Defensive Responses in Groundnut Against Chewing and Sap-Sucking Insects

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Defensive responses in groundnut against chewing and sap sucking insects

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Abstract

Induced resistance is one of the important components of host plant resistance to insects. We studied the induced defensive responses in groundnut genotypes with different levels of resistance to the leaf defoliator, *Helicoverpa armigera* and a sap sucking insect, *Aphis craccivora* to gain an understanding of the induced resistance to insects, and its implications for pest management. The activity of the defensive enzymes [peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT)], and the amounts of total phenols, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and proteins were recorded at six days after infestation. Induction of enzyme activities and the amounts of secondary metabolites were greater in the insect-resistant genotypes; ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697 infested with *H. armigera* and *A. craccivora* than in the susceptible check, JL 24. The resistant genotypes suffered lower insect damage and resulted in lower *Helicoverpa* larval survival and weights than those larvae fed on the susceptible check, JL 24. Number of aphids was significantly lower on insect-resistant genotypes than that on the susceptible check, JL 24. The results suggested that groundnut plants respond to infestation by *H. armigera* and *A. craccivora* in almost similar way, however, the degree of the response differed across the genotypes and insects, and this defense response is attributed to various defensive enzymes and secondary metabolites.

Key words: Groundnut, *Helicoverpa armigera*, *Aphis craccivora*, herbivory, induced resistance, oxidative enzymes, secondary metabolites

Introduction

About 6 million insect species are present in the world, of which 50% are herbivorous (Chapman 2006) and are a major threat to the crop production. They cause an estimated loss of over US$14 billion worldwide annually, despite application of insecticides costing over $2 billion annually (Sharma and others 2005). Groundnut (*Arachis hypogaea* L.) is an important oilseed crop and is cultivated on 23.4 million ha with an annual production of 34.9 million metric tons globally (FAO 2007). In India, groundnut is one of the major oil seed crops with an area of 6.21 million ha, production of 6.74 million tones, and an average yield of 1081 kg ha^{-1} (DGR 2011). A large number of insect pests damage this crop, which includes thrips, aphids, *Aphis craccivora* Koch white grubs, leaf miner, leafhoppers, armyworm, *Spodoptera litura* Fab., and cotton bollworm, *Helicoverpa armigera* (Hub.) (Sharma and others 2003). Host plant resistance plays an important role in insect pest management resulting in reduced losses due to the herbivores, less insecticide use, better crops yields, and a safer environment, in addition being cost effective (Sharma and others 2009; Wu and Baldwin 2010). Plants respond to herbivory through various morphological, biochemical and molecular mechanisms to counter/offset the effects of herbivore attack. This form of defense (induced resistance) adversely affects insect feeding, growth, and survival (Howe and Jander 2008; Wu and Baldwin 2010; War and others 2011a). It is a key component of plant defense against insect herbivory (Sethi and others 2009; Chen and others 2009; Karban 2011). Induced resistance in plants is mediated through various defensive enzymes such as, peroxidases (PODs), polyphenol oxidases (PPO), phenyalanine amino lyase (PAL), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase and secondary metabolites including phenols, condensed tannins etc., and through hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) (Usha Rani and Jyothsna 2010; Gulsen and others 2010; War and others 2011a; War and others 2012).

Cotton bollworm/legume pod borer, *H. armigera* is a polyphagous pest, and is widely distributed in Asia, Africa, southern Europe, and Australasia (Sharma and others 2003, 2005). It is a major pest of cereals, grain legumes, cotton, vegetable, and fruit crops, including groundnut (Sharma and others 2005). Cowpea or groundnut aphid, *A. craccivora* is a polyphagous pest, and feeds on a number of crops worldwide (Minja and others 1999; Ahmed and others 2007). It causes severe damage to groundnut by sucking plant sap and by acting as a vector of at least seven viral diseases including groundnut rosette virus and peanut stripe (Padgham and others 1990). Plants respond differentially to insects with different modes of feeding. The chewing insects cause extensive damage to plant

tissues and induce defense system different from that of the sap sucking insects. The chewing insects (caterpillars) cause an extensive damage to the plant tissue and release a wide range of elicitors that induce specific defense responses that are different from general mechanical damage. On the other hand, aphids with piercing and sucking type of mouth parts use stylets for feeding, and cause minimum physical injury to the plant tissue. They cause serious losses in crops worldwide by draining plant nutrients, injecting plant elicitors, and transmitting pathogenic viruses (Han and others 2009; He and others 2011). Aphids probe into the leaf epidermis cells immediately upon infestation and once probe is initiated; they insert their stylets into the epidermis cell wall and membrane, followed by saliva injection and ingestion of cell wall contents (Tjallingi 2006). The stylet is then inserted further and the insect feeds on phloem (and xylem) sap. Plant defense against herbivory is mediated through both SA and JA-dependant pathways (Walling 2000; Moran and others 2002; Zhu-Salzman and others 2004).The present studies were carried out to understand the defensive responses of groundnut genotypes with differential levels of resistance to insect pests with different modes of feeding, and their implications for pest management. The results obtained could serve as the important biochemical markers for plants resistance against insect pests.

Material and methods

Chemicals

The chemicals used in this study were of analytical grade. Ethylene diamine tetra aceticacid (EDTA), bovine serum albumin (BSA), guaiacol, polyvinyl pyrolidone (PVP), jasmonic acid, tannic acid, vanillin, linoleic acid, dithiothretol (DTT), disodium hydrogen phosphate, sodium dihydrogen phosphate, nitro-blue tetrazolium salt (NBT), methionine, L-phenylalanine, Glucose, potassium iodide (KI), sodium carbonate (Na_2CO_3), and vanillin were obtained from Sigma Aldrich, USA. Catechol was obtained from Glaxo Laboratories, Mumbai, India. Coomassie brilliant blue-G250, tris-HCl, and trichloroacetic acid (TCA) were obtained from Sisco Research Lab., Mumbai, India. 2-mercaptoethanol, gallic acid and Folin-Ciocalteau reagent were obtained from Merck, Mumbai, India. Thiobarbituric acid (TBA) and linoleic acid were obtained from HiMedia Pvt. Ltd., Mumbai, India. Ammonium sulphate was obtained from Qualigens Fine Chemicals, Mumbai.

Groundnut plants

Five groundnut genotypes were grown under greenhouse conditions at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, to study their defensive responses towards *H. armigera*– a chewing type of insect and *A. craccivora*- a sap sucking type insect. These included: ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 (with moderate to high levels of resistance to insects) and JL 24 (susceptible check) (Sharma and others 2003). The plants were grown in plastic pots (30 cm diameter and 39 cm deep), filled with a mixture of soil, sand, and farmyard manure (2:1:1). After 10 d of seedling emergence, only two seedlings of similar growth were retained in each pot. The Desert coolers were used to maintain the temperature at 28 ± 5 ºC and RH 65 ± 5% in greenhouse. Twenty day old plants were infested with ten newly emerged *H. armigera* larvae or 20 nymphs of *A. craccivora*. Ten replications were maintained for each treatment/genotype in a randomized block design.

Insect infestation

Newly emerged larvae of *H. armigera* were obtained from the stock culture maintained on chickpea based artificial diet under laboratory conditions (26 ± 1 ºC; 11 ± 0.5 h photoperiod and 75 ± 5% relative humidity) from the insect rearing laboratory (Armes and others 1992). Ten larvae were gently placed on each 20-day-old plant by using a camel hair brush. The *A. craccivora* wingless adults were obtained from the culture maintained on groundnut plants in the greenhouse, and 10 aphids were released on each plant using a moistened camel hair brush. The insects were allowed to feed on plants for six days after which the leaves were collected randomly from plants for the biochemical assays.

Enzyme extraction

Fresh leaves (0.5 g) were ground in 3 ml of ice cold 0.1M Tris-HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol, 1% polyvinylpyrolidone (PVP), 1 mM Dithiothretol (DTT), and 0.5 mM EDTA. The homogenate was centrifuged at 16,000× g for 25 min and the supernatant was used as enzyme source. The supernatant was further processed for partial purification of proteins.

Precipitation of proteins and partial purification

Proteins were precipitated by ammonium sulphate. To obtain 80% of saturation, initially 40% saturation was carried out and finally 80 %. Ammonium sulphate (1.2 g) was added to 5 ml of the protein extract to obtain 40% saturation.

The solution was kept overnight at 4 ºC and then centrifuged at 12,000 rpm for 30 min. The pellet was collected, and the supernatant was used for further precipitation. For 80% saturation, ammonium sulphate was added at the rate of 0.28 g ml^{-1}. The solution was stirred overnight at 4 ºC and salt precipitated proteins were collected after centrifugation at 12,000 rpm for 30 min. The pellets were pooled together and dissolved in buffer (0.1 M Tris-HCl buffer, pH 7.5, containing 0.5mM EDTA and 1 mM DTT). The protein solution was dialyzed using a dialysis bag. For dialysis, the bag was washed with distilled water, sealed with a plastic clip on one end, and again washed with distilled water. The bag was filled with the precipitated protein sample and sealed on the other end with plastic clip. The dialysis was carried out for 18 h in the preceding buffer at 4 ºC. The buffer was changed after every 3 h.

Enzyme assays

Peroxidase activity was estimated as per the method of Shannon and others (1966) with slight modification. Enzyme activity was expressed as IU g^{-1} FW. One unit of POD activity was defined as the change in absorbance by 0.1 unit per minute under conditions of assay. For the estimation of PPO activity, method described by Mayer and Harel (1979) was followed with some modifications. Enzyme activity was expressed as IU g^{-1} FW. One unit of PPO was defined as the change in absorbance by 0.1 unit per minute under conditions of assay. The activity of SOD was assayed as described by Beauchamp and Fridovich (1971) with slight modifications. SOD activity was expressed in units (IU g^{-1} FW), where 1 IU is the change in 0.1 units of absorbance min^{-1}. LOX activity was measured by following the method of Hildebrand and Hymowitz (1981) with slight modifications. One unit of enzyme activity was defined as the increase in absorbance by 0.01 per min and was expressed as IU g^{-1} FW. Catalase activity was determined using the method of Zhang and others (2008). Phenylalanine ammonia lyase was estimated as described by Campos-Vergas and Saltveit (2002) with slight modifications and the activity was expressed as µmol Cinnamic acid min^{-1} mg^{-1} protein.

Ascorbate peroxidase assay

To determine the APX activity method of Asada and Takahashi (1987) was followed with slight modifications. Leaf tissue (0.2 g) was homogenized in a pestle and mortar with 3 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1mM EDTA, 1% PVP and 1mM ascorbic acid. After filtering through a double-layered cheese cloth the homogenate centrifuged at 15,000 rpm for 20 min at 4 ºC. The supernatant after precipitation and dialysis was used

as enzyme source. The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 0.2 ml of partially purified enzyme extract. Decrease in absorbance at 290 nm due to ascorbate oxidation was measured against the blank and the enzyme activity was expressed as IU g^{-1} FW, where 1 IU is the change in 0.1 units of absorbance min^{-1}.

Total phenols, condensed tannins, H_2O_2, MDA and protein contents

Phenolic content was estimated as per Zieslin and Ben-Zaken (1993) method with some modifications. Phenolic concentration was determined from standard curve prepared with gallic acid and was expressed as µg Gallic acid Equivalents g^{-1} FW (µg GAE g^{-1} FW). Condensed tannin content was estimated by vanillin-hydrochloride method as described by Robert (1971) with some modifications. Catechin was used as the standard and the total amount of condensed tannins was expressed as µg catechin equivalents g^{-1} FW (µg CE g^{-1} FW). Hydrogen peroxide content was estimated by the method of Noreen and Ashraf (2009). H_2O_2 concentration was determined by using an extinction coefficient of 0.28 µM cm^{-1} and expressed as µmol g^{-1} FW. The level of lipid peroxidation was determined in terms of thiobarbituric acid-reactive substances (TBARS) concentration as described by Carmak and Horst (1991) with minor modification. The concentration of TBARS was calculated using the absorption coefficient 155 $mmol^{-1}cm^{-1}$ and expressed as µmol g^{-1}FW. Total protein content was determined according to the method of Lowry and others (1951), using bovine serum albumin as standard.

Plant damage and insect biology

After six days of infestation, plants were assessed for *Helicoverpa* damage by visually rating them to a scale 1-9, where, 1 = <10% and 9 = >80% damage (Sharma and others 2003), and *A. craccivora* damage was evaluated visually on a 1-5 scoring scale (1 = highly resistant, and 5 = highly susceptible). Number of *Helicoverpa* larvae and aphids survived were recorded. The *Helicoverpa* larvae collected were starved for 4 h and their weights (mg) were recorded using a digital balance (Mettler Toledo, AB304-S).

Statistical analysis

The data was analyzed by analysis of variance (ANOVA) using SPSS (15.1). Tukey's test was used to separate the means, when the treatment effects were statistically significant ($p \leq 0.05$). The differences across the treatments and genotypes were shown by using Dunnett's't' test.

Results

POD activity

Infestation with *H. armigera* and *A. craccivora* resulted in greater POD activity in all the five groundnut genotypes (Fig. 1). Plants infested with *H. armigera* and *A. craccivora* showed significantly greater POD activity [ICGV 86699 ($F_{(2,8)} = 34.3$, $P < 0.001$), ICGV 86031 ($F_{(2,8)} = 25.4$, $P < 0.01$), ICG 2271 ($F_{(2,8)} = 28.2$, $P < 0.05$), ICG 1697 ($F_{(2,8)} = 19.3$, $P < 0.01$), and JL 24 ($F_{(2,8)} = 25.9$, $P < 0.05$)] as compared to the uninfested control plants. Across the genotypes, ICGV 86699 showed a strong induction of POD activity in all the plants infested with insects [*H. armigera* ($F_{(4,14)} = 45.4$, $P < 0.01$); *A. craccivora* ($F_{(4,14)} = 23.5$, $P < 0.05$), as well as the uninfested control plants ($F_{(4,14)} = 12.3$, $P < 0.05$)] than rest of the genotypes. JL 24 also exhibited increased POD activity following insect infestation, but the activity was lower than in the insect resistant genotypes. The POD activity was greater in the uninfested insect-resistant genotypes than in the susceptible check, JL 24.

PPO activity

Greater induction in PPO activity was observed in *H. armigera* and *A. craccivora* infested plants of all the groundnut genotypes [ICGV 86699 ($F_{(2,8)} = 45.3$, $P < 0.001$), ICGV 86031 ($F_{(2,8)} = 89.4$, $P < 0.001$), ICG 2271($F_{(2,8)} = 32.3$, $P < 0.05$), ICG 1697 ($F_{(2,8)} = 19.5$, $P < 0.01$), and JL 24 ($F_{(2,8)} = 15.9$, $P < 0.05$) than in the uninfested control plants (Fig. 2). ICGV 86699 and ICGV 86031 plants infested with *H. armigera* showed significantly greater PPO activity ($F_{(4,14)} = 78.4$, 67.2, respectively for ICGV 86699 and ICGV 86031, $P < 0.001$) than that of ICG 2271, ICG 1697 and JL 24. Insect resistant genotypes had higher PPO activity ($F_{(4,14)} = 23.8$, $P < 0.05$) in *A. craccivora* infested plants than that of JL 24.

PAL activity

A strong induction of PAL activity was observed in response to insect infestation (Fig. 3). Both *H. armigera* and *A. craccivora* infested plants had greater PAL activity [ICGV 86699 ($F_{(2,8)} = 34.5$, $P < 0.001$), ICG 2271($F_{(2,8)} = 12.6.7$,

P < 0.001), ICG 1697 ($F_{(2,8)}$ = 18.9, P < 0.05), and JL 24 ($F_{(2,8)}$ = 11.5, P < 0.05)] than the uninfested control plants. However, in ICGV 86031, *H. armigera* infestation elicited significantly greater PAL activity ($F_{(2,8)}$ = 33.3, P < 0.01) than *A. craccivora* infested and uninfested control plants. ICGV 86699 and ICGV 86031 plants infested with *H. armigera* exhibited greater PAL activity ($F_{(4,14)}$ = 23.2, P < 0.05) than the other genotypes. The PAL activity in *A. craccivora* infested plants of ICGV 86699, ICG 2271, and ICG 1697 was significantly higher ($F_{(4,14)}$ =18.6, P < 0.05) than that of the susceptible check. JL 24. The constitutive levels of PAL in insect-resistant genotypes were significantly greater than in the susceptible genotype, JL 24.

CAT activity

Insect infestation resulted in increased activity of CAT (Fig 4). Plants infested with *H. armigera* and *A. craccivora* had significantly greater CAT activities ($F_{(2,8)}$ = 12.2, 18.9, 17.7, and 9.5, respectively for ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697, P < 0.05) than the uninfested control plants. ICGV 86699 and ICGV 86031 showed significantly greater CAT activity in plants infested with *H. armigera* ($F_{(2,8)}$ = 23.6, P < 0.05) and *A. craccivora* ($F_{(2,8)}$ = 14.2, P < 0.05) infested plants than in ICG 2271, ICG 1697, and JL 24. Constitutive CAT activity was higher in insect-resistant genotypes than in the susceptible check, JL 24.

SOD activity

Both *H. armigera* and *A. craccivora* infestation increased the SOD activity in all the groundnut genotypes (Fig. 5). The induction was significantly greater in *H. armigera* infested plants in ICGV 86699 ($F_{(2,8)}$ = 68.7, P < 0.01) and ICG 2271 ($F_{(2,8)}$ = 23.5, P < 0.05) than *A. craccivora* infested, and uninfested control plants. There was no significant difference in SOD activity between *H. armigera* and *A. craccivora* infested plants in ICGV 86031, ICG 1697, and JL 24. Constitutive levels of SOD activity were lower in JL 24 than in the insect-resistant genotypes. ICGV 86699 and ICG 1697 had greater SOD activity in both *H. armigera* ($F_{(4,14)}$ = 98.1, P < 0.001) and *A. craccivora* ($F_{(4,14)}$ = 34.7, P < 0.05) infested plants than that of ICGV 86031, ICG 2271, and JL 24. .

APX activity

The APX activities were significantly greater in *H. armigera* infested plants of ICGV 86699 ($F_{(2,8)}$ = 43.8, P < 0.01), ICGV 86031 ($F_{(2,8)}$ = 27.8, P < 0.01), and ICG 1697 ($F_{(2,8)}$ = 12.3, P < 0.05) than those infested with *A. craccivora*

and the uninfested control plants (Fig. 6). The *H. armigera* and *A. craccivora* infested plants of ICG 2271 and JL 24 had greater APX activities (both, $P > 0.05$) than their uninfested control plants ($P < 0.05$). Across the genotypes, *H. armigera* infested plants of ICGV 86699, ICGV 86031 and ICG 1697 showed significantly greater APX activity ($F_{(4,14)} = 32.4$, $P < 0.05$) than that of ICG 2271, and JL 24. ICGV 86699 plants infested with *A. craccivora* had higher APX activities ($F_{(4,14)} = 19.1$, $P < 0.001$) than the *A. craccivora* infested plants of ICGV 86031, ICG 2271, ICG 1697, and JL 24. Constitutive levels of APX were significantly higher than that in ICGV 86031, ICG 2271 followed by ICGV 86699 and ICG 1697 and JL 24.

LOX activity

Insect infestation resulted in increased levels of LOX in all the genotypes (Fig. 7). The induction was significantly greater in plants infested with *H. armigera* and *A. craccivora* in resistant genotypes [ICGV 86699 ($F_{(2,8)} = 6.8$, $P < 0.01$), ICGV 86031 ($F_{(2,8)} = 8.9$, $P < 0.05$), and ICG 1697 ($F_{(2,8)} = 11.6$, $P < 0.05$) than the uninfested control plants. In ICGV 2271, LOX activity in *H. armigera* infested plants were significantly greater ($F_{(2,8)} = 18.5$, $P < 0.01$) than those infested with *A. craccivora* and the uninfested control plants. Insect- resistant ones showed greater increase in LOX activity in plants infested with *H. armigera* ($F_{(4,14)} = 9.1$, $P < 0.05$), and *A. craccivora* ($F_{(4,14)} = 5.2$, $P < 0.05$) than in JL 24.

Total phenols

Insect damage resulted in a tremendous increase in the amounts of phenolic compounds than the uninfested control plants (Fig. 8). Increase in phenolic content was significantly greater in *H. armigera* infested plants than those infested with *A. craccivora* ($F_{(2,28)} = 39.4$, 16.8, 28.1, and 13.6, respectively for ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697, all $P < 0.001$). No significant difference was observed between *H. armigera* and *A. craccivora* infested plants of the susceptible check, JL 24 ($P > 0.05$). Across the genotypes, the insect infested plants of ICGV 86699 showed higher phenolic content [*H. armigera* infested ($F_{(4,14)} = 16.2$, $P < 0.01$, and *A. craccivora* infested ($F_{(4,14)} = 14.3$, $P < 0.01$)] than ICGV 86031, ICG 2271, ICG 1697, and JL 24. Constitutive levels of phenolic compounds were similar among the resistant genotypes, but significantly higher ($F_{(4,14)} = 9.3$, $P < 0.05$) than the susceptible genotype, JL 24.

Condensed tannins

The *H. armigera* infested plants had greater amounts of tannins in the insect-resistant genotypes ($F_{(2,28)}$ = 13.7, 21.1, 7.4, and 11.6, respectively for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697, all $P < 0.001$) than those infested with *A. craccivora* and the uninfested control plants (Fig. 9). Insect-resistant genotypes, in general, had significantly higher tannin content [*H. armigera* infested ($F_{(4,14)}$ = 11.4, $P < 0.01$, *A. craccivora* infested ($F_{(4,14)}$ = 18.3, $P < 0.05$) and the uninfested controls ($F_{(4,14)}$ = 21.4, $P < 0.05$)] across the genotypes.

H_2O_2 content

Greater amounts of H_2O_2 were observed in insect-infested plants of all the genotypes (Fig. 10). H_2O_2 content was significantly greater in *H. armigera* infested plants of ICGV 86031, ICG 1697 and JL 24 than the *A. craccivora* infested and uninfested control plants ($F_{(2,28)}$ = 11.2, 14.4, 23.1, respectively for ICGV 86031, ICG 1697, and JL 24, all $P < 0.05$). ICGV 86699 and ICG 2271 had greater H_2O_2 content in *H. armigera* and *A. craccivora* infested plants than the uninfested controls ($F_{(2,28)}$ = 17.5 and 9.6, respectively for ICGV 86699 and ICG 2271, all $P < 0.01$). Similarly greater induction of H_2O_2 was recorded in *A. craccivora* infested plants of resistant genotypes ($F_{(4,14)}$ = 13.3, $P < 0.05$) than those of the susceptible check, JL 24. Constitutive levels of H_2O_2 were greater in the insect-resistant genotypes than in JL 24.

MDA content

A significant increase in MDA content was observed in insect-infested plants as compared to the uninfested controls (Fig. 11). Greater MDA content was observed in *H. armigera* infested plants ($F_{(2,28)}$ = 12.5, 17.3, and 45.5, respectively for ICGV 86031, ICG 2271, and JL 24, all $P < 0.01$) than in *A. craccivora* infested, and the uninfested control plants. JL 24 had greater amounts of MDA in *H. armigera* infested plants ($F_{(4,14)}$ = 78.3, $P < 0.05$) than that of ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697. No significant differences were recorded in MDA content of *A. craccivora* infested plants among the genotypes tested.

Protein content

Significant increase in protein content was observed in insect infested plants as compared to the control plants (Fig. 12). There were no significant differences between the plants infested with *H. armigera* and *A. craccivora*. Insect-

resistant genotypes had higher protein content in the insect infested plants [*H. armigera* infested ($F_{(4,14)} = 24.3$, $P < 0.01$, *A. craccivora* infested ($F_{(4,14)} = 19.4$, $P < 0.05$)] than the susceptible genotype, JL 24.

Plant damage and insect biology

Greater leaf damage by *H. armigera* was observed in susceptible check, JL 24 as compared to that on ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697. After 6 DAI, leaf damage rating by *H. armigera* ranged from 2.8 in ICGV 86699 to 7.5 in JL 24 (Table 1). Survival of *H. armigera* larvae was significantly lower in resistant genotypes ICGV 86699 (33.5%), ICGV 86031 (39.4%), ICG 2271 (45.6%) and ICG 1697 (48.3%) than on the susceptible check, JL 24 (77.5%). The genotypes exhibiting low susceptibility to *H. armigera* were also less susceptible to the aphid, *A. craccivora*, and least aphid damage was recorded in ICGV 1697 (DR 2.0) as compared to 4.2 in the susceptible check, JL 24. Similar trend was observed in terms of numbers of aphids. ICG 1697 had the least number of aphids (19 per plant), while the susceptible check, JL 24 had the highest (56.5) number of aphids per plant. Weights of *H. armigera* larvae were significantly lower (55.5 – 68.9 mg/5 larvae) on ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697 than those fed on the susceptible check, JL 24 (95.5 mg/5 larvae).

Discussion

The evolutionary race between plants and insects has resulted in the development of an elegant defense system in plants that recognizes the non-self molecules or signals from the damaged plant parts/cells or the insect regurgitants and in turn activates the plant defense response against the herbivores (Howe and Jander 2008; Karban 2011; Smith and Clement 2012). When damaged by herbivorous insects, plants produce increased amounts of antinutritive and toxic proteins and secondary metabolites that interfere with oviposition, feeding, digestion and absorption of essential nutrients by the insects (Howe and Jander 2008; He and others 2011; Wu and Baldwin 2010; Smith and Clement 2012). The successful defense of plants against the biotic stresses depends on their ability to quickly perceive the incoming stimuli, decode it, and build a strong morphological, physiological, and/or biochemical shield against the invaders. The oxidative state of the host plants, an important component of host plant resistance to insects results in the production of ROS and toxic secondary metabolites (Howe and Jander 2008; Zhao and others 2009; Wu and Baldwin 2010; He and others 2011). Different defensive systems are activated in response to different modes of feeding by the insects.

Antioxidative enzymes such as POD, PPO, LOX, SOD, PAL, and CAT are induced in plants in response to herbivory (Felton and others 1994; Zhao and others 2009; He and others 2011). Infestation of groundnut plants by *H. armigera* and *A. craccivora* resulted in a strong induction of defensive enzymes including POD, PPO, PAL, CAT, SOD, APX, and LOX in all the genotypes, however, the strength of induction varied across insects and genotypes. There were no significant differences in the activities of POD, PAL and CAT in groundnut genotypes infested by *H. armi*gera and *A. craccivora*, except in ICGV 86699 and ICGV 86031, where the *H. armigera* infested plants exhibited greater POD and PPO activities, respectively, than the *A. craccivora* infested plants. In the susceptible check, JL 24, the *H. armigera* infested plants resulted in greater induction of CAT activity than *A. craccivora* infested plants, and the uninfested control plants. The *H. armigera* infested plants of ICGV 86699, ICGV 86031, and ICG 2271 exhibited greater PPO activity as compared to those infested by *A. craccivora*.

In general, greater SOD activity was observed in insect infested plants than the uninfested control plants across the genotypes. However, ICGV 86699 and ICG 2271 showed significantly greater SOD activity in *H. armigera* infested plants than *A. craccivora* infested and the uninfested control plants. Overall, the insect resistant genotypes exhibited greater CAT activity in *H. armigera* and *A. craccivora* infested plants than the uninfested control plants. Greater APX activity was observed in *H. armigera* infested plants than *A. craccivora* infested plants, and the uninfested control plants, except in ICG 2271 and JL 24. The LOX activity increased significantly in both *H. armigera and A. craccivora* infested plants in all the genotypes, and there were no significant differences in the levels of LOX activity between the two treatments in all the genotypes, except ICG 2271. Overall, the insect-resistant genotypes exhibited greater induction of LOX activity than the susceptible check, JL 24.

The enzymes POD, PPO, PAL, CAT, SOD, APX, and LOX play a great role in plant defense against different stresses, including insect herbivory (Bhonwong and others 2009; Zhao and others 2009; Chen and others 2009; Gulsen and others 2010; Usha Rani and Jyothsna 2010, He and others 2011; War and others 2011a,b,c). The role of POD in production of semiquinone free radicals and subsequent formation of quinones has been attributed to its direct post ingestive toxicity against insects (Zhu-Salzman and others 2008; Barbehenn and others 2010). In addition, it also mediates the oxidation of hydroxylcinnamyl alcohols into free radical intermediates, oxidation of phenols, cross-linking of polysaccharides and monomers, lignifications, and suberization (Zhang and others 2008; Chen and others 2009), which in turn lead to the production of anti-nutritive compounds (Gulsen and others 2010;

He and others 2011). Induction of POD activity was greater in the insect-resistant genotypes than the susceptible check, JL 24. The PPO plays an important role in plant defense against insect herbivory as an antinutritional enzyme, and reduces the food quality (Mahanil and others 2008; Bhonwong and others 2009). It oxidizes phenols to highly reactive and toxic quinines that interact with the nucleophilic side chain of amino acids, leading to cross-linking of proteins, and thereby, reducing their availability to insect pests (Zhang and others 2008; Bhonwong and others 2009). In addition to their role in digestibility and palatability of plant tissues, melanin formation by PPOs increases the cell wall resistance to insects and pathogens (Zhao and others 2009).

The *de novo* synthesis and increased activity of PAL is an initial plant defensive response to insect damage (Campos-Vargas and Saltveit 2002), and leads to accumulation of phenolic compounds in plants that are sequestered in cell vacuole (Zhao and others 2009), and forms toxic compounds upon oxidation (Bhonwong and others 2009). A negative correlation has been observed between PAL activity and growth and development of insect pests (Sethi and others 2009). The SOD acts as the first line of defense by catalyzing the dismutation of superoxide into oxygen and H_2O_2 (Raychaudhuri and Deng 2000). It scavenges the toxic free radicals produced in plants on account of stresses, including herbivory (Khattab and Khattab 2005; Usha Rani and Jyothsna 2010). CAT is an important component of the oxygen-scavenging systems, scavenges the toxic and unstable ROS and converts them into less toxic and more stable components such as O_2 and water (Khattab and Khattab 2005). Increased CAT activity in plants increases cell wall resistance, and also acts as a signal for the induction of defensive genes (Chen and others 1993). Higher levels of APX activity decrease the availability of ascorbate in plant tissues, which in turn reduces the insect growth and development (Barbehenn and others 2005). In addition, non-availability of ascorbate in insect midgut increases the oxidative stress that leads to the generation of highly unstable ROS, including semiquinone, peroxides, and hydroxyl radicals (Barbehenn and others 2005). APX also reduces excessive H_2O_2 to water, and oxidizes phenolic compounds to quinones, which inhibit insect feeding (Felton and others 1994; Barbehenn and others 2005). LOX catalyze hydroperoxidation of polyunsaturated fatty acids resulting in the formation of fatty acid hydroperoxides, which are degraded to unstable and highly reactive aldehydes, γ-ketols, epoxides (Bruinsma and others 2009). These interact with proteins, and forms protein-protein cross linking and also cause amino acid damage (Maffei and others 2007). Lipid peroxidation end products act as insect repellents (Bruinsma and others 2009), which are directly toxic to insect pests (Maffei and others 2007; Bhonwong and others 2009). *Nicotiana attenuata* (Torr. ex Wat.) plants

deficient in LOX have been found to be susceptible to *Manduca sexta* (L.) (Rayapuram and Baldwin 2007). Greater induction of plant defensive enzymes in groundnut plants in response to *H. armigera* infestation could be attributed to the more tissue damage by the chewing insect.

Amounts of total phenols and condensed tannins were greater in *H. armigera* infested plants than those infested by *A. craccivora* in insect resistant genotypes. Increase in amounts of phenols and condensed tannins were higher in insect-resistant genotypes than in the susceptible check, JL 24. This could be ascribed to the extensive tissue damage by the chewing insects. Phenolic compounds induced in plants are either directly toxic to insects (Walling 2000; Bhonwong and others 2009) or mediate the signaling of various transduction pathways, which in turn, produce toxic secondary metabolites and activates the defensive enzymes (Walling 2000; Maffei and others 2007; Bhonwong and others 2009). Quinones formed by oxidation of phenols bind covalently to leaf proteins, and inhibit protein digestion in herbivores (Bhonwong and others 2009). Tannins have been reported to reduce the growth and survivorship in many insect pests (Grayer and others 1992; Bernards and Bastrup-Spohr 2008; Sharma and others 2009). They precipitate proteins nonspecifically (including the digestive enzymes of herbivores) by hydrogen bonding or covalent bonding of protein $-NH_2$ groups, thereby, reducing the nitrogen mineralization and/or digestion in herbivore midgut (Bernards and Bastrup-Spohr 2008). Sharma and others (2009) reported higher quantity of polyphenols and condensed tannins in insect resistant genotypes of pigeonpea that are resistant to *H. armigera*.

Greater amounts of H_2O_2 were recorded in insect infested plants, and the insect-resistant genotypes responded strongly than the susceptible check, JL 24. The H_2O_2 acts directly as a toxicant to the insects or as a secondary messenger, where it serves as an important component of intra- and intercellular signal transduction pathways, which in turn lead to the formation of various defensive proteins (Howe and Jander 2008; Maffei and others 2007; Torres 2010). The H_2O_2 induces various defense signaling pathways in plants in response to insect attack (Meffai and others 2007; Torres 2010). Induction of H_2O_2 in plants in response to herbivory could be highly advantageous, since the timing of induction of defensive responses is an important factor for defending the plants against subsequent insect and pathogen invasion (Torres and others 2010; Barbehenn and others 2010; He and others 2011). Increase in MDA content was observed in plants infested with insect pests; however, *H. armigera* infestation showed greater induction than the *A. craccivora* infested plants. An important lipid oxidation, MDA is involved in singnaling the plant defense against a variety of stresses (Huang and others 2007). Lipid peroxidation stimulates the

green leaf volatile emission in plants in response to herbivory that attract the natural enemies of the herbivores (Arimura and others 2009).

The present findings indicated that feeding by *H. armigera* and *A. craccivora* resulted in an increase in protein content. Increase in protein concentration due to *H. armigera* and *A. craccivora* feeding might be partly due to the increase in antioxidative enzyme activities after herbivory. Protein based compounds mediate a wide ranging defense responses in plants. On insect infestation, the production of defensive protein based compounds following insect infestation, including enzymes, are one the important strategies of plant defense (Ni and others 2001; Chen and others 2009). However, there were considerable differences in protein content in *H. armigera* and *A. craccivora* infested plants, which might be due to the extent of the stress caused by the insects due to different modes of feeding.

Genotypes with insect resistance affect both growth and development of herbivores (Sharma and others 2003). Insect-resistant genotypes suffered lower leaf damage by *H. armigera* larvae. The *H. armigera* larvae fed on resistant genotypes exhibited lower larval survival and weights than those larvae fed on the susceptible check, JL 24. Rate of increase of *A. craccivora* population was significantly lower on the insect-resistant genotypes than that on the susceptible check, JL 24. Amongst these, ICG 1697 suffered the lowest aphid damage; because of a dense covering of trichomes on the leaves (War and others unpublished data). Furthermore, reduced plant damage and high larval mortality on insect-resistant genotypes could be due to increased enzyme activities (Mahanil and others 2008; Bhonwong and others 2009; Usha Rani and Jyothsna 2010; Gulsen and others 2010; He and others 2011), and greater amounts of secondary metabolites (Sharma and others 2009; Bhonwong and others 2009; Chen and others 2009; Usha Rani and Jyothsna 2010; War and others 2011a,b).

Conclusions

Plant damage by *H. armigera* feeding induced strong response than the sucking pest *A. craccivora*. Although many reports have suggested that plants respond differently to chewing and sap sucking insects, our results revealed that groundnut plants respond in a similar manner to both the chewing and sap sucking insects, although the degrees of the induced response varied among the genotypes and the insects. Lower induction of plant defensive compounds by *A. craccivora* infestation as compared to *H. armigera* might be due to the greater tissue damage in leaves by *H.*

armigera larvae. However, defensive responses induced by *A. craccivora* could be due to the damage caused due to stylet probing, and the elicitors in the oral secretions released on the leaf. There is a need for in-depth studies on plant response to arthropod herbivores to gain a better understanding of the signal transduction, co-evolution between plants and insects, and the mechanisms of plant resistance to insects to use this information for crop protection and sustainable crop production.

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Figure captions

Figure 1

POD activity (IU g^{-1}FW) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control; FW = fresh weight of leaf tissue.

Figure 2

PPO activity (IU g^{-1}FW) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control; FW = fresh weight of leaf tissue.

Figure 3

PAL activity (µmol cinnamic acid mg^{-1} protein) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control

Figure 4

Catalase activity (µmol min^{-1} mg^{-1} protein) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control.

Figure 5

SOD activity (IU g^{-1}FW) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control; FW = fresh weight of leaf tissue.

Figure 6

APX activity (IU mg^{-1}protein) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control.

Figure 7

LOX activity (IU g^{-1}FW) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control; FW = fresh weight of leaf tissue.

Figure 8

Total phenols (µmol GAE g^{-1}FW) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control; GAE = gallic acid equivalents; FW = fresh weight of leaf tissue.

Figure 9

Condensed tannins (µmol CE g^{-1}FW) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control; CE = Catechin equivalents; FW = fresh weight of leaf tissue.

Figure 10

H_2O_2 content (µmol g^{-1}FW) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control.

Figure 11

MDA content (µmol g^{-1}FW) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control

Figure 12

Protein content (mg g^{-1}FW) of groundnut genotypes infested with *H. armigera* and *A. craccivora*

Bars (Mean ± SE) of same colors with similar alphabets are not statistically different at ($P < 0.05$).

H = *H. armigera* infested*;* A = *A. craccivora* infested; C= non-infested control

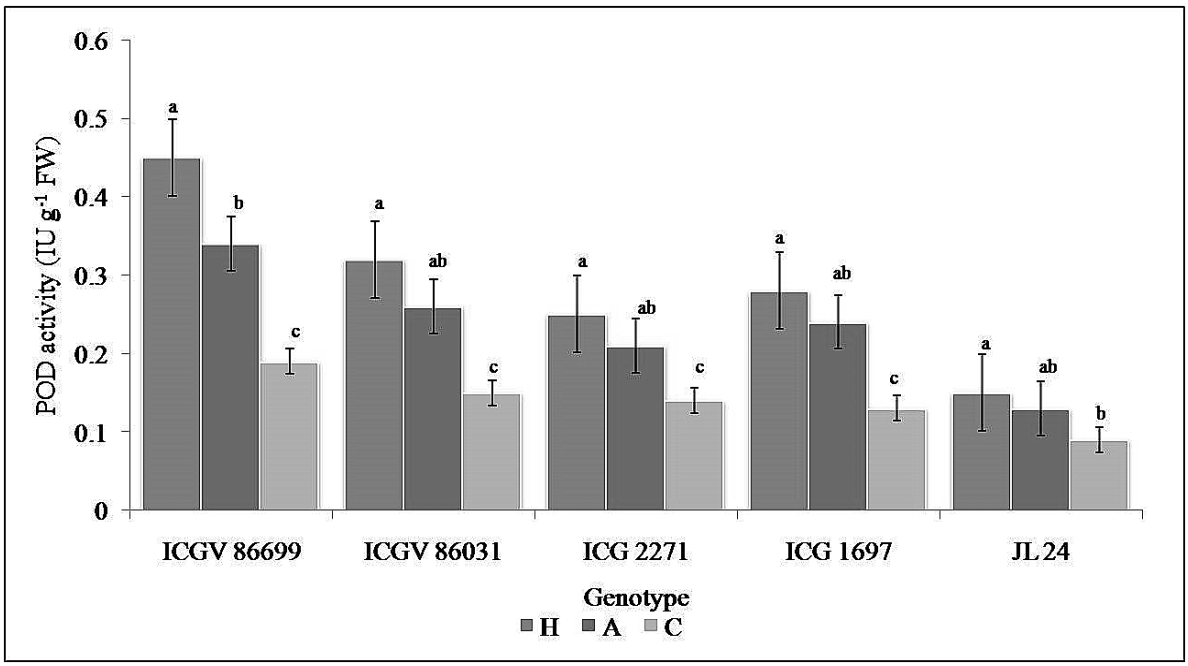
0.6
0.5
0.4
0.3
0.2
0.1
0
POD activity (IU g-1 FW)
a
b
c
a
ab
c
a
ab
c
a
ab
c
a
ab
b
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotype
H
A
C

Fig. 1

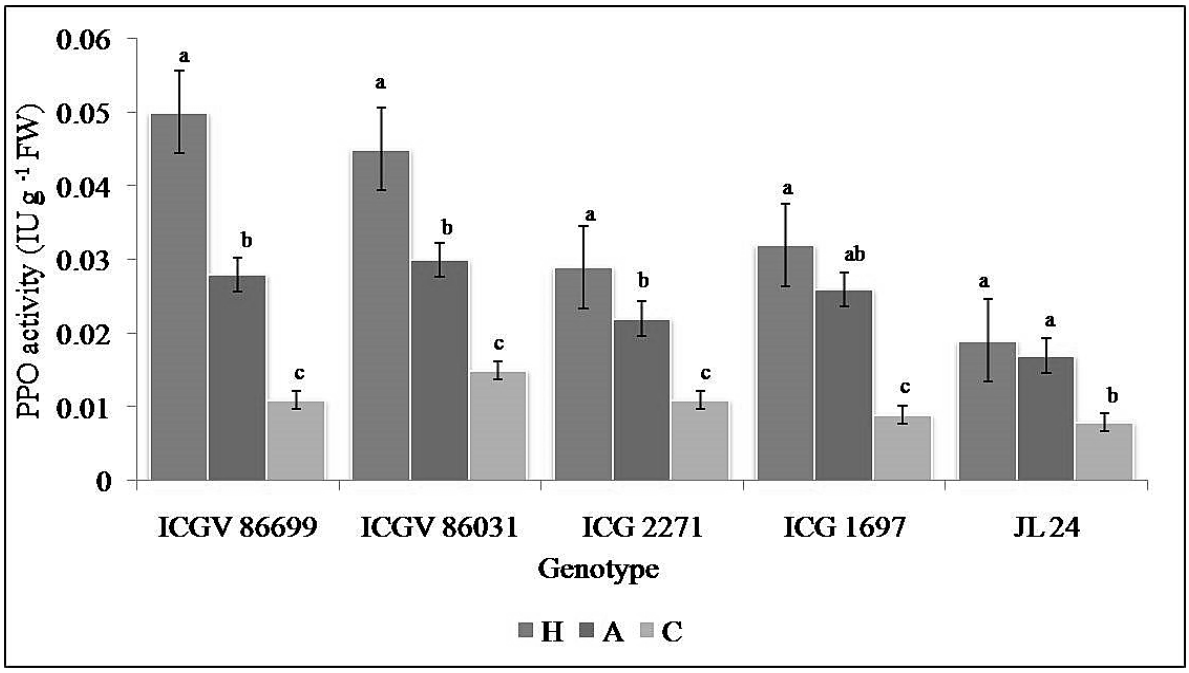
PPO activity (IU g-1 FW)
0.06
0.05
0.04
0.03
0.02
0.01
0
a
b
c
a
b
c
a
b
c
a
ab
c
a
a
b
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotype
H
A
C

Fig. 2

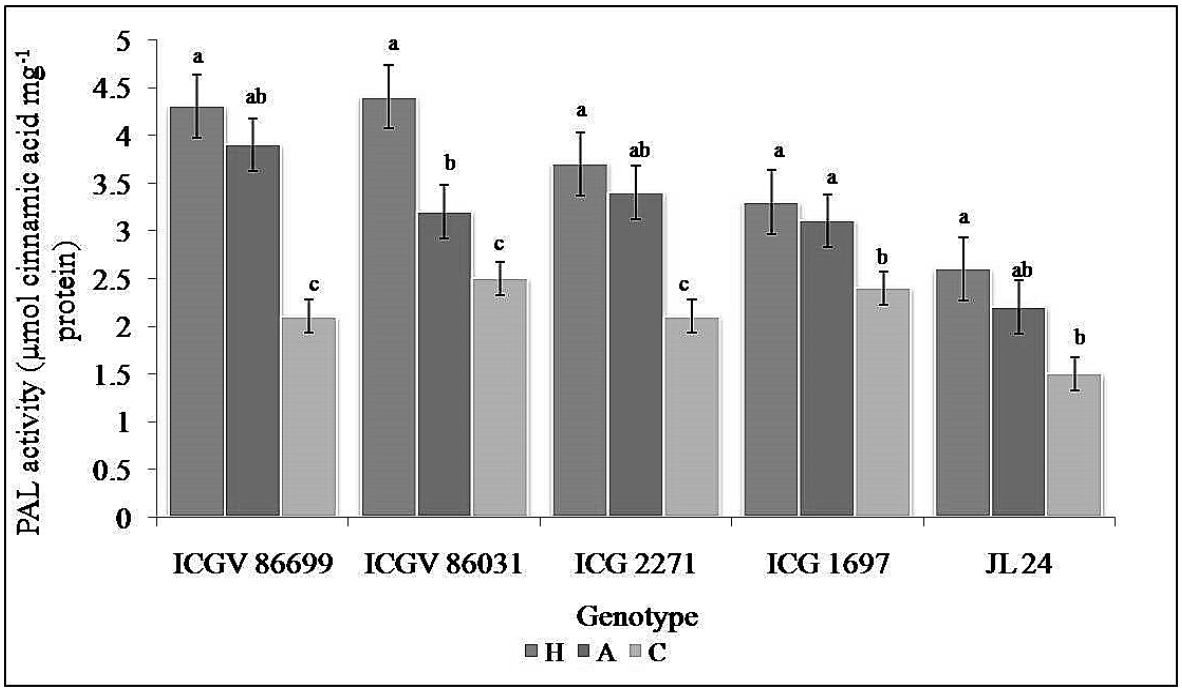
PAL activity (µmol cinnamic acid mg-1 protein)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
a
ab
c
a
b
c
a
ab
c
a
a
b
a
ab
b
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotype
H
A
C

Fig. 3

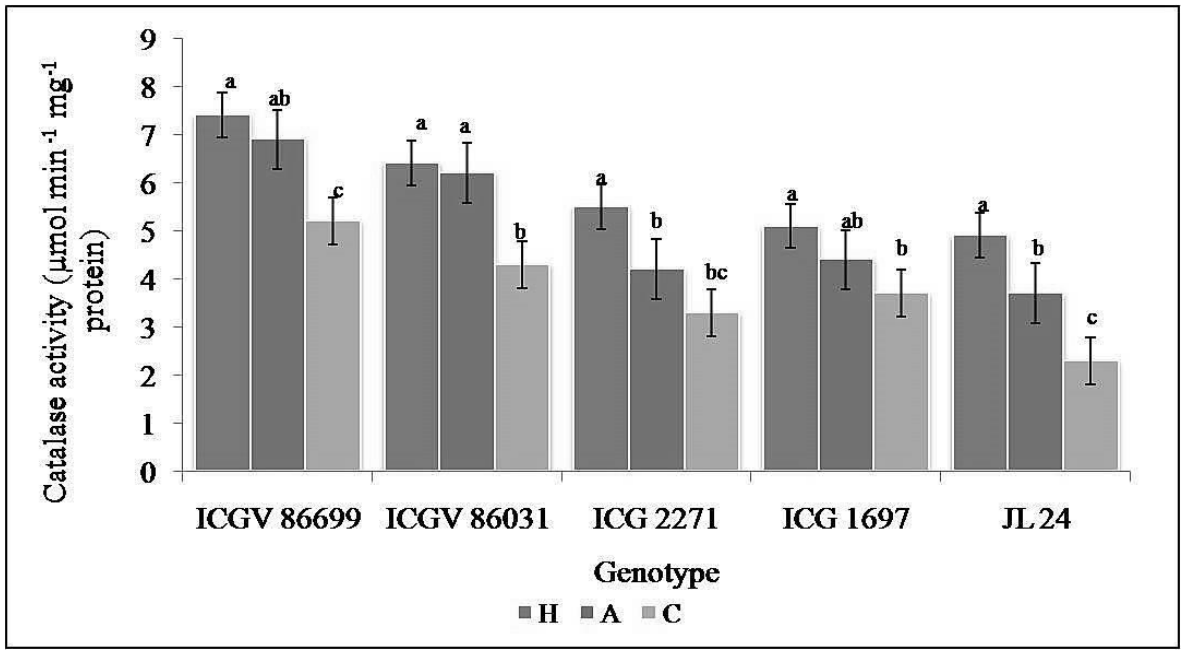
Catalase activity (µmol min-1 mg-1 protein)
9
8
7
6
5
4
3
2
1
0
a
ab
c
a
a
b
a
b
bc
a
ab
b
a
b
c
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotype
H
A
C

Fig. 4

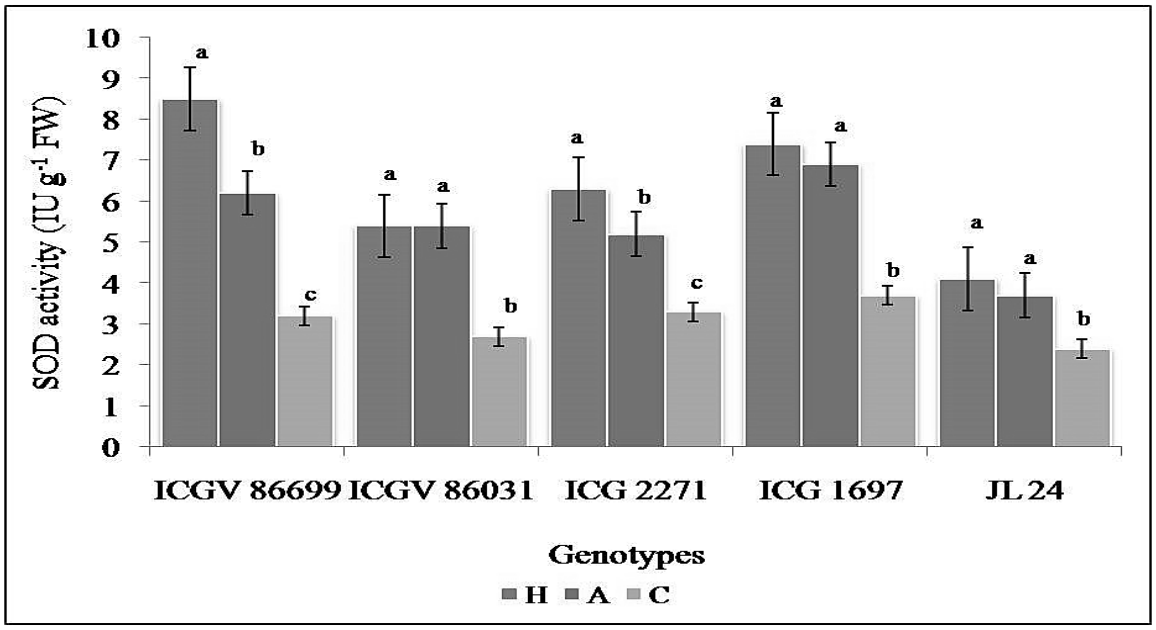
SOD activity (IU g-1 FW)
10
9
8
7
6
5
4
3
2
1
0
a
b
c
a
a
b
a
b
c
a
a
b
a
a
b
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotypes
H
A
C

Fig. 5

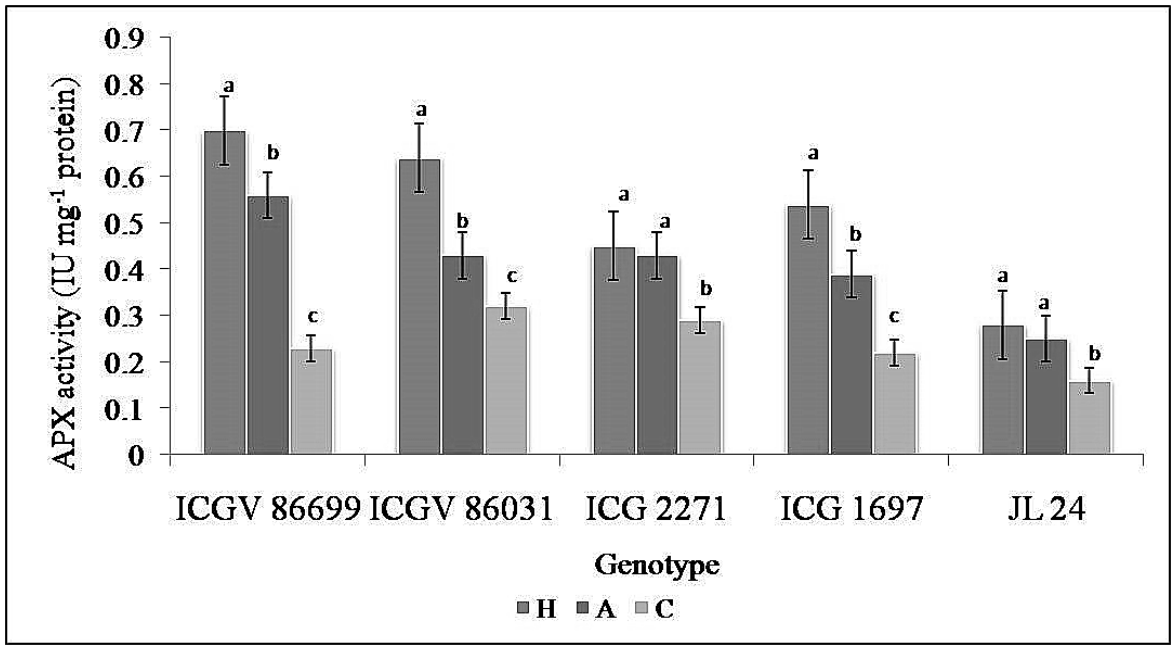
APX activity (IU mg-1 protein)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
b
c
a
b
c
a
a
b
a
b
c
a
a
b
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotype
H
A
C

Fig. 6

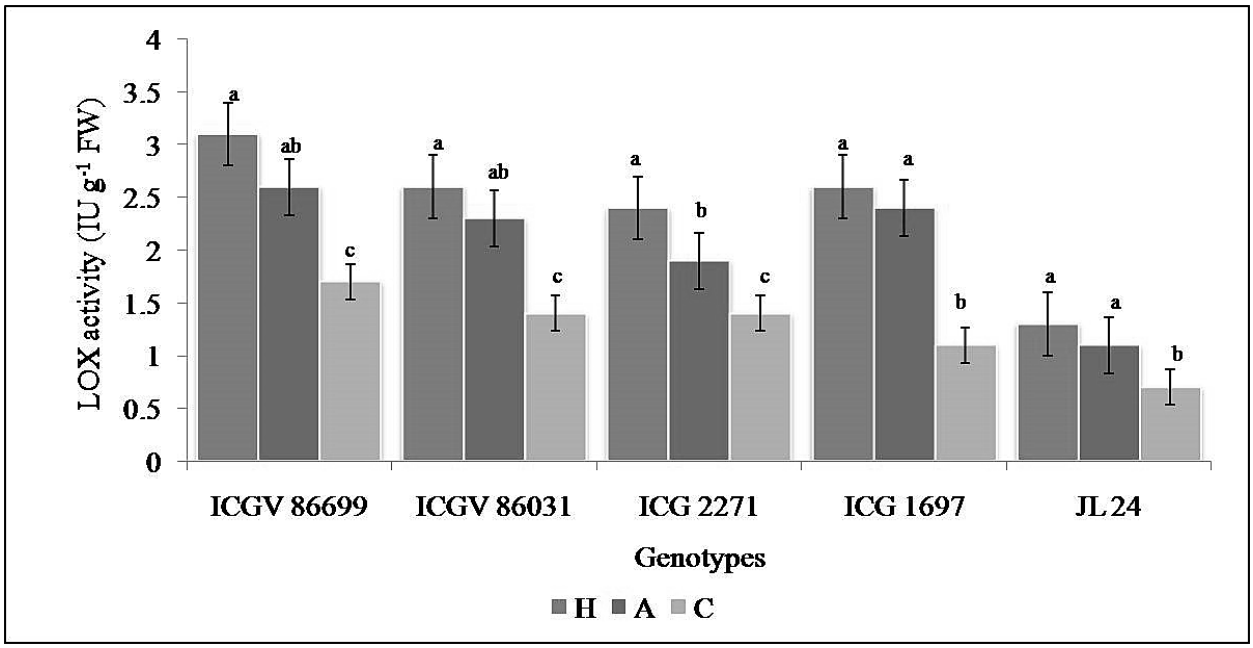
LOX activity (IU g-1 FW)
4
3.5
3
2.5
2
1.5
1
0.5
0
a
ab
c
a
ab
c
a
b
c
a
a
b
a
a
b
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotypes
H
A
C

Fig. 7

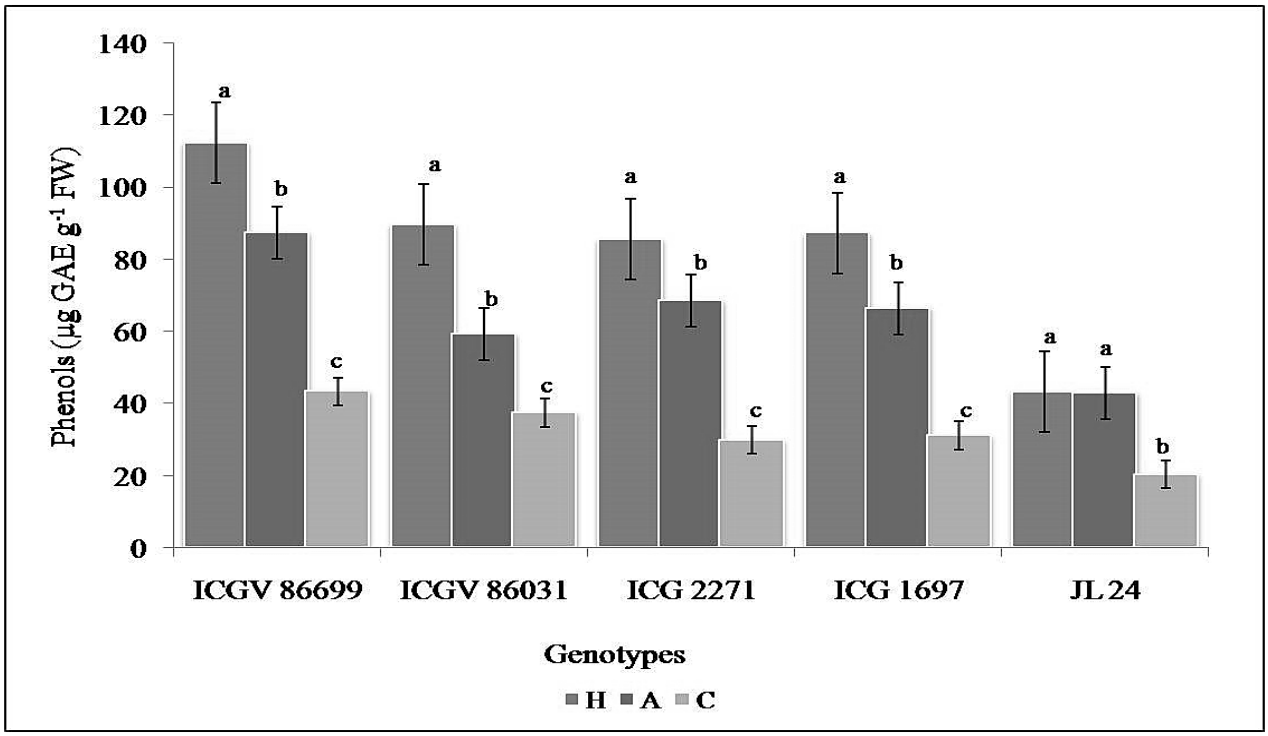
140
120
100
80
60
40
20
0
Phenols (µg GAE g-1 FW)
a
b
c
a
b
c
a
b
c
a
b
c
a
a
b
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotypes
H A C

Fig. 8

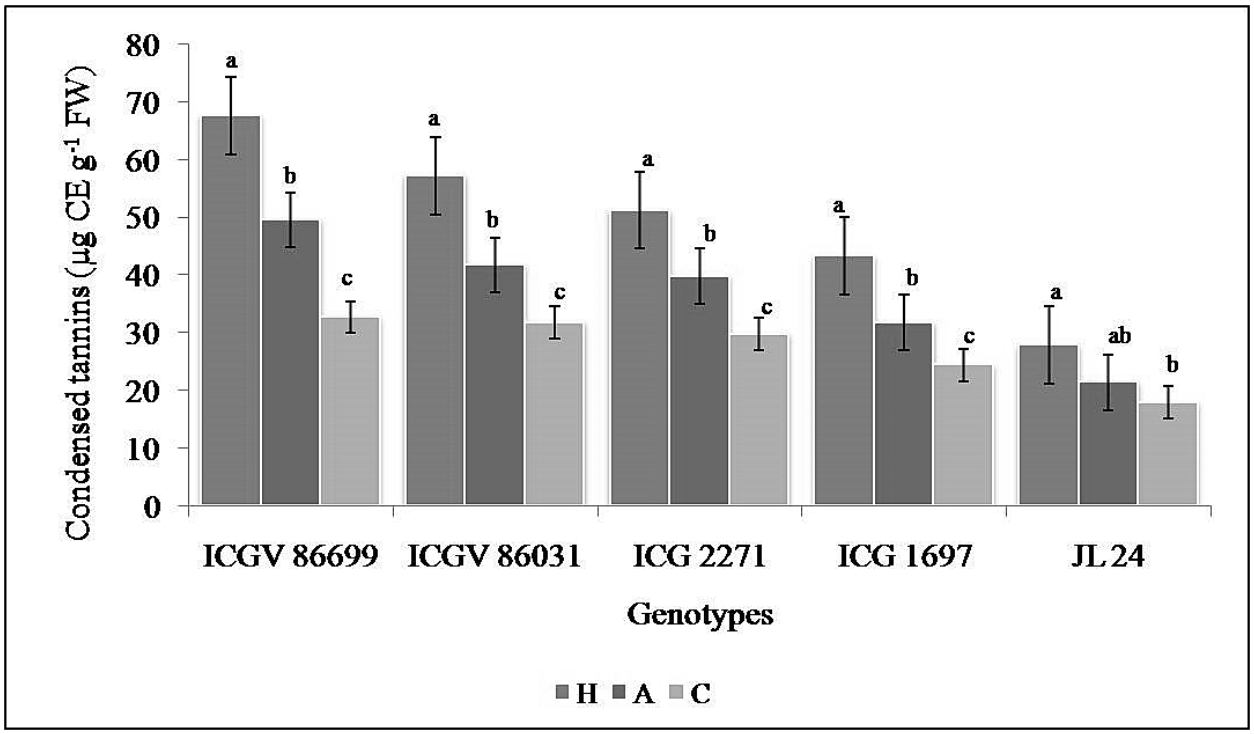
Condensed tannins (µg CE g-1 FW)
80
70
60
50
40
30
20
10
0
a
b
c
a
b
c
a
b
c
a
b
c
a
ab
b
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotypes
H
A
C

Fig. 9

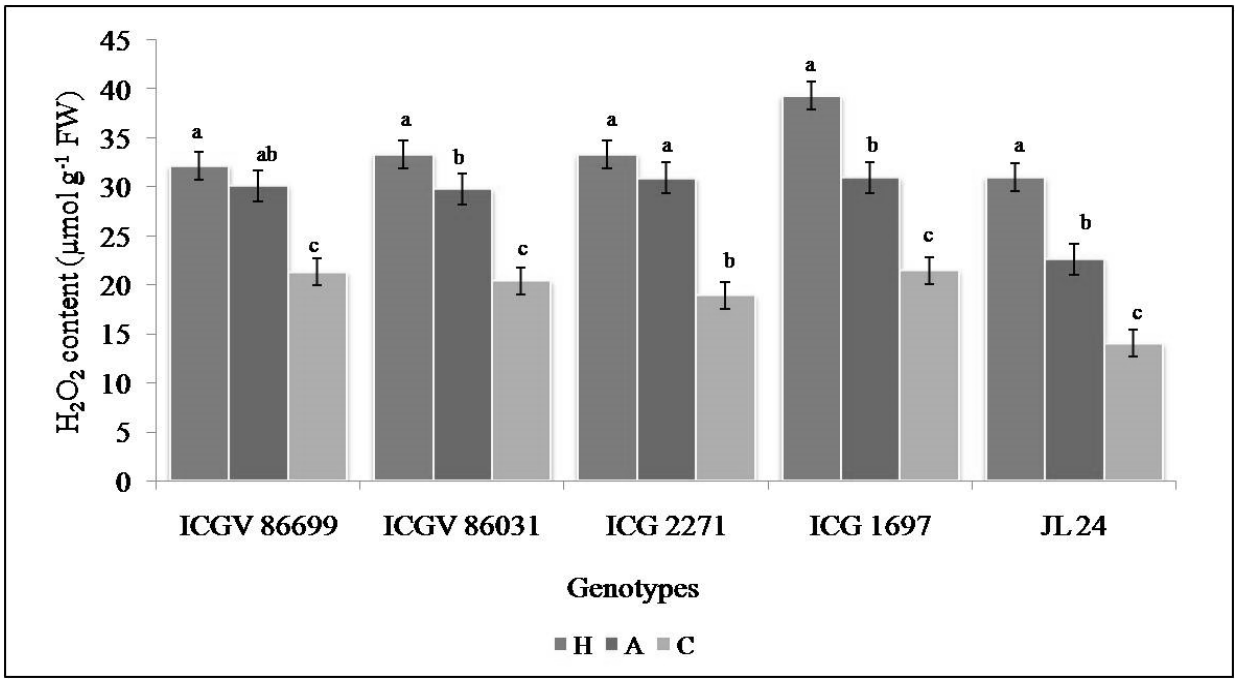
45
40
35
30
25
20
15
10
5
0
H_2O_2 content (µmol g^{-1} FW)
a
ab
c
a
b
c
a
a
b
a
b
c
a
b
c
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotypes
H
A
C

Fig. 10

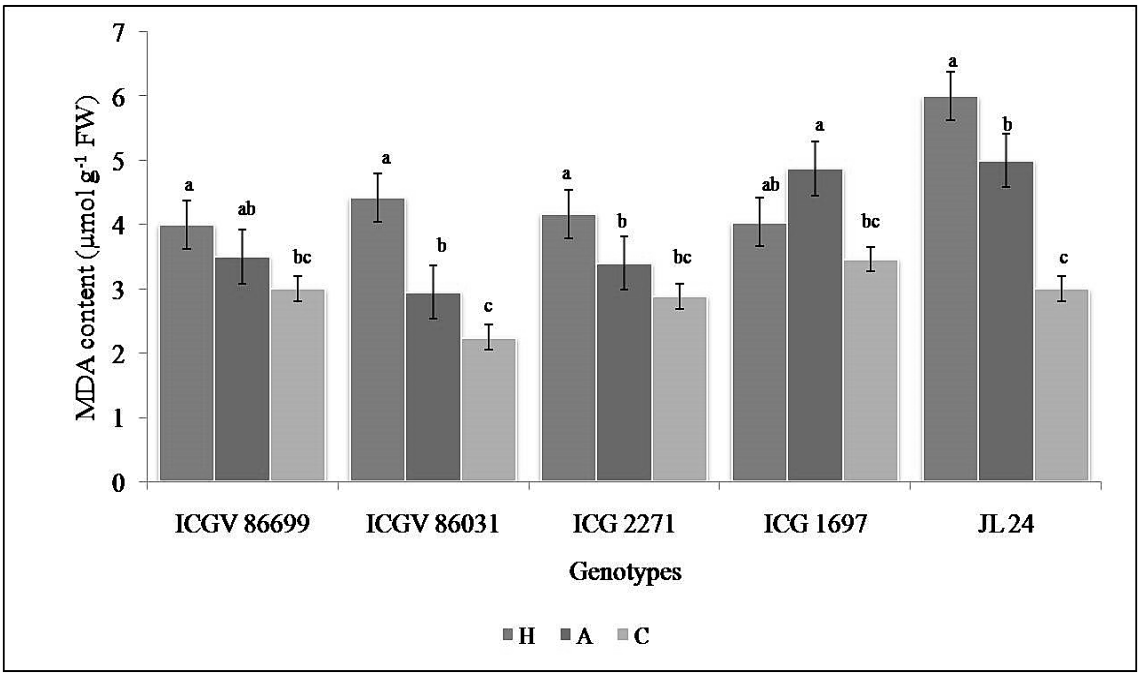
MDA content (µmol g-1 FW)
0
1
2
3
4
5
6
7
a
ab
bc
a
b
c
a
b
bc
ab
a
bc
a
b
c
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotypes
H
A
C

Fig. 11

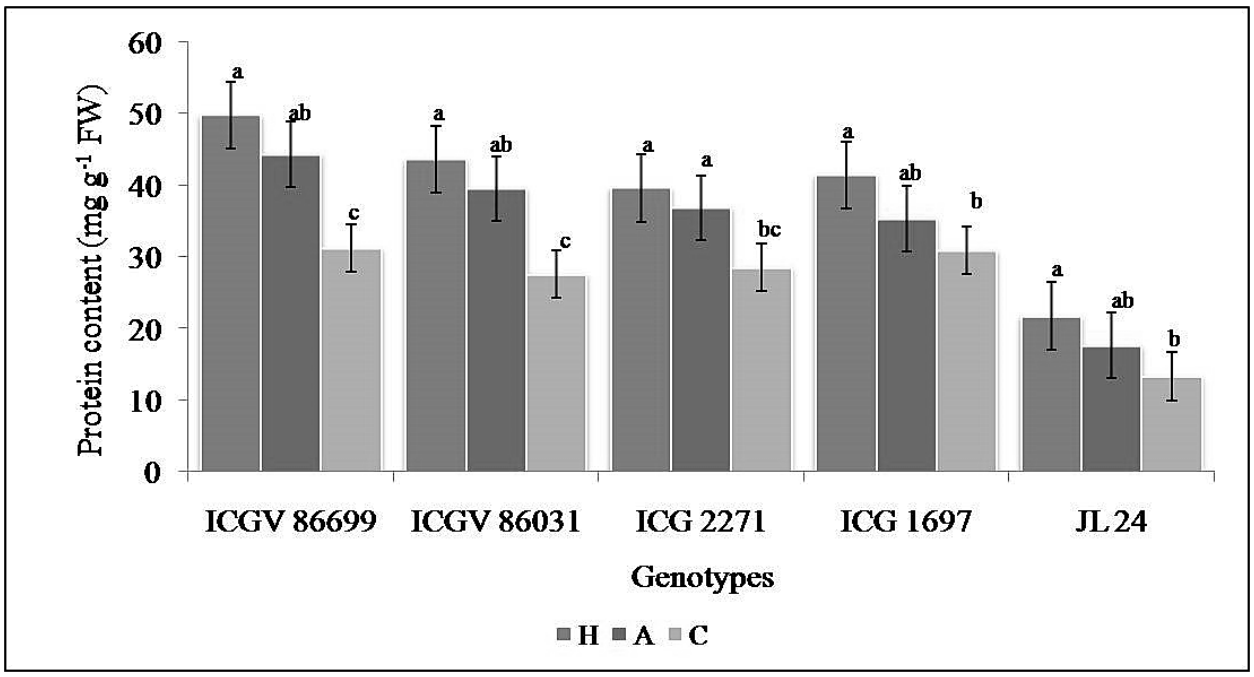
Protein content (mg g-1 FW)
60
50
40
30
20
10
0
a
ab
c
a
ab
c
a
a
bc
a
ab
b
a
ab
b
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotypes
H A C

Fig. 12

Table 1: Plant damage, larval survival and weight of *H. armigera* and *A. craccivora* after feeding on groundnut genotypes

Genotypes	*Helicoverpa* DR[x]	Larval survival (%)	Larval weight (mg)*	Aphid DR[y]	No. of aphids
ICGV 86699	2.8[bc]	33.5 ± 2.4[c]	55.5 ± 3.1[bc]	2.5[b]	31.5 ± 3.5[b]
ICGV 86031	3.5[b]	39.4 ± 2.8[bc]	68.9 ± 6.9[b]	2.6[b]	27.8 ± 2.8[b]
ICG 2271	4.2[b]	45.6 ± 4.6[b]	65.6 ± 5.2[b]	2.3[b]	37.8 ± 4.6[b]
ICG 1697	3.8[b]	48.3 ± 3.4[b]	67.4 ± 4.7[b]	2.0[b]	19.0 ± 3.3[c]
JL 24	7.5[a]	77.5 ± 7.6[a]	95.5 ± 6.8[a]	4. 2[a]	56.5 ± 6.2[a]

Values (Mean ± SEM) carrying same alphabet(s) within a column are not significantly different.

[x] DR = *Helicoverpa* damage rating to a scale 1-9 ($1 \leq 10$ % and $9 \geq 90$ %) 6 days after infestation

[*] Weight per five larva at the time of recovery.

[y] = Aphid damage rating to a scale 1-5 (1 = highly resistant, and 5 = highly susceptible)