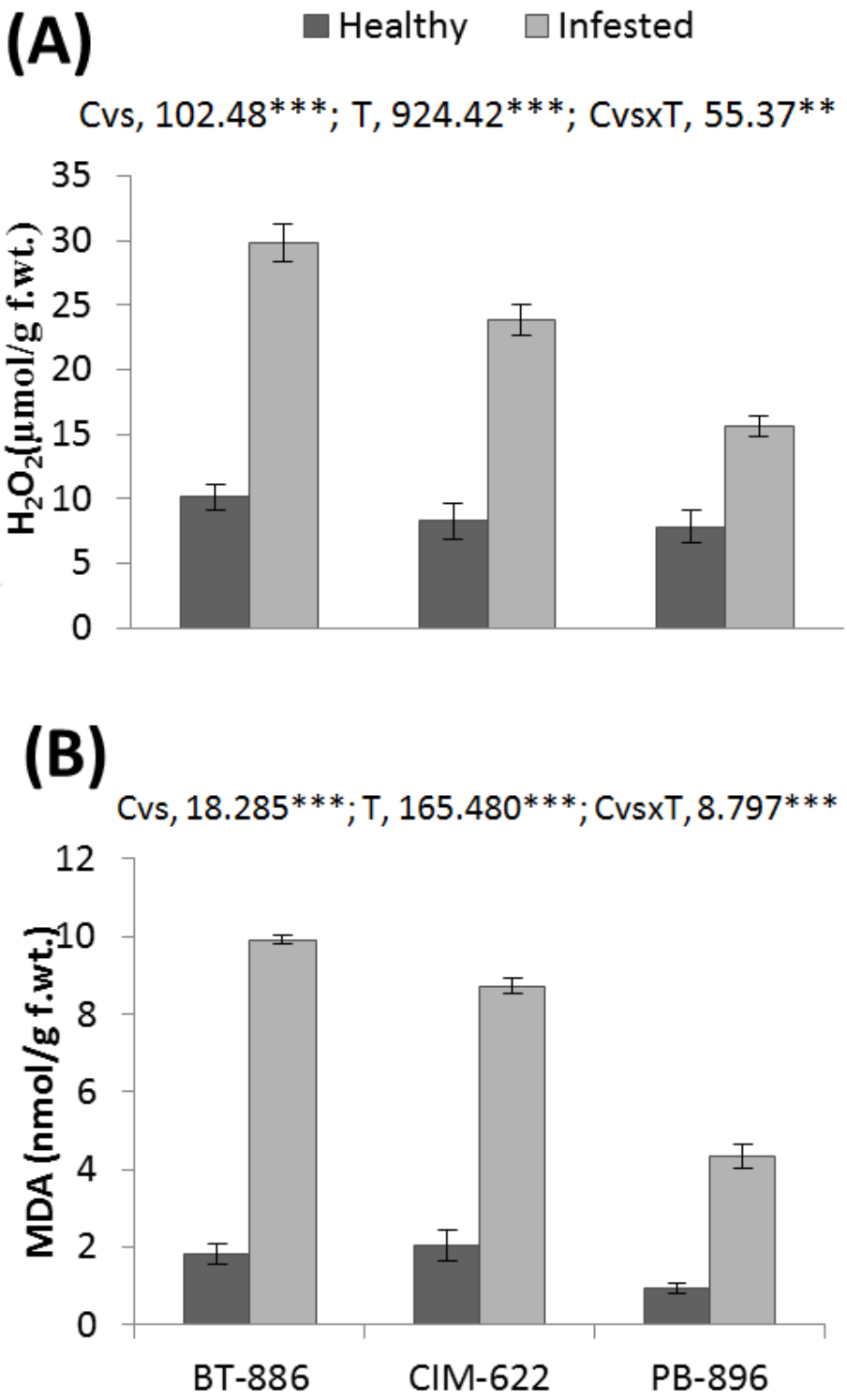


Figure 1

A-B Effect of Spodoptera litura infestation on hydrogen peroxide (H2O2) and malondialdehyde (MDA) concentration in three cotton cultivars. Values are means ± SE (n=3). Cvs, cultivars; T, Treatment (Infestation); ns, non-significant; *, ** and *** = significant at 0.05, 0.01 and 0.001 levels, respectively.

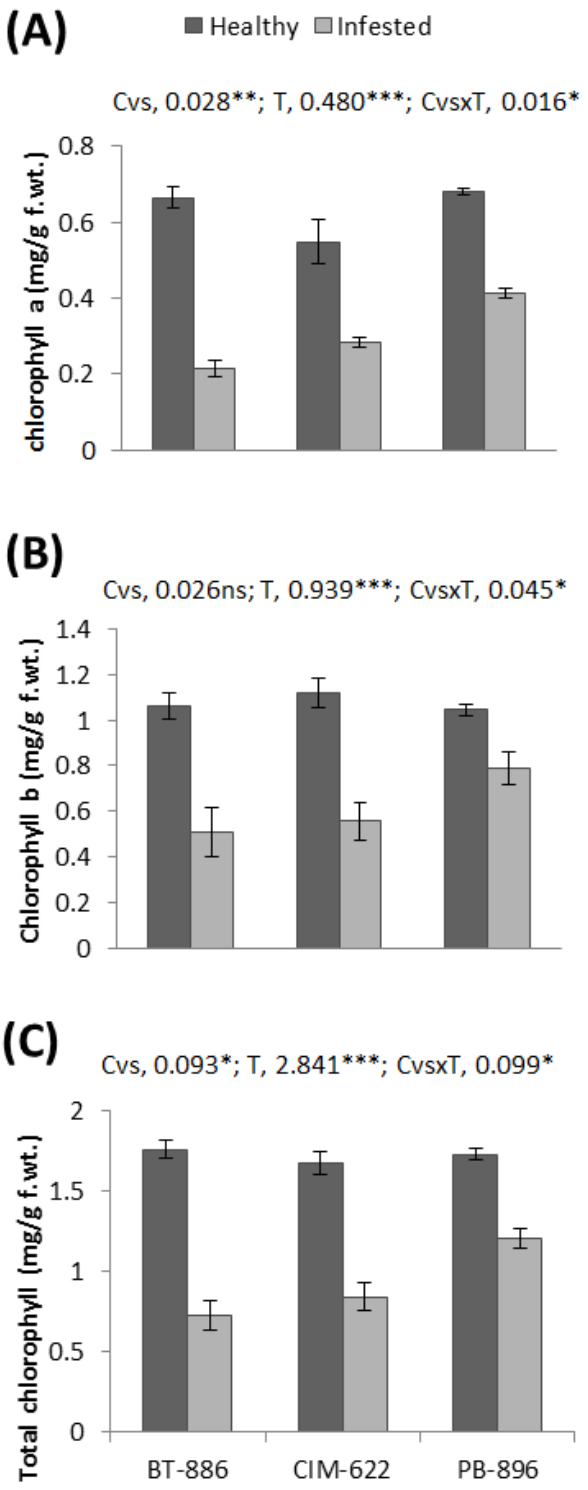


Figure 2

A-C Effect of Spodoptera litura infestation on photosynthetic pigments in three cotton cultivars. Bars represent the means ± SE (n=3). Cvs, cultivars; T, Treatment (Infestation); ns, non-significant; *, ** and *** = significant at 0.05, 0.01 and 0.001 levels, respectively.

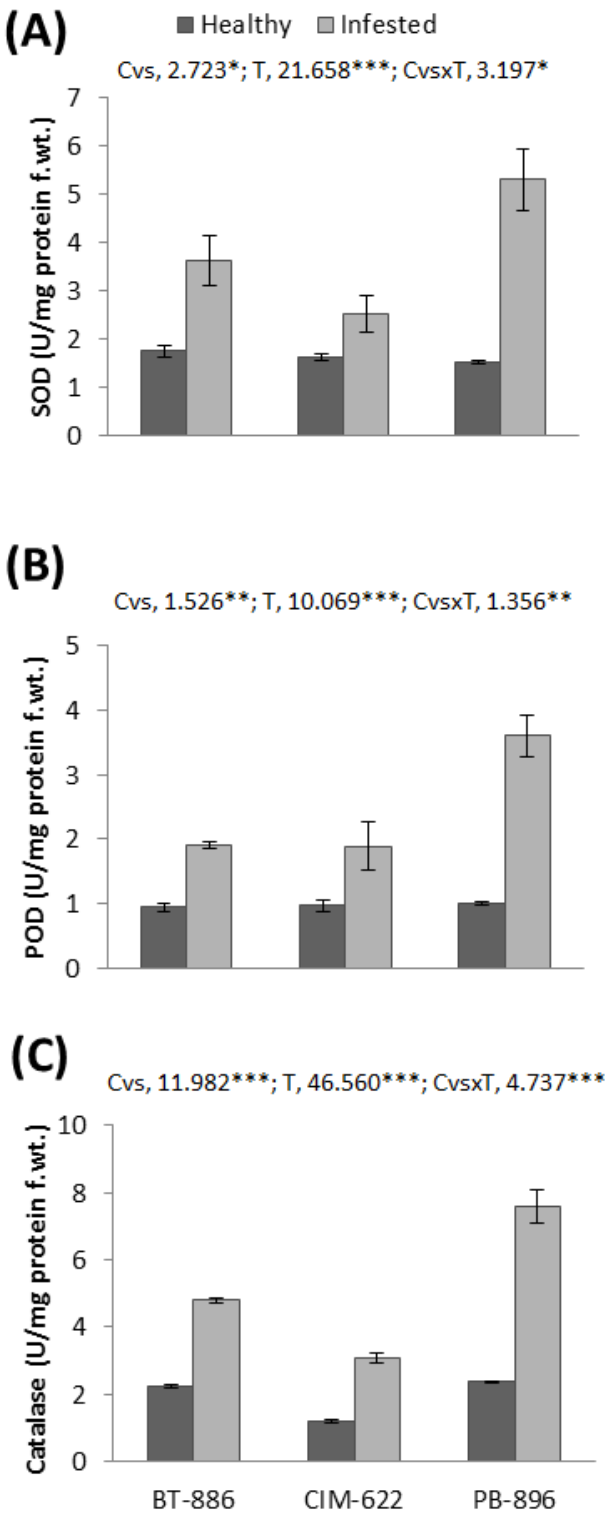


Figure 3

A-C Effect of Spodoptera litura infestation on superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities in three cotton cultivars. Bars represents Mean ± SE (n=3). Values are means ± SE (n=3). Cvs, cultivars; T, Treatment (Infestation); ns, non-significant; *, ** and *** = significant at 0.05, 0.01 and 0.001 levels, respectively.

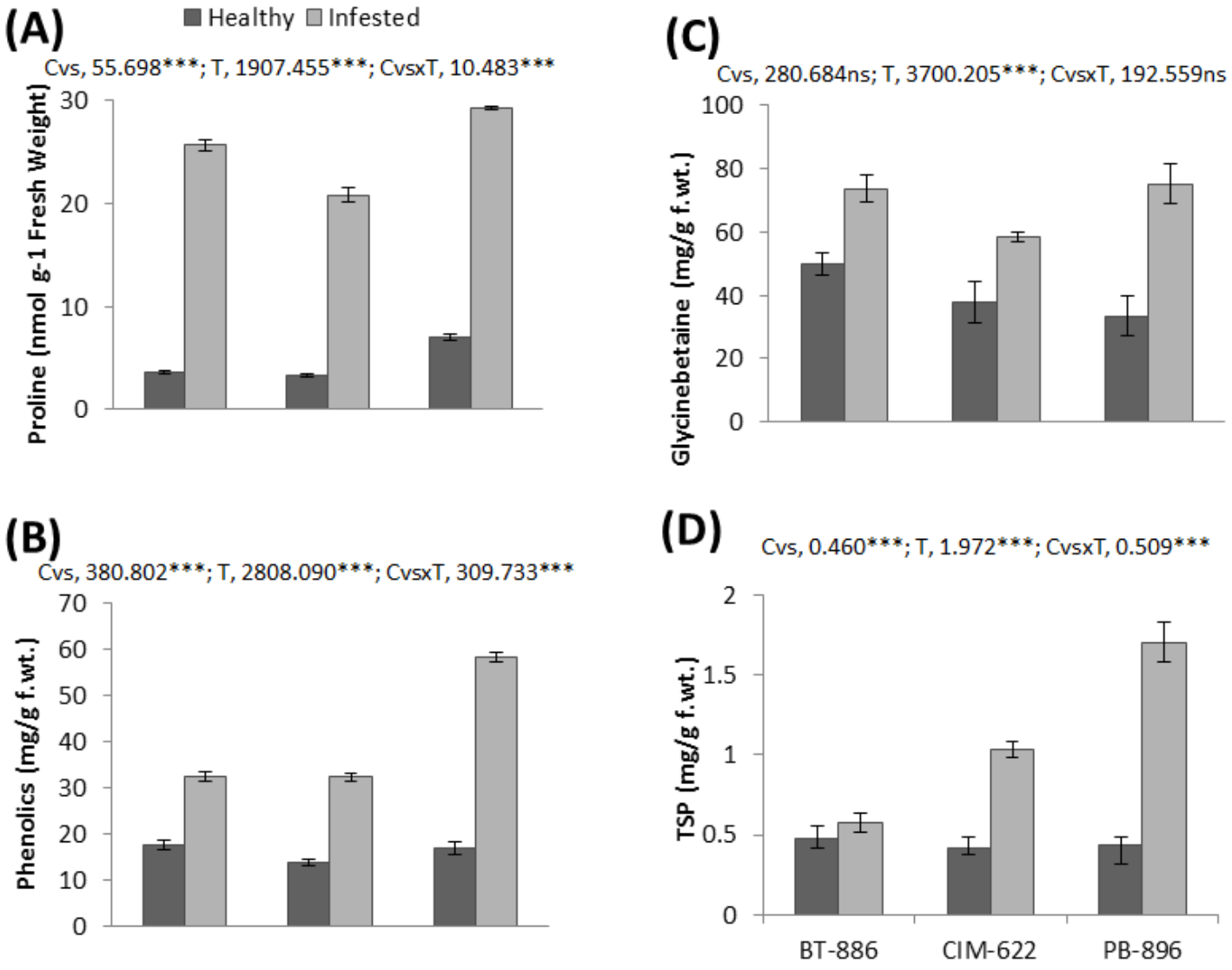


Figure 4

A-D Effect of Spodoptera litura infestation on leaf free proline content, phenolics, glycine betaine (GB) and total soluble proteins (TSP) in three cotton cultivars. Bars represent the means ± SE (n=3). Cvs, cultivars; T, Treatment (Infestation); ns, non-significant; *, ** and *** = significant at 0.05, 0.01 and 0.001 levels, respectively.

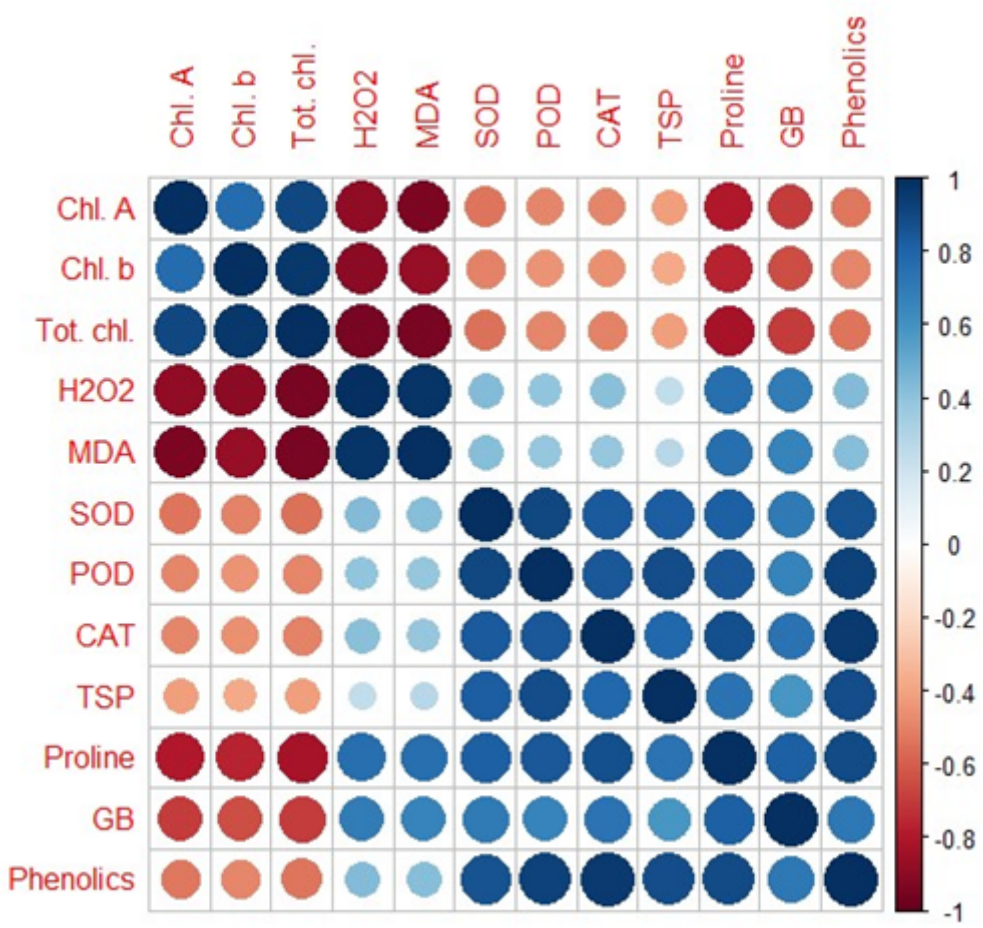


Figure 5

Correlation of S. litura infestation with chlorophyll pigments and biochemical attributes in cotton (Gossypium hirsutum L.) plants. Chl. a (Chlorophyll a), Chl. b (Chlorophyll b), Tot. chl. (Total chlorophyll contents), H2O2 (Hydrogen peroxide), MDA (Malondialdehyde), SOD (Superoxide dismutase), POD (Peroxidase), CAT (Catalase), TSP (Total soluble proteins) and GB (Glycine betaine).