CrossMark

Expression of different mechanisms of resistance to insects in groundnut under field conditions

Abdul Rashid War · Rajendra Sudhakar Munghate · Hari Chand Sharma

Abstract Host plant resistance is an important component of pest management, and information on contribution of different mechanisms of resistance is important for developing cultivars with resistance to the target pests. Therfore, we studied the contribution of different components of resistance in five groundnut genotypes to three insect species occurring in India under field conditions. Plant damage by the larvae of *Helicoverpa armigera, Spodoptera litura*, and leafhoppers (*Empoasca kerri*) was evaluated visually on a 1 – 9 damage rating (DR) scale (1 being <10 % leaf damage, and 9 being >80 % leaf damage). Further, the activities of various plant defensive enzymes [peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), superoxide dismutase (SOD), ascorbate peroxidase (APX), lipoxygenase (LOX) and catalase (CAT)], and the amounts of total phenols, condensed tannins, hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and proteins were also recorded. The genotypes ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 suffered lower leaf damage by *H. armigera* and *S. litura* (DR 2.6 – 3.2) and *E. kerri* (DR 2.0 - 3.2) as compared to JL 24 (DR 7.2 and 6.0, respectively). ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 exhibited greater enzymatic activity, and had more amounts of phenols, condensed tannins, hydrogen peroxide and proteins than the susceptible check, JL 24. There was a positive association between leaf damage and the activity of the defensive enzymes, and the amounts of phenols, condensed tannins and H_2O_2. These results suggested that the plant defensive enzymes such as POD, PPO, LOX, PAL, SOD, APX and CAT were involved in genotypic resistance to insects, and the resistant genotypes accumulated phenols, condensed tannins, and H_2O_2 to impart resistance to insects. This information will be useful for developing groundnut genotypes with resistance to insects for sustainable crop production.

Key words Groundnut · host plant resistance · resistance mechanisms · *Helicoverpa armigera* · *Spodoptera litura* · *Empoasca kerri* · pest management

A. R. War · R. S. Munghate · H. C. Sharma (✉)
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India 502 324
e-mail: H.Sharma@cgiar.org

Introduction

Plants face innumerable challenges from biotic and abiotic stresses such as insect attack, pathogen infection, temperature fluctuations and drought. Insect pests take away a heavy toll of crop yields, and cause an estimated loss of over US$14 billion worldwide annually (Oerke 2006). Furthermore, enormous and indiscriminate use of insecticides has led to adverse effects on non-target organisms (such as parasitoids and predators), pesticide residues in food, pest-resurgence, development of insect resistance, toxic effects on human beings, and environmental pollution (Sharma 2007). In this context, considerable efforts have been made to develop crop cultivars with enhanced resistance to insect pests (Sharma *et al.* 2003). Host plant resistance (HPR) is one of the most

economic and environmentally friendly methods of controlling insect pests. Improving host plant defense to insects will result in reduced losses due to the herbivores, less insecticide use, better crops yields, and a safer environment (Sharma 2007; Howe and Jander 2008).

Groundnut (*Arachis hypogaea* L.), also known as peanut, is an annual herbaceous plant belonging to the family Fabaceae. It occupies about 9 % of the world's oilseed crop area, and contributes to about 5 % of vegetable oil production (Birthal *et al*. 2010). It is cultivated mostly in the semi-arid tropical and sub-tropical regions (Sharma *et al*. 2003). Groundnut is a principal source of digestible proteins, cooking oil and vitamins (Savage and Keenan 1994).

Insect pests are one of the major constraints for groundnut production. In India, the annual yield losses by insect pests in groundnut are about 15 %, which accounts for about 1.6 million tonnes and 25.27 billion rupees (Dhaliwal *et al*. 2010). Groundnut is damaged by a large number of insects (Wightman and Amin 1988; Sahayaraj and Raju 2003). Armyworms, cotton bollworm, white grubs, aphids, thrips, leafhoppers, red hairy caterpillar and leaf miner are the most important pests of groundnut worldwide (Wightman and Amin, 1988; Sahayaraj and Raju 2003). Insect damage at an early stage of the crop leads to severe losses in crop yield (Wightman and Ranga Rao 1994).

The tobacco armyworm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) is a polyphagous pest, which feeds on more than 200 crop species, including groundnut (Wightman and Ranga Rao 1994; Sahayaraj and Raju 2003). The cotton bollworm, *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae) is also considered a key pest of groundnut (Sharma *et al*. 2003; Sharma 2005). Leafhopper, *Empoasca kerri* Pruthi (Homoptera: Cicadellidae) is an important sap sucking pest that causes heavy damage in groundnut (Wightman and Amin, 1988; Wightman and Ranga Rao 1994).

Plants possess a number of oxidative enzymes, which are involved in resistance against insect pests and pathogens. These include peroxidases (POD), polyphenol oxidase (PPO), superoxide dismutase (SOD), phenylalanine ammonia lyase (PAL), lipoxygenase (LOX), catalase (CAT) and ascorbate peroxidase (APX) (Khattab and Khattab 2005; Bhonwong *et al*. 2009; War *et al*. 2011, 2012, 2014). Insect damage induces the *de novo* synthesis of PAL (Campos-Vergas and Saltveit, 2002), which then leads to the phenolic compounds accumulation (Bhonwong *et al*. 2009). POD and PPO act as

scavengers of highly reactive and unstable free radicals and also oxidize phenols to semiquinones that are highly toxic to insect pests (Bhonwong *et al*. 2009; Barbehenn *et al*. 2010; War *et al*. 2012, 2014). Further, PPO acts as an anti-nutritional enzyme by reducing the food quality of the plant tissues (Bhonwong *et al*. 2009; War *et al*. 2012). LOX catalyzes the formation of fatty acid hydroperoxides, which in turn forms highly reactive aldehydes, γ-ketols, epoxides and reactive oxygen species (ROS) (Maffei *et al*. 2007; War *et al*. 2012).The SOD and CAT convert the free radicals to H_2O_2, which is a less reactive and stable ROS (Khattab and Khattab 2005; Howe and Jander 2008). The oxidized products of phenols such as quinones affect the insect growth and development (Maffei *et al*. 2007; War *et al*. 2013). H_2O_2 acts as a second messenger in plant defensive signal transduction pathways that result in the production of many toxic chemicals (Maffei *et al*. 2007), while malondialdehyde (MDA) is a plant defense indicator against insect pests (Gechev *et al*. 2002; War *et al*. 2013).

Considerable information is available on identification of insect-resistant germplasm, but characterization of physiological and biochemical mechanisms of resistance remains limited (Heng-Moss *et al*. 2004; War *et al*. 2013, 2014).There is inadequate information on the magnitude and mechanisms of resistance in groundnut against insect pests, which is the most important oilseed crop grown in the tropics under rainfed conditions in Asia and Africa. The present studies were carried out to understand the mechanism(s) of resistance in groundnut genotypes to key insect pests.

Material and Methods

Chemicals

The chemicals used in this study were of analytical grade. The chemicals and instruments used in the present study, and their source are shown in Table 1.

Evaluation of groundnut genotypes for resistance to insects under field conditions

Five genotypes of groundnut were evaluated for resistance to insects under field conditions, including four genotypes earlier known to be resistant to *H. armigera* and *S. litura* [(ICGV 86699, ICGV 86031, ICG 2271

Table 1 Chemicals and instruments used in the study and their providers

Chemical/instrument	Source
Ethylene diamine tetra acetic acid (EDTA), bovine serum albumin (BSA) guaiacol, polyvinylpyrolidone (PVP), tannic acid, vanillin, linoleic acid, dithiothretol (DTT), disodium hydrogen phosphate, sodium dihydrogen phosphate, nitro-blue tetrazolium salt (NBT), methionine, L-phenylalanine, potassium iodide (KI), sodium carbonate (Na_2CO_3), vanillin	Sigma Aldrich, USA
Catechol	Glaxo Laboratories, Mumbai, India
Glycine, trichloroacetic acid (TCA)	Sisco Research Lab., Mumbai, India
2-mercaptoethanol, gallic acid, Folin-Ciocalteau reagent	Merck, Mumbai, India
Thiobarbituric acid (TBA), sucrose, linoleic acid	HiMedia Pvt. Ltd., Mumbai, India
Ammonium sulphate	Qualigens Fine Chemicals, Mumbai
Ethrel	Imperial Chemical Industries, Berks, UK
Spectrophotometer Hitachi UV – 2900	Hitachi, Japan

(NCAc 343), ICG 1697 (NCAc 17090)], and a susceptible check, JL 24 (Sharma *et al.* 2003). The crop was grown during the 2010/11 and 2011/12 rainy seasons at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India (17^0 25'N latitude, 78^0 00'E longitude). There were three replications in a randomized complete block design. Each plot had 2 rows, 2 m row length, and the material was planted on ridges 75 cm apart. For breaking dormancy, the seeds were treated with Ethrel before sowing. The seeds were sown at a depth of 5-7 cm below the soil surface by hand with a spacing of 15 cm between the plants. The experimental plots were not sprayed with any insecticide. Weeds were removed manually. Resistance/susceptibility of groundnut genotypes to *H. armigera*, *S. litura* and *E. kerri* was measured in terms of plant damage based on a 1 - 9 visual damage rating (DR) (1being no or slight damage, *i.e.*, ≤ 10 % and 9 being ≥ 80 % damage) (Sharma *et al.* 2003).

2.3. Biochemical profile of groundnut genotypes raised in the field

Leaves were randomly collected from the groundnut plants at 20 days after germination to study the activities of various defensive enzymes such as POD, PPO, SOD, APX, LOX, CAT, PAL, PI, and total amounts of phenols, condensed tannins, flavonoids, carbohydrates, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA).

Evaluation of enzyme activity

Enzyme extraction Fresh leaves (0.5 g) were ground in 3 ml of ice cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol, 1 % polyvinylpyrrolidone (PVP), 1 mM DTT, and 0.5 mM EDTA. The homogenate was centrifuged at 14,000 rpm for 20 min and the supernatant was collected. The supernatant was subjected to protein precipitation and dialysis. Proteins were precipitated using ammonium sulphate (NH_4SO_2) and dialyzed using dialysis bags (Sigma-Aldrich, USA).

Peroxidase (POD) assay Peroxidase activity was estimated as per the method of Shannon *et al.* (1966) with slight modification. To 2.9 ml of reaction mixture [0.1 M sodium phosphate buffer (pH 6.5), 0.8 mM H_2O_2 and 5 mM guaiacol], 0.1 ml of partially purified enzyme source was added. The absorbance was read at 470 nm for 2 min at 15 sec intervals and the enzyme activity was expressed as IU g^{-1} FW. The change in absorbance by 0.1 unit per minute was equal to one unit of POD activity under conditions of assay.

Polyphenol oxidase (PPO) assay Polyphenol oxidase activity was estimated following the method of Mayer and Harel (1979) with some modifications. 0.1 ml of partially purified enzyme extract and 0.1 ml of 0.05 M catechol were added to 2.9 ml 0.1 M sodium phosphate buffer (pH 6.8). The absorbance was read at 420 nm for 3 min at 30 sec intervals and the 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 the assay.

Phenylalanine ammonia lyase (PAL) assay Phenylalanine ammonia lyase was estimated by the method as described by Campos-Vergas and Saltveit (2002) with slight modifications. 0.2 ml of partially purified enzyme source and 0.4 ml of 50 mM potassium phosphate buffer (pH 8.8) were added to 0.4 ml of 50 mM L-phenylalanine (dissolved in 20 mM potassium phosphate buffer, pH 8.8). After incubation of the reaction mixture at 40 °C for 30 min, change in absorbance was read at 290 nm and PAL activity was expressed as µmol cinnamic acid min^{-1} mg^{-1} protein.

Lipoxygenase (LOX) assay Lipoxygenase activity was measured according to the method of Hildebrand and Hymowitz (1983) with slight modifications. To the reaction mixture (0.95 ml) containing 1 mM linoleic acid dispersed in 0.1 M sodium phosphate buffer (pH 7.0), 0.05 ml of partially purified enzyme extract was added. Absorbance was read at 234 nm for 2-3 min. The enzyme activity was expressed as IU g^{-1} FW.

Superoxide dismutase (SOD) assay The activity of SOD was assayed as described by Beauchamp and Fridovich (1971) with slight modifications. To 3 ml of 0.05 M sodium phosphate buffer with 0.1 % NaCl (pH 7.8), 0.3 ml of 0.1 mM EDTA, 0.3 ml of 0.13 mM methionine, 0.1 ml of 0.02 mM KCN, 0.3 ml of 0.75 mM nitroblue tetrazolium salt (NBT), 0.3 ml of 0.02 mM riboflavin and 0.1 ml of enzyme extract were added. The reaction mixture was illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes for 1 hour. Identical solutions that were kept under dark served as blanks. Absorbance was read at 560 nm against the blank. SOD activity was expressed in IU g^{-1} FW.

Ascorbate peroxidase (APX) assay The APX activity was determined by following the method of Asada and Takahashi (1987) 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 1 mM EDTA, 1 % polyvinylpyrrolidone (PVP) and 1 mM ascorbic acid. After filtering through a double-layered cheese cloth, the homogenate was centrifuged at 18,000× g for 20 min at 4 °C. The

supernatant was collected and subjected to precipitation and dialysis. The partially purified sample was used as the 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$_2$O$_2$ 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.

Catalase (CAT) assay Catalase activity was determined as per the method of Zhang *et al*. (2008). The reaction mixture consisted of 1 ml of Tris- HCl buffer (pH 7.0), 0.1 ml of partially purified enzyme extract and 0.2 ml of H$_2$O$_2$. Absorbance was read at 240 nm for 2 min and the enzyme activity was expressed as µmol min^{-1} mg^{-1} protein.

Estimation of secondary metabolites

Phenolic content Fresh leaves (0.5 g) were homogenized in 3 ml of 80 % methanol and agitated for 15 min at 70 °C. The solution was centrifuged at 10, 000 rpm for 10 min and the supernatant collected. The supernatant was used for the estimation of total phenols, condensed tannins and total flavonoids. The Phenolic content was estimated as per Zieslin and Ben-Zaken (1993) method with some modifications. To 2 ml of 2 % sodium carbonate (Na$_2$CO$_3$), 1 ml of methanol extract was added. The solution was incubated for 5 min at room temperature after which 0.1 ml of 1 N Folin-Ciocalteau reagent was added. The solution was incubated again for 10 min and absorbance of the blue color measured at 760 nm. 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 tannins Condensed tannins were estimated by using vanillin-hydrochloride method as described by Robert (1971), with some modifications. The 0.5 ml of supernatant was added to 2.5 ml of vanillin-HCl reagent [equal volumes of 8 % HCl (in methanol) and 4 % vanillin (in methanol) and the solutions mixed just before use]. The reaction mixture was incubated at room temperature for 20 min and the absorbance read at 500 nm against a blank containing the reagent alone. Catechin was used as the standard. The total amount of

condensed tannins was expressed as µg catechin equivalents g^{-1} FW (µg CE g^{-1} FW).

Estimation of hydrogen peroxide (H_2O_2) content

Hydrogen peroxide (H_2O_2) content was estimated by the method of Noreen and Ashraf (2009). Fresh leaf tissue (0.1 g) was homogenized in 2 ml of 0.1 % (w/v) trichloroacetic acid (TCA) in a pre-chilled pestle and mortar, and the homogenate was centrifuged at 10,000 rpm for 15 min. To 0.5 ml of supernatant, 0.5 ml of phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide (KI) were added. The absorbance was read at 390 nm. H_2O_2 concentration was determined by using an extinction coefficient of 0.28 µM cm^{-1} and expressed as µmol g^{-1} FW.

Estimation of malondialdehyde (MDA) content

The level of lipid peroxidation was determined in terms of thiobarbituric acid-reactive substances (TBARS) as described by Carmak and Horst (1991) with minor modifications. Fresh leaf tissue (0.2 g) was homogenized in 3 ml 0.1 % (w/v) trichloroacetic acid (TCA) at 4 °C. The homogenate was centrifuged at 20,000 × g for 15 min. 0.5 ml of supernatant was added to 3 ml 0.5 % (v/v) thiobarbituric acid (TBA) in 20 % TCA. The mixture was incubated at 95 °C in a shaking water bath for 50 min and the reaction stopped by cooling the tubes in an ice water bath. Then samples were centrifuged at 10,000 rpm for 10 min and the absorbance of the supernatant read at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using the absorption coefficient 155 $mmol^{-1}cm^{-1}$ and expressed as µmol g^{-1}FW.

Estimation of protein content

Total protein content was determined by following the method of Lowry et al. (1951), using bovine serum albumin as standard, and expressed as mg g^{-1}FW.

Statistical analysis

The data were subjected to 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 ≤ 0.05). Correlation coefficients were computed to determine the association between plant damage and biochemical traits of the genotypes studied using MS Excel 2007. The significance of the correlation coefficients was judged at (P ≤ 0.05).

Results

Evaluation of groundnut genotypes resistance to insects under open field conditions

Leaf damage

The leaf damage due to foliage feeders, especially *H. armigera* and *S. litura* was significantly lower in ICGV 86699 (2.6), ICGV 86031 (3.1), ICG 2271 (2.9) and ICG 1697 (3.2) ($F_{(4,14)}$ = 54.4, P ≤ 0.01) than in JL 24 (7.2) (Table 2). The *E. kerri* damage was greater in JL 24 (6.0) than in ICGV 86699 (2.0), ICGV 86031 (3.2), ICG 2271 (3.1) and ICG 1697 (2.9) ($F_{(4,14)}$ = 36.2, P ≤ 0.001).

Biochemical profile of the groundnut plants grown under field conditions

The biochemical constituents, namely POD, PPO, PAL, LOX, CAT, SOD and APX (Table 3), and the secondary metabolites such as total phenols, condensed tannins, flavonoids and H_2O_2, MDA and total proteins of groundnut genotypes showed considerable variability (Table 4). Amongst the

Table 2 Relative resistance/ susceptibility of five groundnut genotypes to *Helicoverpa armigera*, *Spodoptera litura* and *Empoasca kerri* under field conditions

Genotypes	Damage rating[x]	
	H. armigera and *S. litura*	*Empoasca kerri*
ICGV 86699	2.6 ± 0.09[b]	2.0 ± 0.04[c]
ICGV 86031	3.1 ± 0.06[b]	3.2 ± 0.06[b]
ICG 2271	2.9 ± 0.06[b]	3.1 ± 0.08[b]
ICG 1697	3.2 ± 0.09[b]	2.9 ± 0.05[bc]
JL 24	7.2 ± 0.09[a]	6.0 ± 0.09[a]

Values (Mean ± SD) carrying same alphabet(s) within a column are not significantly different at P < 0.05 (Tukey's HSD test).
[x] Damage rating (1=being no, slight damage or ≤ 10 % leaf damage, and 9=being ≥ 80 % leaf damage).

Table 3 Activity of defensive enzymes in five genotypes of groundnut grown under field conditions

Genotypes	POD (IU g^{-1} FW)	PPO (IU g^{-1} FW)	PAL (μmol cinnamic acid mg^{-1} protein)	LOX (IU g^{-1} FW)	SOD (IU g^{-1} FW)	APX (IU g^{-1} FW)	CAT (μmol min^{-1} mg^{-1} protein)
ICGV 86699	0.24 ± 0.03^a	0.044 ± 0.001^a	6.4 ± 0.9^a	4.1 ± 0.1^a	7.6 ± 1.03^a	0.54 ± 0.02^a	6.8 ± 0.7^a
ICGV 86031	0.21 ± 0.06^a	0.034 ± 0.004^a	6.8 ± 0.9^a	3.4 ± 0.7^{ab}	6.3 ± 0.08^b	0.46 ± 0.04^b	7.3 ± 0.3^a
ICG 2271	0.19 ± 0.02^a	0.038 ± 0.004^a	5.5 ± 0.7^a	2.7 ± 0.3^b	5.9 ± 0.05^b	0.34 ± 0.01^c	5.2 ± 0.4^b
ICG 1697	0.18 ± 0.07^a	0.035 ± 0.003^a	5.3 ± 0.3^a	3.1 ± 0.4^b	5.4 ± 0.05^b	0.37 ± 0.07^c	5.8 ± 1.0^b
JL 24	0.09 ± 0.01^b	0.021 ± 0.001^b	3.2 ± 0.1^b	1.7 ± 0.5^c	4.1 ± 1.01^c	0.26 ± 0.04^d	4.1 ± 0.5^c

Values (Mean ± SD) with same letter (s) in a column are not significantly different at $P < 0.05$ (Tukey's HSD test).

SOD = Superoxide dismutase; POD = Peroxidase, PPO = Polyphenol oxidase; LOX = Lipoxygenase; APX = Ascorbate peroxidase; PAL = Phenylalanine ammonia lyase; CAT = Catalase; and FW = fresh weight.

genotypes tested, greater POD, PPO and PAL activities were observed in ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697 ($F_{(4,14)}$ = 24.2, 46.8 and 32.4, respectively, $P < 0.05$) than in JL 24. The LOX activity was considerably greater in ICGV 86699 and ICGV 86031 and ICG 1697 ($F_{(4,14)}$ = 98.3, $P < 0.01$) than ICG 2271, ICG 1697, and JL 24. The SOD and APX activities were significantly greater in ICGV 86699 ($F_{(4,14)}$ = 34.6, 23.4 for SOD and APX, respectively, $P < 0.01$) than in ICGV 86031, ICG 2271, ICG 1697 and JL 24. ICGV 86699 and ICGV 86031 had significantly greater CAT activities ($F_{(4,14)}$ = 49.7, $P < 0.01$) than ICG 2271, ICG 1697 and JL 24).

Total phenols and condensed tannins did not exhibit any significant differences among ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697 ($P < 0.05$). However, the amounts of total phenols and condensed tannins were significantly greater ($F_{(4,14)}$ = 34.9 and 25.8 for phenolic and tannins, respectively,

$P < 0.01$) than that of JL 24 (Table 4). Moreover a strong negative correlation was observed between plant damage and amounts of total phenols (r = -0.90 and -0.91 for *H. armigera* and *S. litura* damage, and leafhopper damage, respectively), and condensed tannins (r = -0.98 and -0.94 for *H. armigera* and *S. litura* damage, and leafhopper damage, respectively). The H_2O_2 content was significantly high in ICGV 86699 ($F_{(4,14)}$ = 76.1, $P < 0.01$). Furthermore, a strong negative correlation was observed between plant damage and H_2O_2 content (r = -0.88). The ICG 2271, ICG 1697 and JL 24 had greater MDA content ($F_{(4,14)}$ = 65.3, $P = 0.05$) than that of ICGV 86031 and ICGV 86699. Protein content was significantly higher in ICGV 86699, ICGV 86031 and ICG 2271 ($F_{(4,14)}$ = 34.6 , $P < 0.05$) than in ICG 1697 and JL 24. A strong negative correlation was observed between protein levels and plant damage by *H. armigera*, *S. litura* (r = -0.83) and leafhoppers (-0.86).

Table 4 Concentrations of plant defensive compounds in five groundnut genotypes grown under field conditions

Genotype	Phenols (μg GAE g^{-1} FW)	Condensed tannins (μg TAE g^{-1} FW)	H_2O_2 (μmol g^{-1} FW)	MDA (μmol g^{-1} FW)	Protein (mg g^{-1}FW)
ICGV 86699	87.4 ± 2.6^a	9.5 ± 1.3^a	60.7 ± 2.3^a	8.9 ± 0.9^a	10.2 ± 1.02^a
ICGV 86031	79.4 ± 2.4^a	8.8 ± 0.9^{ab}	42.9 ± 3.7^b	8.6 ± 1.0^a	7.9 ± 0.09^{ab}
ICG 2271	73.7 ± 3.5^a	8.3 ± 1.3^b	38.6 ± 2.0^b	6.5 ± 0.9^b	8.5 ± 0.1^{ab}
ICG 1697	65.2 ± 2.6^b	8.6 ± 1.1^{ab}	31.4 ± 1.9^{bc}	5.3 ± 0.1^{bc}	6.2 ± 0.07^{bc}
JL 24	45.3 ± 1.9^c	3.9 ± 0.2^c	15.9 ± 0.9^d	7.7 ± 0.7^{ab}	4.4 ± 0.01^c

Values (Mean ± SD) with the same letter (s) in a column are not significantly different at $P < 0.05$ (Tukey's HSD test). GAE = Gallic acid equivalents; TAE = Tannic acid equivalents; H_2O_2 = Hydrogen peroxide; MDA = Malondialdehyde; and FW = Fresh weight.

Discussion

Host plant resistance is the most effective, economic and environment friendly strategy for pest management (Sharma 2007), and is manifested through morphological, physiological and biochemical features of the host plant (Howe and Jander 2008; Sharma et al. 2009; War et al. 2012).

In the present study, there were significant differences in leaf damage by *H. armigera*, *S. litura* and *E. kerri* in different groundnut genotypes under field conditions. Leaf damage by *H. armigera, S. litura* and *E. kerri* was lower in ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 as compared to JL 24. These results are similar to the earlier observations by Sharma et al. (2003). The enzymes such as POD, PPO and PAL showed greater activities in ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697 than in JL 24. However, the activity of SOD, LOX, APX and CAT varied across genotypes, irrespective of their level of resistance to the insect pests. Phenols, condensed tannins, H_2O_2, and total proteins were also significantly greater in insect resistant genotypes than in JL 24. The insect-resistant genotypes have been reported to possess higher levels of antioxidative enzymes and secondary metabolites, and they respond strongly to different stresses (Heng-Moss et al. 2004; Chen et al. 2009; Gulsen et al. 2010; War et al. 2011). The differential levels of resistance in groundnut genotypes might be due to the differential activities of enzymes such as POD, PPO, PAL, LOX, SOD, CAT and APX, and total amounts of phenols, tannins, and H_2O_2, and these could be used as biochemical markers to select plants with resistance to insects (Chaman et al. 2001; Sankar et al. 2007; War et al. 2011, 2012).

Plant secondary metabolites play an important role in host plant resistance against insect pests. The present studies showed that insect-resistant genotypes suffered lower levels of leaf damage by *H. armigera, S. litura* and/or *E. kerri*, and had greater levels of total phenols and condensed tannins than the susceptible genotypes (Table 4). A significant and negative correlation was observed between plant damage and amounts of total phenols and condensed tannins. Phenolic compounds are either directly toxic to insect pests or activate the production of various toxic secondary metabolites by mediating the transduction pathways, and also activate various defensive enzymes (Walling 2000; Maffei et al. 2007; Bhonwong et al. 2009). Oxidation of phenols produces toxic quinones, which covalently bind to leaf proteins, thereby inhibiting, protein digestion in herbivores (Bhonwong et al. 2009). Tannins are considered as important plant defensive compounds and negatively affect the growth and survivorship in many insect pests (Bernards and Bastrup-Spohr 2008; Sharma et al. 2009). Precipitation of proteins by tannins reduces the nitrogen mineralization and/or digestion in herbivore midgut (Bernards and Bastrup-Spohr 2008). It has been reported that higher levels of condensed tannins, in addition to other factors, makes pigeonpea resistant to *H. armigera* (Sharma et al. 2009). A strong negative correlation was observed between various enzyme activities and the severity of damage by *H. armigera, S. litura* and *E. kerri*.

Groundnut genotypes that showed lower levels of damage by *H. armigera, S. litura* and *E. kerri* had greater amounts of H_2O_2 than those suffering more insect damage. The H_2O_2 is one of the most important reactive oxygen species involved in plant defense against insect pests. It acts as a secondary messenger and mediates various transduction pathways, which produces various plant defensive compounds (Maffei et al. 2007; Howe and Jander 2008). In addition, H_2O_2 causes oxidative damage to insect midgut (Maffei et al. 2007).

There were no significant differences in MDA content among ICGV 86699, ICGV 86031 and JL 24. However, ICG 2271 and ICG 1697 had reduced levels of MDA. Although the direct role of MDA in plant defense has not been established, it has been suggested that it signals the plant defensive response against various stresses (Huang et al. 2007), and stimulates the release of green leaf volatiles, which attract the natural enemies of the insect pests (Arimura et al. 2009). Significantly greater levels of proteins were observed in ICGV 86699, ICGV 86031and ICG 2271 as compared to ICG 1697 and JL 24. The higher amounts of proteins could be attributed to the greater activity of plant defensive enzymes, and the production of other plant defensive proteins.

Plant defensive enzymes such as POD, PPO, LOX, PAL, SOD, and the secondary metabolites such as total phenols and condensed tannins, and H_2O_2 play an important role in host plant resistance to *H. armigera, S. litura* and *E. kerri*. These

biochemical traits could be used to select groundnut genotypes for insect resistance for use in integrated pest management.

References

Arimura, G., Matsui, K., & Takabayashi, J. (2009). Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. *Plant Cell Physiology, 50*(5), 911–923.

Asada, K., & Takahashi, M. (1987). Production and scavenging of active oxygen in photosynthesis. In D. J. Kyle, C. B. Osmond, & C. J. Arntzen (Eds.), *Photoinhibition* (pp. 227–287). Amsterdam: Elsevier.

Barbehenn, R., Dukatz, C., Holt, C., Reese, A., Martiskainen, O., Salminenm, J. P., Yip, L., Tran, L., & Constabel, C. P. (2010). Feeding on poplar leaves by caterpillars potentiates foliar peroxidase action in their guts and increases plant resistance. *Oecologia, 164*, 993–1004.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improve assay and an assay applicable to acrylamide gels. *Annals of Biochemistry, 44*, 276–287.

Bernards, M., & Bastrup-Spohr, L. (2008). Phenylpropanoid metabolism induced by wounding and insect herbivory. In A. Schaller (Ed.), *Induced plant resistance to herbivory* (pp. 189–211). NY: Springer-Verlag.

Bhonwong, A., Stout, M. J., Attajarusit, J., & 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.

Birthal, P.S., Parthasarathy, Rao P., Nigam, S.N., Bantilan, M.C.S., & Bhagavatula, S. (2010). Groundnut and Soybean Economies in Asia: Facts, Trends and Outlook. Patancheru 502 324, Andhra Pradesh, India: International Crops Research lnstitute for the Semi-Arid Tropics. 92 pp. ISBN: 978-92-9066-531-1. Order code: BOE 050.

Campos-Vergas, R., & Saltveit, M. E. (2002). Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. *Physiologia Plantarum, 114*, 73–84.

Carmak, I., & Horst, J. H. (1991). Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean. (*Glycine max*). *Physiologia Plantarum, 83*, 463–468.

Chaman, M. E., Corcueram, L. J., Zuniga, G. E., Cardemil, L., & Argandona, V. H. (2001). Induction of soluble and cell wall peroxidases by aphid infestation in barley. *Journal of Agricultural Food Sciences, 49*, 2249–2253.

Chen, Y., Ni, X., & Buntin, G. D. (2009). Physiological, nutritional and biochemical bases of corn resistance to foliage-feeding fall Armyworm. *Journal of Chemical Ecology, 35*, 297–306.

Dhaliwal, G. S., Jindal, V., & Dhawan, A. K. (2010). Insect pest problems and crop losses: Changing trends. *Indian Journal of Ecology, 37*, 1–7.

Gechev, T., Gadjev, I., Van Breusegem, F., Inze, D., Dukiandjiev, S., Toneva, V., & Minkov, I. (2002). Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. *Cellular and Molecular Life Sciences, 59*, 708–714.

Gulsen, O., Eickhoff, T., Heng-Moss, T., Shearman, R., Baxendale, F., Sarath, G., et al. (2010). Characterization of peroxidase changes in resistant and susceptible warm-season turfgrasses challenged by *Blissus occiduus. Arthropod-Plant Interactions, 4*, 45–55.

Heng-Moss, T. M., Sarath, G., Baxendale, F., Novak, D., Bose, S., Ni, X., Quisenberry, S., et al. (2004). Characterization of oxidative enzyme changes in buffalograsses challenged by *Blissus occiduus. J. Econ. Entom., 97*, 1086–1095.

Hildebrand, D., & Hymowitz, T. (1983). Lipoxygenase activities in developing and germinating soybean seeds with and without Lipoxygenase-1. *Botanical Gazette, 144*, 212–216.

Howe, G. A., & Jander, G. (2008). Plant immunity to herbivores. *Annual Review of Plant Biology, 59*, 41–66.

Huang, W., Zhikuan, J., & Qingfang, H. (2007). Effects of herbivore stress by *Aphis medicaginis* Koch on the melondialdehyde contents and activities of protective enzymes in different alfalfa varieties. *Acta Ecologica Sinica, 27*(6), 2177–2183.

Khattab, H., & Khattab, M. (2005). Responses of Eucalypt trees to the insect feeding (Gall-forming psyllid). *International Journal of Agricultural Biology, 7 (6),* DOI: 1560–8530/ 2005/07–6–979–984.

Lowry, O. H., Rosebrough, N. I., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Maffei, M. E., Mithofer, A., & Boland, W. (2007). Insects feeding on plants: Rapid signals and responses preceding the induction of photochemical release. *Phytochemistry, 68*, 2946–2959.

Mayer, A. M., & Harel, E. (1979). Polyphenol oxidases in plant. *Phytochemistry, 18*, 193–215.

Noreen, Z., & Ashraf, M. (2009). Change in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.). *Environmental and Experimental Botany, 67*, 395–402.

Oerke, E. C. (2006). Crop losses to pests. *Journal of Agricultural Sciences, 144*, 31–43.

Robert, E. B. (1971). Method for estimation of tannin in grain sorghum. *Agrochemical Journal, 63*, 511.

Sahayaraj, K., & Raju, G. (2003). Pest and natural enemy complex of groundnut in Tuticorin and Tirunelveli districts of Tamil Nadu, India. *International Arachis Newsletter, 233*, 25–29.

Sankar, B., Abdul Jaleel, C., Manivannan, P., Kishore Kumar, A., Somasundaram, R., & Panneerselvam, R. (2007). Effect of paclobutrazol on water stress amelioration through antioxidants and free radical scavenging enzymes in *Arachis hypogaea* L. *Colloids Surfaces. B: Biointeractions, 60*, 229–235.

Savage, G. P., & Keenan, J. I. (1994). The composition and nutritive value of groundnut kernels. In J. Smartt (Ed.), *The groundnut crop: A scientific basis for improvement* (pp. 173–213). London, UK: Chapman and Hall.

Shannon, L. M., Kay, E., & Lew, J. Y. (1966). Peroxidase isozymes from horse radish roots. Isolation and physical properties. *Journal of Biological Chemistry, 241*, 2166–2172.

Sharma, H. C. (2007). Host plant resistance: Modern approaches and limitations. *Indian Journal Plant Protection, 35*, 179–184.

Sharma, H. C. (2005). *Heliothis/Helicoverpa Management: Emerging trends and strategies for future research*. New Delhi, India: Oxford and IBH Publishing Co. Pvt.Ltd.. 469 pp.

Sharma, H. C., Pampathy, G., Dwivedi, S. L., & Reddy, L. J. (2003). Mechanism and diversity of resistance to insect pests in wild relatives of groundnut. *Journal of Economic.Entomology, 96*(6), 1886–1897.

Sharma, H. C., Sujana, G., & Rao, D. M. (2009). Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. *Arthropod-Plant Interactions, 3*(3), 151–161.

Walling, L. L. (2000). The myriad plant responses to herbivores. *Journal of Plant Growth Regulation, 19*, 195–216.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., & Sharma, H. C. (2012). Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavior, 7*(10), 1306–1320.

War, A. R., Paulraj, M. G., Hussain, B., Buhroo, A. A., Ignacimuthu, S., & Sharma, H. C. (2013). Effect of plant secondary metabolites on *Helicoverpa armigera*. *Journal of Pest Science, 86*, 399–408.

War, A. R., Paulraj, M. G., Ignacimuthu, S., & Sharma, H. C. (2014). Induced resistance to *Helicoverpa armigera* through exogenous application of jasmonic acid and salicylic acid in groundnut, *Arachis hypogaea*. *Pest Management Science*. doi:10.1002/ps.3764.

War, A. R., Paulraj, M. G., War, M. Y., & Ignacimuthu, S. (2011). Jasmonic acid- mediated induced resistance in groundnut (*Arachis hypogaea* L.) against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Journal of Plant Growth Regulation, 30*, 512–523.

Wightman, J. A., & Amin, P. W. (1988). Groundnut pests and their control in the semi-arid tropics. *Tropical pest Management, 34*, 218–266.

Wightman, J. A., & Ranga Rao, G. V. (1994). Groundnut pests. In J. Smart (Ed.), *The groundnut crop: A scientific basis for improvement* (pp. 395–479). London: Chapman and Hall publishers.

Zhang, S. Z., Hau, B. Z., & 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.

Zieslin, N., & Ben-Zaken, R. (1993). Peroxidase activity and presence of phenolic substances in penduncles of rose flowers. *Plant Physiology and Biochemistry, 31*, 333–339.