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Effects of *Helicoverpa armigera* (Hubner) infestation on metabolic sensors dynamics in chickpea

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ABSTRACT

We studied the dynamics of oxidative stress biomarkers and biochemical constituents of chickpea (*Cicer arietinum*) genotypes against gram pod borer (*Helicoverpa armigera*). Selected genotypes *viz.*, NBeG - 786, GL -13001, ICC - 3137 (susceptible check), ICCL - 86111 (resistant check), GL - 13042 and RSG - 959 were screened for enzymatic, stress biomarkers, nutritional and anti-nutritional compounds at different time intervals (24, 48, 72 and 96 h). Data on leaf consumption, damage rating, pest susceptibility or resistance (%) and pest susceptibility/resistance rating were recorded. Results indicated that activity of all metabolic sensors (including nutritional and anti-nutritional factors) increased except catalase activity in response to *H. armigera* feeding, this suggested that biochemical compounds and their regulating enzymes and antioxidant defence system played important role in plant defence. Clustering and heat map analysis revealed that superoxide dismutase, catalase, polyphenol oxidase, total phenols and tannins were predominant in chickpea under insect pest stress and their over expression in chickpea genotypes will increase the tolerance to pod borer. The chickpea genotypes *viz.*, GL -13001, GL- 13042 and RSG- 959 possessed antibiosis mechanism of resistance and were found tolerant against *H. armigera* in field screening.

Keywords: Antioxidant enzymes, biotic stress, *Cicer arietinum*, chickpea, defence system, *Helicoverpa armigera*, oxidative biomarkers.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is major pulse crop worldwide and Sub-Saharan Africa and South Asia, accounts for 75 % Area and production. Globally, in recent years chickpea cultivation area has increased (23,46). The major barrier for increasing its production and productivity are gram pod borer, *Helicoverpa armigera* (Hubner), beet armyworm, *Spodoptera exigua* (Hubner), *Fusarium* wilt, root rots, *Ascochyta* blight, *Botrytis* grey mold and drought (20), of these *H. armigera* causes yield losses upto 80 % (54). This pest is active throughout the year but in India its damage to chickpea is during the months of November to March. The migratory nature, polyphagy, short life cycle, multivoltinism and insecticide resistance in *H. armigera* make its management a very difficult task (50). However to manage this pest, an integrated approach has to be followed which includes use of resistant varieties, plant products, biopesticides, natural enemies, following good agronomic practices, monitoring of pest through pheromone traps and judicious use of chemical pesticides. Among all these options host plant resistance plays an important role in pest management with a potential to reduce loss by herbivore and minimize

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insecticide use thereby, ensuring higher crop yield, safer environment and cost effective (56,73).

Plants respond to herbivore damage either through morphological or biochemical or molecular mechanisms to ward off the effects of herbivore, of which biochemical mechanisms are dynamic. The biochemical defensive compounds are produced either constitutively or induced in response to pest damage. Constitutive defence mechanism work in the plant system independent of stress, while induced defence mechanism is activated only in response to stress and it protects the plant from further damage. Infestation by herbivorous insects results in accumulation of defensive compounds through physiological, morphological and biochemical changes in plant system (2,47,66). The insect herbivory damage causes the changes in plant cell metabolic machinery such as respiration or photosynthesis which vleads to the production of reactive oxygen species (ROS). The increased production of ROS leads to oxidative stress causing damage to nucleic acids, lipids and proteins (19,26), so plants must maintain a balance between ROS production and ROS-scavenging to minimize plant tissue damage due to oxidative stress.

To maintain homeostasis, plants initiate the signalling cascade under oxidative stress by inducing the activity of defensive enzymes like superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), polyphenol oxidases (PPO) and secondary metabolites such as phenols, hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and nitric oxide (NO) (69). Considerable progress has been made in increasing the tolerance levels in different crops against both biotic and abiotic stress by simultaneous expression of antioxidant enzymes.

As a survival strategy, insects also employ variety of resistance mechanisms viz., avoidance, detoxification, target site modification against phytochemicals. However, the co-evolution of plants with herbivores has made plants to recognize the key metabolic process in insects such as digestive, nervous, endocrine systems and produce specialized defensive products which interfere with key processes. So, host plant resistance mediated through induced resistance can be exploited as an important tool in pest management programme.

The induced defence mechanism of plants against the biotic stress depends upon their ability to quickly perceive the incoming stimuli, decoding it and building it into a strong morphological and biochemical shield against insects (72). To develop resistant cultivars against *H. armigera*, it is necessary to understand the role of defensive mechanisms adapted by plants in response to insect attack. Since little information is available on biochemical dynamics occurring in chickpea in response to *H. armigera* infestation, present study was done to determine the activity of key metabolic sensors like antioxidant enzymes, oxidative stress biomarkers, nutritional and anti- nutritional components both at constitutive and induced level in different chickpea genotypes including resistant and susceptible checks at different time intervals after infestation.

MATERIALS AND METHODS

The experiments were conducted during November 2019- March 2020 in polyhouse, Entomology Division and Division of Biochemistry, ICAR- Indian Agricultural Research Institute, New Delhi (28°38’23”N, 77°12’27”E, 228.61m above MSL, 708.7 annual rainfall). Maximum and minimum temperature during study period was 31.8 and 2.2 ^{o}C.

Chemicals

All the chemicals used in this study were of analytical grade and all solutions were prepared in deionized water of resistivity not less than 18.2 M Ω/cm.

Test insect

H. armigera larvae were collected from pigeon pea fields (IARI, New Delhi) and reared on chickpea based semi-synthetic diet (18) in our insect rearing laboratory under optimum conditions (27±1 ºC, 65-75 % relative humidity and photoperiod of 12: 12 [L:D] h). F_4 neonates were used to study the defensive response of chickpea genotypes.

Chickpea plant

Chickpea leaf twig

Helicoverpa armigera larvae

Helicoverpa armigera adult

Plate 1. Chickpea plant, larva and adult of *Helicoverpa armigera*

Chickpea plants (*Cicer arietinum* L.)

Six chickpea genotypes [ICCL -86111 (resistant check), ICC - 3137 (susceptible check), GL- 13001, GL - 13042, RSG - 959 and NBeG-786] including resistant and susceptible checks were grown in polyhouse. Briefly, 15-seeds were sown in each plastic

pot (27 cm height and 31 cm dia) filled with the potting mixture (soil and farm yard manure in 3:1 ratio), watered as and when needed and applied with fertilizers. Plants were covered in cages with nylon mesh. The genotypes viz., GL- 13001, GL - 13042, RSG - 959 and NBeG-786 were found tolerant against *H. armigera* under in earlier field studies in All India co-ordinated research trials, so these genotypes were selected to study their induced resistance mechanism under biotic stress. Each genotype was sown in 10 pots in completely randomized design and 5 seeds were sown per pot. The leaf samples from top, middle and bottom portion of seedlings of all chickpea genotypes under uninfested and infested conditions were collected at 24, 48, 72 and 96 h after infestation and stored at -80 °C for downstream analysis.

H. ARMIGERA INFESTATION

Newly emerged (4 h old) starved *H. armigera* larvae were released on 21-day old chickpea seedlings @ 10 larvae/plant using camel hairbrush. Un-infested plants were considered as control for respective genotypes.

BIOCHEMICAL ANALYSIS

Biochemical estimations of enzymatic (superoxide dismutase, peroxidase, catalase and polyphenol oxidase), non-enzymatic (hydrogen peroxide and malondialdehyde content), nutritional compounds (reducing sugar and protein content), and anti-nutritional compounds (total phenols and tannins content) were done in triplicates using microplate reader (Bio Tek Instruments, Inc/USA) and UV-Vis spectrophotometer (Bench Top, India).

I. Enzyme extraction: Enzyme extraction for superoxide dismutase, peroxidase and catalase assay was done by homogenizing 200 mg of leaf sample in ice-cold 2 ml extraction buffer (0.1 M phosphate buffer, pH 7.5). The extract was then centrifuged for 20 min at 12000 rpm at 4 ºC and the supernatant was used as an enzyme source. Likewise for polyphenol oxidase the leaf samples (100 mg) was homogenized in 1 ml of ice-cold extraction buffer (0.1 M phosphate buffer, pH 7.8) and the extract was centrifuged for 10 min at 10000 rpm at 4 ºC and the supernatant was used for estimation.

(i). Superoxide dismutase (SOD) assay: Superoxide dismutase activity was estimated according to Dhindsa *et al.* (21). The 3 ml reaction mixture contained 13.33 mM methionine, 75 µM NBT, 0.1 mM EDTA, 0.1 mM phosphate buffer (pH 7.8), 50 mM sodium carbonate, enzyme extract (10 µl) and distilled water. The reaction was started by adding 2 µM riboflavin and tubes were placed under two 15 W fluorescent lamps for 15 min, while a non-irradiated complete reaction mixture of each sample served as blank. The absorbance was recorded at 560 nm on a microplate reader and enzyme activity was expressed as units /g FW.

(ii). Peroxidase (POX) assay: The peroxidase activity was determined as per Castillo *et al.* (17). The 2.5 ml reaction mixture contained 50 mM phosphate buffer pH 6.1, 16 mM Guaiacol, 2 mM H_20_2, enzyme extract (10µl) and water. Absorbance of formed tetra-guaiacol was recorded at 470 nm for 3 min on the microplate reader and enzyme activity was calculated as per the extinction co-efficient of its oxidation product, tetra-guaiacol £= 26.6 $mm^{-1}cm^{-1}$. Enzyme activity was expressed as units per g fresh weight of sample.

(iii). Catalase (CAT) assay: The catalase activity was determined according to Sinha (61) following the ability of the enzyme to split H_2O_2 within 1 min of incubation time. The reaction mixture consisted of phosphate buffer (0.01 mM, pH 7), enzyme extract,

H_20_2 and water. The absorbance was measured at 570 nm on a microplate reader. Enzyme activity was expressed as micromoles of H_2O_2 decomposed per minute /g FW.

(iv). Polyphenol oxidase (PPO) assay: The PPO activity was determined according to Kruger *et al.* (42) with minor modifications. The reaction was initiated by adding 0.2 ml of enzyme extract to 2.8 ml of substrate catechol solution (0.01 M). This mixture was incubated at 37 °C for 30 min. The absorbance was measured at 410 nm on a microplate reader and enzyme activity was expressed as units per g fresh weight of sample.

Hydrogen peroxide (H_2O_2) content

The hydrogen peroxide (H_2O_2) content was determined as per Loreto and Velikova (43) using H_2O_2 as a standard. The extract was prepared by grinding 100 mg leaf sample in liquid nitrogen and 1ml of chilled TCA (0.1 %) was added. The mixture was then centrifuged at 12000 rpm for 15 min at 4 ºC. The supernatant was used to determine hydrogen peroxide content. To 750 µl supernatant, 750 µl of potassium phosphate buffer (10 mM, pH 7) and 1.5 ml of potassium iodide (1 M) were added. The optical density was recorded at 390 nm on a UV-Vis spectrophotometer and H_2O_2 content was expressed as µmol /g FW.

Lipid peroxidation / Malondialdehyde (MDA) content

The MDA content was determined as per Cakmak and Horst (16). Extract was prepared by grinding (500 mg) leaf sample in 10 ml of TCA (0.1 %) and shaken for 15 min at room temperature. The extract was centrifuged for 10 min at 12000 rpm at 4 ºC and the supernatant was used for estimation. The reaction mixture consisted of 1 ml of supernatant and 4 ml of 20 % TCA + 0.6 % TBA mixture. After incubation in boiling water for 30 min, the absorbance was recorded at 532 and 600 nm wavelengths on a microplate reader. The MDA content was computed using extinction coefficient of 155 mM^{-1} cm^{-1} with the formula: [MDA level (nmol) = ΔA (532 - 600) nm / 1.56 x 10^5] and expressed as nmol/g FW of the sample.

Reducing sugar, protein, total phenols and tannins content estimation

Reducing sugar content was estimated by as per Nelson (48) and modified method Somogyi (63) using glucose as a standard. The reducing sugar content was calculated using glucose standard curve and expressed as mg g^{-1} FW. The protein content was estimated according to Bradford (14) using Bovine serum albumin (BSA) as standard. The amount of protein concentration was expressed as mg/g of plant tissue. Total phenol content (TPC) of chickpea leaves was estimated by the method of Singleton *et al.* (60) using gallic acid as a standard and was expressed as mg of gallic acid equivalents/g extract. The tannins content was determined by the Folin Ciocalteu method (4) and expressed as mg/g FW, calculated with the help of gallic acid standard curve.

Damage rating

Detached leaf assay method was used to determine the extent of damage from *H. armigera* on different chickpea genotypes (57). In brief, terminal leaf twigs of each genotypes were excised, washed, shade dried and weighed on an analytical balance (Mettler Toledo, Switzerland) and immediately placed in plastic Petri dish containing 2 % agar medium in a slanting manner. Entire experiment set up was maintained at 27±1 ºC, 65-75 % relative humidity. Neonates of *H. armigera* (≤ 12 h) were released @ 10 larvae/ leaf twig. There were four replications for each genotype in a completely randomized design. The experiments were terminated 4 days after infestation and observations were recorded on

final leaf weight and damage rating. Leaf twigs were visually rated for *H. armigera* damage on chickpea genotypes using 1-9 scale (55), where 1 = ≤ 10, 2 = 11-20, 3= 21- 30 and 9 = ≥ 80 % leaf damage. Based on the leaf weight consumed in each test genotype, pest susceptibility or resistance (%) was calculated using the following formula (1).

$$\text{Pest susceptibility or resistance (\%)} = \frac{\text{Leaf weight consumed in susceptible check - leaf weight consumed in test genotype}}{\text{Leaf weight consumed in susceptible check}} \times 100$$

The pest susceptibility or resistance (%) was converted into pest susceptibility/ resistance rating (PSRR) scale of (1-9) according to Kooner and Cheema (41).

Statistical analysis

The data of the biochemical constituents of chickpea leaves of different genotypes under control and infested conditions, and genotype x treatment combinations were subjected to analysis of variance (ANOVA) in factorial design and data on detached leaf assay in completely randomized design. The treatment means were compared by least significant difference (LSD) at P = 0.05 using the statistical software SAS ® version 9.3. Cluster analysis and heatmap were generated for all the ten biochemical constituents of six chickpea genotypes at 48 h after infestation using R- version 3.6.2.

RESULTS AND DISCUSSION

We quantified changes in the metabolic sensors such as enzymatic (SOD, POX, CAT and PPO), non-enzymatic biomarkers (H_2O_2 and MDA content), nutritional (reducing sugar and protein content) and anti-nutritional compounds (TPC and tannins content) of chickpea leaves at four intervals viz., 24, 48, 72 and 96 h after *H. armigera* infestation as well as in un-infested plants. *H. armigera* infested chickpea seedlings showed higher biochemical activity than the uninfested seedlings. The response of metabolic sensors *viz.*, SOD, POX and PPO, and H_2O_2, MDA, protein, reducing sugar, total phenols and tannin content increased, while, catalase activity decreased upon *H. armigera* feeding in all the chickpea genotypes.

Induced resistance is an important component of plant defence that makes plants phenotypically plastic to face different types of stresses and is more economical, effective and environmentally friendly (71). Plants have developed an elegant defence system due to the evolutionary race between plants and insects. The plant defence system recognizes the non-self molecules, which elicit the defence response to insect attack (34). Plants produce higher amounts of proteins and secondary metabolites to function as anti-nutritive, toxins or deterrents against insect attack (31,32,62,73). Herbivore damage induces rapid signals and responses in plants like oxidative burst, accumulation and release of secondary metabolites and antioxidative enzymes *viz.*, peroxidase, polyphenol oxidase, lipoxygenase, superoxide dismutase, phenyl-ammonia lyase and catalase (26,31,77). In host plant resistance to insects, host plants oxidative state is important in the production of ROS and toxic secondary metabolites (31,32,73,77). To manage insect pests, the plants defence mechanism is an attractive area of research all over the world. Here, we studied the induced defensive biochemical response of six chickpea genotypes against *H. armigera* feeding.

Superoxide dismutase (SOD) activity

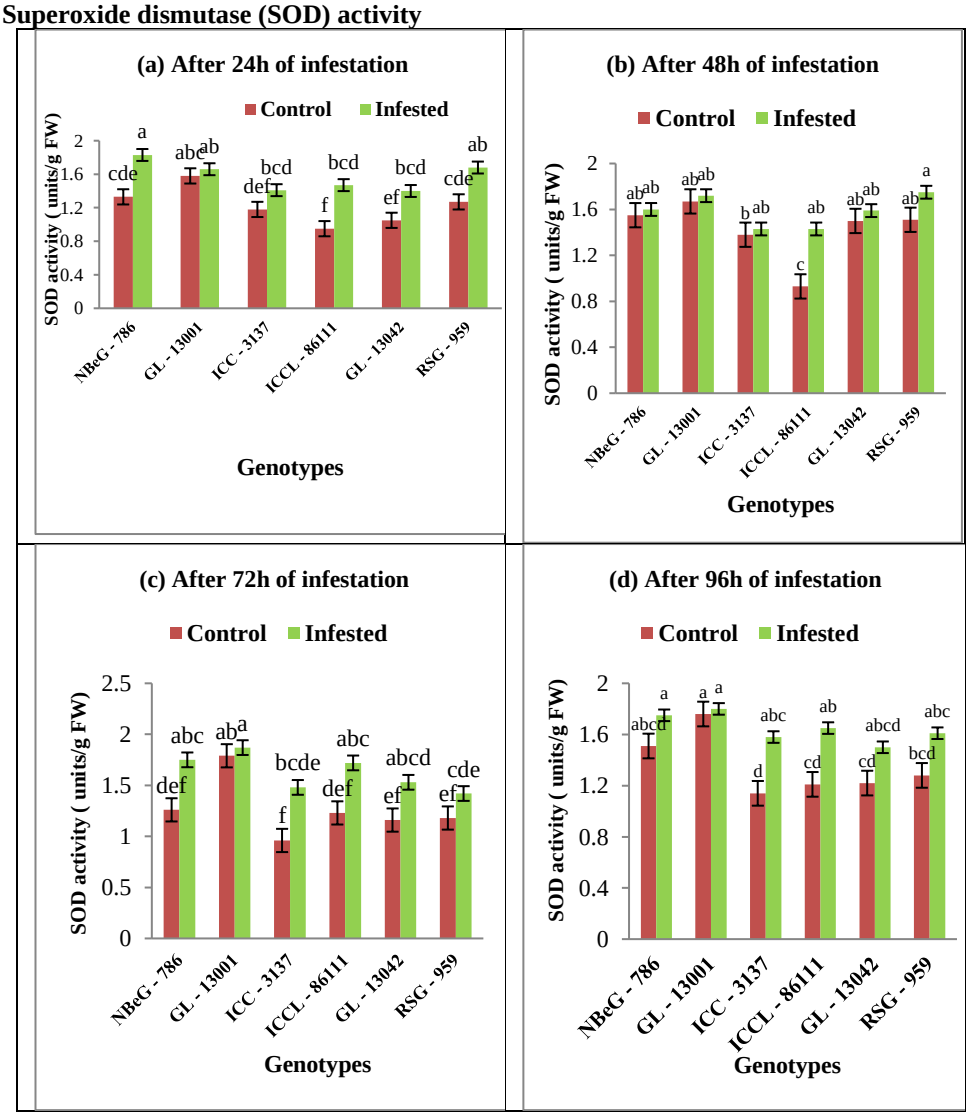

Figure 1. Superoxide dismutase (SOD) activity (units/g FW) in the leaves of different chickpea genotypes in response to damage by *H. armigera* at different hours (a-d) after infestation. Bars (Mean ± SE) with different superscripts are significantly different at p ≤0.05.

A significant increase in SOD activity was observed in infested plants than in the non-infested plants across the genotypes and durations indicates the upregulation of antioxidant defence system in chickpea plants after insect infestation. The SOD activity increased from 1.02 to 1.55 folds in *H. armigera* damaged plants from 24 to 72h but decreased thereafter. Among test genotypes, significantly higher SOD activity in both control and infested plants was observed in GL -13001, followed by NBeG - 786, RSG-959 than resistant check, ICCL-86111 (Fig. 1). The variations in SOD activity might be due to genotypic differences in upregulation response against insect pest damage. It has been reported earlier that, SOD is the early detoxification enzyme that act as a component of first line defence system against ROS by catalyzing the dismutation of superoxide radicals into oxygen and H_2O_2 (66). Likewise, superoxide anion, a biologically active and one of the major reactive oxygen species triggers a cascade of events that lead to hypersensitive cell death (53). Kaur *et al.* (36) opined that increased SOD activity after pest damage in resistant genotypes will be helpful in reducing membrane damage by preventing the lipid peroxidation that scavenges free radicals. War *et al.* (71) observed that the SOD activity was higher in infested plants than in non-infested groundnut genotypes and the constitutive levels of SOD activity was lower in susceptible genotype, JL 24 than the resistant genotype. Similarly, increased SOD activity was exhibited in pigeon pea test genotypes after infestation by *H. armigera* that showed the upregulation of defence mechanism (35).

Peroxidase (POX) activity

Chickpea plants infested with *H. armigera* showed significantly higher POX activity among genotypes (P=0.0001, 0.0230 and 0.0389 at 48, 72 and 96 h respectively), treatments (infested and uninfested) (P=0.0388, <0.0001, <0.0001 and 0.0006) and in the genotype x treatment combinations (P=0.0005, 0.0106 at 48 & 72h respectively). The POX activity increased from 1.02 to 2.41 folds in all test chickpea genotypes after *H. armigera* infestation. The highest POX activity among test genotypes was recorded in NBeG-786 (5.45) followed by RSG - 959 (5.09), GL - 13001(4.66) and GL - 13042(3.19) after infestation by *H. armigera* at different sampling periods than susceptible check (Fig. 2). Peroxidase takes part in the detoxification of H_2O_2 and also in production of quinones that are toxic to insects after ingestion (78). It mediates the oxidation of hydroxycinnamic alcohols which produces the anti-nutritive compounds in stressed plants (31). The higher POX activity in test genotypes might be for protection against insect damage and up-regulation response of defensive response in chickpea germplasms to biotic stress. Earlier it has been observed that, increasing POX activity may detoxify the peroxides, thereby, reducing plant tissue damage in insect infested plants (28). Apart from antioxidative, POX also plays role in cell wall strengthening and producing toxic secondary metabolites in response to different stresses (31). Higher POX activity against herbivore attack in plants could be attributed to lignification, suberization, somatic embryogenesis and wound healing (3,29). Present findings are in agreement with other studies (21,28,29,31,66,69,71,76) that the POX activity was higher after herbivore infestation in different crops.

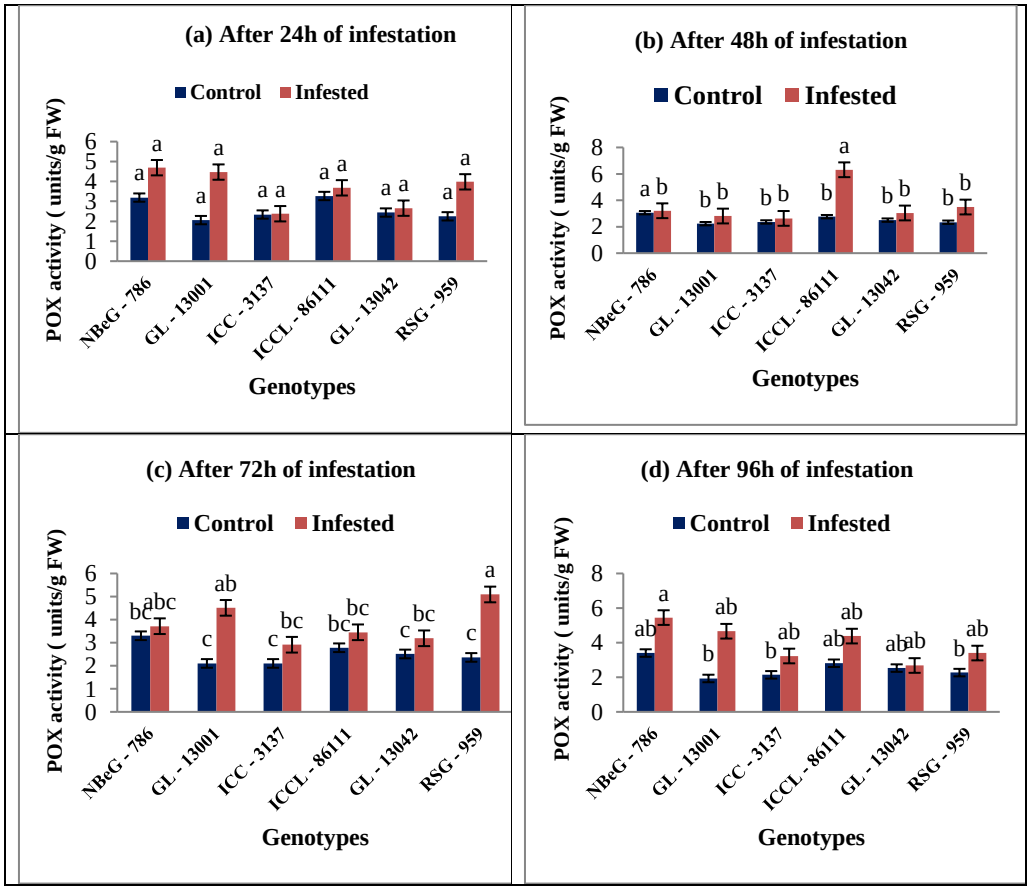

Figure 2. Peroxidase (POX) activity (units/g FW) in response to *H. armigera* damage in the leaves of different chickpea genotypes at different hours (a-d) after infestation. Bars (Mean ± SE) with different superscripts are significantly different at p ≤0.05

Catalase (CAT) activity

One more efficient enzyme in scavenging ROS is catalase, which converts hydrogen peroxide into molecular oxygen and water (39). It plays a critical role in elimination of H_2O_2 which is involved in β-oxidation of fatty acids in peroxisomes, photorespiration, and purine catabolism at the time of oxidative stress (67). We found that infestation by gram pod borer, *H. armigera* decreased the CAT activity in plants. It was observed that the CAT activity was increased in susceptible check (ICC-3137) after infestation by *H. armigera*. Among test genotypes, minimum decline in CAT activity was recorded in RSG-959 (Fig. 3). Earlier, similar pattern of decline in catalase activity after insect infestation has been noticed in pigeon pea and black gram (35,64). The decline in CAT activity might be due to high level of superoxide radical generation during oxidative damage as superoxide radical is small enough to gain access to the hemes of CAT and would convert the enzyme into inactive

ferro-oxy state (40). Present studies showed greater decrease in CAT activity in susceptible check compared to test genotypes, earlier Kaur *et al.* (35) and Taggar *et al.* (64) reported greater decrease in CAT activity in susceptible genotypes than the resistant genotypes. Kaur *et al.* (37) reported that decline in CAT activity in the leaves, pod wall and developing seeds of chickpea after *H. armigera* infestation might be due to H_2O_2 accumulation and catalase mediated detoxification of H_2O_2.

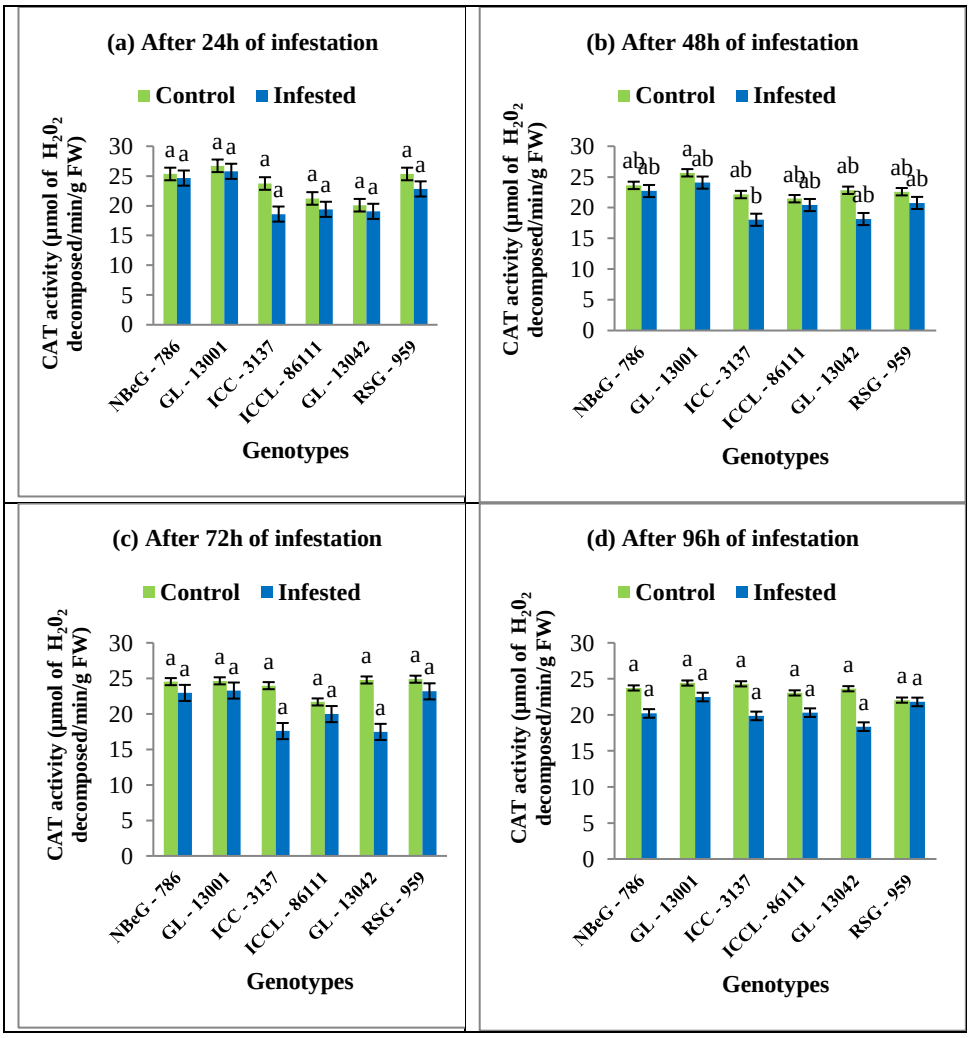

Figure 3. Activity of catalase (CAT) (µmol of H_20_2 decomposed /min/g FW) in response to *H. armigera* damage in the leaves of different chickpea genotypes at different hours (a-d) after infestation. Bars (Mean ± SE) with different superscripts are significantly different at $p \leq 0.05$

Polyphenol oxidase (PPO) activity

After *H. armigera* infestation, PPO activity was increased in all test chickpea genotypes. There were significant differences among genotypes, treatments and in genotype x treatment interactions throughout the test period. The PPO activity increased in infested plants from 1.06 to 2.45 folds like SOD activity, the PPO activity showed increasing trend from 24 to 72 h but decreased thereafter. The induced PPO activity was significantly greater in GL-13001 (2.69 folds) and NBeG-786 (2.64 folds) at 72 h. Among the test genotypes, the PPO activity was significantly higher in NBeG - 786 (0.47) and GL - 13001(0.43) but significantly lower in GL - 13042 (0.36) and RSG - 959 (0.27) as compared to susceptible check, ICC - 3137 (0.39) after *H. armigera* feeding (Fig. 4). Earlier it has been reported that, in response to insect attack PPO participates in the plant defence as an antinutritional enzyme by reducing plant nutrition thereby reducing herbivore palatability and digestion (12). PPO oxidizes monophenols and o-diphenols to quinones, which interact with the nucleophilic side

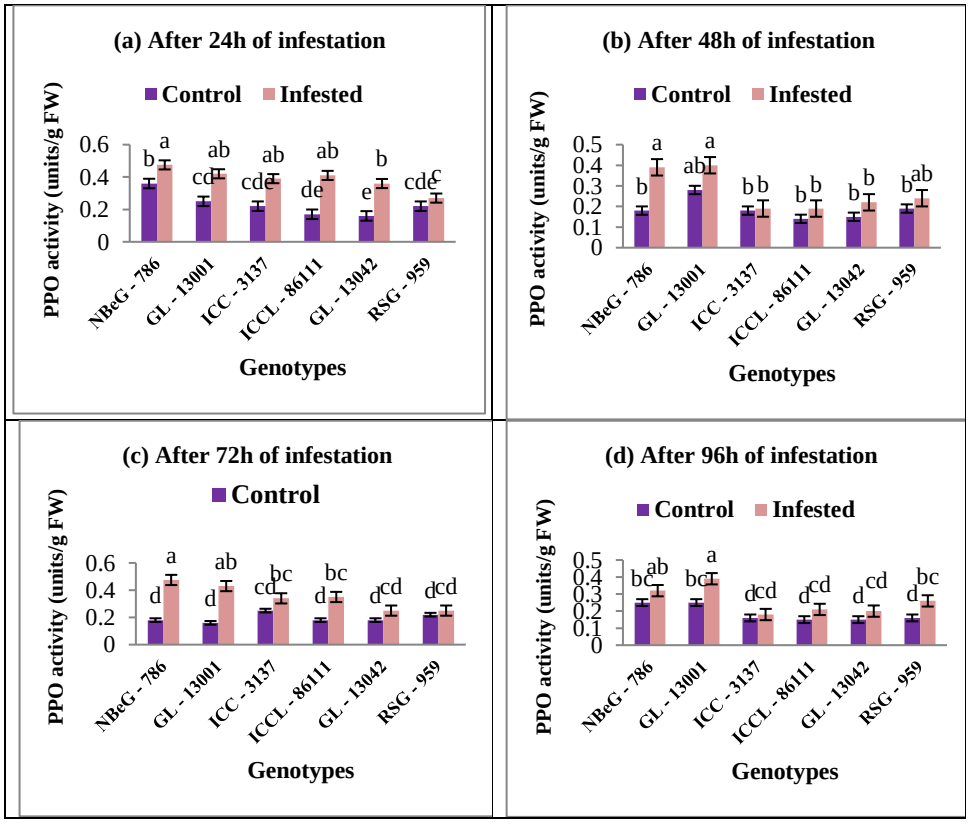

Figure 4. Polyphenol oxidase (PPO) activity (units/g FW) in response to *H. armigera* damage in the leaf of different chickpea genotypes at different hours (a-d) after infestation. Bars (Mean±SE) with different superscripts are significantly different at p ≤0.05

chain of amino acids that lead to proteins cross-linking and reducing their availability to insect pests (12,76). The melanin formation by PPO increases the cell wall resistance to insects and pathogens (77). Present results are in line with other studies in the sense that PPO activity was increased after herbivore infestation (12,24,51,70,71).

Hydrogen peroxide content (H_2O_2) content

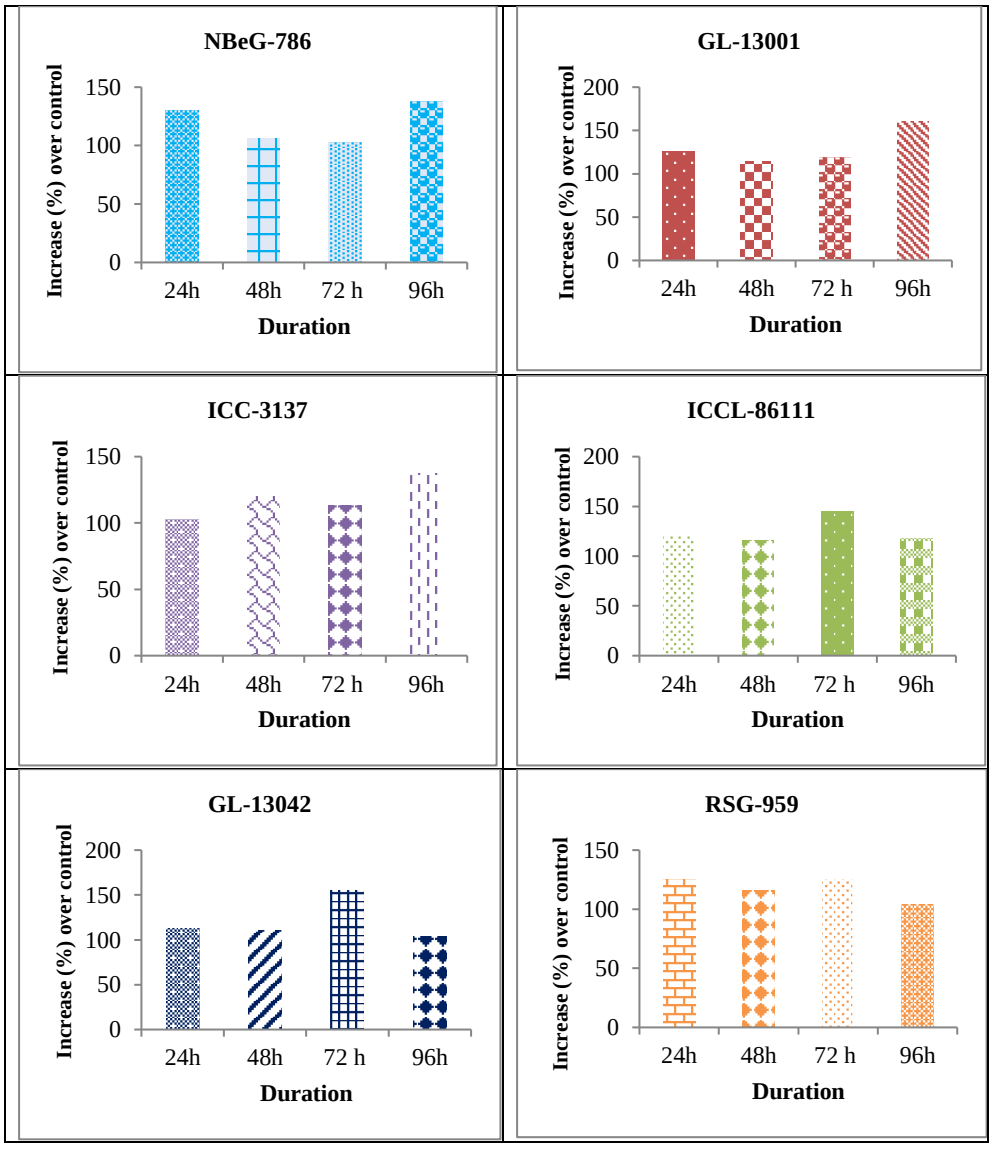

Figure 5. Changes in hydrogen peroxide (H_2O_2) content (µmol /g FW) in response to damage by *Helicoverpa armigera* in leaves of chickpea genotypes at different hours after infestation

We found that plants infested with *H. armigera* showed greater H_2O_2 content than healthy plants and all the test genotypes proved more tolerant than resistant check, ICCL - 86111 against *H. armigera* due to increased production of H_2O_2. The H_2O_2 contents increased from 1.03 to 1.61 folds by the pest damage during test periods. Among the test genotypes, the H_2O_2 content was significantly higher in NBeG - 786 (5.45) followed by GL -13001(5.37), RSG - 959 (5.25) and GL - 13042 (4.59) after *H. armigera* infestation at different sampling periods (Fig. 5). Earlier reports suggest that plants showed the rapid production of ROS as basic response to various stresses (44) and a close interaction was observed between the H_2O_2 production and activation of defence related pathways against herbivory in plant systems (7,45,68,70,71). H_2O_2 is a vital molecule that plays a major role in signalling pathway of metabolic sensors and toxic to insects as it triggers different molecular and physiological processes leading to generation of various defensive compounds and enzymes (69). It has been reported earlier that, pod borer damage increases the production of H_2O_2 in leaves, developing seeds and pod wall of pigeon pea genotypes (35) and Gechev *et al.* (25) opined that it also stimulates the activation of defence-related genes that lead to the production of antioxidative enzymes and toxic secondary metabolites. H_2O_2 acts as a secondary messenger as the accumulation of defensive proteins is directly toxic to the insects (32,44,65). Plants respond to herbivory by the induction of H_2O_2, this might benefit the plant system, when it is perceived quickly for the induction of defensive responses for protecting the plants against insect pests (10,31,65).

Lipid peroxidation/ malondialdehyde (MDA) content

MDA content increased from 1.02 to 2.7 folds in *H. armigera* damaged plants. Higher induction of MDA content was observed at initial stages of infestation (24 and 48 h) wherein, the highest MDA content was observed in susceptible check, ICC - 3137 (2.30 and 2.07 folds) than healthy plants (Fig. 6). Similar results were observed with higher MDA content in leaves, pod wall and developing seeds of susceptible chickpea genotypes ICC-3137 and L-550 after *H. armigera* infestation (38). MDA is important in defence related signalling processes in plants against herbivory (33). It occurs naturally and used as biomarker for oxidative stress like lipid peroxidation and membrane permeability in plants against herbivory (9). Earlier it has been found that MDA has been widely used as a convenient biomarker among secondary products for lipid peroxidation due to its facile reaction with thiobarbituric acid (74). Lipid peroxidation stimulates the plants volatile emission, which attracts the natural enemies in response to insects (8) and it also decreases the membrane fluidity, increases the membrane leakage and damages its proteins (9).

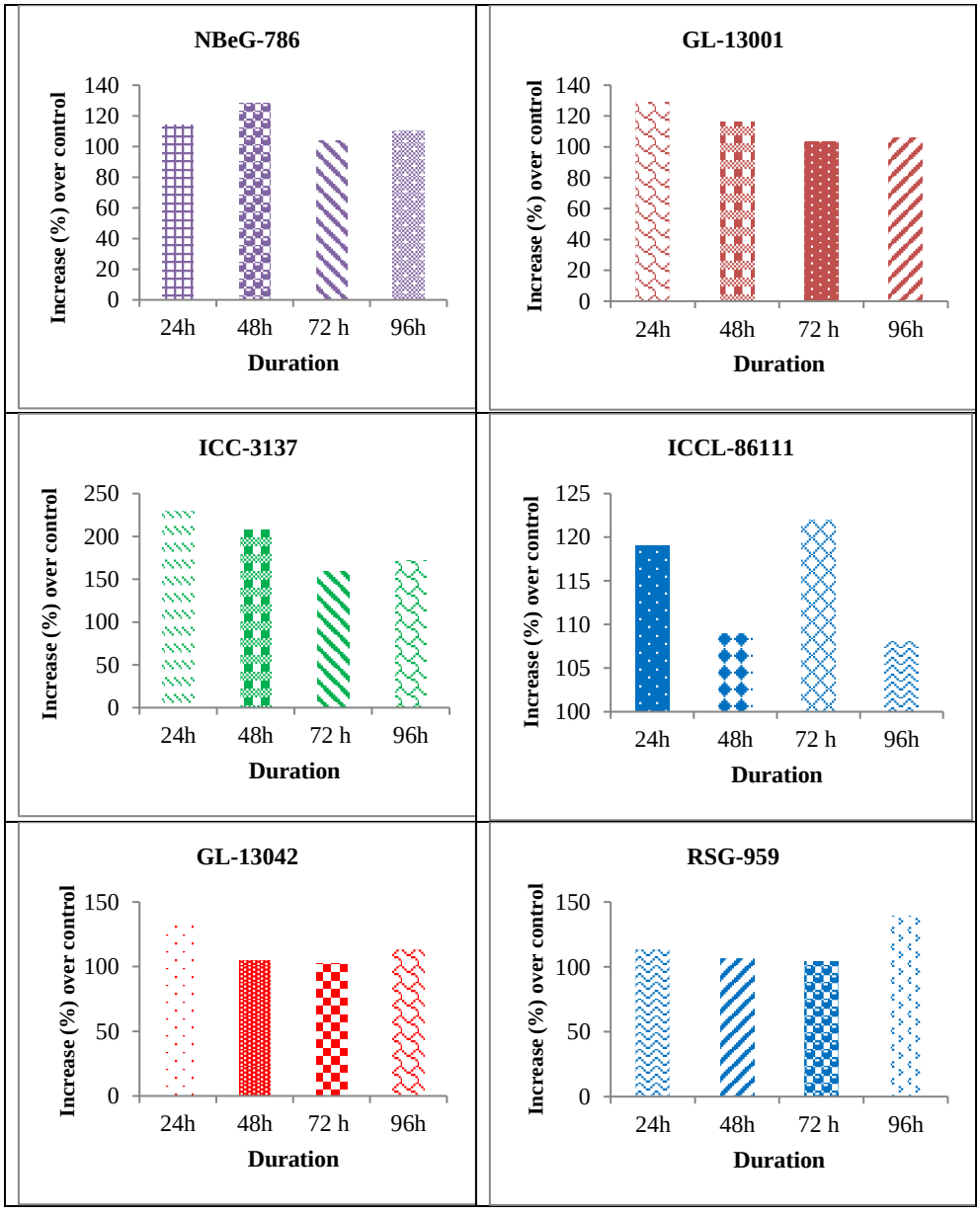

Figure 6. Changes in lipid peroxidation/ malondialdehyde (MDA) content (nmol/g FW) in the leaves of different chickpea genotypes in response to damage by *Helicoverpa armigera* at different hours after infestation

Table 1. Reducing sugar content (mg /g FW) in the leaves of different chickpea genotypes in response to damage by *Helicoverpa armigera* at different hours after infestation

Genotypes	24h			48h			72h			96h		
	Control	Infested	Change in fold	Control	Infested	Change in fold	Control	Infested	Change in fold	Control	Infested	Change in fold
NBeG-786	0.82^{cd}	0.88^{cd}	1.07	0.64^{b}	0.90^{b}	1.40	1.06^{abcd}	1.35^{abc}	1.28	0.90^{cd}	1.07^{cd}	1.19
GL-13001	0.97^{cd}	1.00^{cd}	1.03	0.62^{b}	0.84^{b}	1.35	0.82^{cde}	1.75^{a}	2.13	1.22^{bcd}	1.66^{ab}	1.36
ICC-3137 (S)	0.59^{de}	1.18^{c}	2.00	0.64^{b}	0.85^{b}	1.33	0.78^{cde}	0.95^{cde}	1.22	0.28^{ef}	1.02^{cd}	3.64
ICCL-86111(R)	1.34^{bc}	2.05^{a}	1.53	0.70^{b}	1.81^{a}	2.59	0.57^{de}	1.00^{bcd}	1.75	1.41^{bc}	2.16^{a}	1.53
GL-13042	0.14^{e}	1.78^{ab}	12.71	0.81^{b}	1.00^{b}	1.23	0.25^{e}	1.69^{ab}	6.76	0.17^{f}	0.76^{de}	4.47
RSG-959	0.24^{e}	1.05^{cd}	4.38	0.72^{b}	1.87^{a}	2.60	0.45^{de}	1.17^{abcd}	2.60	0.95^{cd}	1.16^{bcd}	1.22
Factors	**P-value**	**LSD at 5%**		**P-value**	**LSD at 5%**		**P-value**	**LSD at 5%**		**P-value**	**LSD at 5%**	
Genotypes (G)	<0.0001	0.200		0.0007	0.247		0.0089	0.280		<0.0001	0.215	
Treatment (T)	<0.0001	0.115		<0.0001	0.142		<0.0001	0.161		<0.0001	0.124	
G x T	<0.0001	0.283		0.001	0.350		0.0038	0.396		0.0479	0.304	

Data represent mean±SD of triplicates; genotypes with different superscripts are significantly different at $p \leq 0.05$.

Reducing sugar content

In our study, *H. armigera* infestation increased the reducing sugar content than uninfested plants. The reducing sugar content increased from 1.03 to 12.71 folds in *H. armigera* damaged plants. Among the test genotypes, the reducing sugar content was significantly higher in RSG - 959 followed by GL - 13042, GL -13001 and NBeG - 786 after *H. armigera* infestation. All the test genotypes showed significantly lower reducing sugar content than resistant check, ICCL - 86111 (Table 1). These observations were in conformity with Singh *et al.* (59) who reported lower amount of reducing sugars in the leaves of leafhopper resistant varieties of cotton in comparison to susceptible check. The results are also in agreement with earlier findings of Rani (52) and Bommesha *et al.* (13) who reported that the amount of total proteins and total reducing sugars were comparatively low in flower buds, pods and seeds of the tolerant varieties with lower pod borer damage than susceptible pigeon pea varieties. Haralu *et al.* (30) reported that the lowest reducing sugar content was noticed in BGD 111-01 genotype, and that can be exploited in breeding programme as resistance source for pod borer, *H. armigera* in chickpea ecosystem.

Protein content

Protein content significantly increased in infested plants than control plants. The protein content increased from 1.03 to 2.74 folds in *H. armigera* damaged plants. Among the test genotypes, the significantly higher protein content was observed in GL -13001 followed by NBeG - 786, RSG - 959 while it was lower in GL - 13042 as compared to resistant check, ICCL - 86111 after *H. armigera* infestation (Fig. 7). After herbivore attack, increased protein content could be ascribed to higher antioxidative enzyme activities. The insect-resistant genotypes had higher protein content than susceptible genotype after *H. armigera* infestation. It has been reported earlier that, protein-based compounds mediate wide-ranging defence responses in plants (72). War *et al.* (71) reported that the higher protein content may be attributed to the higher activity of plant defensive enzymes and also other defensive protein production. After insects attack, plant produce protein-based defensive compounds, which are one of the important defence mechanism against herbivory (21,49).

Total phenol content (TPC)

The plants infested with *H. armigera* showed higher total phenol content than healthy plants. Across the genotypes, the total phenol content was significantly higher in both control and infested plants of NBeG-786 throughout the test period. Among the test genotypes, the significantly higher TPC was observed in NBeG - 786 (1.21) followed by RSG - 959(0.91), GL - 13042(0.89) and GL -13001(0.85) after the pest damage (Fig. 8). Earlier it has been found that, plants respond to insect herbivory by accumulation of phenolic compounds (56,66) and phenolic compounds were higher in resistant genotypes than in intermediate and susceptible pigeon pea genotypes, it might be the reason for insect resistance (72). The phenolics (phenols, o-dihydroxyphenols, flavonols) accumulation in plants against herbivores are toxic to insects (12,68) because they reduce free radicals (15) and stimulate the signalling of various transduction pathways by up regulating various defensive enzymes (12,44). Phenols are involved in cyclic reduction of reactive oxygen species (ROS) and activates the cascade of reactions, which in turn activate defensive enzymes (44,58). Quinones resulting from phenols oxidation bind covalently to leaf proteins and reduces insect digestibility. The phenols act as reducing agents, hydrogen donators and quenchers of singlet oxygen (75).

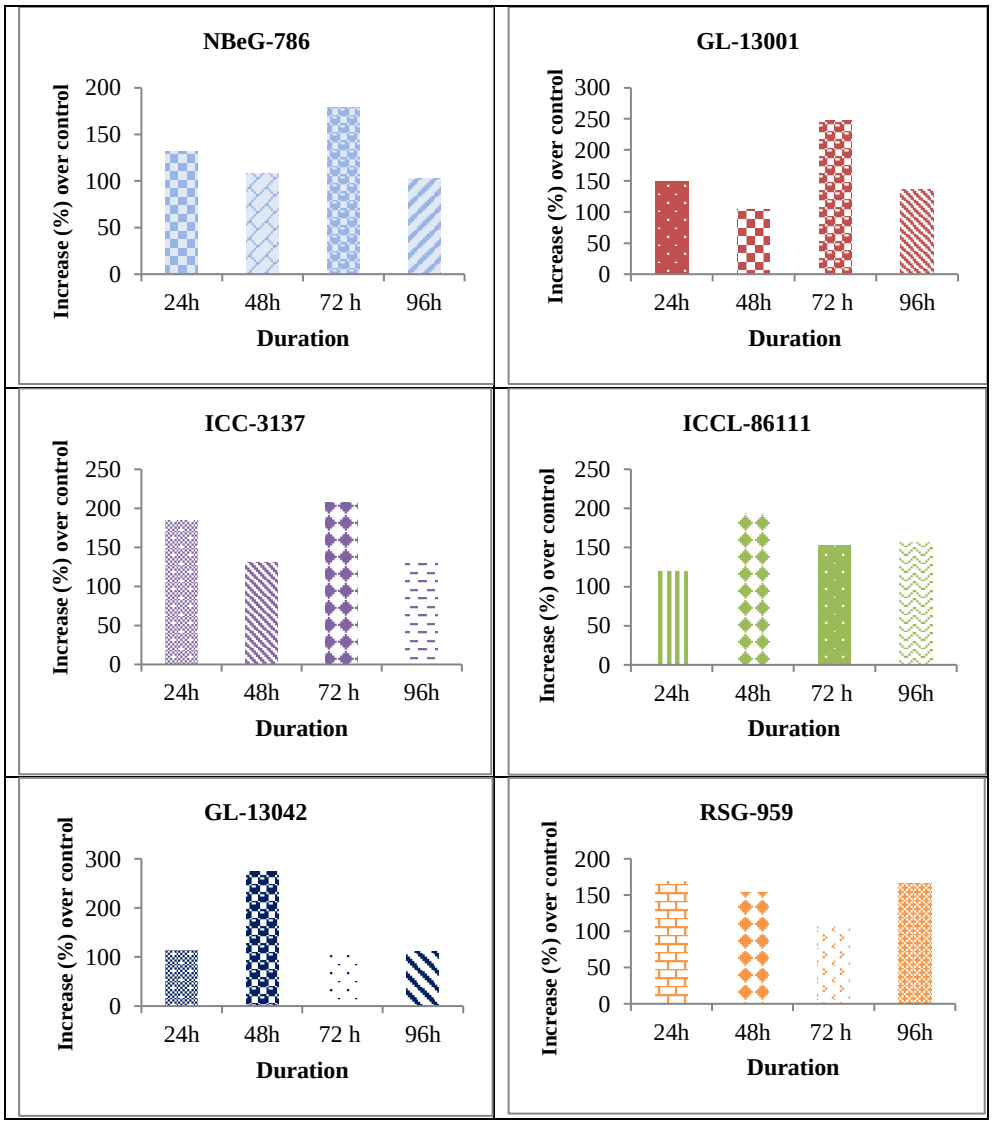

Figure 7. Effects of *Helicoverpa armigera* damage on protein content change (mg /g FW) in the leaves of different chickpea genotypes at different hours after infestation.

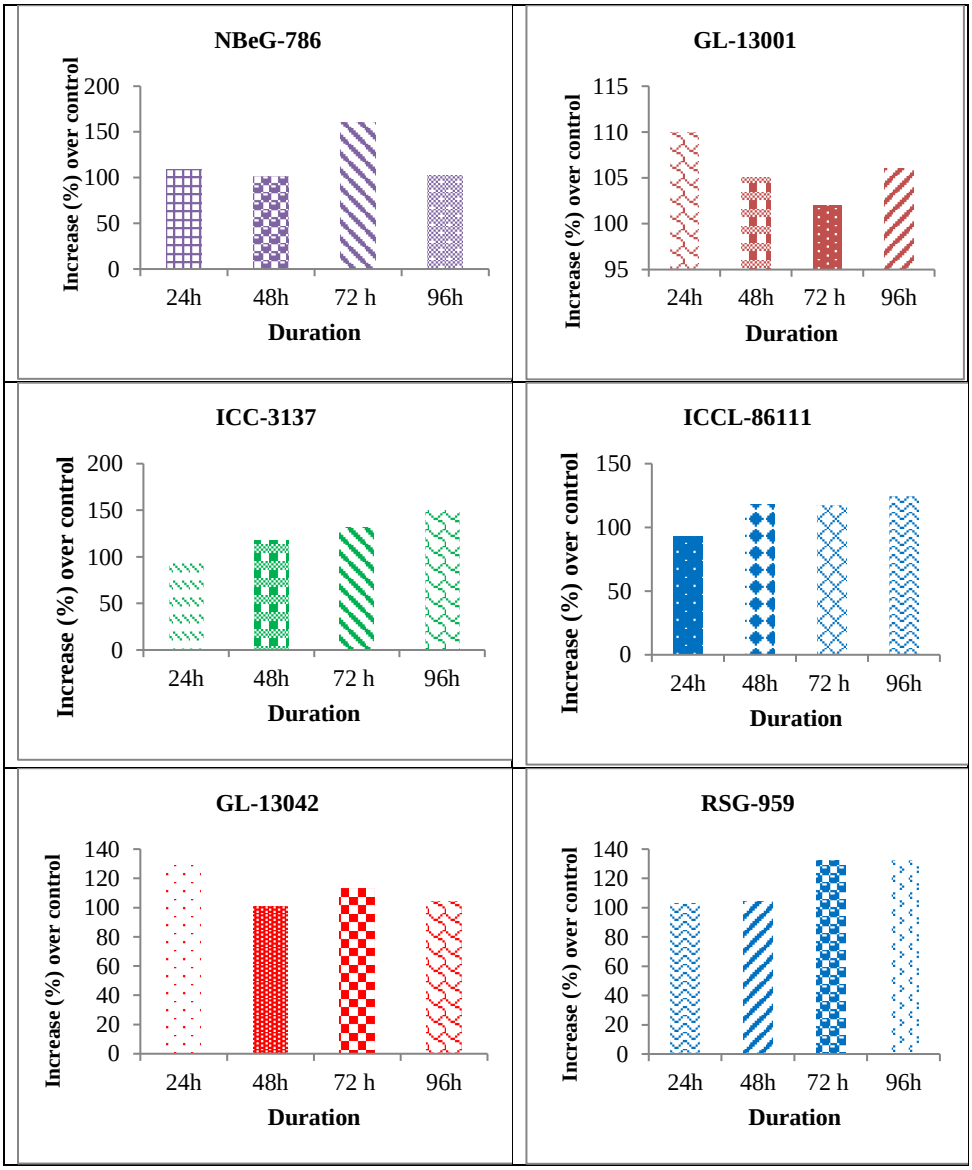

Figure 8. Changes in total phenol content (mg /g GAE) in response to damage by *Helicoverpa armigera* in the leaves of different chickpea genotypes at different hours after infestation.

Tannins content

The *H. armigera* infested plants showed higher tannins content than undamaged plants. The tannins content increased from 1.08 to 2.14 folds in *H. armigera* damaged plants. Like phenols, significantly higher tannin content was found both in control and infested plants of NBeG - 786 throughout the test period. Among the test genotypes, 72 h after

infestation, GL - 13042 exhibited significantly higher increase in tannin content (1.75 folds) than control plants and highest tannin content was observed in NBeG - 786 (3.27) followed by GL -13042 (3.16), GL -13001 (2.77) and RSG - 959(2.68) after *H. armigera* damage (Fig. 9). After *H. armigera* infestation, increased tannins content might be important to deter

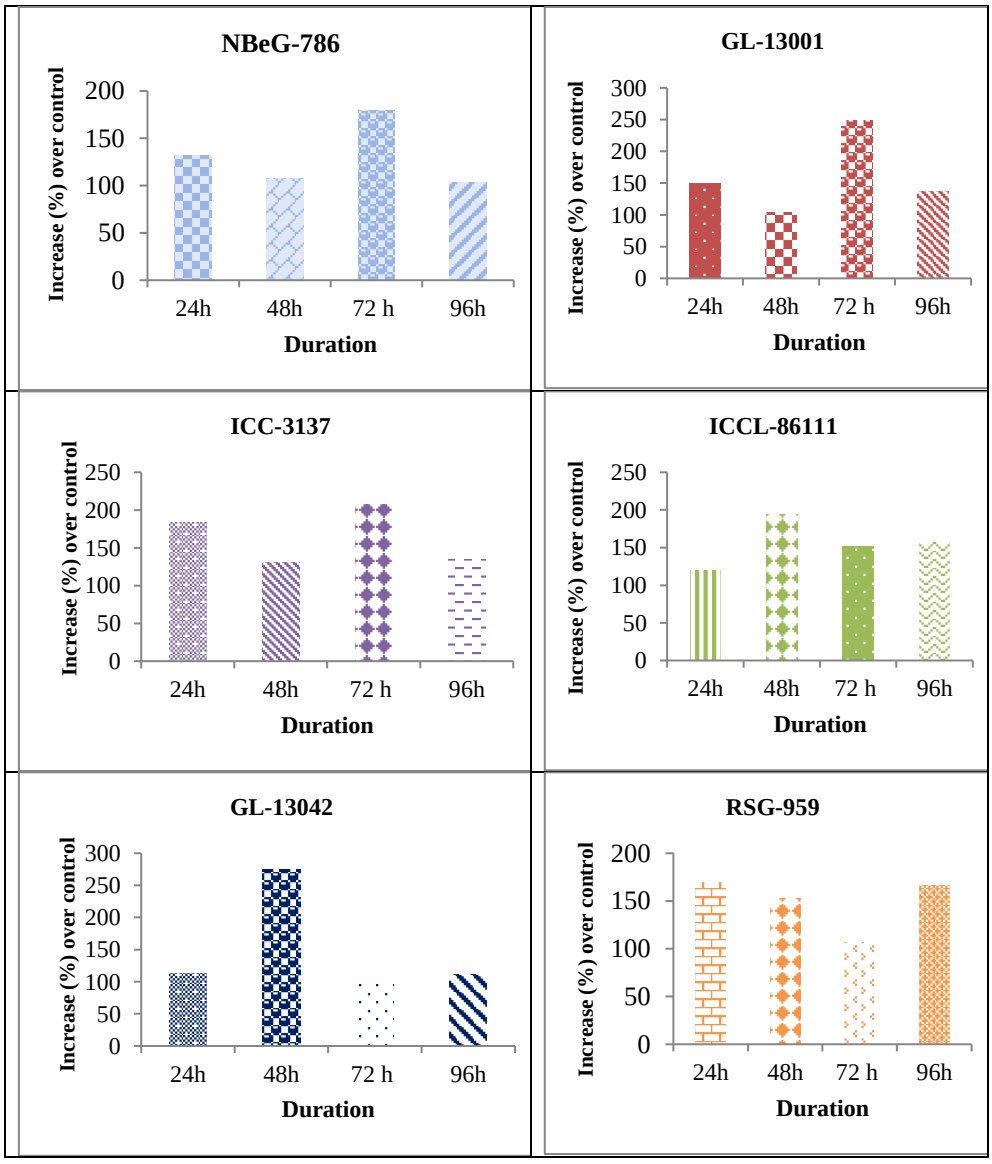

Figure 9. Effects of *Helicoverpa armigera* damage on tannins content (mg/g FW) in the leaves of different chickpea genotypes at different hours after infestation.

herbivore feeding. Earlier, many researchers reported that the tannins adversely affects the development and survival of many insect pests (27,56) by non-specific reducing nitrogen mineralization and/or digestion in the midgut of herbivore (11). Likewise it has also been reported that, after infestation of *H. armigera* in pigeon pea, the tannins accumulation was higher in the leaves of intermediate and moderately susceptible genotypes than moderately resistant genotypes, which helps in reducing the damage of plant tissue by feeding deterrence (35).

Heat Map

Hierarchical clustering was done for 10-biochemical constituents of six chickpea genotypes (Fig. 10), wherein, rows referred to the chickpea genotypes and the columns referred to biochemical activities. The dark and pale colour respectively indicates increase and decrease in biochemical activities. The six genotypes were grouped into three clusters, cluster-1 consisting of NBeG-786 and GL-13001, cluster-2 having RSG-959, ICCL-86111 and cluster-3 comprising of GL-13042 and ICC-3137. Among the test genotypes, CAT and PPO activities were highest in GL - 13001, tannins content highest in GL - 13042, TPC &

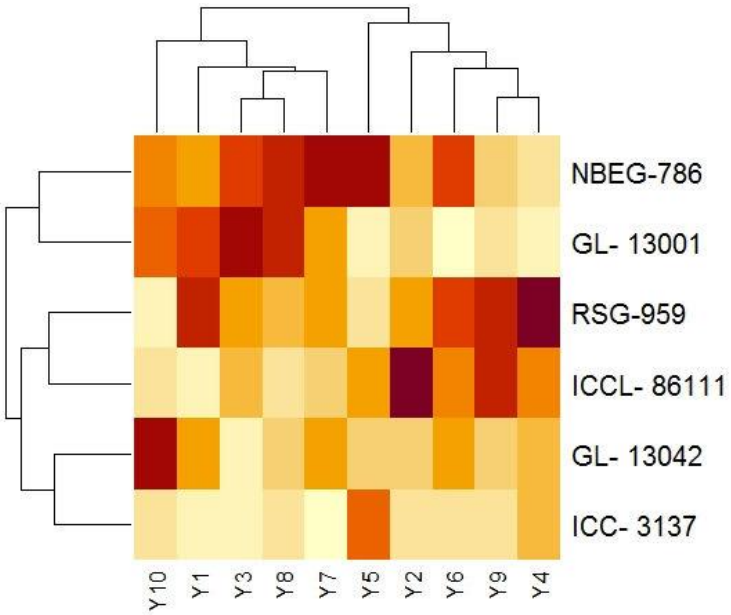

Figure 10. Different patterns of ten biochemical constituents *viz.*, (Y_1) SOD, (Y_2) POX, (Y_3) CAT, (Y_4) H_2O_2, (Y_5) MDA, (Y_6) Protein, (Y_7) TPC, (Y_8) PPO, (Y_9) Reducing sugar and (Y_{10}) Tannins content from the leaf extracts of chickpea genotypes *viz.*, NBeG - 786, GL -13001, ICC - 3137 (susceptible check), ICCL - 86111 (resistant check), GL- 13042 and RSG- 959 after 48h of infestation by *H. armigera* obtained through hierarchical clustering and heatmap analysis using R software.

MDA contents were highest in NBeG - 786, and SOD activity, POX activity, reducing sugar, protein and H_2O_2 content were highest in RSG - 959. Clustering and heat map analysis showed that biochemical constituents (superoxide dismutase, catalase, polyphenol oxidase, total phenols and tannins) were affected similarly across the genotypes and they were predominant components in chickpea under insect pest stress and can be used in developing the pod borer tolerant varieties of chickpea.

Leaf damage rating

The leaf weight consumed by *H. armigera* larvae was significantly higher in NBeG - 786 (200.8 mg) followed by susceptible check, ICC - 3137 (200.13 mg); resistant check, ICCL - 86111(164.75 mg); RSG - 959 (148.2 mg) and GL - 13001(132.13 mg), whereas, it was lowest in GL - 13042 (108.88 mg). Among the test genotypes, the leaf damage rating was highest in NBeG - 786 (6.5), followed by RSG 959 (5.0) and it was lowest in GL - 13001(4.0) and GL -13042 (3.5) as compared to susceptible check, ICC - 3137 (7.0). The PRSR rating varied from 4 to 6 and it was 6 in NBeG- 786 and 5 in resistant check, ICCL - 86111 while the rest had 4 rating (Table 2).

Table 2. Expression of resistance in different chickpea genotypes to *H. armigera* by detached leaf assay method

Genotypes	Leaf weight consumed(mg)	Damage rating scale	Pest susceptibility/ resistance (%)	PRSR
NBeG - 786	200.80^{a}	6.5	-0.34	6
GL-13001	132.13^{ab}	4	33.98	4
ICC-3137 (S)	200.13^{a}	7	-	-
ICCL - 86111(R)	164.75^{ab}	4	17.68	5
GL-13042	108.88^{b}	3.5	45.58	4
RSG-959	148.20^{ab}	5	25.95	4
Mean	159.15	-	-	-
P-value	0.0185	-	-	-
Tukey HSD at 5%	86.818	-	-	-

Data represent mean ± SE; genotypes with different superscripts are significantly different ($P \leq 0.05$) , PRSR = Percent Resistance / Susceptibility Rating

Overall among the test genotypes, the lower leaf damage and leaf consumption by *H. armigera* was observed in genotypes GL-13001 and GL-13042 followed by RSG-959. Earlier it has been reported that, genotypes with insect resistance affect the growth and development of herbivores (55). Anonymous (6) reported that the entries GL-13001, GL-13042 and RSG-959 scored ≤ 5 PRSR rating in both detached leaf and detached pod assay. The reduced leaf consumption by *H. armigera* in test genotypes might be due to increased activity of defensive enzymes and anti-nutritional constituents (12,21,31,35,36,38,56,66,71,72).

CONCLUSIONS

The *H. armigera* infestation strongly inducted the activity of antioxidant enzymes and anti nutritional factors that showed a quick response to herbivory by the chickpea genotypes. The increase in enzyme activities in all test chickpea genotypes due to

H. armigera infestation possibly caused physiological and biochemical changes through accumulation of plant defence compounds. Thus several biochemical compounds and their regulating enzymes played role in plant defence against *H. armigera* especially antioxidant defence system in chickpea. Clustering and heat map analysis revealed that biochemical constituents (superoxide dismutase, catalase, polyphenol oxidase, total phenols and tannins) are predominant biochemical component in chickpea under insect pest stress and their over expression increased the tolerance to pod borer. In summary chickpea genotypes *viz.*, GL - 13001, GL- 13042 and RSG- 959 possessed antibiosis mechanism of resistance.

The genotypes GL-13001, GL-13042, RSG-959 and NBeG-786 tolerant against *H. armigera* may be recommended as potential resistant donors in breeding chickpea varieties with stable resistance against gram pod borer.

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DECLARATION

D. Sagar and V. Krishnan designed and planned experiment. Su Htet San and M. Awana did enzyme activity experiments and bioassay. Su Htet San, D. Sagar, A. Singh and A. Bhowmik wrote article. R. Singh and S. Chander corrected the manuscript. The authors have declared that no competing interests exist.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

REFERENCES

1. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265-267.
2. Agrawal, A.A., Fishbein, M., Jetter, R., Salminen, J.P., Goldstein, J.B., Freitag, A.E. and Sparks, J.P. (2009). Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepias* spp.): Chemistry, ecophysiology, and insect behavior. *New Phytologist* **183**: 848-867.
3. Allison, S.D. and Schultz, J.C. (2004). Differential activity of peroxidase isozyme in response to wounding, gypsy moth and plant hormones in northern red oak (*Quercus rubra* L.). *Journal of Chemical Ecology* **30**(7): 1363-1379.

4. Amorim, E.L.C., Nascimento, J.U., Monteiro, J.M., Sobrinho, T.J.S., Araujo, T. and Albuquerque, U.P. (2008). A simple and accurate procedure for the determination of tannin and flavonoid levels and some applications in Ethnobotany and Ethnopharmacology. *Functional Ecosystems and Communities* **2**: 88-94.
5. Anonymous (2021) Project Co-ordinators report 2020-21. All Indian Co-ordinated Research project on chickpea, ICAR-Indian Institute of Pulses Research. pp.1-52
6. Anonymous (2019) Annual report 2018-19. All Indian Co-ordinated Research project on chickpea, ICAR-Indian Institute of Pulses Research. pp.1-379
7. Argandona, V.H., Chaman, M., Cardemil, L., Munoz, O., Zuniga, G.E. and Corcuera, L.J. (2001). Ethylene production and peroxidase activity in aphid-infested barley. *Journal of Chemical Ecology* **27**: 53-68.
8. Arimura, G., Matsui, K. and Takabayashi, J. (2009). Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. *Plant Cell Physiology* **50**: 911-923.
9. Ayala, A., Munoz, M.F. and Argüelles, S. (2014). Lipid peroxidation: Production, metabolism, and signalling mechanisms of malondialdehyde and 4-hydroxy-2-nonena*l*. *Oxidative Medicine and Cellular Longevity* **2014**: 360-438.
10. Barbehenn, R., Dukatz C., Holt, C., Reese, A., Martiskainen, O., Salminen, J. P., Yip, L., Tran, L. and Constable, C.P. (2010). Feeding on poplar leaves by caterpillars potentiates foliar peroxidase action in their guts and increases plant resistance. *Oecologia* **164**: 993-1004.
11. Bernards, M.A. and Bastrup-Spohr, L. (2008). Phenylpropanoid metabolism induced by wounding and insect herbivory. In: Induced Plant Resistance to Herbivory (Ed., A. Schaller). Springer, Berlin, pp 189-211.
12. Bhonwong, A., Stout, M.J., Attajarusit, J. and Tantasawat, P. (2009). Defensive role of tomato polyphenol oxidase against cotton bollworm (*Helicoverpa armigera*) and Beet armyworm (*Spodoptera exigua*). *Journal of Chemical Ecology* **35**: 28-38.
13. Bommesha, B., Naik, M.I., Mutthuraju, G.P., Arati, P., Imran, S. and Prashantha, C. (2012). Effect of organic manures on biochemical components of pigeon pea, *Cajanus cajan* (L.) Millsp. and their impact on the incidence of insect pests. *Current Biotica* **6**(2): 171-180.
14. Bradford, M.M. (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**: 248-254.
15. Buettner, G.R. (1993). The pecking order of free radicals and antioxidants: Lipid peroxidation, a-tocopherol and ascorbate. *Archives of Biochemistry and Biophysics* **300**: 535-543.
16. Cakmak, I. and Horst, J.H. (1991). Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Plant Physiology* **83**: 463-468.
17. Castillo, F.I., Penel, I. and Greppin, H. (1984). Peroxidase release induced by ozone in *Sedum album* leaves. *Plant Physiology* **74**: 846-851.
18. Chakravarty, S., Srivastava, C.P. and Keval, R. (2018). Biology of *Helicoverpa armigera* on chickpea-based artificial diet under laboratory conditions. *Annual Review of Plant Science* **26** (2): 265-269.
19. Chen Z., Silva, H. and Klessig, D.F. (1993). Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science* **262**: 1883-1886.
20. Chen, W., Sharma, H.C. and Muehlbauer, F.J. (2011). *Compendium of Chickpea and Lentil Diseases and Pests*. American Phytopathological Society, St Paul.pp.1-350.
21. Chen, Y., Ni, X. and Buntin, G.D. (2009). Physiological, nutritional and biochemical bases of corn resistance to foliage-feeding fall armyworm. *Journal of Chemical Ecology* **35**: 297-306.
22. Dhindsa, R.A., Plumb-Dhindsa, P. and Thorpe, T.A. (1981). Leaf senescence: Correlated with increased permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany* **126**: 93-101.
23. FAOSTAT (2016) Food and Agriculture Organization of United Nations (FAO) Statistical Databases, http: //faostat.fao.org.
24. Felton, G. and Korth, K. (2000). Trade-offs between pathogen and herbivore resistance. *Current Opinion in Plant Biology* **3**: 309-314.
25. Gechev, T., Gadjev I., Breusegem, F., Inze, D., Dukiandjiev, S., Toneva, V. and Minkov, I. (2002). Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. *Cell and Molecular Life Sciences* **59**: 708-714.
26. Gill, R.S., Gupta, A.K., Taggar, G.K. and Taggar, M.S. (2010). Role of oxidative enzymes in plant defenses against insect herbivory. *Acta Phytopathologica et Entomologica Hungarica* **45**: 277-290.
27. Grayer, R.J., Kimmins, F.M., Padgham, D.E., Harborne, J.B. and Ranga Rao D.V. (1992). Condensed tannin levels and resistance in groundnuts *Arachis hypogoea* (L.) against *Aphis craccivora* (Koch). *Phytochemistry* **31**: 3795-3799.

28. Gulsen, O., Eickhoff, T., Heng-Mos,S. T., Shearman, R., Baxendale, F., Sarath, G. and Lee D. (2010). Characterization of peroxidase changes in resistant and susceptible warm-season turf grasses challenged by *Blissus occiduus*. *Arthropod Plant Interactions* **4**: 45-55.
29. Han, Y., Wang, Y., Bi, J.L., Yang, X.Q., Huang, Y., Zhao, X., Hu, Y. and Cai, Q. N. (2009). Constitutive and induced resistance in aphid-resistant and aphid-susceptible cultivars of wheat. *Journal of Chemical Ecology* **35**: 176-182.
30. Haralu, S., Karabhantanal, S.S., Naidu, G.K. and Jagginavar, S.B. (2018). Biophysical and biochemical basis of resistance to pod borer, *Helicoverpa armigera* (Hubner) in chickpea. *Journal of Entomology and Zoology Studies* **6**(5): 873-878.
31. He, J., Chen, F., Chen, S., Lv, G., Deng, Y., Fang, Z., Guan, Z. and He, C. (2011). Chrysanthemum leaf epidermal surface morphology and antioxidant and defence enzyme activity in response to aphid infestation. *Journal of Plant Physiology* **168**(7)**:** 687-693.
32. Howe, G.A. and Jander, G. (2008). Plant immunity to herbivores. *Annual Review of Plant Biology* **59**: 41-66.
33. Huang, W., Zhikuan, J. and Qingfang, H. (2007). Effects of herbivore stress by *Aphis medicaginis* Koch on the malondialdehyde contents and activities of protective enzymes in different alfalfa varieties. *Acta Ecological Sinica* **27**(6): 2177-2183.
34. Karban, R. (2011). The ecology and evolution of induced resistance against herbivores. *Functional Ecology* **25**: 339-347.
35. Kaur, R., Gupta, A.K. and Taggar, G.K. (2014). Role of catalase, H_2O_2 and phenolics in resistance of pigeon pea towards *Helicoverpa armigera* (Hubner). *Acta Physiologiae Plantarum* **36**(6): 1513-1527.
36. Kaur, A., Grewal, S.K., Singh R., Rachana D. and Bhardwaj, (2017). Induced defence dynamics in plant parts is requisite for resistance to *Helicoverpa armigera* (Hubner) infestation in chickpea. *Phytoparasitica* **45**: 559-576.
37. Kaur, A., Grewal, S.K., Singh, R. and Kaur, J. (2017a). defence system in chickpea genotypes differing in tolerance to *Helicoverpa armigera* infestation. *Indian Journal of Plant Physiology* **22** (3): 324-331.
38. Kaur, R., Gupta, A.K. and Taggar, G.K. (2015). Induced resistance by oxidative shifts in pigeonpea (*Cajanus cajan* L.) following *Helicoverpa armigera* (Hubner) herbivory. *Pest Management Science* **71**: 770-782.
39. Khattab, H. and Khattab, M. (2005). Responses of Eucalyptus trees to the insect feeding (gall-forming psyllid*). International Journal of Agricultural Biology* **7**(6): 979-984.
40. Kono, Y. and Fridovich, I. (1982). Superoxide radical inhibits catalase. *Journal of Biological Chemistry* **257**: 5751-5754.
41. Kooner, B.S. and Cheema H.K. (2006). Evaluation of pigeon pea genotypes for resistance to pod borer complex. *Indian Journal of Crop Science* **1**(1-2): 194-196.
42. Kruger, J.E., Hatcher, D.W. and Depauw, R. (1994). A whole seed assay for polyphenol oxidase in Canadian prairie spring wheat and its usefulness as a measure of noodle darkening. *Cereal Chemistry* **71**(4): 324-326.
43. Loreto, F. and Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology* **127**(4): 1781-1787.
44. Maffei, M.E., Mithofer, A. and Boland, W. (2007). Insects feeding on plants: Rapid signals and responses preceding the induction of phytochemical release. *Phytochemistry* **68**: 2946-2959.
45. Maffei, M.E., Mithofer, A., Arimura, G.I., Uchtenhagen, H., Bossi, S., Bertea, C.M., Cucuzza, L.S., Novero, M., Volpe, V., Quadro, S. and Boland, W. (2006). Effects of feeding *Spodoptera littoralis* on Lima bean leaves. III. Membrane depolarization and involvement of hydrogen peroxide. *Plant Physiology* **140**: 1022-1035.
46. Merga, B. and Haji, J. (2019). Economic importance of chickpea: Production, value and world trade, *Cogent Food & Agriculture* **5**(1): 1615-1718.
47. Mishra, P., Singh, S., Rathinam, M., Kumar, N.R., Thangraj, A., Thimmegowda, V., Krishnan, V., Mishra, V., Jain, N., Rai, V., Pattanayak, D. and Sreevathsa, R. (2017). Comparative proteomic and nutritional composition analysis of independent transgenic pigeon pea seeds harboring Cry1AcF and Cry2aa genes and their nontransgenic counterparts. *Journal of Agriculture and Food Chemistry* **65(**7): 1395-1400.
48. Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry* **153**: 375-380.
49. Ni, X., Quisenberry, S.S., Heng-Moss, T., Markwell, J., Sarath, G., Klucas, R. and Baxendale, F.P. (2001). Oxidative responses of resistant and susceptible cereal leaves to symptomatic and nonsymptomatic cereal aphid (Hemiptera: Aphididae) feeding. *Journal of Economic Entomology* **94**: 743-751.

50. Parde, V.D., Sharma, H.C. and Kachole, M.S. (2012). Protease inhibitors in wild relatives of pigeon pea against the cotton bollworm/legume pod borer *Helicoverpa armigera*. *American Journal of Plant Sciences* **3**: 627-635.
51. Ramiro, D.A., Guerreiro-Filho, O. and Mazzafera, P. (2006). Phenol contents, oxidase activities and the resistance of coffee to the leaf miner *Leucoptera coffeella*. *Journal of Chemical Ecology* **32**: 1977-1988.
52. Rani, S.S.N. (2005). *Biochemical Basis of Tolerance in Pigeon Pea to the Pod Borer* (*Helicoverpa armigera) Infestation*. M.Sc. (Agri.). Thesis, University of Agricultural Sciences, Bengaluru, India, 2005; 165.
53. Raychaudhuri, S. and Deng, X.W. (2000). The role of superoxide dismutase in combating stress in higher plants. *Botany Review* **66**: 89-98.
54. Ruttoh, E.K., Mulwa, R.M.S., Ngode, L., Gohole, L., Towett, B. and Njogu, N., Silim, S., Rao, G.V.R. and Kimurto, P.K. (2013). Screening for host plant resistance to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in selected chickpea (*Cicer arietinum* L.) genotypes in Kenya. *Egerton Journal of Science & Technology* **13**: 39-55.
55. Sharma, H.C., Pampapathy, G., Dwivedi, S.L and Reddy, L.J. (2003). Mechanisms and diversity of resistance to insect pests in wild relatives of groundnut. *Journal of Economic Entomology* **96**(6): 1886-1897.
56. Sharma, H.C., Sujana, G. and Rao, D.M. (2009). Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeon pea. *Arthropod Plant Interactions* **3**(3): 151-161.
57. Sharma, H.C., Pampathy, G., Dhillon, M.K. and Ridsdill-Smith, J.T. (2005). Detached leaf assay to screen for host plant resistance to *Helicoverpa armigera*. *Journal of Economic Entomology* **98**(2): 568-576.
58. Sharma, P., Jha, A.B., Dubey, R.S. and Pessarakli, M. (2012). Reactive oxygen species, oxidative damage and antioxidative defence mechanism in plants under stressful conditions. *Journal of Botany* **12**: 1-26.
59. Singh, T.H., Singh, G., Sharma, K.P. and Gupta, S.P. (1972). Resistance of cotton (*Gosspium hirsutum* L.) to cotton jassid, *Amrasca devastans* Distant (Homoptera: Jassidae). *Indian Journal of Agricultural Sciences* **42**(5): 521-525.
60. Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. *Methods in Enzymology* **299**: 152-178.
61. Sinha, A.K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry* **47**: 389-394.
62. Smith, C.M. and Clement, S.L. (2012). Molecular bases of plant resistance to arthropods. *Annual Review of Entomology* **57**: 309-328.
63. Somogyi, M.J. (1952) Notes on sugar determination. *Journal of Biological Chemistry* **195**: 19-23.
64. Taggar, G.K., Gill, R.S., Gupta, A.K. and Sandhu, J.S. (2012). Fluctuations in peroxidase and catalase activities of resistant and susceptible blackgram (*Vigna mungo* (L.) Hepper) genotypes elicited by *Bemisia tabaci* (Gennadius) feeding. *Plant Signalling and Behaviour* **7**: 1321-1329.
65. Torres, M.A. (2010). ROS in biotic interactions. *Physiologia Plantarum* **138:** 414-429.
66. Usha Rani, P. and Jyothsna, Y. (2010). Biochemical and enzymatic changes in rice as a mechanism of defense. *Acta Physiologiae Plantarum* **32**: 695-701.
67. Vellosillo, T., Vicente, J., Kulasekaran, S., Hamberg, M. and Castresana, C. (2010). Emerging complexity in reactive oxygen species production and signalling during the response of plants to pathogens. *Plant Physiology* **154**: 444-48.
68. Walling, L.L. (2000). The myriad plant responses to herbivores. *Journal of Plant Growth Regulation* **19**: 195-216.
69. War, A.R., Paulraj, M.G., War, M.Y. and Ignacimuthu, S. (2011). Role of salicylic acid in induction of plant-defence system in chickpea (*Cicer arietinum* L.). *Plant-Signalling and Behaviour* **6**: 1787-1792.
70. War, A.R., Paulraj, M.G., War, M.Y. and Ignacimuthu, S. (2011a). Jasmonic acid mediated induced resistance in groundnut (*Arachis hypogaea* L.) against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Journal of Plant Growth Regulation* **30**: 512-523.
71. War, A.R., Paulraj, M.G., War, M.Y. and Ignacimuthu, S. (2012). Differential defensive response of groundnut germplasm to *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Journal of Plant Interactions* **7**(1): 45-55.
72. War, A.R., Paulraj, M.G., War, M.Y., Ignacimuthu, S. and Sharma, H.C. (2013). Defensive responses in groundnut against chewing and sap sucking insects. *Journal of Plant Growth Regulation* **32**: 259-272.
73. Wu, J. and Baldwin, I.T. (2010). New insights into plant responses to attack from insect herbivores. *Annual Review of Genetics* **44**: 1-24.
74. Yadav, N. and Sharma, S. (2016). Reactive oxygen species, oxidative stress and ROS scavenging system in plants. *Journal of Chemical and Pharmaceutical Research* **8**: 595-604.

75. Yildiz-Aktas, L., Dagnon, S., Gurel, A., Gesheva, E. and Edreva, A. (2009). Drought tolerance in cotton: Involvement of non-enzymatic ROS scavenging compounds. *Journal of Agronomy and Crop Science* **195**: 247-253.
76. Zhang, S.Z., Hau, B.Z. and Zhang, F. (2008). Induction of the activities of antioxidative enzymes and the levels of malondialdehyde in cucumber seedlings as a consequence of *Bemisia tabaci* (Hemiptera: Aleyrodidae) infestation. *Arthropod Plant Interactions* **2**: 209-213.
77. Zhao, L.Y., Chen, J.L., Cheng, D.F., Sun, J.R., Liu, Y. and Tian, Z. (2009). Biochemical and molecular characterizations of *Sitobion avenae* induced wheat defence responses. *Crop Protection* **28**: 435-442.
78. Zhu-Salzman, K., Luthe, D.S. and Felton, G.W. (2008). Arthropod-induced proteins: broad spectrum defences against multiple herbivores. *Plant Physiology* **146**: 852-885.

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