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Jasmonic Acid-Mediated-Induced Resistance in Groundnut (*Arachis hypogaea* L.) Against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)

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Abstract Jasmonic acid (JA) acts as a signal molecule to induce resistance in plants against herbivores and its levels are elevated in plants after wounding or insect damage. Groundnut is an important crop in many tropical and subtropical regions worldwide, but there is surprisingly little knowledge on its induced defenses against herbivores. The effect of JA as a spray on induced resistance in three groundnut genotypes, namely, ICGV 86699 (resistant), NCAc 343 (resistant), and TMV 2 (susceptible), against *Helicoverpa armigera* was studied. The activity of oxidative enzymes [peroxidase (POD) and polyphenol oxidase (PPO)] and the amounts of other host plant defense components [total phenols, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and protein content] were recorded at 24, 48, 72, and 96 h after pretreatment (1 day) with JA followed by infestation with *H. armigera* (PJA + HIN) and *H. armigera* infestation with simultaneous JA application (HIN + JA) to understand the consequences of induced resistance in groundnut. The plant damage, larval survival, and larval weights were also recorded. There was a significant increase in POD and PPO activities and in the amounts of total phenols, H_2O_2, MDA, and proteins in PJA + HIN- and JA + HIN-treated plants as compared to the plants treated with JA and infested with *H. armigera* individually and to untreated control plants. Among all the genotypes, the strongest induction of defense was observed in the ICGV 86699 genotype. It is concluded that pretreatment with JA and its application during low levels of insect infestation can increase the levels of host plant resistance against herbivorous insects and reduce the pest-associated losses in groundnut.

Keywords Antioxidant enzymes · Herbivory · Chemical defense · Hydrogen peroxide · Malondialdehyde · Peroxidase · Polyphenol oxidase

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Introduction

Cotton bollworm (*Helicoverpa armigera* Hubner) is a major polyphagous pest and reduces the yield in many crops, including cotton, groundnut, sorghum, maize, chickpea, and pigeonpea in Asia, Africa, Australia, and Mediterranean Europe (Sharma and others 2005). This insect has developed resistance to a number of synthetic insecticides (Kranthi and others 2002). The yield of groundnut is severely affected by *H. armigera*. Insecticides used against *H. armigera* are harmful to beneficial insects and natural enemies (Wu and others 2004) and also lead to the development of pesticide resistance in pests (Kranthi and others 2002). Therefore, there is a general need for alternative methods of pest control. The induction of host plant resistance to insects is one of the most important and widespread defenses adopted by plants against herbivory (Karban 2011). Improving host plant resistance to insects will result in reduced losses due to herbivores, less insecticide use, better crop yields, and a safer environment to live. Although induced responses have some metabolic costs (Agrawal 2000), they are highly important when aimed at the stress of immediate concern. Moreover, many of these chemicals are produced when on demand (Miranda and others 2007; Karban 2011).

Plants possess several physical and chemical characteristics to defend themselves against herbivores (Sharma and others 2009). The resistance strategies could be constitutive, that is, they are always present in the plant independent of herbivore attack (Franceschi and others 2005), or inducible, activated only when the plant is attacked (Kessler and Baldwin 2002). Induced resistance can be direct or indirect. Direct induced resistance is mediated by the accumulation of defense-related proteins to protect the plants against insects or the plants may produce noxious chemicals to reduce herbivore feeding, oviposition, growth, and development (Senthil-Nathan and others 2009). Indirect induced resistance results in release of a blend of volatile compounds that specifically attract natural enemies (predators or parasitoids) of the herbivore species feeding on the plant (Bruinsma and Dicke 2008).

In recent decades, several plant chemical elicitors such as jasmonic acid (JA), methyl jasmonate, salicylic acid, methyl salicylate, and ethylene have been used to induce defensive responses in plants against insect herbivory and/or pathogen damage (Moreira and others 2009; Venu and others 2010). These endogenous plant phytohormones are involved in triggering induced defense responses after insect damage (Halitschke and Baldwin 2005; Moreira and others 2009). Jasmonic acid is considered to be one of the most important elicitors of plant defenses against herbivores, mediating the expression of both direct and indirect defenses (Scott and others 2010; Shivaji and others 2010) and accumulating rapidly in plant tissues near the site of herbivore attack (Moreira and others 2009). JA, a ubiquitous regulator of the wound-induced response in plants, is an essential component of the signaling pathway in crop plants, and when applied exogenously, it induces several defense-related responses, including the activation of oxidative enzymes, proteinase inhibitors, and alkaloids, and production of volatile compounds (Wasternack 2007; Scott and others 2010).

The patterns of induction by JA have been reported to be similar to herbivory for polyphenol oxidase (PPO), peroxidase (POD), LOX, proteinase inhibitors, and other defensive compounds. Because of their potential roles in the synthesis of defense compounds and/or in oxidative stress tolerance, JA and related compounds are being implicated in host plant resistance against insect herbivores (Thaler and others 2001; Wasternack 2007).

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop cultivated worldwide in tropical and subtropical regions and is valued for high-quality edible oil and easily digestible protein in its seeds. Globally, groundnut cultivation occupies about 23.4 million ha, with an annual production of 34.9 million metric tons (Food and Agriculture Organization 2007). India is the largest groundnut producer in the world. Large numbers of insect pests damage this crop, including thrips, aphids, white grubs, leafhoppers, armyworm (*Spodoptera litura*), and cotton bollworm (*H. armigera*) (Sharma and others 2005).

Although considerable progress has been made in identifying genotypes resistant to insect pests, progress toward characterization of physiological and biochemical mechanisms conferring resistance remains limited (Heng-Moss and others 2004). There are several reports of induced resistance to insects in many plant species by exogenous application of JA. However, such interactions have not been studied in detail in groundnut. Therefore, the aim of the present study was to investigate whether exogenous application of JA induces plant defense in groundnut against *H. armigera*. The study focused on the oxidative enzymes such as POD and PPO and other defensive components (total phenols, H_2O_2, MDA, and total proteins) in relation to plant damage, survival, and development of the insect.

Materials and Methods

Chemicals

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

Groundnut Plants (*Arachis hypogaea*)

Seeds of three groundnut genotypes were obtained from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Andhra Pradesh, India. The three groundnut genotypes were ICGV 86699 (resistant), NCAC 343 (moderately resistant), and TMV 2 (susceptible) (Sharma and others 2003). Groundnut plants were grown in pots with a mixture of soil, sand, and vermicompost (2:1:1) as substrate. The plants were watered as needed. Utmost care was taken to prevent the plants from insect attack other than the experimental insect by enclosing them in cages. Twenty-day-old groundnut plants were used for the study.

Helicoverpa armigera Infestation

Newly emerged (1 or 2 days old) larvae of *H. armigera* were obtained from the stock culture maintained on artificial diet under laboratory conditions (26 ± 1°C, 11 ± 0.5 h photoperiod, and 75 ± 5% relative humidity) from the insectary of the Entomology Research Institute, Loyola College, Chennai, India. Ten larvae were gently placed on each 20-day-old plant by using a camel hair brush.

Jasmonic Acid Application

To prepare 1 mM JA solution, 21 mg of JA was dissolved in 1 ml of ethanol and the JA/ethanol solution was dispersed in 100 ml of distilled water to make the desired concentration (Hamm and others 2010). Plants were sprayed with 1 mM JA until runoff, followed by insect infestation. Plants in each genotype were grouped into five sets with ten replicates in each:

Group I: One day pretreatment with JA and infested with *H. armigera* (PJA + HIN)
Group II: Infested with *H. armigera* (HIN)
Group III: *H. armigera* + JA (1 mM) spray (HIN + JA)
Group IV: JA (1 mM) spray only (JA)
Group V: control (sprayed with ethanol dissolved in water)

After 24, 48, 72, and 96 h of treatment, leaves were collected from the plants for evaluating induced resistance.

Enzyme Extraction

Fresh leaves (0.5 g) were frozen in liquid nitrogen and ground in 3 ml of ice cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol, 1% polyvinylpyrolidone (PVP), and 0.5 mM EDTA. The homogenate was centrifuged at 16,000 *g* for 25 min and the supernatant was used as an enzyme source. All spectrophotometric analyses were performed on a Hitachi UV-2010 spectrophotometer.

Peroxidase Assay

Peroxidase activity was estimated as per the method of Shannon and others (1966) with slight modification. The reaction mixture (2.9 ml) containing 0.1 M sodium phosphate buffer (pH 6.5), 0.8 mM H_2O_2, and 5 mM guaiacol was taken in a test tube. A total of 0.1 ml of enzyme source was added to it and the absorbance was read at 470 nm for 2 min at 15-s intervals. Enzyme activity was expressed as IU g^{-1} fresh weight (FW). One unit of POD activity was defined as the change in absorbance by 0.1 unit per minute under conditions of assay.

Polyphenol Oxidase Assay

Polyphenol oxidase 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 catechol) were added. Absorbance was read at 420 nm for 3 min at 30-s intervals. 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.

Phenolic Content

Phenolic content of treated leaves was estimated as per the Zieslin and Ben-Zaken (1993) method with some modifications. Fresh leaves (0.5 g) were homogenized with 3 ml of 80% methanol and agitated for 15 min at 70°C. To 2 ml of 2% sodium carbonate (Na_2CO_3), 1 ml of methanol extract was added. The solution was incubated for 5 min at room temperature after which 0.1 ml of Folin-Ciocalteau reagent was added. The solution was incubated again for 10 min and absorbance of the blue color was measured at 760 nm. Phenolic concentration was determined from a standard curve prepared with gallic acid and was expressed as µg gallic acid equivalents per gram FW (µg GAE g^{-1} FW).

Hydrogen Peroxide Content

Hydrogen peroxide 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) with a prechilled 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 substance (TBARS) concentration as described by Carmak and Horst (1991) with minor modification. Fresh leaf tissue (0.2 g) was homogenized in 3 ml of 0.1% (w/v) trichloroacetic acid (TCA) at 4°C. The homogenate was centrifuged at 20,000×*g* for 15 min. A total of 0.5 ml of supernatant was added to 3 ml of 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 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.

Protein Content

Protein was determined according to the method of Bradford (1976) using bovine serum albumin as substrate.

Larval Survival and Larval Weight

After 96 h of infestation, larvae were recovered from the plants, counted, and weighed to record data on insect survival and larval weights. Larvae were again carefully released on the same plants to allow feeding for two more days. At 6 days after infestation (DAI), larvae were again recovered and weighed. The larvae recovered were starved for 4 h, after which their weights were recorded using a digital balance (Mettler Toledo, AB304-S). Plants were assessed for insect damage at 6 DAI by visually rating using a scale of 1-9, with 1 being no or slight damage (≤10%) and 9 being at least 90% damage.

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) to compare the differences between treatments within a genotype and among the genotypes using SAS ver. 9.2 (SAS Institute, Cary, NC, USA).

Results

Peroxidase Activity

Plants treated with PJA + HIN exhibited significantly higher POD activity in all three genotypes [ICGV 86699 ($F_{(2,\ 11)}$ 45.5, 89.9, 143.5, and 165.9 at 24, 48, 72, and 96 h, respectively, $P < 0.001$); NCAc 343 ($F_{(2,\ 11)}$ 24.4, 57.2, 45.4, and 72.1 at 24, 48, 72, and 96 h, respectively, $P < 0.001$); and TMV 2 ($F_{(2,\ 11)}$ 31.2, 23.1, 65.8, and 76.5 at 24, 48, 72, and 96 h, respectively, $P < 0.01$)] compared to other treatments (Fig. 1). Of the three genotypes tested, ICGV 86699 exhibited significant differences in POD activity in all the treatments at 24 h ($F_{(2,\ 18)}$ 67.4, 56.5, 45.4, 27.4, and 12.3 with PJA + HIN, HIN, HIN + JA, JA, and control, respectively, $P < 0.01$), 48 h ($F_{(2,\ 18)}$ 124.6, 101.4, 67.5, 174.4, and 50.6 with PJA + HIN, HIN, HIN + JA, JA, and control, respectively, $P < 0.001$), 72 h ($F_{(2,\ 18)}$ 214.7, 171.8, 76.7, 98.4, and 30.3 with PJA + HIN, HIN, HIN + JA, JA, and control, respectively, $P < 0.001$), and 96 h after treatment ($F_{(2,\ 18)}$ 114.3, 99.3, 123.5, 84.9, and 2.1 with PJA + HIN, HIN, HIN + JA, JA, and control, respectively, $P < 0.001$).

Polyphenol Oxidase Activity

The PJA + HIN-treated plants showed significantly greater PPO activity in ICGV 86699 ($F_{(2,\ 11)}$ 8.9, 19.3, 33.4, and 65.4 at 24, 48, 72, and 96 h, respectively, $P < 0.05$), NCAc 343 ($F_{(2,\ 11)}$ 12.2, 5.4, 7.3, and 16.4 at 24, 48, 72, and 96 h, respectively, $P < 0.05$), and TMV 2 ($F_{(2,\ 11)}$ 4.9, 32.2, 46.5, and 42.1 at 24, 48, 72, and 96 h, respectively, $P < 0.05$) compared to the plants treated with HIN and JA (Fig. 2). Across the three genotypes tested, ICGV 86699 exhibited significantly higher PPO activity than NCAc 343 and TMV 2 in PJA + HIN, HIN, HIN + JA, and control treatments at 24 h, in PJA + HIN, HIN, HIN + JA, and JA treatments at 48 h, and in PJA + HIN and HIN + JA at 72 and 96 h.

Total Phenols

Plants treated with PJA + HIN had higher amounts of phenolic compounds in ICGV 86699 ($F_{(2,\ 11)}$ 99.2, 63.5, 120.3, and 132.4 at 24, 48, 72, and 96 h, respectively, $P < 0.001$), NCAc 343 ($F_{(2,\ 11)}$ 24.6, 74.3, 121.2, 187.9 at 24, 48, 72, and 96 h, respectively, $P < 0.01$), and TMV 2 ($F_{(2,\ 11)}$ 101.2, 45.4, 37.8, and 154.9 at 24, 48, 72, and 96 h, respectively, $P < 0.01$) compared to the other treatments (Fig. 3). ICGV 86699 had significantly higher amounts of phenolic compounds in all the treatments at 24 h ($P < 0.001$), 48 h ($P < 0.001$), 72 h ($P < 0.01$), and 96 h ($P < 0.001$) than did TMV 2 and NCAc 343. However, NCAc 343 also exhibited significantly higher phenolic content at 72 and 96 h in HIN-treated plants than those of TMV 2 ($P < 0.01$).

Hydrogen Peroxide Content

The PJA + HIN-treated plants had significantly higher H_2O_2 content in ICGV 86699 ($F_{(2,\ 11)}$ 18.4, 43.3, 18.2, and 76.6 at 24, 48, 72, and 96 h, respectively, $P < 0.001$), NCAc 343 ($F_{(2,\ 11)}$ 6.5, 34.6, 19.8, and 46.2 at 24, 48, 72, and 96 h, respectively, $P < 0.001$), and TMV 2 ($F_{(2,\ 11)}$ 6.3, 11.5, 28.7, and 54.8, respectively, at 24, 48, 72, and 96 h, $P < 0.05$) than in other treatments (Fig. 4). ICGV 86699 had significantly higher H_2O_2 content in PJA + HIN, HIN + JA, and JA treatments at 24 h than did NCAc 343 and TMV 2. Both ICGV 86699 and NCAc 343 showed higher H_2O_2 levels in PJA + HIN, HIN, HIN + JA, and control plants at 48, 72, and 96 h than the respective treatments of TMV 2. However,

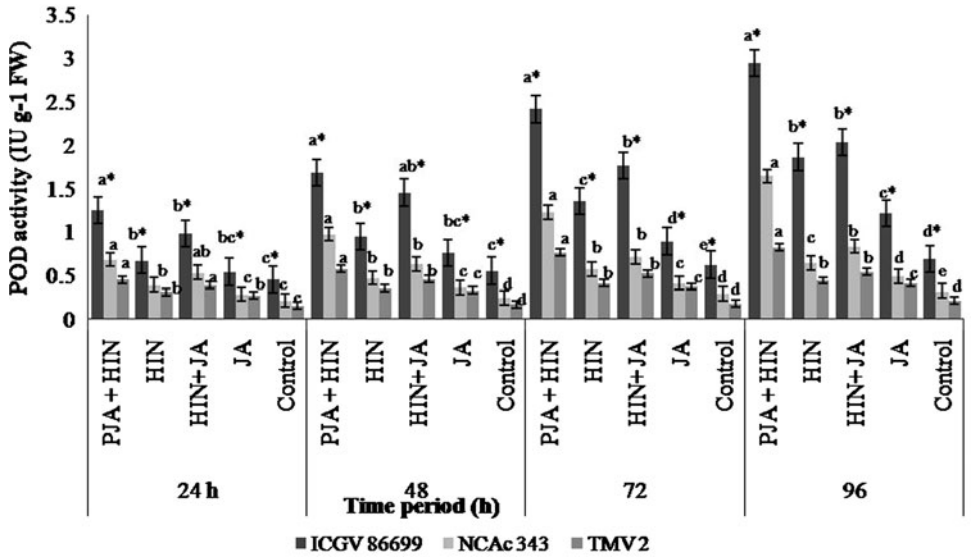

Fig. 1 POD activity (IU g^{-1} FW) of three groundnut genotypes after *H. armigera* infestation and JA application. *Bars* (mean ± SE) of same color with similar *letters* within a time interval are not statistically different at $P < 0.05$. * on *bars* within a treatment shows significant difference among the genotypes ($P < 0.05$). HIN *H. armigera*-infested, *PJA + HIN* pretreatment with JA and infested with *H. armigera*, *HIN + JA H. armigera*-infested + jasmonic acid sprayed, *JA* jasmonic acid sprayed, *control* noninfested plants sprayed with ethanol in water, *FW* fresh weight of leaf tissue

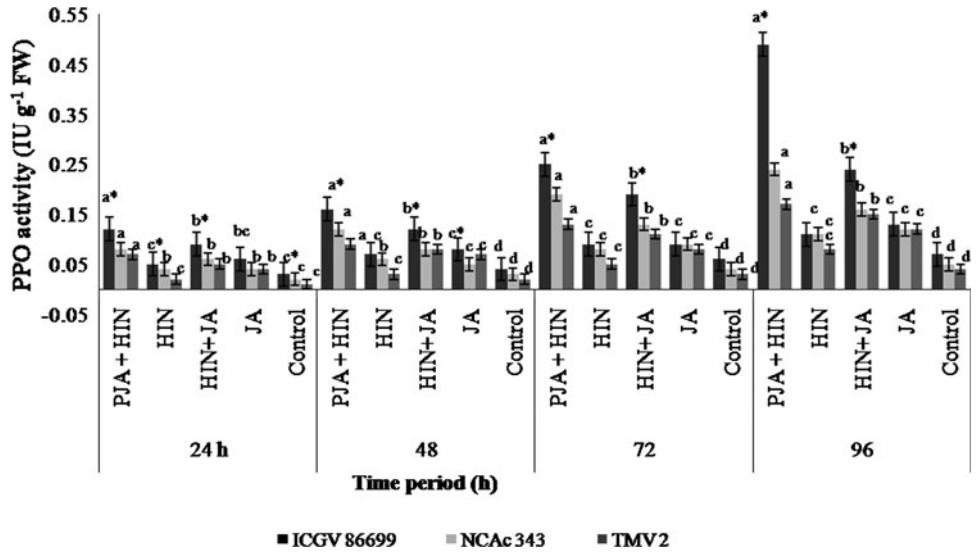

Fig. 2 PPO activity (IU g^{-1} FW) of three groundnut genotypes after *H. armigera* infestation and JA application. *Bars* (mean ± SE) of same colors with similar *letters* within a time interval are not statistically different at $P < 0.05$. * on *bars* within a treatment shows significant difference among the genotypes ($P < 0.05$). *HIN* *H. armigera*-infested, *PJA + HIN* pretreatment with JA and infested with *H. armigera*, *HIN + JA H. armigera*-infested + jasmonic acid sprayed, *JA* jasmonic acid sprayed, *control* noninfested plants sprayed with ethanol in water, *FW* fresh weight of leaf tissue

all the treatments of ICGV 86699 exhibited higher H_2O_2 levels at 72 and 96 h (all, $P < 0.01$). Furthermore, H_2O_2 content of NCAc 343 plants treated with JA was at par with that of ICGV 86699 at 72 h ($P < 0.05$).

Malondialdehyde Content

The MDA content increased on account of various treatments at different time intervals (Fig. 5). Treatment with PJA + HIN induced significantly higher MDA levels in all the tested genotypes at 48, 72, and 96 h compared to the other respective treatments. HIN +JA-treated NCAc 343 plants showed higher MDA content which was on par with that of ICGV 86699 at 72 and 96 h. However, at 96 h, HIN + JA induced significantly higher MDA content ($F_{(2,\ 11)}$ 72.3, $P < 0.001$) in NCAc 343 than that in ICGV 86699 and TMV 2. There were no significant differences in MDA content among the three genotypes in all the

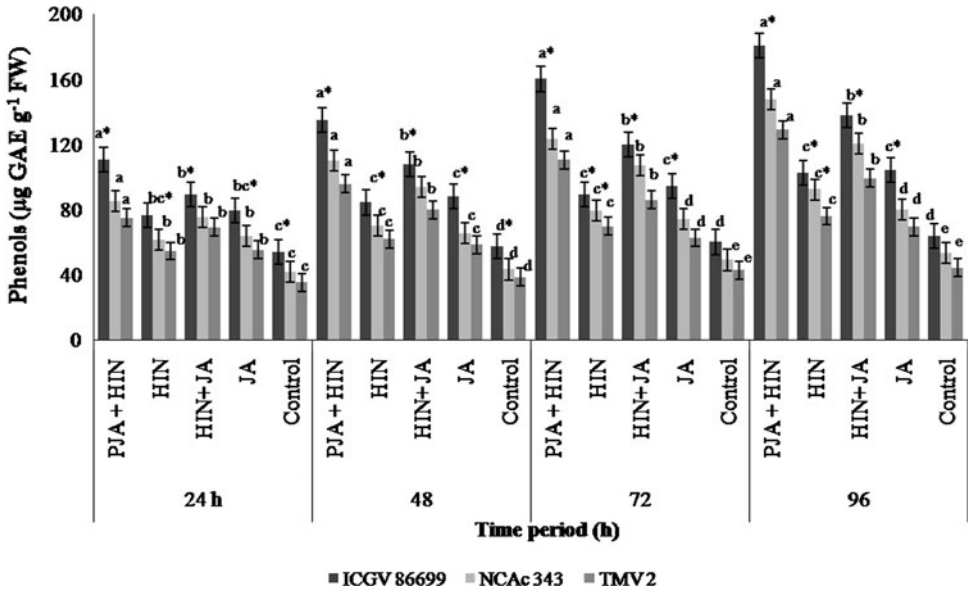

Fig. 3 Total phenols (µg GAE g^{-1} FW) of three groundnut genotypes after *H. armigera* infestation and JA application. *Bars* (mean ± SE) of same colors with similar *letters* within a time interval are not statistically different at $P < 0.05$. * on *bars* within a treatment shows significant difference among the genotypes ($P < 0.05$). *HIN H. armigera*-infested, *PJA + HIN* pretreatment with JA and infested with *H. armigera*, *HIN + JA H. armigera*-infested + jasmonic acid sprayed, *JA* jasmonic acid sprayed, *control* noninfested plants sprayed with ethanol in water, *FW* fresh weight of leaf tissue, *GAE* gallic acid equivalents

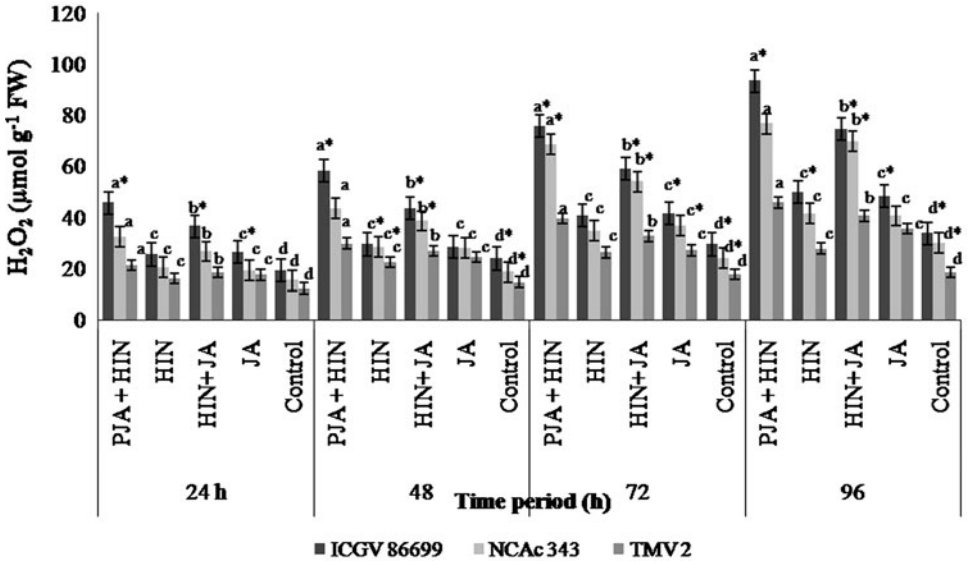

Fig. 4 H_2O_2 content (µmol g^{-1} FW) of three groundnut genotypes after *H. armigera* infestation and JA application. *Bars* (mean ± SE) of same colors with similar *letters* within a time interval are not statistically different at $P < 0.05$. * on *bars* within a treatment shows significant difference among the genotypes ($P < 0.05$). *HIN H. armigera*-infested, *PJA + HIN* pretreatment with JA and infested with *H. armigera*, *HIN + JA H. armigera*-infested + jasmonic acid sprayed, *JA* jasmonic acid sprayed, *control* noninfested plants sprayed with ethanol in water, *FW* fresh weight of leaf tissue

treatments at 24 h (all, $P > 0.5$). However, significant differences in MDA content were observed in HIN + JA-treated ICGV 86699 at 48 h compared to that of NCAc 343 and TMV 2. ICGV 86699 showed significantly higher MDA levels in PJA + HIN-treated plants at 72 h. At 96 h, ICGV 86699 showed higher MDA content in all the treatments than that in NCAc 343 and TMV 2, except in JA-alone-treated and untreated control plants where both ICGV 86699 and TMV 2 showed higher MDA content.

Proteins

The PJA + HIN and HIN + JA treatments resulted in greater protein content in ICGV 86699 and NCAc 343 at

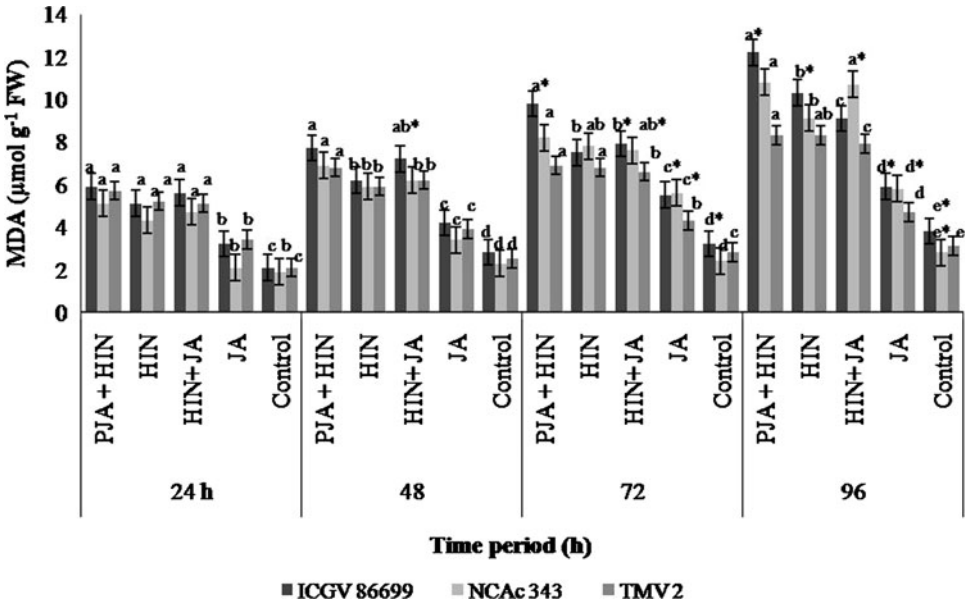

Fig. 5 MDA content (μmol g^{-1} FW) of three groundnut genotypes after *H. armigera* infestation and JA application. *Bars* (mean ± SE) of same colors with similar *letters* within a time interval are not statistically different at $P < 0.05$. * on *bars* within a treatment shows significant difference among the genotypes ($P < 0.05$). *HIN H. armigera*-infested, *PJA + HIN* pretreatment with JA and infested with *H. armigera*, *HIN + JA H. armigera*-infested + jasmonic acid sprayed, *JA* jasmonic acid sprayed, *control* noninfested plants sprayed with ethanol in water, *FW* fresh weight of leaf tissue

24 h than the other treatments (Fig. 6). Plants treated with PJA + HIN had significantly higher protein content in all the genotypes at all the time intervals than the other treatments. ICGV 86699 had significantly greater protein content at 24 h in plants treated with PJA + HIN, HIN +JA, and HIN (all, $P < 0.05$). Furthermore, significantly greater protein contents were observed in all the treatments in ICGV 86699 at 48, 72, and 96 h than those of NCAc 343 and TMV 2.

Plant Damage, Larval Survival, and Larval Weight

TMV 2 suffered greater insect damage than ICGV 86699 and NCAC 343. After 6 DAI, the leaf damage was low in PJA + HIN-treated ICGV 86699 (3.5) and NCAc 343 (5.1) compared to that in TMV 2 (5.9) (Table 1). However, leaf damage of plants treated with HIN + JA and HIN were 4.0 and 4.6, 6.1 and 7.4, and 6.9 and 8.4, respectively in ICGV 86699, NCAc 343, and TMV 2. Larval survival was

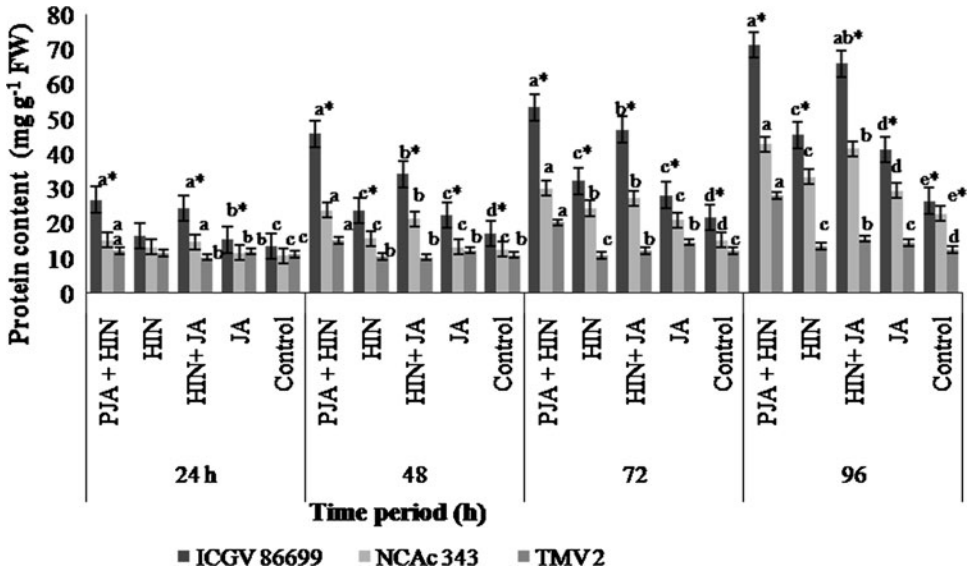

Fig. 6 Protein content (mg g^{-1} FW) of three groundnut genotypes after *H. armigera* infestation and JA application. *Bars* (mean ± SE) of same colors with similar *letters* within a time interval are not statistically different at $P < 0.05$. * on *bars* within a treatment shows significant difference among the genotypes ($P < 0.05$). *HIN H. armigera*-infested, *PJA + HIN* pretreatment with JA and infested with *H. armigera*, *HIN + JA H. armigera*-infested + jasmonic acid sprayed, *JA* jasmonic acid sprayed, *control* noninfested plants sprayed with ethanol in water, *FW* fresh weight of leaf tissue

Table 1 Plant damage, *H. armigera* larval survival, and weight after feeding on three groundnut genotypes

Groundnut genotypes	Plant damage (6 DAI)[A]			Survival (%) (6 DAI)			Larval weight (mg)[B]					
							96 h			6 DAI		
	PJA + HIN	HIN	HIN + JA	PJA + HIN	HIN	HIN + JA	PJA + HIN	HIN	HIN + JA	PJA + HIN	HIN	HIN + JA
ICGV 86699	3.5^{c}	4.6^{c}	4.0^{c}	20.4 ± 2.1^{b}	38.3 ± 2.3^{b}	33.8 ± 4.6^{b}	28.3 ± 3.4^{b}	47.5 ± 2.1^{b}	45.4 ± 4.3^{b}	37.1 ± 1.9^{b}	62.5 ± 3.1^{b}	50.2 ± 2.7^{b}
NCAc 343	5.1^{b}	7.4^{b}	6.1^{b}	40.7 ± 3.2^{b}	49.8 ± 5.6^{b}	44.2 ± 7.2^{b}	32.5 ± 2.5^{b}	44.7 ± 4.9^{b}	40.6 ± 5.0^{b}	44.5 ± 2.8^{b}	69.6 ± 4.5^{c}	49.6 ± 5.8^{c}
TMV 2	5.9^{a}	8.4^{a}	6.9^{a}	63.3 ± 6.1^{a}	79.4 ± 6.8^{a}	75.9 ± 6.3^{a}	55.6 ± 5.8^{a}	70.3 ± 9.9^{a}	67.4 ± 3.9^{a}	67.6 ± 3.3^{a}	98.4 ± 3.8^{a}	90.6 ± 7.8^{a}

Values (mean ± SEM) carrying same letter(s) within a column are not significantly different

DAI days after infestation, *PJA + HIN* pretreatment with JA for 1 day and infested with *H. armigera*, *HIN H. armigera*-infested, *HIN + JA H. armigera*-infested + jasmonic acid sprayed

[A] Damage rating on a scale of 1–9 (1 = ≤10% and 9 = ≥90%) 6 days after infestation

[B] Weight per five larvae at the time of recovery

significantly lower in ICGV 86699 (20.4, 33.8, and 38.3%) and NCAc 343 (40.7, 44.2, and 49.8%) plants treated with PJA + HIN, HIN + JA, and HIN, respectively (Table 1). In TMV 2, larval survival was 63.3, 75.9, and 79.4% in PJA + HIN-, HIN + JA-, and HIN-treated plants, respectively, at 6 DAI. The weights of larvae recovered at 6 DAI were lower in ICGV 86699 (37.1, 62.5, and 50.2 mg per five larvae) and NCAc 343 (44.5, 69.6, and 49.6 mg per five larvae) than in TMV 2 (67.6, 98.4, and 90.6 mg per five larvae) treated with PJA + HIN, HIN, and HIN + JA, respectively.

Discussion

Induced resistance in plants against herbivores and pathogens is viewed as an enviable crop protection strategy with relatively benign environmental impact. It allows plants to act dynamically and makes them phenotypically plastic in order to face different stresses (Karban 2011). Secondary metabolites and defensive proteins accumulate in plant tissues as a result of insect damage or pathogen infestation and defend the plants against further damage by insects and/or pathogens (Torres and others 2006). The octadecanoid pathway(s) regulated by JA is one of a plant's important defenses against insect herbivores (Scott and others 2010), and several classes of compounds that confer resistance to plants against a number of insect pests are induced by JA (Cipollini and others 2004; Shivaji and others 2010).

Significantly greater peroxidase activity was observed in PJA + HIN-treated plants followed by plants treated with HIN + JA in all the tested genotypes. Across the genotypes, ICGV 86699 exhibited significantly greater POD activity in all the treatments throughout the test period compared to NCAc 343 and TMV 2. However, a progressive increase in POD activity was observed in all the treatments. POD activity kept increasing in all three genotypes; however, the elevated levels of POD activity were lower overall in NCAc 343 and TMV 2 than in ICGV 86699. Crop cultivars resistant to insect pests would have either a higher upregulation capacity for peroxidase or a more sensitive upregulation response or both (Heng-Moss and others 2004; Gulsen and others 2010). POD activity has been regarded as part of the immediate response to insect damage (Moloi and van der Westhuizen 2006; He and others 2010). Induction of POD activity in response to JA application or insect attack might enhance the cell lignifications, wound healing, and production of secondary metabolites, besides increasing the amounts of detoxifying peroxides that defend the plants against insects, pathogens, and other stresses (Allison and Schultz 2004; Heng-Moss and others 2004; Han and others 2009; Gulsen and others

2010). Our study shows that pretreatment with JA and infestation with *H. armigera* induced high levels of POD activity. This is in line with results of earlier studies (Cipollini and others 2004; Gould and others 2009; Shivaji and others 2010) and has been associated with host plant resistance against insect pests (Huang and others 2007; Chen and others 2009; Usha Rani and Jyothsna 2010; Gulsen and others 2010; Barbehenn and others 2010).

An increase in PPO activity in response to stresses is a common phenomenon (Zhao and others 2009). Greater PPO activity was observed in PJA + HIN-treated plants followed by HIN + JA-treated plants. Pretreatment with JA strongly induced the PPO activity in all three genotypes and was very high at 96 h after insect infestation. Although all the genotypes showed increased PPO activity, overall levels were high in ICGV 86699. PPO is the key secondary metabolism enzyme and its activity has been associated with bioprotection of plants against insect damage (Zhang and others 2008). PPO reduces the nutrient quality, digestibility, and palatability of plant tissues to insects and catalyzes the oxidation of phenols leading to the production of toxic quinines, a response induced by herbivory (Thipyapong and others 2006; Zhang and others 2008; Bhonwong and others 2009), pathogen infection (Raj and others 2006; Ballhorn and others 2010; Ballhorn 2011), wounding, or treatment with methyl jasmonate (Wang and Constabel 2004). Quinines and quinine-generated ROS can alkylate amino acids in proteins, rendering them indigestible (Mahanil and others 2008; Bhonwong and others 2009). Exogenous application of JA has been reported to induce PPO activity in many plants (Cipollini and others 2004; Zhao and others 2009; Gould and others 2009; Bhonwong and others 2009). Induction of these enzymes in response to insect damage stimulates the biosynthesis of phenylpropanoids and other secondary metabolites that confer resistance/tolerance to herbivory (He and others 2010).

A high concentration of foliar phenolics generally provides plant resistance against insect herbivores. Treatment with PJA + HIN induced higher phenols in ICGV 86699, NCAc 343, and TMV 2 as compared to the other treatments. Among all the genotypes tested, ICGV 86699 had high amounts of phenols and there was a progressive increase with time. Increased production of phenols as a result of PJA + HIN treatment might be due to the signaling of defensive pathways by insect damage and JA. Accumulation of phenols is a common reaction of plants to herbivory that affects insect feeding and development adversely (Kessler and Baldwin 2002; Ramiro and others 2006; Sharma and others 2009; Usha Rani and Jyothsna 2010). Furthermore, quinines and ROS such as superoxide ($O^{\cdot -}$), hydroxyl radicals (HO^{-}), hydrogen peroxide (H_2O_2), and singlet oxygen (O^{2-}) formed by the oxidation of phenols activate the defensive enzymes that induce resistance in plants against insects (Johnson and Felton 2001).

The production of ROS is a preliminary response of plants to biotic stress that provides a signal in insect–plant interactions (Maffei and others 2007). The highly stable and freely diffusible nature of H_2O_2 makes it an important component of plant defense against oxidative stress through signal transduction pathways which lead to the expression of defense genes (Maffei and others 2007). Hydrogen peroxide content was significantly greater in all the plants treated with PJA + HIN followed by HIN + JA. Among the genotypes tested, H_2O_2 was more elevated in ICGV 86699 in all the treatments and time intervals; however, H_2O_2 content of NCAc 343 plants was at par with ICGV 86699 at 24 h in HIN + JA, in all the treatments (except HIN) at 72 h, and in HIN + JA-treated and control plants at 96 h. Because accumulation of H_2O_2 was higher in PJA + HIN and HIN + JA treatments, this suggests that pretreatment with JA exogenously stimulates higher production of ROS after initial damage by insects, which in turn signals the production of defense compounds (Maffei and others 2006; Guo and others 2010). Hydrogen peroxide has been shown to act as a second messenger in JA-mediated defense signaling that acts downstream from JA and corresponds with oxidative damage in the midgut of insects feeding on previously wounded plants (Orozco-Cárdenas and others 2001; Usha Rani and Jyothsna 2010). Many studies have demonstrated the induction of resistance in plants against insects by elevated levels of H_2O_2 (Walling 2000; Maffei and others 2006; Barbehenn and others 2010; Guo and others 2010; He and others 2010; Usha Rani and Jyothsna 2010).

Malondialdehyde has been used as a suitable biomarker for oxidative stress (Gechev and others 2002). An increase in MDA content was observed in different treatments; however, significant differences were found only at later stages. Higher MDA content was induced by PJA + HIN in all the genotypes tested, followed by HIN + JA. One of the best-known lipid oxidation products is MDA, which is commonly used to determine lipid peroxidation. It is an important step in host plant defense against biotic stresses (Huang and others 2007). Lipid peroxidation under biotic stress may be due to higher H_2O_2 levels, which in turn could function as a signal for induction of defense systems and enhance the production of secondary metabolites (Zhang and others 2008). Jasmonic acid is regarded as a cellular signal for activation of plant defense responses, including lipid peroxidation (Reymond and Farmer 1998). Stimulation of emission of green leaf volatiles by lipid peroxides in response to plant damage has been reported (Arimura and others 2009). An increase in MDA levels in insect-resistant groundnut after PJA + HIN treatment most

probably results in synthesis of more complex defense compounds. Increased levels of MDA have also been found to induce plant resistance against many insect pests (Huang and others 2007; Boka and others 2007).

Increased levels of defense-related proteins are a general phenomenon in plants on account of biotic and abiotic stresses (Chen and others 2009). The present study showed that there was a significant elevation of proteins in PJA + HIN- followed by HIN + JA-treated ICGV 86699 plants at all the time intervals, and the highest levels were reached at 96 h. Among the tested genotypes, ICGV 86699 had higher protein content than NCAc 343 and TMV 2 in plants treated with PJA + JA and HIN + JA. An increase in protein concentration may be due to increased antioxidative enzymes after treatment. Differences in induction of proteins in different genotypes might be due to the difference in their response to biotic stress. Defense-related enzymes and other protein-based defensive compounds accumulate in plants in response to oxidative stress and defend them from biotic and abiotic stresses (Chen and others 2009). Elevation in protein concentration in plants might be due to changes in physiological processes resulting in increased production of protein-based defensive compounds. Induction of proteins and their role in induced resistance against insect pests has been demonstrated in many plants (Lawrence and Koundal 2002; Zavala and others 2004; Chen and others 2009; Piotrowska and others 2010).

Survival and development of insects are important components of host plant resistance to insect pests (Sharma and others 2005). Insect damage was less in ICGV 86699 and NCAc 343 than in TMV 2. Greater larval mortality was recorded for insects that fed on ICGV 86699 and NCAc 343 than for those that fed on TMV 2. Larval weights were also lower in insects that fed on ICGV 86699 and NCAc 343 than those that fed on TMV 2. There were significant differences in the larval weights of insects reared on plants treated with PJA + JA, HIN + JA, and HIN. Reduced damage, decreased larval survival, and low larval weights might be due to higher induction of secondary metabolites and other defensive compounds in the insect-resistant genotypes on account of insect damage and JA application (Lawrence and Koundal 2002; Sharma and others 2005; Bhonwong and others 2009; Chen and others 2009). Induction of resistance due to increased activity of POD in tomato, barley, and lettuce (Stout and others 1999; Chaman and others 2001; Sethi and others 2009) and of PPO in tomato, poplar, barley, and lettuce (Wang and Constabel 2004; Chaman and others 2001; Bhonwong and others 2009; Sethi and others 2009) has been correlated with reduction of insect growth and development. Plant defensive compounds induced in insect-resistant genotypes reduce the survival and development of *S. frugiperda* larvae (Chen and others 2009). Sharma and others (2005) observed that resistant genotypes resulted in reduced weight gain in *H. armigera* larvae as compared to susceptible genotypes.

Conclusion

The present study suggests that the pretreatment of groundnut with JA followed by insect infestation induced higher levels of resistance in plants as is evident from reduced plant damage, low larval survival, and larval weights. This seemed to be mediated through POD, PPO, and other defensive compounds such as phenols, H_2O_2, and MDA, which were also elevated in JA-pretreated plants. A quick response was shown by all genotypes to PJA + HIN treatment by increasing POD and PPO activities and concentrations of other defensive compounds. Systemic induction of oxida tive enzymes and other defensive compounds in PJA + HIN- and HIN + JA-treated plants suggested that pretreatment with elicitors such as JA can play an important role in induced resistance in plants to avoid subsequent pest attack and thus serves as an important component of insect pest management for sustainable crop protection.

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