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ORIGINAL PAPER

Herbivore-induced resistance in different groundnut germplasm lines to Asian armyworm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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Abstract Three groundnut germplasm lines, ICGV86699 (resistant), NCAC 343 (resistant) and TMV 2 (susceptible), were examined for *Spodoptera litura* (Fab.) resistance. Biochemical parameters such as oxidative enzyme activities, peroxidase (POD) and polyphenol oxidase (PPO), other defensive components such as total phenols, hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and protein contents were evaluated in these germplasm lines after 24, 48, 72 and 96 h following *S. litura* infestation to characterize the mechanism of resistance. Enzyme activities and total phenols, H_2O_2, MDA and protein contents were increased following infestation; however, significance varied at different time intervals and among germplasm lines depending upon the induced level of resistance. The three germplasm lines differed in resistance mechanisms to *S. litura* and the resistance may be partly due to higher enzyme activities, and other components studied. Among the three germplasms tested, ICGV86699 showed greater elevation in POD and PPO activities and in phenolic and H_2O_2 contents at different time intervals as compared to NCAC 343 and TMV 2.

Keywords Induced resistance · Antioxidant enzymes · Peroxidase · Polyphenol oxidase · Hydrogen peroxide · Malondialdehyde

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Abbreviations

ANOVA	Analysis of variance
BSA	Bovine serum albumin
EDTA	Ethylenediaminetetraacetic acid
FW	Fresh weight
GAE	Gallic acid equivalents
H_2O_2	Hydrogen peroxide
KI	Potassium iodide
LOX	Lipoxygenase
MDA	Malondialdehyde
Na_2CO_3	Sodium carbonate
POD	Peroxidase
PPO	Polyphenol oxidase
PVP	Polyvinyl pyrolidone
ROS	Reactive oxygen species
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substance
TCA	Trichloroacetic acid
Tris–HCl	Tris–hydrochloride

Introduction

Plants are always under the biotic stress due to herbivores, which cause severe damage to crops worldwide. In order to defend themselves against these herbivores, plants have developed a wide range of physical and chemical mechanisms (Rasman and Agrawal 2009; Sharma et al. 2009; War et al. 2011a, b). The resistance strategies adopted by plants can be constitutive, meaning that they are always present in the plant independent of herbivore attack; or inducible, meaning that they are only activated when the plant is under threat (Karban and Baldwin 1997, 2001).

Induced resistance acts either directly or indirectly. Direct resistance aims at the accumulation of substantial amounts of defense proteins and/or production of noxious chemicals in damaged plants that reduce feeding, oviposition, growth and development of herbivores (Heil et al. 2004). Indirect resistance is mediated by the emission of volatile blend that specifically attract natural enemies of herbivores (Arimura et al. 2005; Bruinsma and Dicke 2008). Although constitutive resistance has its own role to play in plant protection, induced resistance is of higher energy utilization efficiency and is more economic and effective to protect plants from damage especially when aimed at the stress of immediate concern (Zhao et al. 2009). Therefore, induced resistance has received more attention recently.

Plants respond to the herbivore damage through physiological, morphological, and chemical changes (Agrawal et al. 2009). However, direct defense provided by accumulated defensive compounds is very important (Sharma et al. 2009; Usha Rani and Jyothsna 2010). One of the most prominent plant responses to insect herbivory is the induction of oxidative enzymes such as peroxidase (POD), polyphenol oxidase (PPO), lipoxygenase (LOX), catalase (CAT) and reactive oxygen species (ROS) (Zhang et al. 2008; Usha Rani and Jyothsna 2010; War et al. 2011a, b). These enzymes, because of their potential roles in synthesis of defense compounds and/or in oxidative stress tolerance, are being implicated in plant resistance to insect herbivores. POD and PPO play important role in plant defense against a number of biotic and abiotic stresses (Zhao et al. 2009; Gulsen et al. 2010; Usha Rani and Jyothsna 2010; War et al. 2011a, b). Plant phenols comprise a structurally diverse and ubiquitous group of plant compounds that has been suggested to play variety of roles in plant defense (Usha Rani and Jyothsna 2010). Hydrogen peroxide (H_2O_2) plays a central role in generation of defense response in plants through the activation of signaling pathways (Boka et al. 2007). Malondialdehyde (MDA) is a decomposition product of polyunsaturated fatty acid hydroperoxides, the concentration of which is related to the degree of membrane lipid peroxidation and is an important indicator of plant response to oxidative stress (Zhang et al. 2008; Arimura et al. 2005).

Groundnut (*Arachis hypogaea* L.) is an annual herbaceous plant belonging to family Fabaceae and is grown mainly for the production of edible plant oil and protein (Freeman et al. 1999). It is attacked by many defoliating, subterranean and stem boring insect pests. Asian armyworm *Spodoptera litura* (Fab.), a polyphagous insect, is economically an important pest of many agricultural crops including groundnut (Sharma et al. 2003). It is distributed in many parts of the world including Asia, North Africa, Japan, Australia, and New Zealand (Mallikarjuna et al. 2004). *Spodoptera litura* has developed resistance to a number of synthetic insecticides (Kranthi et al. 2002). ICGV 86699 and NCAC 343 differ in the maturity period (Prasad and Gowda 2006) and levels of resistance to different insects (Sharma et al. 2003).

Although considerable progress has been made in identifying insect-resistant germplasms, however, characterization of physiological and biochemical mechanisms of resistance remains limited (Heng-Moss et al. 2004). The role of phenolic compounds, POD and PPO in induced resistance in groundnut germplasms in response to infections by bacterial and fungal pathogens has been well studied (Rathna Kumar and Balasubramanian 2000). However, induced resistance in response to insect attack has not been thoroughly studied in this crop. Hence this study was undertaken to compare the biochemical responses of different germplasm lines of groundnut to the damage by *S. litura.* Our study focused on the oxidative enzymes like peroxidase and polyphenol oxidase and other defensive components such as total phenols, hydrogen peroxide, malondialdehyde and protein content in these germplasm lines. The effect of different germplasm lines on larval weight was also assessed.

Materials and methods

Chemicals

The chemicals used in this study were of analytical grade. Tris–HCl, polyvinyl pyrolidone (PVP), EDTA, disodium hydrogen phosphate, sodium dihydrogen phosphate, guaiacol and thiobarbituric acid (TBA) were obtained from HiMedia Lab. Pvt. Ltd. Mumbai and 2-mercaptoethanol was procured from Loba Chemie, Mumbai, India. Pyrocatechol was obtained from Central Drug House, Mumbai, India. Coomassie brilliant blue-G250 was obtained from Sisco Research Lab., Mumbai, India. Bovine serum albumin (BSA), potassium iodide (KI) and sodium carbonate (Na_2CO_3) were obtained from S.d. Fine Chemicals Ltd. Mumbai, India. Gallic acid and Folin–Ciocalteau reagent were obtained from Merck, Mumbai, India. Trichloroacetic acid (TCA) was obtained from Qualigens Fine Chemicals, Mumbai, India.

Groundnut plants *(Arachis hypogaea* L.*)*

Seeds of three groundnut germplasm lines namely ICGV 86699, NCAC-343 and TMV 2 were obtained from International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Andhra Pradesh, India. ICGV 86699 and NCAC 343 represented the resistant varieties, while TMV

2 represented the susceptible variety. The seeds were sown in the field and the plants were maintained by following regular farmer's practices. Utmost care was taken to prevent the plants from insect attack other than the experimental insect by enclosing them in net cages. Twenty days old groundnut plants were used for the study. The plants were grouped into two sets. One set was infested with *S. litura* and the other set was maintained as control.

Spodoptera litura infestation

First instar larvae of *S. litura* were obtained from the stock culture maintained on castor leaves at laboratory conditions (26 ± 1°C; 11 ± 0.5 h photoperiod and 75 ± 5% relative humidity) from the insectary of the Entomology Research Institute. Five neonates were gently placed on each 20 days old plant by using a camel hair brush.

Enzyme extraction

About 0.5 g of fresh leaves from control and experimental plants were collected, frozen in liquid nitrogen separately and ground in 3 ml of ice cold 0.1 M Tris–HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol, 1% polyvinyl pyrolidone (PVP) and 0.5 mM EDTA. The homogenate was centrifuged at 14,000 rpm for 25 min at 4°C and the supernatant was used as enzyme source. All spectrophotometric analyses were carried out on HITACHI UV-2010 spectrophotometer.

Peroxidase assay

POD activity was estimated as per the method of Shannon et al. (1966) with slight modifications. To 2.9 ml of reaction mixture containing 0.1 M sodium phosphate buffer (pH 6.5), 0.8 mM H_2O_2 and 5 mM Guaiacol, 0.1 ml of enzyme source was added. Absorbance was read at 470 nm for 2 min at 15 s interval. Enzyme activity was expressed as IU g^{-1} FW, where one unit of POD activity is equal to 6.46 µmol g^{-1} FW min^{-1} (extinction coefficient for Guaiacol is 26.6 mM^{-1} cm^{-1}).

Polyphenol oxidase assay

PPO activity was estimated as per the method of Mayer and Harel (1979) with some modifications. To 2.9 ml of 0.1 M sodium phosphate buffer (pH 6.8), 0.1 ml of enzyme source and 0.1 ml of substrate (0.05 M Pyrocatechol) were added. Absorbance was read at 420 nm for 3 min at 30 s interval. Enzyme activity was expressed as IU g^{-1} FW, where one unit of POD activity is equal to 0.23 µmol g^{-1} FW min^{-1} (extinction coefficient for catechol is 0.95 mM^{-1} cm^{-1}).

Phenolic content

Phenolic content was estimated as per Zieslin and Ben-Zaken (1993) method with some modifications. About 500 mg of fresh leaf was homogenized with 3 ml of 80% methanol and agitated for 15 min at 70°C. The extract (0.1 ml) was added to 2 ml of 2% sodium carbonate (Na_2CO_3). After incubation for 5 min, 0.1 ml of Folin–Ciocalteau reagent was added and the solution was again incubated for 10 min. The absorbance of the blue color was measured using a spectrophotometer 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).

Hydrogen peroxide content

H_2O_2 content was estimated by the method of Noreen and Ashraf (2009). Fresh leaf tissue (0.1 g) was homogenized with 2 ml of 0.1% (w/v) trichloroacetic acid (TCA) in a pre-chilled pestle and mortar and the homogenate was centrifuged at 12,000×*g* 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.

Malondialdehyde content

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 modifications. Fresh leaf (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 was stopped by cooling the tubes in an ice water bath. Then samples were centrifuged at 10,000×*g* for 10 min, and the absorbance of the supernatant was read at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using the absorption coefficient 155 mM^{-1} cm^{-1} and was expressed as µmol g^{-1} FW.

Protein determination

Protein was determined according to the method of Bradford (1976). To 3 ml of Bradford's reagent, 10 µl of the supernatant was added and absorbance was read at 595 nm after 20 min of incubation and the protein content was

determined from standard curve established using known quantities of bovine serum albumin and the above reagent.

Larval weight

After 24, 48, 72 and 96 h of infestation, larvae were collected from the infested plants and weighed by using digital balance (Mettler Toledo, AB304-S). After weighing, the larvae were again released on the same plants. Weight of larvae was expressed as mg per five larvae.

Statistical analysis

The replication data were pooled together and mean and standard error were calculated. All data were analyzed by repeated analysis of variance (ANOVA) using SAS (version 9.2). Student's *t* test was applied for the evaluation of differences between the controls and infested for all parameters.

Results

POD activity

Infestation with *S. litura* resulted in greater POD activity in NCAC 343 and TMV 2 than their respective controls at 24 h after infestation (Table 1). After 48 h, NCAC 343 and TMV 2 infested plants exhibited higher POD activity than their respective control plants. Differences were significant between control and infested plants of ICGV 86699 at 72 h, and in all the germplasms at 96 h after infestation. Among the germplasms, ICGV 86699 had significantly higher POD activity both in control and infested plants at all the time intervals than that of NCAC 343 and TMV 2. However, at 24 and 96 h, POD activity of NCAC 343 infested plants was at par with ICGV 86699.

PPO activity

PPO activity was significantly higher in ICGV 86699 infested plants at 24 and 48 h than the control plants (Table 2). Significant differences were observed between control and infested plants of all the three germplasms at 72 and 96 h after infestation. When comparing germplasms, PPO activity was significantly higher in ICGV 86699 germplasm both in control and infested plants at all the time intervals than the respective treatments of NCAC 343 and TMV 2.

Phenolic content

Although there was induction in phenolic content following *S. litura* infestation, however, statistically significant difference was recorded only at 96 h of infestation in ICGV 86699 between control and infested (Table 3). Among the three germplasms ICGV 86699 infested plants showed higher phenolic content than that of NCAC 343 and TMV 2 at 24 h after infestation. Phenolic content was significantly higher in control plants of ICGV 86699 after 48 h than control plants of NCAC 343 and TMV 2. However, at 96 h, ICGV 86699 exhibited significantly higher phenolic content both in control and infested plants as compared to control plants of TMV 2 and infested plants of NCAC 343 and TMV 2.

H_2O_2 content

Infested plants showed significantly higher H_2O_2 content at 24 h in NCAC 343 and TMV 2 as compared to their respective controls (Table 4). Differences between control and infested plants were significant in all the three germplasms at 48 and 72 h after infestation, however, at 96 h, significant differences were observed between control and infested plants of ICGV 86699 and TMV 2. Among the

Table 1 Peroxidase activity (IU g^{-1} FW) of three groundnut germplasm lines after *S. litura* infestation

Groundnut germplasm	Time after insect release (h)							
	24		48		72		96	
	Control	Infested	Control	Infested	Control	Infested	Control	Infested
ICGV 86699	0.24 ± 0.01^{a}	0.27 ± 0.04^{a}	0.32 ± 0.04^{a}	0.44 ± 0.05^{a}	0.34 ± 0.01^{a}	0.68 ± 0.03^{a}**	0.47 ± 0.07^{a}	0.69 ± 0.08^{a}*
NCAC 343	0.13 ± 0.02^{b}	0.25 ± 0.01^{a}**	0.15 ± 0.01^{b}	0.38 ± 0.02^{b}**	0.19 ± 0.02^{b}	0.40 ± 0.07^{b}*	0.21 ± 0.03^{b}	0.56 ± 0.04^{ab}**
TMV 2	0.14 ± 0.01^{b}	0.21 ± 0.01^{b}*	0.14 ± 0.01^{b}	0.25 ± 0.03^{c}**	0.15 ± 0.01^{b}	0.37 ± 0.04^{b}***	0.16 ± 0.02^{b}	0.45 ± 0.02^{b}*

One IU = 6.46 μmol g^{-1} FW min^{-1}

Figures (mean ± SEM) with the same letter(s) in a column within a time interval are not significantly different at $P \leq 0.05$

FW fresh weight of leaf tissue, *Control* non-infested plants

Asterisk indicates the levels of statistical significance between control and infested plants within a germplasm at each time interval

*, **, *** Significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively, by students "*t* test"

Table 2 Polyphenol oxidase activity (IU g^{-1} FW) of three groundnut germplasm lines after *S. litura* infestation

Groundnut germplasm	Time after insect release(h)							
	24		48		72		96	
	Control	Infested	Control	Infested	Control	Infested	Control	Infested
ICGV 86699	0.026 ± 0.005^{a}	$0.042 \pm 0.003^{a**}$	0.031 ± 0.002^{a}	$0.062 \pm 0.007^{a*}$	0.063 ± 0.01^{a}	$0.082 \pm 0.009^{a**}$	0.052 ± 0.01^{a}	$0.103 \pm 0.03^{a**}$
NCAC 343	0.016 ± 0.007^{b}	0.018 ± 0.004^{b}	0.016 ± 0.005^{b}	0.026 ± 0.007^{b}	0.018 ± 0.008^{b}	$0.032 \pm 0.01^{b*}$	0.022 ± 0.007^{b}	$0.042 \pm 0.002^{c**}$
TMV2	0.013 ± 0.007^{b}	0.019 ± 0.002^{b}	0.015 ± 0.003^{b}	0.024 ± 0.004^{b}	0.012 ± 0.006^{b}	$0.042 \pm 0.007^{b***}$	0.013 ± 0.008^{b}	$0.061 \pm 0.02^{b*}$

One IU = 0.23 μmol g^{-1} FW min^{-1}

Figures (mean ± SEM) with the same letter(s) in a column within a time interval are not significantly different at $P \leq 0.05$

FW fresh weight of leaf tissue, *Control* non-infested plants

Asterisk indicates the levels of statistical significance between control and infested plants within a germplasm at each time interval

*, **, *** Significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively, by students "*t* test"

three germplasms tested, at 48 h after infestation, ICGV 86699 showed significantly higher H_2O_2 content in infested plants than that of NCAC 343 and TMV 2. However, at 96 h, ICGV 86699 and TMV 2 infested plants showed significantly higher H_2O_2 content than that of NCAC 343.

MDA content

NCAC 343 had significantly higher MDA content in *S. litura* infested plants at 24 h after infestation than uninfested control plants (Table 5). At 48 h, significant differences were observed in MDA content between control and infested plants of all the germplasm lines. Significantly higher MDA contents were recorded in infested ICGV 86699 and TMV 2 plants at 72 h after infestation as compared to their respective controls. At 96 h, difference in MDA content was significant between control and infested plants of TMV 2. Among the three germplasms tested, NCAC 343 had significantly higher MDA content both in control and infested plants at 24 and 48 h than the respective treatments of ICGV 86699 and TMV 2. At 72 h, significantly higher MDA content was observed in NCAC 343 control plants as compared to the respective treatments of ICGV 86699 and TMV 2. Significantly higher MDA content was recorded in NCAC 343 control and TMV 2 infested plants at 96 h after infestation compared with the respective treatments of other germplasms.

Protein content

Plants infested with *S. litura* had significantly greater protein content in ICGV 86699 at 24, 48 and 72 h after infestation as compared to control plants (Table 6). TMV 2 also exhibited significant difference between control and infested plants at 24 h after infestation. Significant differences were observed in protein content between control and infested plants in all the three germplasms tested at 48, 72 and 96 h. When comparing germplasms, at 24 h of infestation, control plants of ICGV 86699 showed significantly greater protein content than the control plants of NCAC 343 and TMV 2, however, infested plants of both ICGV 86699 and TMV 2 exhibited significantly higher protein content than that of NCAC 343. Protein levels were significantly higher both in control and infested plants of ICGV 86699 at 48 and 72 and 96 h after infestation than control and infested plants of NCAC 343 and TMV 2.

Larval weight

Larval weight was much lower on ICGV 86699 at 24, 48, 72 and 96 h of infestation than the larvae on NCAC 343 and TMV 2 (Fig. 1).

Table 3 Phenolic content (μg GAE g^{-1} FW) of three groundnut germplasm lines after *S. litura* infestation

Groundnut germplasm	Time after insect release (h)							
	24		48		72		96	
	Control	Infested	Control	Infested	Control	Infested	Control	Infested
ICGV 86699	73.1 ± 5.2^{a}	75.2 ± 3.8^{a}	72.7 ± 4.3^{a}	79.4 ± 5.9^{a}	75.2 ± 6.9^{a}	85.6 ± 9.0^{a}	79.3 ± 4.6^{a}	120.2 ± 11.4^{a}**
NCAC 343	54.5 ± 6.8^{b}	57.2 ± 7.9^{b}	59.3 ± 7.8^{b}	61.9 ± 6.9^{a}	67.3 ± 3.8^{ab}	73.7 ± 7.5^{ab}	72.3 ± 4.9^{ab}	81.2 ± 7.7^{b}
TMV 2	56.3 ± 6.0^{b}	59.0 ± 6.8^{b}	57.2 ± 4.3^{b}	63.8 ± 3.9^{a}	59.8 ± 6.6^{b}	69.3 ± 6.9^{b}	64.2 ± 9.3^{b}	78.3 ± 8.7^{b}

Figures (mean ± SEM) with the same letter(s) in a column within a time interval are not significantly different at $P \leq 0.05$

FW fresh weight of leaf tissue, *GAE* Gallic acid equivalent, *Control* non-infested plants

Asterisk indicates the levels of statistical significance between control and infested plants within a germplasm at each time interval

*, **, *** Significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively, by students "*t* test"

Table 4 Hydrogen peroxide content (μmol g^{-1} FW) of three groundnut germplasm lines after *S. litura* infestation

Groundnut germplasm	Time after insect release (h)							
	24		48		72		96	
	Control	Infested	Control	Infested	Control	Infested	Control	Infested
ICGV 86699	22.9 ± 3.2^{a}	28.6 ± 5.1^{a}	24.3 ± 2.9^{a}	35.9 ± 4.9^{a}**	27.6 ± 5.7^{a}	34.9 ± 6.2^{a}**	28.8 ± 5.3^{a}	38.7 ± 3.9^{b}**
NCAC 343	16.4 ± 1.9^{a}	23.6 ± 3.4^{a}*	19.8 ± 3.0^{a}	29.4 ± 2.9^{b}*	21.4 ± 2.9^{b}	27.0 ± 7.0^{b}**	26.0 ± 5.1^{a}	27.5 ± 4.8^{c}
TMV 2	14.4 ± 2.7^{a}	26.3 ± 6.2^{a}*	19.7 ± 5.9^{a}	30.0 ± 4.5^{b}*	20.3 ± 5.6^{b}	35.9 ± 6.0^{a}**	23.5 ± 6.2^{b}	44.5 ± 8.4^{a}**

Figures (mean ± SEM) with the same letter(s) in a column within a time interval are not significantly different at $P \leq 0.05$

FW fresh weight of leaf tissue, *Control* non-infested plants

Asterisk indicates the levels of statistical significance between control and infested plants within a germplasm at each time interval

*,**,*** Significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively, by students "*t* test"

Table 5 Malondialdehyde content (μmol g^{-1} FW) of three groundnut germplasm lines after *S. litura* infestation

Groundnut germplasm	Time after insect release (h)							
	24		48		72		96	
	Control	Infested	Control	Infested	Control	Infested	Control	Infested
ICGV 86699	4.9 ± 1.5^{b}	6.2 ± 2.0^{b}	6.5 ± 0.5^{b}	10.1 ± 0.9^{b}**	8.3 ± 2.2^{b}	14.7 ± 3.5^{b}**	8.7 ± 1.2^{b}	13.2 ± 3.8^{b}**
NCAC 343	8.5 ± 1.1^{a}	10.1 ± 2.9^{a}*	9.3 ± 2.1^{a}	13.7 ± 3.3^{a}**	10.3 ± 3.2^{a}	12.6 ± 2.0^{b}*	10.6 ± 2.8^{a}	12.1 ± 4.3^{b}*
TMV 2	3.3 ± 0.8^{b}	4.6 ± 1.2^{c}	4.4 ± 0.7^{c}	7.01 ± 2.1^{c}***	5.5 ± 1.9^{c}	20.4 ± 4.2^{a}**	6.5 ± 2.2^{c}	18.8 ± 2.9^{a}***

Figures (mean ± SEM) with the same letter(s) in a column within a time interval are not significantly different at $P \leq 0.05$

FW fresh weight of leaf tissue, *Control* non-infested plants

Asterisk indicates the levels of statistical significance between control and infested plants within a germplasm at each time interval

*, **, *** Significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively, by students "*t* test"

Discussion

In recent years increased emphasis has been placed on the development of effective, non-chemical strategies for managing insect pests attacking the crops. Induced resistance to pathogens and insects is viewed as a desirable crop protection strategy with relatively benign environmental impacts. It allows plants to be phenotypically plastic in order to face different stresses. Utilization of plant's own defense mechanism is an attractive area of research practiced all over the world to manage plant insect pests and diseases. In this study we examined the defensive biochemical response of three germplasm lines of groundnut to *S. litura* feeding.

The activities of POD and PPO, and protein content increased upon insect attack in all the germplasm lines. Significant differences in POD activity were observed between control and infested plants of the tested germplasm lines (Table 1). Similar results were obtained for PPO, where infested plants showed higher activity than the

Table 6 Protein content (mg g^{-1} FW) of three groundnut germplasm lines after *S. litura* infestation

Groundnut germplasm	Time after insect release (h)							
	24		48		72		96	
	Control	Infested	Control	Infested	Control	Infested	Control	Infested
ICGV 86699	26.5 ± 3.2[a]	29.2 ± 4.9[a]**	32.5 ± 8.3[a]	36.8 ± 3.2[a]*	33.0 ± 3.0[a]	39.5 ± 5.9[a]***	33.3 ± 7.0[a]	37.8 ± 4.3[a]*
NCAC 343	20.1 ± 4.4[b]	20.0 ± 5.0[b]	24.8 ± 7.5[b]	28.5 ± 4.4[b]*	27.2 ± 5.4[b]	34.2 ± 2.8[b]**	29.2 ± 6.4[b]	33.8 ± 2.0[b]*
TMV 2	23.6 ± 2.5[b]	28.6 ± 6.2[a]*	25.7 ± 4.4[b]	30.9 ± 6.0[b]*	26.8 ± 7.5[b]	31.7 ± 4.0[b]*	30.7 ± 2.7[b]	34.5 ± 9.1[b]*

Figures (mean ± SEM) with the same letter(s) in a column within a time interval are not significantly different at $P \leq 0.05$

FW fresh weight of leaf tissue, *Control* non-infested plants

Asterisk indicates the levels of statistical significance between control and infested plants within a germplasm at each time interval

*, **, *** Significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively, by students "*t* test"

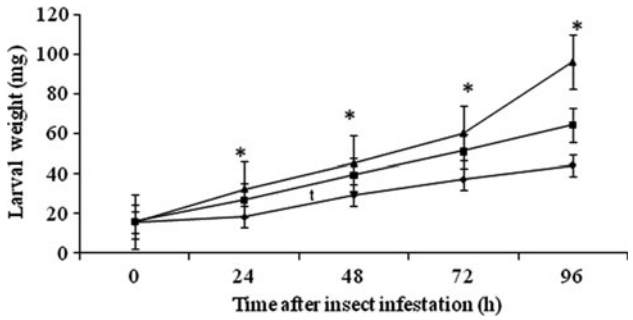

Fig. 1 *S. litura* larval weight* (mg) on three groundnut germplasm lines after infestation, *closed triangle* TMV 2; *closed square* NCAC 343; *closed diamond* ICGV 86699. Values (mean ± SEM); weight per five larvae. *Asterisk* indicates the significant difference in larval weights among the germplasms within a time interval

uninfested controls (Table 2). POD and PPO activities were activated by *S. litura* infestation; however, the expression rhythm varied among the genotypes. This might be due to the difference in sensitive up-regulation response of germplasms to biotic stress. An increase in POD activity in insect infested plants may detoxify the peroxides, thus reducing plant tissue damage (Gulsen et al. 2010). In addition to its antioxidative role, POD participates in integrated defense response of plants to a variety of stresses through cell wall toughening and production of toxic secondary metabolites (He et al. 2011). Increase in POD activity in response to insect attack can be attributed to the participation of these enzymes in lignification, suberization, somatic embryogenesis, and wound healing, as well as, defense against pathogens and other biotic and abiotic stresses (Allison and Schultz 2004; Han et al. 2009; He et al. 2011). PPO plays a pivotal role in the plant defense against insect pests by reducing the nutrient quality, digestibility and palatability of plant tissues to insects and PPO catalyzed quinones alkylate amino acids like lysine, histidine, cysteine and methionine of proteins, rendering them indigestible (Bhonwong et al. 2009). Our results agree with many previous studies, where an increase in POD (Zhang et al. 2008; Han et al. 2009; Chen et al. 2009; Gulsen et al. 2010; Usha Rani and Jyothsna 2010; He et al. 2011; War et al. 2011a, b), and PPO (Felton and Korth 2000; Ramiro et al. 2006; Bhonwong et al. 2009; He et al. 2011; War et al. 2011a, b) activities after herbivore infestation have been reported.

Total phenolic content was increased in *S. litura* infested plants in all the three germplasms, however, no statistically significant differences were recorded within the germplasms between infested and control plants except in ICGV 86699 at 96 h of infestation (Table 3). Increase in total phenols is a common reaction of plants to herbivory (Karban and Baldwin 1997). Higher accumulation of phenols in groundnut on account of bacterial and fungal infestations has been reported earlier (Rathna Kumar and Balasubramanian 2000). Phenolic compounds have been reported to impart negative effects on growth and development of insect larvae (Green et al. 2003). Moreover, the oxidation of phenols by PPO leads to the formation of quinones, ROS such as superoxide anion and hydroxyl radicals, H_2O_2, and singlet oxygen that can activate defensive enzymes in plants (Johnson and Felton 2001; Maffei et al. 2006; Howe and Jander 2008). Genotypes with insect resistance showed greater accumulation of phenols in response to insect attack, and similar results have been reported earlier (Sharma et al. 2009; Usha Rani and Jyothsna 2010; He et al. 2011; War et al. 2011a, b).

Production of ROS is a very early response to biotic stress and provides a signal in insect–plant interaction (Maffei et al. 2007). Among all the ROS, H_2O_2 has been found to play an important role in plant defense against oxidative stress due to its high stability and freely diffusible property, and acts through signal transduction pathways which lead to the expression of defense genes (Orozco-Cardenas and Ryan 1999; Foreman et al. 2003; Maffei et al. 2007). Moreover, H_2O_2 has been reported to stimulate the cascade of events that trigger physiological and molecular plant responses to prevent or minimize the

insect attack (Powell et al. 2006; Maffei et al. 2006, 2007), and also defends plants against subsequent insect and pathogen invasion (Dangl and Jones 2001; Torres et al. 2006; Maffei et al. 2007). Following *S. litura* infestation, H_2O_2 content increased in all the germplasm lines, however, significance varied at different time intervals. Overall, ICGV 86699 showed higher H_2O_2 content (Table 4). H_2O_2 has been investigated to defend plants against insects both directly and indirectly (Maffei et al. 2007; Boyko et al. 2006). Our results correlate with the findings of many workers who observed elevation in the levels of H_2O_2 in plants after herbivore feeding (Argandona et al. 2001; Walling 2000; Maffei et al. 2006; War et al. 2011a, b).

MDA content increased in all the three germplasm lines after infestation. However, induction was more in TMV 2 at 96 h than ICGV 86699 and NCAC 343 (Table 5). This might be due to the severe oxidative stress suffered by TMV 2 plants. Accumulation of MDA content after herbivore attack indicates higher stress levels and most probably results in synthesis of more complex defense compounds and activates antioxidative enzymes (Berglund and Ohlsson 1995; Gechev et al. 2002; Zhang et al. 2008). Furthermore, lipid peroxidation has been reported to induce emission of green leaf volatiles in response to plant damage (Arimura et al. 2005). Similar results have been reported earlier where MDA levels were induced by insect damage (Huang et al. 2007; Zhang et al. 2008; War et al. 2011a, b).

Proteins play an important role in plant defense. There was a significant increase in protein content on account of *S. litura* infestation (Table 6). Increase in protein concentration might be partly due to the increase in antioxidative enzyme activities after *S. litura* infestation. Plants under various biotic and abiotic stresses try to defend themselves by producing defense related enzymes and other protein based defensive compounds, thereby increasing protein concentration (Lawrence and Koundal 2002; Zavala et al. 2004; Chen et al. 2009). Elevation of protein concentration in response to insect attack has been reported in many plants (Zavala et al. 2004; Chen et al. 2009; War et al. 2011a, b).

Reduction of larval growth is an important aspect of the plant resistance to insect pests. Larval weights were lower in insects that fed on ICGV 86699 and NCAC 343 than those fed on TMV 2. Among the three germplasms ICGV 86699 significantly reduced larval weight at all the time intervals. The reduction in larval weight might be partly due to the increased levels of the PPO, POD activities, phenolics and other compounds, on account of insect attack. Alteration in digestibility and palatability of plant tissues by the induced compounds in response to insect attack affect insect growth and development adversely (Sharma et al. 2005a, b; Chen et al. 2009; Bhonwong et al. 2009; Senthil-Nathan et al. 2009; War et al. 2011a, b).

Conclusion

A considerable increase in the enzyme activities of POD, PPO and total phenols, H_2O_2, MDA, and protein contents were recorded in infested plants as compared to the control plants. These results suggest that POD, PPO, total phenols and H_2O_2 might play an important role in elevated resistance in groundnut plants against insect attack. In addition, other factors such as phenylalanine ammonia lyase, superoxide dismutase, lipoxygenase, catalase (Heng-Moss et al. 2004; Zhang et al. 2008; Chen et al. 2009; Zhao et al. 2009; Usha Rani and Jyothsna 2010), and structural components such as trichomes, wax, gland cells, and main stem thickness etc., also contribute to genotypic resistance to insects (Sharma et al. 2003; Agrawal et al. 2009; He et al. 2011). These results highlight the mechanism of response of three germplasm lines of groundnut against *S. litura* by way of induced resistance and offer a perspective on plant resistance in insect–plant interaction. The study of plant response to arthropod herbivores can help to better understand the basic mechanisms of chemical communication and plant–animal co-evolution that in turn may open new avenues for crop protection and improvement.

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