Physiological Entomology

Physiological Entomology (2014) **39**, 136–142 DOI: 10.1111/phen.12057

Effect of jasmonic acid and salicylic acid induced resistance in groundnut on *Helicoverpa armigera*

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Abstract. Induced resistance in plants affects insect growth and development as a result of the up-regulation of defence-related secondary metabolites or enzyme-binding proteins. In the present study, the effects of jasmonic acid (JA) and salicylic acid (SA) induced resistance in groundnut on *Helicoverpa armigera* (Hübner) are examined. Larval survival, larval weights and the activities of digestive enzymes (total serine protease and trypsin) and of detoxifying enzymes [glutathione *S*-transferase (GST) and esterase (EST)] are studied in insects fed on four groundnut genotypes with moderate levels of resistance to *H. armigera* (ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697) and a susceptible genotype (JL 24). The plants are pre- and/or simultaneously treated with JA and SA, and then infested with *H. armigera*, which are allowed to feed for 6 days. Significantly lower serine protease and trypsin activities are observed in *H. armigera* fed on plants treated with JA. Greater GST activity is recorded in insects fed on JA and SA treated plants, whereas EST activity is low in *H. armigera* larvae fed on plants treated with JA and SA. Serine proteases, trypsin and GST activities and larval weights ($r = 0.74–0.95$) and larval survival ($r = 0.77–0.93$) are positively correlated, whereas EST activity and larval weight ($r = -0.55$) and larval survival ($r = -0.65$) are negatively correlated. The results suggest that midgut digestive and detoxifying enzymes can be used as indicators of the adverse effects of constitutive and/or induced resistance in crop plants on the insect pests and the role of JA and SA in insect pest management.

Key words. Groundnut, gut enzymatic activity, *Helicoverpa armigera*, induced resistance, phytohormones.

Introduction

Host plant resistance to insects is expressed constitutively and/or is activated in response to biotic and abiotic stresses. Induced resistance is also activated in plants in response to the exogenous application of elicitors such as jasmonic acid (JA) and salicylic acid (SA) (Zhao *et al.*, 2009; Shivaji *et al.*, 2010). Jasmonic acid and SA mediated plant signalling pathways produce various secondary metabolites that inhibit insect growth and development, as well as volatiles that attract natural enemies of the insect pests (Peng *et al.*, 2004; Shivaji *et al.*, 2010). Induced resistance is an important component of plant defence against insect pests, and it makes plants phenotypically plastic and unpalatable to the insects. It defends plants against insect pests either directly through antixenosis and antibiosis mechanisms of resistance or indirectly by recruiting natural enemies (Bhonwong *et al.*, 2009; Zhao *et al.*, 2009; Shivaji *et al.*, 2010; War *et al.*, 2012).

The ability of insect pests to obtain sufficient amounts of essential amino acids from dietary protein is very important for optimal growth and development. Any variation in nutritional quality and quantity will have drastic effects on insect growth and development, as observed in *Manduca sexta* L., *Helicoverpa armigera* (Hub.) and *Spodoptera frugiperda* (J.E. Smith) (Chen *et al.*, 2005; Bhonwong *et al.*, 2009). Serine proteases are important insect digestive enzymes and comprise the main targets of the toxic plant secondary metabolites (Pearce *et al.*, 1991; Luo *et al.*, 2009). Trypsin plays an important role in peptide bond hydrolysis of the proteins. The role of insect detoxification enzymes such as glutathione *S*-transferase (GST) and esterase (EST) in the metabolism of insecticides, allelochemicals and other xenobiotics has been well established (Ortego *et al.*, 1999; Scott *et al.*, 2010).

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The cotton bollworm/legume pod borer *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is widely distributed in Asia, Africa, Australia and Mediterranean Europe, and feeds on more than 300 plant species worldwide (Sharma, 2005). The main characteristics for being a severely damaging pest are polyphagy, high fecundity, mobility and facultative diapauses (Sharma, 2005).

To develop induced resistance as an effective control strategy for this pest, a thorough understanding of post-ingestive effect on insect pests is very important. Phytohormones induce plant resistance through the up- or down-regulation of genes, which leads to the production of a number of plant secondary metabolites and volatiles. However, studies describing the direct effect of such induced resistance on insect pests are limited. To test the hypothesis that JA and SA induced resistance in groundnut has adverse effects on growth and development of insects by affecting the insect gut enzymes/proteins, the response of *H. armigera* fed on groundnut plants pre- and/or simultaneously treated with JA and SA is examined. This provides insights about the implication of phytohormones in induced resistance in plants and helps with understanding the mechanisms of induced resistance in groundnut plants against *H. armigera.*

Materials and methods

Chemicals

The chemicals used in the present study were of analytical grade. Ethylenediaminetetraacetic acid (EDTA), bovine serum albumin, jasmonic acid, salicylic acid, tannic, disodium hydrogen phosphate, sodium dihydrogen phosphate, 4-chloronapthol, glucose, 1-napthol, glycine, GSH, trypsin inhibitor, sodium hydroxide, *N*-α-benzoyl-D$_L$-arginyl-*p*-nitroanilide (BA*p*NA) and sodium carbonate (Na_2CO_3), were obtained from Sigma Aldrich (St Louis, Missouri). Acetic acid was procured from Sisco Research Laboratory (India). 1-chloro-2, 4-dinitrobenzene (CDNB) was obtained from HiMedia Pvt. Ltd (India).

Groundnut plants (Arachis hypogaea L.)

Five groundnut genotypes [ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 (with moderate to high levels of resistance to insects)] and JL 24 (susceptible) were grown under greenhouse conditions at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India (Sharma *et al.*, 2003). The plants were grown in plastic pots (diameter 30 cm, depth 39 cm) containing a mixture of soil, sand and farmyard manure (2 : 1 : 1). Twenty days after germination, plants were used for the experiment.

Insect infestation

Helicoverpa armigera neonates were obtained from the stock culture maintained in the insect rearing laboratory at ICRISAT. The culture was maintained on chickpea based semi-synthetic diet (Armes *et al.*, 1992) under laboratory conditions (26 ± 1 °C; LD 12 : 12 h and 75 ± 5% relative humidity). Ten larvae were released on each plant with the help of a camel hair brush.

Treatments with exogenous application of JA and SA in groundnut against H. armigera

To prepare the 1 mM JA solution, 21 mg of JA was dissolved in 1 mL of ethanol, and then the JA/ethanol solution was dispersed in 100 mL of water to make 1 mM concentration. The 1 mM SA was prepared by dissolving 0.069 g of SA in 5 mL of ethanol, and then dissolved in water to form a 1 mM concentration (Hamm *et al.*, 2010; War *et al.*, 2011a, b). Plants were sprayed with JA and SA until run-off. There were five treatments, with 10 plants in each treatment in a completely randomized design. (i) Plants sprayed with JA (1 mM) for 24 h before infestation with *H. armigera* (PJA); (ii) plants sprayed with SA (1 mM) for 24 h before infestation with *H. armigera* (PSA); (iii) plants sprayed with JA (1 mM) and simultaneously infested with *H. armigera* (JA); (iv) plants sprayed with SA (1 mM) and simultaneously infested with *H. armigera* (SA); and (v) plants infested with *H. armigera* (HA).

Effect of JA and SA induced resistance in groundnut plants on larval survival, larval weights and midgut enzymes of H. armigera

At 6 days after treatment (6 DAT), surviving larvae were collected from the plants. The larvae were counted and weighed to record the data on insect survival and larval weights. Surviving larvae from each treatment were dissected, and the midguts were isolated to study the activities of important midgut enzymes such as serine proteases, trypsin, EST and GST.

Total serine protease assay

The midguts collected from the dissected larvae were homogenized in 0.1 M glycine-NaOH buffer (pH 10), containing 1 mM EDTA. The homogenate was centrifuged at 11 200 ***g*** for 20 min at 4 °C. The supernatant was used as enzyme source for serine protease and trypsin activity. Activity of serine protease was estimated described previously by Hegedus *et al.* (2003) using azocasein as a substrate. Absorbance was read at 495 nm using an ultraviolet spectrophotometer 2900 (Hitachi, Japan). Serine protease activity (SP) was calculated by subtracting the azocasein blank absorbance (Abs_{blank}) from sample absorbance (Abs_{sample}) divided by incubation time (min) multiplied by 1000:

$$SP = \frac{Abs_{sample} - Abs_{blank}}{\text{Incubation time (min)}} \times 100$$

The enzyme activity was expressed as mU per min of incubation per mg protein (mU min^{-1} mg^{-1} protein).

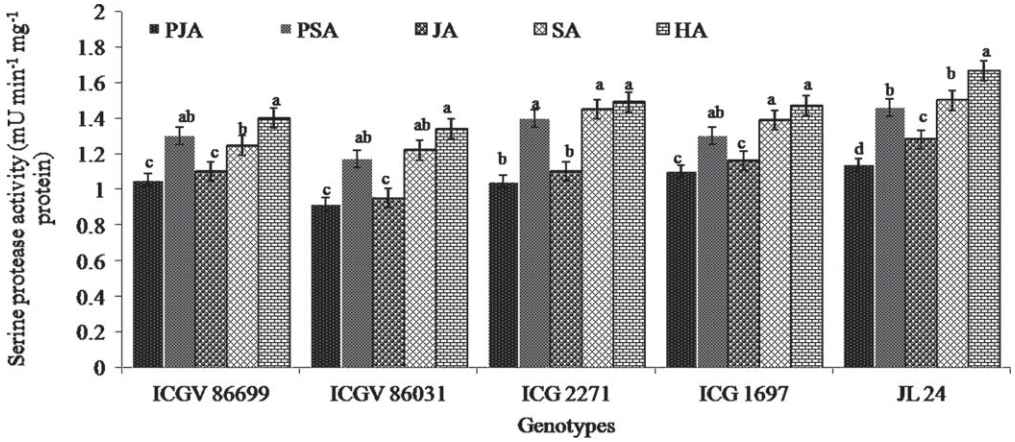

Fig. 1. Total serine protease activity (mU min^{-1} mg^{-1} protein) of *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants. Bars (mean ± SEM) of same colour with similar letters within a genotype are not statistically different at $P \leq 0.05$. PJA, pre-treatment with jasmonic acid (JA) 1 day before *H. armigera* infestation; PSA, pre-treatment with salicylic acid (SA) 1 day before *H. armigera* infestation; JA, simultaneous application of JA and *H. armigera* infestation; SA, simultaneous application of SA and *H. armigera* infestation; HA, *H. armigera* infested plants.

Trypsin assay

The method of Colowick & Kaplan (1970) was followed to determine the trypsin activity of the insect midgut. To 1 mL of 1 mM BApNA (in 0.2 M glycine–NaOH buffer, pH 10), 0.15 mL of midgut extract was added. After incubation for 10 min at 37 °C, the reaction was terminated by adding 0.2 mL of 30% acetic acid. Absorbance was read at 410 nm and the enzyme activity was expressed as μmol min^{-1} mg^{-1} protein.

GST and EST assays

The larvae were dissected in 0.1 M sodium phosphate buffer (pH 7.5), and then the midguts were removed and homogenized in 0.1 M sodium phosphate buffer (pH 7.5) containing 1 mM EDTA. The homogenate was filtered through three layered cheese cloth and centrifuged at 15 000 ***g*** for 15 min at 4 °C. The supernatant was collected and used as enzyme source for GST and EST. GST activity was determined using CDNB and reduced GSH as substrates according to Habig *et al.* (1974). Absorbance was recorded at 340 nm. The enzyme activity was calculated with an extinction coefficient of 9.6 mM cm^{-1} for CDNB. Specific activity was expressed as nmol of CDNB conjugate formed min^{-1} mg^{-1} protein. The EST activity was determined according to the method of Van Asperen (1962) with slight modifications. Absorbance was recorded at 490 nm and the specific activity was expressed as μmol of 1-napthol formed min^{-1} mg^{-1} protein.

Protein content

Total protein content was estimated by the method of Lowery *et al.* (1951) using bovine serum albumin as a standard.

Statistical analysis

Data were analyzed by analysis of variance using SPSS, version 15.1 (SPSS Inc., Chicago, Illinois). Tukey's multiple comparisons/regression tests were used to separate the means, when the treatment effects were statistically significant ($P \leq 0.05$).

Results

Effect of JA and SA induced resistance in plants on midgut enzymes of H. armigera

Total serine protease activity. The serine protease activity of *H. armigera* larvae fed on plants treated with PJA and JA was significantly lower than the larvae fed on PSA, SA and the untreated plants in groundnut genotypes ($F_{4,14} = 16.8$, 13.6, 19.2, 14.3 and 11.9 for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively, $P < 0.05$) (Fig. 1). Larvae fed on untreated JL 24 plants had significantly greater serine protease activity ($F_{4,14} = 13.4$, $P < 0.05$) compared with the larvae fed on untreated plants of ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697. There were no significant differences in serine protease activity of the larvae in the rest of the treatments across the genotypes.

Trypsin activity. Significantly lower trypsin activity was recorded in the larvae fed on plants treated with PJA, PSA and JA in ICGV 86699, ICGV 86031, ICG 2271 and JL 24 ($F_{4,14} = 7.8$, 10.4, 9.9 and 8.5, respectively, $P < 0.05$) than in the larvae fed on SA treated and untreated plants (Fig. 2). However, in ICG 1697, significantly lower trypsin activity was recorded in larvae fed on PJA and JA treated plants ($F_{4,14} = 23.8$, $P < 0.01$) followed by those fed on the plants treated with PSA

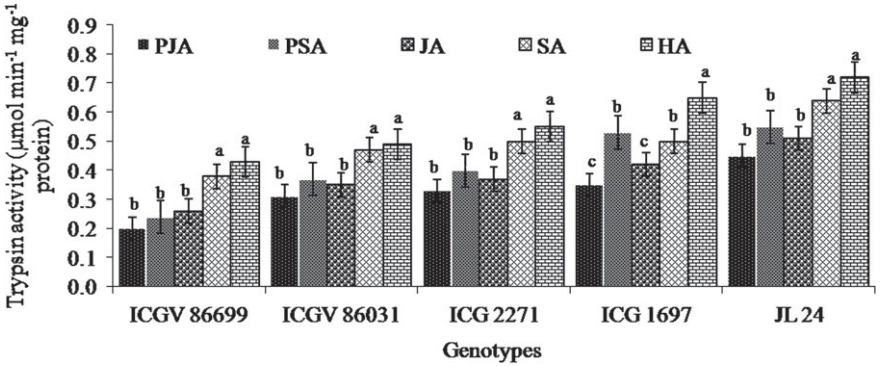

Fig. 2. Trypsin activity (µmol min^{-1} mg^{-1} protein) of *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants. Bars (mean ± SEM) of same colour with similar letters within a genotype are not statistically different at $P \leq 0.05$. PJA = Pre-treatment with jasmonic acid (JA) 1 day before *H. armigera* infestation; PSA, pre-treatment with salicylic acid (SA) 1 day before *H. armigera* infestation; JA, simultaneous application of JA and *H. armigera* infestation; SA, simultaneous application of SA and *H. armigera* infestation; HA, *H. armigera* infested plants.

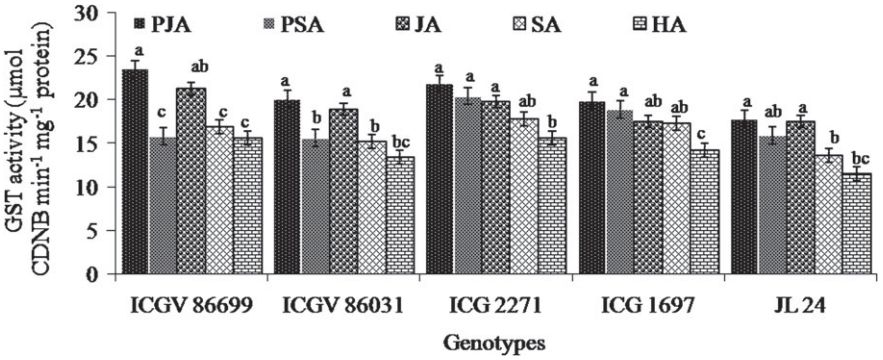

Fig. 3. Glutathione *S*-transferase (GST) activity [µmol 1-chloro-2, 4-dinitrobenzene (CDNB) min^{-1} mg^{-1} protein] of *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants. Bars (mean ± SEM) of same colour with similar letters within a genotype are not statistically different at $P \leq 0.05$. PJA, pre-treatment with jasmonic acid (JA) 1 day before *H. armigera* infestation; PSA, pre-treatment with salicylic acid (SA) 1 day before *H. armigera* infestation; JA, simultaneous application of JA and *H. armigera* infestation; SA, simultaneous application of SA and *H. armigera* infestation; HA, *H. armigera* infested plants.

and SA. Across the genotypes, larvae fed on PJA, PSA and JA treated plants of ICGV 86699 had significantly lower trypsin activity ($F_{4,14} = 35.6$, 27.8 and 32.6, respectively, $P < 0.01$) than those fed on the respective treatments in ICGV 86031, ICG 2271, ICG 1697 and JL 24. Larvae fed on SA treated plants of the insect-resistant genotypes had significantly lower trypsin activity than the larvae fed on respective treatments in JL 24 ($P < 0.05$). The trypsin activity of the larvae fed on untreated plants of ICGV 86699, ICGV 86031 and ICG 2271 was lower than those fed on untreated plants of ICG 1697 and JL 24 ($F_{4,14} = 14.2$, $P < 0.05$).

GST activity

Helicoverpa armigera larvae fed on PJA and JA treated plants of ICGV 86699 and ICGV 86031 had greater GST activity ($F_{4,14} = 13.9$ and 9.9, respectively, $P < 0.05$) than those fed on plants treated with PSA, JA, SA and HA (Fig. 3). However, in ICG 2271, ICG 1697 and JL 24, the GST activity in the larvae fed on PSA and SA treated plants was on a par with that for those fed on PJA and JA treated plants but significantly greater than those fed on untreated plants. There were no significant differences in GST activity of the larvae across genotypes ($P > 0.05$).

EST activity

Helicoverpa armigera larvae fed on groundnut plants with different treatments did not show any significant differences in EST activity of *H. armigera* larvae across the treatments (Fig. 4). However, in JL 24, the larvae fed on untreated plants had significantly greater activity ($F_{4,14} = 10.1$, $P < 0.05$) than the larvae fed on plants treated with PJA and PSA. There were no significant differences in EST activity among the treatments across the genotypes (all $P > 0.05$), except in larvae fed on untreated JL 24 plants ($F_{4,14} = 6.9$, $P < 0.05$).

Total protein content

The protein content of the larvae fed on plants treated with PJA and JA was significantly lower (5.5 mg mL^{-1}) than for those

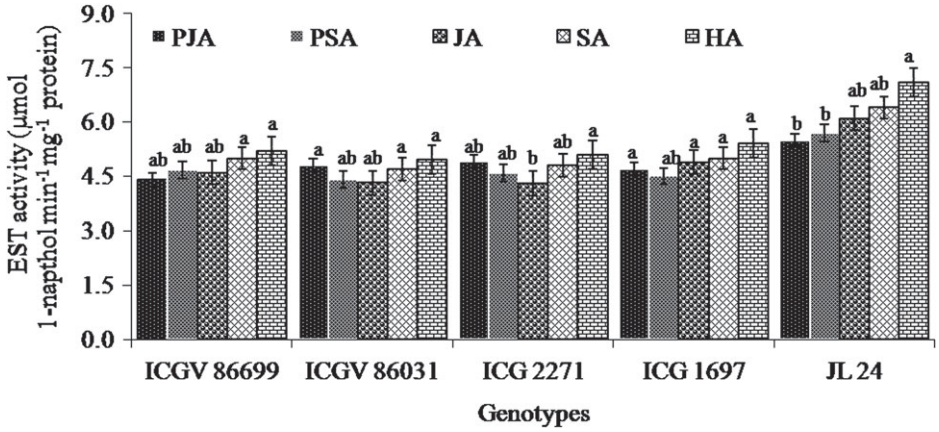

Fig. 4. Esterase (EST) activity (µmol 1-napthol min^{-1} mg^{-1} protein) of *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants. Bars (mean ± SEM) of same colour with similar letters within a genotype are not statistically different at $P \leq 0.05$. PJA, pre-treatment with jasmonic acid (JA) 1 day before *H. armigera* infestation; PSA, pre-treatment with salicylic acid (SA) 1 day before *H. armigera* infestation; JA, simultaneous application of JA and *H. armigera* infestation; SA, simultaneous application of SA and *H. armigera* infestation; HA, *H. armigera* infested plants.

Table 1. Protein concentrations (mg mL^{-1} tissue) of *Helicoverpa armigera* larvae fed on jasmonic acid (JA) and salicylic acid (SA) treated groundnut plants.

	Treatments				
Genotypes	PJA	PSA	JA	SA	HA
ICGV 86699	$5.5 \pm 0.1^{b*}$	7.7 ± 0.8^{b}	$6.1 \pm 0.6^{b*}$	7.9 ± 0.5^{b}	8.2 ± 0.6^{b}
ICGV 86031	$6.2 \pm 0.8^{b*}$	7.3 ± 0.7^{b}	$6.8 \pm 0.7^{b*}$	7.7 ± 0.7^{b}	7.9 ± 0.3^{b}
ICG 2271	$6.4 \pm 0.7^{b*}$	$6.9 \pm 1.3^{b*}$	$6.6 \pm 0.4^{b*}$	8.1 ± 0.7^{b}	8.4 ± 0.8^{b}
ICG 1697	$5.9 \pm 0.7^{b*}$	7.0 ± 1.4^{b}	6.8 ± 1.3^{b}	7.8 ± 0.3^{b}	8.0 ± 0.3^{b}
JL 24	$10.7 \pm 1.3^{a*}$	13.3 ± 1.6^{a}	12.6 ± 1.7^{a}	14.0 ± 1.5^{a}	17.4 ± 1.6^{a}

Values (mean ± SD) with same superscript letter(s) within a column are not significantly different at $P \leq 0.05$ (Tukey's honestly significant difference test). An asterisk (*) appearing in a row indicates statistical significance across treatments.

PJA, pre-treatment with JA 1 day before *H. armigera* infestation; PSA, pre-treatment with SA 1 day before *H. armigera* infestation; JA, simultaneous application of JA and *H. armigera* infestation; SA, simultaneous application of SA and *H. armigera* infestation; HA, *H. armigera* infested plants.

fed on PSA, SA and untreated plants of ICGV 86699 and ICG 2271 ($F_{4,14} = 21.3$ and 17.2, respectively, $P < 0.05$) (Table 1). In ICGV 86031, ICG 1697 and JL 24, larvae fed on PJA plants had a significantly lower protein content ($F_{4,14} = 11.6$, $P < 0.05$) than those fed on PSA, JA, SA and HA plants. Across the genotypes, there were no significant differences in total protein content of the larvae fed on various treatments in insect-resistant genotypes, although there was a statistically significantly difference compared with the susceptible JL 24 (all $P < 0.05$).

Effect of JA and SA induced resistance in plants on larval weight and larval survival of H. armigera

Helicoverpa larvae showed significant differences in larval weights and survival when fed on plants pre and/or simultaneously treated with SA and JA and untreated control (Table 2). Less larvae were recovered from the PJA treated plants in all the genotypes. Across the genotypes, larval survival was lower on ICGV 86699 and ICGV 86031 than on JL 24. Similarly, larvae collected from PJA treated plants had lower weights compared with those collected from PSA, PSA, SA and JA treated plants. Activities of serine proteases, trypsin and GST were positively associated with the larval weights ($r = 0.54–0.98$) and larval survival ($r = 0.67–0.93$), whereas esterase activity was significantly and negatively associated with larval weight ($r = -0.55$) and larval survival ($r = -0.65$), suggesting that the mid gut enzymes of *H. armigera* can be used as an indicators of stress in insect larvae in response to constitutive and/or induced resistance to insects.

Discussion

The post-ingestive interaction between insect pests and plant toxins plays a significant role in determining the resistance/susceptibility of plant tissues to insects. Induced resistance is an important component in this respect because it leads to the production of various toxic secondary metabolites and other compounds, which affect insect physiology, and growth and development. The negative effects of induced resistance on insect pests are attributed to the lower nutritional value of plant tissues and the toxicity of allelochemicals, proteins and protease inhibitors (Bhonwong *et al.*, 2009; Barbehenn *et al.*, 2010).

The results reported in the present study show that JA- and SA-mediated induced resistance in groundnut affects the digestive and detoxifying enzymes in *H. armigera.* The *H. armigera* larvae fed on plants pre- and/or simultaneously treated with JA show reduced serine protease activity compared with those fed on plants pre- and/or simultaneously treated with SA and infested with *H. armigera*, as well as the untreated control

Table 2. Survival (%) and weights (mg) of *Helicoverpa armigera* larvae fed on jasmonic acid (JA) and salicylic acid (SA) treated groundnut plants.

	Survival (%)					Larval weight (mg per larva)				
Genotypes	PJA	PSA	JA	SA	HA	PJA	PSA	JA	SA	HA
ICGV 86699	$20.4 \pm 2.1^{c*}$	32.3 ± 2.3^{bc}	30.2 ± 4.6^{c}	36.5 ± 3.4^{bc}	41.2 ± 3.1^{c}	$37.5 \pm 3.1^{d*}$	48.6 ± 5.3^{d}	47.5 ± 5.6^{d}	59.7 ± 3.5^{c}	69.6 ± 3.6^{c}
ICGV 86031	$26.6 \pm 2.1^{bc*}$	$34.3 \pm 2.2^{bc*}$	$35.5 \pm 3.3^{c*}$	39.6 ± 4.4^{bc}	47.4 ± 2.1^{b}	$44.5 \pm 2.8^{bc*}$	60.6 ± 3.7^{c}	75.5 ± 7.7^{bc}	74.4 ± 3.7^{c}	97.7 ± 5.3^{bc}
ICG 2271	$32.4 \pm 1.4^{b*}$	$40.5 \pm 3.8^{b*}$	$40.4 \pm 2.1^{b*}$	44.5 ± 2.1^{b}	48.9 ± 3.1^{b}	$55.4 \pm 3.2^{b*}$	65.6 ± 5.3^{bc}	87.6 ± 3.4^{b}	98.8 ± 4.7^{bc}	110.3 ± 8.8^{b}
ICG 1697	$35.7 \pm 3.2^{b*}$	$44.8 \pm 2.6^{b*}$	48.2 ± 3.2^{b}	50.5 ± 3.6^{b}	54.4 ± 4.7^{b}	$59.6 \pm 2.7^{a*}$	80.6 ± 6.4^{b}	95.5 ± 4.3^{b}	114.4 ± 6.3^{ab}	127.5 ± 7.3^{b}
JL 24	$58.3 \pm 2.1^{a*}$	69.4 ± 3.8^{a}	75.9 ± 2.3^{a}	79.6 ± 4.1^{a}	81.4 ± 6.6^{a}	$73.6 \pm 4.3^{a*}$	102.4 ± 7.6^{a}	120.3 ± 8.7^{a}	129.5 ± 9.5^{a}	159.5 ± 10.0^{a}

Values (mean ± SD) with same superscript letter(s) within a column are not significantly different at $P \leq 0.05$ (Tukey's honestly significant difference test). An asterisk (*) appearing in a row indicates statistical significance across treatments.

PJA, pre-treatment with JA 1 day before *H. armigera* infestation; PSA, pre-treatment with SA 1 day before *H. armigera* infestation; JA, simultaneous application of JA and *H. armigera* infestation; SA, simultaneous application of SA and *H. armigera* infestation; HA, *H. armigera* infested plants.

plants. This may be attributed to changes in JA mediated secondary metabolites produced by the octadecanoid pathway. Serine proteases represent the important digestive endopeptidases in insects. A lower activity of trypsin is observed in insects fed on plants treated with PJA, PSA and JA compared with insects fed on SA treated and untreated plants. A decrease in trypsin activity may be a result of the secondary metabolites induced by JA and SA. Pre-treatment with SA shows almost similar effects on trypsin activity compared with those of PJA and JA. The results suggest that defensive compounds present in groundnut genotypes constitutively and/or induced by chemical elicitors result in antibiotic effects in *H. armigera* larvae. SA induces resistance against sap sucking insects and pathogens (Zhao *et al.*, 2009). Furthermore, plant defensive enzymes, including peroxidase, are directly toxic to insect gut (Barbehenn *et al.*, 2010). When incorporated into an artificial diet, plant secondary metabolites reduce the serine protease and trypsin activities of *H. armigera* (War *et al.*, 2013). The inhibition of the activities of insect digestive enzymes results in reduced digestion of plant components and an amino acid deficiency in insects, which in turn decreases insect growth and development, fecundity and survival (Lawrence & Koundal, 2002; Azzouz *et al.*, 2005).

Polyphagous insects express a wide range of defensive enzymes to counteract the plant defensive compounds produced in plants. The GST and EST enzymes are the most important detoxifying enzymes produced in insects in response to toxins. A substantial increase in GST activity is observed in larvae fed on treated plants. The *H. armigera* larvae fed on plants treated with PJA and JA show an increased activity of GST in ICGV 86699 and ICGV 86031. However, the induction of GST activity in larvae fed on PSA treated plants of ICG 2271, ICG 1697 and JL 24 is at par with those fed on PJA and JA treated plants. A strong positive correlation is observed between GST and larval weights and larval survival. Insects directly fed on plant defence chemicals possess high levels of GST (Mukherjee, 2003; Vanhaelen *et al.*, 2003). This shows the involvement of GST in plant–herbivore interactions. Barley aphid *Sitobion avenae* (F.) responds to insect resistant wheat with greater phenolic content by increasing the levels of GST (Leszczynski & Dixon, 1992). Similarly, War *et al.* (2013) reported an increase in GST activity in *H. armigera* larvae fed on artificial diet containing plant secondary metabolites.

In general, no significant difference is observed in EST activity of *H. armigera* larvae fed on groundnut plants pre- and/or simultaneously treated with JA and SA and across the groundnut genotypes, except in JL 24. The decrease in EST activity can be linked to the toxicity and/or the inhibition of EST production by the secondary metabolites present in plants. ESTs hydrolyze the ester bonds from various substrates and thereby metabolize the toxic plant xenobiotics into less toxic compounds (Yang *et al.*, 2005). The alteration of insect midgut enzymes confers a direct influence of induced plant compounds on insect metabolism. The activities of serine proteases, trypsin and GST are positively associated with the larval weights and larval survival, whereas esterase activity is significantly and negatively associated with larval weight and larval survival, suggesting that mid gut enzymes of *H. armigera* can be used as indicators of constitutive and/or induced resistance to insects.

The total protein content of the larvae fed on plants pre-treated with JA is significantly lower compared with the rest of the treatments in groundnut genotypes, except in ICG 2271, in which the protein content of the larvae fed on PSA is at par with those fed on PJA. This may be a result of the inhibition of the enzymes by toxic plant components such as protease inhibitors and other antinutritional components induced by JA and/or the toxic effect of plant toxins on insect midgut membrane. Moreover, the non-availability of essential amino acids leads to reduced insect growth and development (Chen *et al.*, 2005).

Host plant resistance directly affects the insect growth and development. Overall, larval survival and larval weights are significantly less when fed on PJA treated plants compared with larvae fed on the rest of the treatments. However, in ICGV 86031, ICG 2271 and ICG 1697, larval survival on PSA treated plants is on a par with that of PJA treated plants. The reduction in survival and weights can be a result of the strong antibiosis mechanisms modulated by the JA/SA pathways in plants. JA and SA mediated signalling pathways result in the production of various toxic secondary metabolites that decrease larval survival and weights (Peng *et al.*, 2004; Chen *et al.*, 2009; War *et al.*, 2011b).

In conclusion, the results indicate that PJA treated plants have a significantly greater effect on digestive and detoxifying enzymes of *H. armigera* than SA treated plants. However, pre-treatment with SA also results in considerable effects on the trypsin and GST activities of *H. armigera* larvae. The effect of induced resistance is greater in larvae fed on the

insect-resistant genotypes than those fed on the susceptible ones. An alteration in insect detoxifying and digestive enzymes by exogenous application of JA and SA, or both, either in pre-/or simultaneous application, increases host plant resistance to *H. armigera*, which can be exploited in insect pest management for sustainable crop production.

Acknowledgements

We thank Mr Rajendra S. Munghate, Mr Suraj P. Sharma and Mr V. V. Rao of Entomology, ICRISAT, for their technical assistance.

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Accepted 1 March 2014
First published online 7 April 2014