Induced resistance to *Helicoverpa armigera* through exogenous application of jasmonic acid and salicylic acid in groundnut, *Arachis hypogaea*

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ABSTRACT

- **BACKGROUND:** Induced resistance to *Helicoverpa armigera* through exogenous application of jasmonic acid (JA) and salicylic acid (SA) was studied in groundnut genotypes (ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697) with different levels of resistance and the susceptible check, JL 24, under greenhouse conditions. Activities of oxidative enzymes and the amounts of secondary metabolites and proteins were quantified at 6 days after JA and SA application/insect infestation. Data was also recorded on plant damage and *H. armigera* larval weights and survival.

- **RESULTS*:*** Higher levels of enzymatic activities and the amounts of secondary metabolites were observed in the insect-resistant genotypes pretreated with JA and then infested with *H. armigera* than in JL 24. The insect-resistant genotypes suffered lower insect damage and resulted in poor survival and lower weights of *H. armigera* larvae than JL 24. In some cases, both JA and SA showed similar effect.

- **CONCLUSION:** JA and SA induced the activity of antioxidative enzymes in groundnut plants against *H. armigera*, and reduced its growth and development. However, induced response to application of JA was greater than SA, and resulted in reduced plant damage, larval weights and survival, suggesting that induced resistance can be used as a component of pest management in groundnut.

Key-words: Groundnut, *Helicoverpa armigera*, induced resistance, secondary metabolites, antioxidant enzymes, jasmonic acid, salicylic acid.

1 INTRODUCTION

Plants have developed an elegant defense system against insect herbivory. The defense systems employed by plants against insects can be constitutive or induced. Constitutive resistance is present in plants all the time, while as, induced resistance occurs in response to various stimuli such as insect herbivory, pathogen infestation and/or elicitor application.[1-3] Induced resistance is very important as it makes plants phenotypically plastic, thereby making it freakish for the insect pests to feed on it.[4,5] Induced resistance could be direct or indirect. The direct induced resistance directly affects the insect pest through antixenosis and/or antibiosis mechanisms,[6,7] while as, the indirect induced resistance is mediated through volatiles emitted by the plants in response to insect damage, which attract the natural enemies (parasitoids and predators) of the insect pests.[4,8,9]

Although many plant hormones act as elicitors of induced resistance, the most important and widely used phytohormones are jasmonic acid (JA) and salicylic acid (SA).[3,10] The use of these phytohormones in inducing plant resistance against insect pests has raised the possibility of their implications for insect pest management. Exogenous application of JA results in the induction of plant responses that are almost similar to herbivore feeding. The JA mediated octadecanoid pathway leads to the production of many defensive components such as plant defensive proteins, oxidative enzymes, glandular trichomes, flavonoids, terpenoids, alkaloids, volatile compounds, etc.[1,4,9] SA, a benzoic acid derivative, is an endogenous plant growth regulator that generates a wide range of metabolic and physiological responses in plants involved in plant growth and development,[11] and defense against various stresses including insect herbivory.[3,10,12]

Groundnut (*Arachis hypogaea* L.) is an annual herbaceous plant belonging to the family Fabaceae. It is cultivated mostly in semi-arid tropical and sub-tropical regions. It is damaged by several insect pests, of which, legume pod borer, *Helicoverpa armigera* (Hubner) is an important defoliator during the vegetative stage. *H. armigera* is widely distributed in Asia, Africa, southern Europe, and Australia.[13] In semi-arid tropics, *H. armigera* causes an estimated loss of over US$2 billion annually, despite US$500 million worth of pesticides applied for controlling this pest.[13] It has developed high levels of resistance to several commonly used insecticides.[14] Therefore, there is a need for alternative methods of pest control to reduce overdependence on insecticides and to conserve biodiversity. It is in this context that host plant resistance, which is economic and environmental friendly, assumes a central role in integrated pest management.[13]

Host plant resistance plays an important role in groundnut defense against a variety of insect pests. Many biochemical parameters have been associated with resistance in groundnut against insect pests. Higher levels of antioxidative enzymes, phenols and tannins contribute to groundnut resistance against *Spodoptera litura* (Fab.) and *H. armigera.*[15-18] Stevenson *et al.*[15] observed that quercetin, caffeoylquinic acids and diglycosides contribute to resistance in groundnut against *S. litura.* Procyanidin in groundnut plants provide resistance against *Aphis craccivora* (Koch).[16,19] Nitrogen, soluble sugars and polyphenols are involved in groundnut resistance against leaf miner, *Aproraema modicella* Dev.[20] Understanding the mechanisms of induced resistance can help us to build up the natural defenses in plants by the application of elicitors and/or mild damage by the herbivores. Although it has been well documented that phytohormones induce plant resistance in plants through the expression of a number of proteins and non protein based compounds, such studies are limited in groundnut. To test the hypothesis, JA and SA were exogenously applied to groundnut plants with differential levels of resistance to *H. armigera* to study the induced resistance. The plants were pre-and/or simultaneously treated with JA and SA and infested with *H. armigera.* Various plant defensive enzymes and plant secondary metabolites were investigated.

2 MATERIALS AND METHODS

2.1 **Chemicals**

The chemicals used in this study were of analytical grade. Ethylene diamine tetra acetic acid (EDTA), bovine serum albumin (BSA), guaiacol, polyvinylpyrolidone (PVP), jasmonic acid, salicylic acid, tannic acid, vanillin, linoleic acid, dithiothretol (DTT), disodium hydrogen phosphate, sodium dihydrogen phosphate, nitro-blue tetrazolium salt (NBT), methionine, L-phenylalanine, sodium carbonate (Na_2CO_3), and vanillin were obtained from Sigma Aldrich, USA. Catechol was obtained from Glaxo Laboratories, Mumbai, India. Tris-HCl, glycine, and trichloroacetic acid (TCA) were obtained from Sisco Research Lab., Mumbai, India. 2-mercaptoethanol, gallic acid and Folin-Ciocalteau reagent were obtained from Merck, Mumbai, India. Thiobarbituric acid (TBA) and linoleic acid were obtained from HiMedia Pvt. Ltd., Mumbai, India. Ammonium sulphate was obtained from Qualigens Fine Chemicals, Mumbai. The spectrophotometer used for the estimation of biochemical parameters was Hitachi UV – 2900 (Hitachi, Japan).

2.2 Groundnut plants

Five groundnut genotypes were grown under greenhouse conditions at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, to study the induction of resistance by exogenous application of JA and SA against *H. armigera.* The genotypes were: ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 (with moderate levels of resistance to insects), and JL 24 (susceptible check).[21] The plants were raised in plastic pots (30 cm diameter and 39 cm deep) containing a mixture of soil, sand, and farmyard manure (2:1:1). Five seeds were planted in each pot, and 2 seedlings were retained in each pot at five days after seedling emergence. The desert coolers were used to maintain the temperature at 28 ± 5 ºC and RH 65 ± 5% in greenhouse. After twenty day of emergence, plants were infested with 10 newly emerged *H. armigera* larvae with a camel hair brush. The experiment was repeated thrice and the data shown is the pooled data.

2.3 Insect infestation

The *H. armigera* neonates were obtained from the stock culture maintained on chickpea based semi-synthetic diet[22] under laboratory conditions (26 ± 1 ºC; 11 ± 0.5 h photoperiod and 75 ± 5% relative humidity) from the insect rearing laboratory at ICRISAT, Patancheru, Andhra Pradesh, India.

2.4 Induction of resistance by exogenous application of JA and SA in groundnut against *H. armigera*

There were six treatments for each genotype, and five replications for each treatment with two plants in each replication. In group I - the plants were pre-treated with JA (1 mM) for 24 h and then infested with *H. armigera* (PJA + HIN); in group II – the plants were pretreated with SA for 24 h and then infested with *H. armigera* (PSA + HIN); in group III – the plants were sprayed with JA (1 mM) and simultaneously infested with *H. armigera* (JA + HIN); in group IV – the plants were sprayed with SA (1 mM) and simultaneously infested with *H. armigera* (SA + HIN); in group V – the plants were infested with *H. armigera* (HIN); and in group VI – the plants were maintained as untreated control (sprayed with ethanol only).

At six days after treatment (6 DAT), plants were assessed for insect damage by visually rating them to a scale 1-9, with 1 showing no or slight damage (< 10%) and 9 shows > 80% damage.[21] Larvae recovered from the plants were counted and weighed to record the data on insect survival and larval weights.

The fully expanded quadrifoliate leaves were collected randomly from the groundnut plants at six days after treatment to study the activities of various defensive enzymes such as peroxidase (POD), polyphenol oxidase (PPO), lipoxygenase (LOX), phenylalanine ammonia lyase (PAL), superoxide dismutase (SOD), ascorbate

peroxidase (APX), catalase (CAT), trypsin proteinase inhibitor (PI), and total amounts of phenols, condensed tannins, flavonoids, carbohydrates, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA).

2.4.1 Enzyme extraction

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

2.4.2 Enzyme assays

Activities of enzymes such as peroxidase,[23] polyphenol oxidase,[24] lipoxygenase,[25] SOD[26] were estimated by adopting standard procedures. The enzyme activity was expressed as IU g^{-1} FW (units per gram fresh weight). One unit of enzyme was defined as the change in absorbance by 0.1 unit per minute under conditions of the assay. Phenylalanine ammonia lyase was estimated as described by Campos-Vergas and Saltveit[27] with slight modifications. The enzyme activity was expressed as µmol cinnamic acid min^{-1} mg^{-1} protein. Catalase activity was determined by using the method of Zhang *et al.*[28] and the enzyme activity was expressed as µmol min^{-1} mg^{-1} protein.

Ascorbate peroxidase (APX) activity was determined by the method of Asada and Takahashi.[29] Leaf tissue (0.2 g) was homogenized in a pestle and mortar with 3 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1% polyvinylpyrrolidone (PVP) and 1mM ascorbic acid. After filtering through a double-layered cheese cloth, the homogenate was centrifuged at 12,000 rpm for 20 min at 4º C. The supernatant was collected and subjected to precipitation and dialysis as mentioned above. The partially purified sample was used as the enzyme source. The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 0.2 ml of partially purified enzyme extract. Decrease in absorbance at 290 nm due to ascorbate oxidation was measured against the blank and the enzyme activity was expressed as IU g^{-1} FW.

2.4.3 Proteinase inhibitor (PI) activity

To measure PI activity, the leaf sample (0.2 g) was homogenized in 4 ml of 50 mM Tris-HCl buffer (pH 7.8) containing 5% PVP, 0.016 M phenyl urea, 0.03 M KCl, 0.05 M EDTA and 0.4 mM ascorbic acid. The homogenate was filtered through three layers of cheese cloth and centrifuged at 12,000 rpm for 15 min at 4 ºC. The supernatant

was collected, the protein precipitated by ammonium sulphate, dialyzed and used as the protein inhibitor source. All the steps were carried out on ice to ensure the lowest possible temperature. The PI activity was estimated by following the method of Kakade *et al.*[30] using *N*-α-benzoyl-D_L-arginyl-*p*-nitroanilide (BA*p*NA) as a substrate, and trypsin as a standard. The PI activity was expressed as percentage inhibition of trypsin.

2.4.4 Estimation of secondary metabolites and other defensive compounds

2.4.4.1 Phenolic content

Fresh leaves (0.5 g) were homogenized in 3 ml of 80% methanol and agitated for 15 min at 70 ºC. The solution was centrifuged at 10, 000 rpm for 10 min and the supernatant collected. The supernatant was used for estimation of total phenols, condensed tannins and total flavonoids. The phenolic content was estimated as per Zieslin and Ben-Zaken[31] method. Amounts of total phenols were determined from the standard curve prepared with gallic acid, and expressed as µg gallic acid equivalents g^{-1} FW (µg GAE g^{-1} FW). Condensed tannins content was estimated by using vanillin-hydrochloride method as described by Robert.[32] Catechin was used as the standard. The total amount of condensed tannins was expressed as µg catechin equivalents g^{-1} FW (µg CE g^{-1} FW). Total flavonoid content was determined by the modified aluminum chloride method as described by Woisky and Salatino.[33] The total amount of flavonoids was expressed as µg catechin equivalents g^{-1} FW (µg CE g^{-1} FW).

2.4.4.2 Hydrogen peroxide

The hydrogen peroxide (H_2O_2) content was estimated by the method of Noreen and Ashraf.[34] The H_2O_2 concentration was determined by using an extinction coefficient of 0.28 µM cm^{-1} and expressed as µmol g^{-1} FW.

2.4.4.3 Melandialdehyde (MDA)

The level of lipid peroxidation was determined in terms of thiobarbituric acid-reactive substances i.e., MDA as described by Carmak and Horst[35] with minor modifications. The concentration of TBARS was calculated using the absorption coefficient 155 $mmol^{-1}cm^{-1}$ and expressed as µmol g^{-1}FW.

2.4.4.4 Protein content

Total protein content was estimated by Lowey method[36] using bovine serum albumin as a standard.

2.5 Statistical analysis

The data was subjected to analysis of variance (ANOVA) using SPSS (15.1). Tukey's/Multiple comparisons test were used to separate the means, when the treatment effects were statistically significant ($p \leq 0.05$).

3 RESULTS

3.1 Induction of enzyme activity and secondary metabolites following exogenous application of JA and SA in groundnut

3.1.1 POD activity

The PJA + HIN treated plants showed significantly greater POD activity in ICGV 86699 and ICG 2271 ($F_{(5,17)}$ = 23.4 and 48.1, respectively, $P < 0.01$) as compared to PSA + HIN, JA + HIN, HIN and untreated control plants. (Fig.1A). In ICGV 86031, PJA + HIN and JA + HIN treated plants exhibited significantly greater POD activity ($F_{(5,17)}$ = 27.4, $P < 0.05$) than those treated with PSA + HIN, SA + HIN, HIN and untreated control plants, however, POD activity of PSA + HIN treated plants was at par with those treated with JA + HIN. In ICG 1697 and JL 24, plants treated with PJA + HIN, PSA + HIN and JA + HIN showed significantly greater POD activity ($F_{(5,17)}$ = 29.3 and 18.1, respectively, $P < 0.05$) than SA + HIN, HIN and untreated control plants. Across, the genotypes, insect resistant genotypes showed significantly greater POD activity in all the treatments ($F_{(4,14)}$ = 36.8, 15.0, 19.6, 9.9, and 12.6 for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and control, respectively, $P < 0.05$) than the susceptible check, JL 24.

3.1.2 PPO activity

Among the treatments, PJA + HIN treated plants had significantly greater PPO activity in ICGV 86699 ($F_{(5,17)}$ = 25.7, $P < 0.01$), ICGV 86031 ($F_{(5,17)}$ = 23.4, $P < 0.01$) and ICG 1697 ($F_{(5,17)}$ = 11.9, $P < 0.05$) than the plants treated with PSA + HIN, JA + HIN, SA + HIN, *H. armigera* infested plants and the untreated control plants (Fig. 1B). In ICG 2271 plants infested with *H. armigera* and pre- and/or simultaneously treated with JA showed significantly greater PPO activity ($F_{(5,17)}$ = 20.1, $P < 0.05$) as compared to the SA + HIN, HIN and the untreated control plants In JL 24, no significant difference was recorded in PPO activities of plants treated with PJA + HIN and JA + HIN($F_{(5,17)}$ = 18.7, $P < 0.05$), however, PPO activity of JA + HIN treated plants was at par with PSA + HIN and SA + HIN treated plants. Among the tested genotypes, ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 had significantly higher PPO activity in PJA + HIN treated plants ($F_{(4,14)}$ = 16.7, $P < 0.05$) than those of JL 24. The PSA

+ HIN treated plants of insect resistant genotypes showed significantly greater PPO activity than JL 24, however, the level of significance varied [ICGV 86699 ($P < 0.001$) and ICGV 86699, ICG 2271 and ICG 1697 (all, $P < 0.05$)]. Significantly greater PPO activity was observed in JA + HIN treated plants of ICGV 86699, ICGV 86031 and ICG 2271 ($F_{(4,14)} = 22.5$, $P < 0.05$) as compared to those of ICG 1697 and JL 24. Constitutive levels of PPO activity were significantly higher in insect-resistant genotypes ($F_{(4,14)} = 8.9$, $P < 0.05$) than JL 24.

3.1.3 PAL activity

The PJA + HIN, PSA + HIN and JA + HIN treated plants showed significantly greater PAL activity ($F_{(5,17)} = 45.7$, 22.9, and16.9for ICGV 86699, ICGV 86031, and ICG 1697, respectively, $P < 0.05$) than the SA + HIN, HIN and the untreated control plants (Fig. 1C). In ICG 2271 and JL 24, the PAL activity of plants treated with PSA + HIN and SA + HIN did not differ significantly. Among the genotypes tested, ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 exhibited significantly greater PAL activity in PJA + HIN, PSA + HIN, JA + HIN, SA + HIN,HIN treated and untreated control plants ($F_{(4,14)} = 21.8$, 11.9, 32.5, 17.9, 28.4 and 16.4, respectively, $P < 0.01$) as compared to those of JL 24.

3.1.4 LOX activity

The plants infested with *H. armigera* and pre- and/or simultaneously treated with JA showed significantly greater LOX activity in all the genotypes tested ($F_{(5,17)} = 32.5$, 21.3, 23.9, 21.9 and 13.2 for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL24, respectively, $P < 0.05$) than the plants treated with PSA + HIN, SA + HIN, HIN and untreated control plants (Fig. 1D). ICGV 86699, ICGV 86031 and ICG 2271 plants treated with PJA + HIN, PSA + HIN, JA + HIN and HIN showed significantly greater LOX activity ($F_{(4,14)} = 32.1$, 24.6, 18.4 and 14.3, respectively, $P < 0.01$) than the respective treatments of ICG 1697 and JL 24. No significant differences were observed in LOX activity of untreated control plants.

3.1.5 SOD activity

The PJA + HIN treated plants had significantly greater SOD activity in ICGV 86699 and ICG 1697 ($F_{(5,17)} = 11.3$ and 15.2, respectively, $P < 0.05$) than PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control plants (Fig. 2A). The PJA + HIN and JA + HIN treated plants showed significantly greater SOD activity in ICGV 86031, ICG 2271 and JL 24 ($F_{(5,17)} = 11.7$, 21.4 and 13.7, respectively, $P < 0.01$) as compared to the respective PSA + HIN, SA + HIN, HIN and the untreated control plants, however, in JL 24, SOD activity of PSA + HIN and JA + HIN plants did

not differ significantly. Insect resistant genotypes exhibited significantly greater SOD activity in all the treatments ($F_{(4,14)}$ = 38.5, 21.4, 17.4, 25.6 and 13.6 for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN and HIN, respectively, $P < 0.05$) as compared to those of JL 24. Untreated control plants did not show any significant difference across the genotypes.

3.1.6 APX activity

The APX activity of plants treated with PJA + HIN, PSA+ HIN and JA + HIN was significantly greater ($F_{(5,17)}$ = 38.5, 21.7, 37.3, 18.6 and 24.9 for ICGV 86699, ICGV 86031, ICGV 2271, ICG 1697, and JL 24, respectively, $P < 0.05$) than SA + HIN, HIN and the untreated control plants (Fig. 2B). In ICG 2271, no significant difference was observed in APX activity of PSA + HIN and SA + HIN plants. Insect resistant genotypes showed significantly greater APX activity in all the treatments ($F_{(4,14)}$ = 30.3, 21.1, 11.5, 9.3, 25.8 and 7.6 for , PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and untreated control, respectively, $P < 0.05$) as compared to that of the susceptible check, JL 24.

3.1.7 CAT activity

The CAT showed altered expression in various treatments and in different genotypes (Fig. 2C). Significantly greater CAT activity was observed in plants infested with *H. armigera* and pre- and/or simultaneously treated with JA in groundnut genotypes ($F_{(5,17)}$ = 33.9, 39.9, 28.5, 31.9 and 17.3 for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL24, respectively, $P < 0.01$) than the plants infested with *H. armigera* and pre- and/or simultaneously treated with SA and the untreated control plants, except in ICGV 86031, where CAT activity of PSA + HIN treated plants was at par with those of JA + HIN treated plants, and in JL 24, where no significant difference was observed in CAT activities of PSA + HIN, JA + HIN and SA + HIN treated plants. The PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and untreated control plants of the insect-resistant genotypes showed significantly greater CAT activity ($F_{(4,14)}$ = 11.3, 15.2, 8.6, 20.6, 17.2 and 10.5, respectively, $P < 0.05$) than in JL 24.

3.1.8 PI activity

Significantly greater *in vitro* PI activity (%) was shown by groundnut plants treated with PJA + HIN and JA + HIN in ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24 ($F_{(5,17)}$ =47.1, 37.9, 32.2, 22.4 and 34.5, respectively, $P < 0.05$) as compared to PSA + HIN, SA + HIN, HIN and the untreated control plants (Fig. 2D). Across the genotypes, insect resistant genotypes showed significantly greater PI activity in PJA + HIN, PSA + HIN, JA + HIN,

SA+ HIN and HIN treated plants ($F_{(4,14)}$ = 9.5, 11.7, 6.8, 8.1 and 10.2, respectively, $P < 0.05$) than JL 24. No significant difference was observed in constitutive levels of PI activity across the tested genotypes.

3.1.9 Total phenols

There were no significant differences in phenolic content of the plants infested with *H. armigera* and pre- and/or simultaneously treated with JA and SA in ICGV 86031, ICG 2271, ICG 1697 and JL 24 ($F_{(5,17)}$ = 30.4, 45.9, 28.3 and 39.8 for respectively, $P < 0.01$) (Fig. 3A). The PJA + HIN and JA + HIN treated plants of ICGV 86699 had significantly greater phenolic content ($F_{(5,17)}$ = 30.4, $P < 0.05$) as compared to the plants treated with PSA + HIN, SA + HIN, HIN and the untreated control plants, however, phenolic content of plants treated with JA + HIN was at par with those of PSA + HIN and SA + HIN plants. Phenolic content of the insect-resistant genotypes was significantly greater in PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control plants ($F_{(4,14)}$ = 25.4, 36.5, 29.7, 42.5, 30.6 and 31.2, respectively, $P < 0.01$) as compared to that of JL 24. The HIN infested plants of ICGV 86699, ICGV 86031 and ICG 1697 had significantly higher phenolic content ($F_{(4,14)}$ = 33.6, $P < 0.05$) than in the ICG 2271and JL 24.

3.1.10 Flavonoids

Flavoniod content was significantly greater in plants treated with PJA + HIN and JA + HIN in ICGV 86699, ICGV 86031 and ICG 2271 ($F_{(5,17)}$ = 12.3 17.5 and 10.9, respectively, $P < 0.01$) than in PSA + HIN, SA + HIN, HIN treated and the untreated control plants (Fig. 3B). In ICG 1697, flavonoid content of JA + HIN plants was at par with those of PSA + HIN, SA + HIN and HIN plants. In JL 24, no significant differences were observed in flavonoid content of plants treated with PJA + HIN, PSA + HIN, JA + HIN, SA + HIN and the HIN (Fig. 10). Insect-resistant plants had greater amounts of flavonoids in all the treatments ($F_{(4,14)}$ = 22.2, 13.5, 26.4, 14.9, 19.2 and 15.3 for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and untreated control, $P < 0.05$) than JL 24.

3.1.11 Condensed tannins

There were significant differences in condensed tannins content across the treatments, and the genotypes tested (Fig. 3C). The PJA + HIN treated plants exhibited greater levels of condensed tannins in ICGV 86699 ($F_{(5,17)}$ = 35.7, $P < 0.01$), ICGV 86031 ($F_{(5,17)}$ = 59.2, $P < 0.001$) and ICG 2271 ($F_{(5,17)}$ = 27.9, $P < 0.05$) as compared to PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control treated plants. In ICG 1697 and JL 24, PJA + HIN and JA + HIN treated plants had significantly greater tannin content ($F_{(5,17)}$ = 21.3, and 19.8, respectively, $P < 0.05$) than PSA +

HIN, SA + HIN, HIN treated and the untreated control plants. The tannin content of PSA + HIN plants was at par with that of JA + HIN in ICG 1697 and JL 24. Insect-resistant genotypes had significantly greater amounts of condensed tannins in all the treatments ($F_{(4,14)}$ = 21.8, 11.7, 10.8, 16.5, 32.5 and 13.3 for PJA+HIN, PSA+HIN, JA+HIN, SA+HIN, HIN and the untreated control, $P < 0.05$) than the respective treatments in JL 24.

3.1.12 H_2O_2 content

The H_2O_2 levels increased in plants in response to various treatments (Fig. 3D). The PJA + HIN, PSA + HIN and JA + HIN treated plants had significantly greater H_2O_2 content in ICGV 86699 ($F_{(5,17)}$ = 27.9, $P < 0.001$), ICGV 86031 ($F_{(5,17)}$ = 15.6, $P < 0.01$), ICG 2271 ($F_{(5,17)}$ = 18.3, $P < 0.05$) and ICG 1697 ($F_{(5,17)}$ = 9.3, $P < 0.05$), than the respective SA + HIN, HIN and the untreated control plants. However in JL 24, no significant difference was observed in H_2O_2 content of PSA + HIN, JA + HIN, SA + HIN and HIN treated plants. The insect-resistant genotypes showed considerable increase in H_2O_2 content in all the treatments ($F_{(4,14)}$ = 10.4, 15.7, 21.4, 13.9, 11.6 and 23.1 for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control, $P < 0.01$) as compared to JL 24.

3.1.13 MDA content

The MDA content varied between plants treated with JA and SA, and insect infested plants (Fig. 4). The PSA + HIN, SA + HIN and HIN treated plants exhibited greater MDA content in ICGV 86031, ICG 2271 and ICG 1697 ($F_{(5,17)}$ = 10.3, 7.5 and 11.6, respectively, $P < 0.05$) as compared to PJA + HIN, JA + HIN and the untreated control plants. In ICGV 86699, MDA content of plants treated with SA + HIN was significantly greater ($F_{(5,17)}$ = 9.7, $P < 0.05$) than rest of the treatments. In JL 24, PSA + HIN treated plants had significantly greater MDA content ($F_{(5,17)}$ = 18.3, $P < 0.05$) than that of PJA + HIN, JA + HIN, SA + HIN, HIN and the untreated control plants. PSA + HIN, PJA + HIN and JA + HIN treated plants of JL 24 exhibited significantly higher MDA content ($F_{(4,14)}$ = 8.6, 11.1 and 7.8, respectively, $P < 0.05$) than that of ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697. No significant differences were observed in MDA content of PSA + HIN, SA + HIN, HIN and the untreated control plants across the genotypes.

3.1.14 Protein content

There was a tremendous increase in total protein content in JA and SA treated and insect infested plants (Fig. 5). The plants pretreated with JA and SA and infested with *H. armigera*, and the plants treated with JA + HIN had

greater protein content ($F_{(5,17)}$ = 12.6, 25.5, 21.3and 6.6 for ICGV 86699, ICGV 86031, ICG 2271, and JL 24, respectively, $P < 0.01$) than the plants treated with SA + HIN, HIN and the untreated control plants. There were no significant differences in protein content in ICG 1697 between JA + HIN and SA + HIN treated plants ($P > 0.05$). Across the genotypes tested, the insect resistant genotypes showed significantly greater accumulation of proteins ($F_{(4,14)}$ = 21.4, 41.9, 33.4, 26.3, 16.9 and 9.5 for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control, $P < 0.01$) than in the susceptible check, JL 24.

3.2 Effect of JA and SA induced resistance on plant damage, larval survival and larval weights

The plant damage by *H. armigera* was significantly lower in plants pre- and/or simultaneously treated with JA in ICGV 86699 ($F_{(4,14)}$ = 7.7, P = 0.05), ICGV 86031 ($F_{(4,14)}$ = 10.5, $P < 0.05$) and ICG 1697 ($F_{(4,14)}$ = 6.9, $P < 0.05$) as compared to PSA + HIN, SA + HIN and the insect-infested plants (Table 1). In ICG 2271, no significant difference was observed in plant damage in PJA + HIN, PSA + HIN, JA + HIN, SA + HIN treated plants, however, was significantly greater ($F_{(4,14)}$ = 7.4, $P < 0.05$) than HIN plants. Among the genotypes tested, the insect-resistant genotypes (ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697) suffered much lower damage in all the treatments as compared to that of the susceptible check, JL 24. There were significant differences in larval weights and larval survival across treatments. Larval survival was significantly lower in PJA + HIN treated plants in all the genotypes [ICGV 86699 ($F_{(4,14)}$ = 15.7, P = 0.05), ICGV 86031 ($F_{(4,14)}$ = 7.4, $P < 0.01$), ICG 2271 ($F_{(4,14)}$ = 6.6, $P < 0.05$), ICG 1697 ($F_{(4,14)}$ = 9.5, $P < 0.01$) and JL 24 ($F_{(4,14)}$ = 5.5, $P < 0.01$)]. Among the genotypes tested, the larvae fed on ICGV 86699 and ICGV 86031 showed significantly lower survivals ($F_{(4,14)}$ = 11.9, 17.4, 9.3, 12.4 and 7.8 for PJA+HIN, PSA+HIN, JA+HIN, SA+HIN and HIN, respectively, $P < 0.05$) than on JL 24 in all the treatments. Larvae fed on PJA + HIN treated plants showed significantly lower weights ($F_{(4,14)}$ = 23.3, 20.2, 15.3, 9.8 and 10.6 for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively, $P < 0.01$) as compared to those fed on PSA + HIN, SA + HIN, JA + HIN and HIN treated plants (Table 2). Across genotypes, larvae fed on ICGV 86699 had lower weights ($F_{(4,14)}$ = 21.2, 11.4, 8.6, and 18.9 for PJA+HIN, PSA+HIN, JA+HIN and HIN, respectively, $P < 0.05$) than those fed on rest of the genotypes. However, no significant differences were observed between weights of the larvae fed on SA + HIN treated plants of ICGV 86699 and ICGV 86031($P > 0.05$).

4 DISCUSSION

Although several phytohormones are involved in host plant defense against biotic and abiotic stresses, JA and SA play an important role in modulating plant defense against insect herbivory.[1,3,4,5,12] The JA and SA mediated induced resistance operates through octadecanoid and phenylpropanoid pathways, respectively, resulting in increased production of secondary metabolites and plant volatiles.[4,37] JA also regulates the activity of calcium-dependent protein kinases involved in plant defense against a variety of biotic and abiotic stresses through signal transduction.[38] JA accumulates in plants in response to insect damage and also by exogenous application. During this process, several secondary metabolites and volatiles are produced.[4] Further, JA also activates the antioxidative enzymes such as POD, PPO, LOX and production of PIs.[4] SA regulates reactive oxygen species (ROS) metabolism in plants, and oxidation of certain substrates of POD, CAT, SOD and other antioxidative enzymes, thus altering the hormonal balance and cell wall lignifications.[3,10-12] Increase in host plant resistance to herbivores has been observed through exogenous application of JA or MeJA[4,37] and SA.[10,12] Elucidation of various defensive responses in plants by exogenous application of JA and SA is essential for gaining an understanding of induced plant resistance against insect pests mediated by these hormones and their implications for insect pest management.

Our results showed that plants pretreated with JA had greater activity of defensive enzymes such as POD and PPO than the plants pretreated with SA. Increase in POD activity is regarded as the initial response of plants to the insect attack.[5,8] Increased activities of these enzymes in response to JA might be due to the greater accumulation of JA after insect infestation, and the subsequent activation of plant defensive pathways, resulting in increased activity of defensive enzymes such as POD and PPO. Induction of POD activity in response to JA and SA application and/or insect attack enhances the cell lignification, wound healing, and production of secondary metabolites, besides detoxifying the peroxides, and thus, defending the plants against insects, pathogens and other stresses.[8,39,40] The reduced nutritional quality of plant tissues on account of PPO has also been reported to play an important role in plant defense against insect herbivory.[10,41,42] Moreover, toxic but highly reactive quinines produced from phenol oxidation interact with nucleophilic side chain of amino acids and cross-link the proteins in plant tissues, thus reducing their digestibility.[42]

The PAL activity is induced by various stresses including insect herbivory.[10] PAL activity of groundnut plants was greater when pretreated with JA and SA, and the plants simultaneously treated with JA as compared to the insect-infested and uninfested control plants. The increase in PAL activity by JA and SA can be attributed to their similar effect on the activation of defensive pathways in response to damage by *H. armigera*. These pathways

produce various plant secondary metabolites, which on oxidation form several defensive compounds.[10] In addition, phenylpropanoid pathway, of which PAL is a central enzyme, also leads to lignin synthesis.[43] Lipoxygenase (LOX) gene expression is regulated by JA, and different biotic/abiotic stresses, including insect herbivory.[46] LOX catalyzes the production of JA from linolenic acid in octadecanoid pathway.[44] It also elicits the production of various plant defensive secondary metabolites and plant volatiles. The present study revealed that PJA + HIN and JA + HIN treated plants had significantly greater levels of LOX activity than rest of the treatments. This increased LOX activity in JA pre- and/or simultaneously treated plants might be due to the signaling of octadecanoid pathway by exogenous application of JA. Oxylipins produced from fatty acid oxidation by LOX play a wide array of functions in plant growth and development, senescence, and defense against biotic and abiotic stresses including insect herbivory.[45] Compounds formed from LOX mediated reactions are either directly deterrent to insect pests and/or produce post-ingestive toxicity in insects.[44]

The antioxidative enzymes involved in plant oxidative stress due to biotic and abiotic factors are SOD, APX and CAT. The present study revealed greater increase in APX activity in plants pretreated with JA and SA, and JA + HIN. Insect-resistant genotypes exhibited significantly greater APX activity than the susceptible check, JL 24. Pretreatment with JA, followed by insect infestation and simultaneous application of JA and insect infestation resulted in greater increase in CAT and SOD activities across the genotypes. Pre- and/or simultaneous treatment with SA also increased the activities of these enzymes; however, the induction was less as compared to that of JA. Insect-resistant genotypes showed greater increase in the activities of antioxidative enzymes as compared to the susceptible check, JL 24, but the levels of induction varied. The differential responses across the genotypes might be due to the differential ability of groundnut genotypes to perceive insect damage and/or the ability to mount the defensive response. Greater increase in SOD, APX and CAT following JA or SA treatment could be due to signaling of transduction pathways modulated by these phytohormones, which lead to the production of antioxidative enzymes to scavenge the toxic free radicals produced by herbivory. The higher constitutive levels of these enzymes in insect-resistant genotypes might protect them from the initial oxidative damage before the induced defense system is activated. APX decreases the ascorbate content in plant tissues by utilizing ascorbic acid as the electron donor in ascorbate-glutathione recycling while catalyzing the reduction of H_2O_2 to water, which in turn reduces the insect growth and development.[47] Greater APX activities in soybean leaves removes ascorbate from *H. zea* larval midgut, thereby, reduce insect growth and development.[47] *Scirpophaga incertulas* (Walk.) and *Cnaphalocrosis medinalis*

(Guenee) damage induced higher levels of CAT in rice.[48] CAT resists the oxidative stress in soybean caused by *H. zea* infestation.[49] The SOD converts the toxic free radicals, especially of oxygen, into less toxic and relatively stable H_2O_2.[50] Induction of SOD activity by SA has been found to reduce plant oxidative damage in maize.[51] *H. zea* infestation increased the SOD activity in tomato[52] and soybean.[49]

Plants produce many non-enzymatic defensive proteins against insect pests. However, PIs are the most exploited plant defensive proteins that confer resistance to insect pests.[53] The *in vitro* PI activity of groundnut plants pre-and/or simultaneously treated with JA and infested with *H. armigera* was significantly greater than the uninfested control plants. Overall, insect resistant genotypes showed greater PI activity than JL 24 in almost all the treatments. The reduction in protein digestibility by PIs and deprivation of insects of essential amino acids leads to retarded growth and development of insects.[53] PIs are strongly up-regulated in plants in response to wounding or herbivore damage and/or elicitor application. For example, exogenous application of MeJA in *Nicotina attenuata* Torr. ex S.Watson results in quick accumulation of JA, and the induction of trypsin proteinase inhibitors against *M. sexta*.[54]

Phenols constitute one of the most important and extensively studied groups of secondary metabolites against insect pests.[7,17,48] An abrupt increase in phenolic content occurs in plants damaged by insects and/or treated with elicitors including JA or SA.[21,22] PJA + HIN, PSA + HIN and HIN treated plants exhibited greater phenolic content than the SA + HIN treated and untreated plants, however, some genotypes such as ICG 2271, ICG 1697 and JL 24 responded similarly to pre- and/or simultaneous treatments of J A and SA. Further, insect-resistant genotypes showed a greater increase as compared to the susceptible check, JL 24. This might be due to the strong induction of the octadecanoid and phenylpropanoid signaling pathways by JA and SA, respectively. Flavonoids have been reported to confer resistance against *Spodoptera frugiperda* (J.E Smith) in *Arabidopsis thaliana* (L.).[55] Higher levels of flavonoids such as, daidezin and genistin have been observed in soybean plants infested with *Nezara viridula* (L.).[56] Tannins have been reported to be systemically induced in insect damaged plants.[54] In *N. attenuata*, application of MeJA induced greater accumulation of JA, which in turn activated the production of phenols, flavonoids, nicotine and trypsin proteinase inhibitors against *M. sexta*.[54]

Oxidative state of the host plants is associated with plant resistance to insects,[5,10] which results in the production of ROS, that are toxic to herbivores. Our results showed that both JA and SA induced higher levels of

H_2O_2 in all the genotypes infested with *H. armigera*. However, the induction was greater in plants pretreated with JA and SA, and in plants simultaneously treated with JA and infested with *H. armigera*. Insect-resistant genotypes showed a strong response in terms of accumulation of H_2O_2. The higher induction of H_2O_2 by pretreatment with JA and SA could be attributed to the increased activity of antioxidative enzymes in the treated plants, and conversion of toxic free radicals into H_2O_2. JA and SA induce oxidative burst in plants,[10,11,12] which happens to be the first and foremost defense against insect herbivory.[5,8,17,48] Transduction pathways signaled by H_2O_2 produce many defensive compounds, which result in oxidation of phenols and other compounds producing many defensive compounds.[11] Oxidative damage in midgut of the insects feeding on pre-wounded plants is due to the accumulation of H_2O_2 through JA and SA mediated pathways.[12,57]

Malondialdehyde is an important lipid peroxidation product, which indicates the extent of plant defensive response to the stress. The plants infested with *H. armigera* and pre- and/or simultaneously treated with SA had higher MDA content. Overall, JL 24 showed higher amounts of MDA among all the genotypes. This could be due to greater stress experienced by this genotype and the higher levels of lipid peroxidation. Lipid peroxidation and hydroxyl ion formation (OH^{-}) have been proposed to play an important role in plant defense by increasing the activity of oxidative enzymes.[49] MDA is also involved in volatile emission, and thus, having role in indirect plant defense as well.[58] Hao *et al.*[59] reported higher amounts of MDA in rice plants in response to rice stripe virus and small brown planthopper, *Nilaparvata lugens* (Stal.). Induction of proteins and their role in induced resistance against insect pests has been well established.[5,41,48] The present studies indicated that there was a significant increase in proteins in plants treated with PJA + HIN, followed by JA + HIN treated plants. Increase in protein concentration may be due to the increase in antioxidative enzymes and other non-enzymatic defensive proteins. Defense related enzymes and other protein based defensive compounds accumulate in plants in response to oxidative stress,[39,41] and on application of elicitors,[4,21,22,37] which defend them from various biotic and abiotic stresses.

Expression of resistance to insects, and insect growth and development are closely related. The PJA + HIN treated plants suffered relatively lower damage due to *H. armigera* across genotypes. The insect-resistant genotypes showed greater reduction in plant damage than the susceptible check, JL 24. Similar results were observed in terms of larval survival and larval weights of *H. armigera*. Reduced damage, lower larval survival and larval weights might be because of the greater production of toxic secondary metabolites in the insect-resistant genotypes by insect damage and JA application[41,42,44,46]. Reduced damage and lower larval growth and development were correlated with

increased activity of POD, PPO and other defensive enzymes induced following insect attack and/or elicitor application. Larvae of *Manduca sexta* (L.) and *Spodoptera exigua* (Hub.) fed on JA deficient mutant (*def1*) tomato plants exhibited higher survival and weight gain as compared to those fed on wild-type tomato.[60,61] Increased levels of POD, PPO and LOX in plants have been correlated with the reduction of insect growth and development.[39,42,52] Plant defensive compounds induced in insect-resistant genotype reduced the survival and development of *S. frugiperda* larvae.[41] Reduced larval weights due to antibiosis and antixenosis against *H. armigera* have also been observed in chickpea.[13]

4 CONCLUSIONS

The present studies showed that both JA and SA induced the antioxidative responses in groundnut plants against *H. armigera*, which in turn reduced insect growth and development, however, the effect of JA was greater than that of SA. The insect resistant genotypes have a better capability to respond to exogenous application of JA and SA than the susceptible check, JL 24. JA resulted in greater induced response than SA. The results suggested that induced resistance can be exploited as a component of pest management.

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REFERENCES

1. Wasternack C, Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann Bot* 100: 681-697 (2007)
2. Chitra K, Ragupathi N, Dhanalakshmi K, Mareeshwari P, Indra N, Kamalakannan A, *et al*, Salicylic acid induced systemic resistant on peanut against *Alternaria alternata. Arch Phytopath Plant Prot* **41**(1):50–56 (2008).

3. Kawazu K, Mochizuki A, Sato Y, Sugeno W, Murata M, Seo S, *et al*, Different expression profiles of jasmonic acid and salicylic acid inducible genes in the tomato plant against herbivores with various feeding modes. *Arthropod-Plant Interact* **6**:221-230 (2012).
4. Scott MI, Thaler SJ and Scott GF, Response of a generalist herbivore *Trichoplusia ni* to jasmonate-mediated induced defense in tomato. *J Chem Ecol* **36**:490-499 (2010).
5. He J, Chen F, Chen S, Lv G, Deng Y, Fang Z, Guan Z, *et al,* Chrysanthemum leaf epidermal surface morphology and antioxidant and defense enzyme activity in response to aphid infestation. *J Plant Physiol* **168**:687-693 (2011).
6. Sharma HC and Norris DM, Chemical basis of resistance in soya bean to cabbage looper, *Trichoplusia ni. J Sci Food Agri*, **55:**353-364 (1991).
7. Sharma HC, Sujana G and Rao DM, Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. *Arthropod-Plant Interact* 3:151-161 (2009).
8. Heng-Moss TM, Sarath G, Baxendale F, Novak D, Bose S, Ni X, *et al*, Characterization of oxidative enzyme changes in buffalograsses challenged by *Blissus occiduus*. *J Econ Entomol* **97**:1086-1095 (2004).
9. Arimura G, Köpke S, Kunert M, Volpe V, David A, Brand P, *et al*, Effects of feeding *Spodoptera littoralis* on Lima bean leaves: IV. Diurnal and nocturnal damage differentially initiate plant volatile emission. *Plant Physiol* 146:965-973 (2008)
10. Zhao LY, Chen JL, Cheng DF, Sun JR, Liu Y and Tian Z, Biochemical and molecular characterizations of *Sitobion avenae*– induced wheat defense responses. *Crop Protect* 28:435-442 (2009).
11. Vicent MRS and Plasencia J, Salicylic acid beyond defense: its role in plant growth and development. *J Exp Bot* DOI:10.1093/jxb/err031 (2011).
12. Peng J, Deng X, Huang J, Jia S, Miao X and Huang Y, Role of salicylic acid in tomato defense against cotton bollworm, *Helicoverpa armigera* Hubner. *Zeitschrift Naturf C* 2004, 59:856-862.
13. Sharma HC(Ed.): *Heliothis/Helicoverpa Management: Emerging Trends and Strategies for Future Research*. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, India, pp. 469 (2005,).
14. Kranthi KR, Jadhav DR, Kranthi, S, Wanjari RR, Ali SS, Russel DA, Insecticide resistance in five major insect pests of cotton in India. *Crop Protect* **21**:449–460 (2002).

15. Stevenson PC, Blaney WL, Simmonds MSJ and Wightman JA: The identification and characterization of resistance in wild species of *Arachis* to *Spodoptera litura* (Lepidoptera: Noctuidae). *Bull Entomol Res* **83**:421-429 (1993).

16. Rao KVR, Influence of host plant nutrition on the incidence of *Spodoptera litura, Helicoverpa armigera* on groundnut. *Indian J Entomol* **65(3):**386-392 (2003).

17. War AR, Paulraj MG, War MY and Ignacimuthu S, Jasmonic acid- mediated induced resistance in groundnut (*Arachis hypogaea* L.) against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *J Plant Growth Regul* **30**:512-523 (2011).

18. War AR, Paulraj MG, War MY and Ignacimuthu S, Herbivore induced resistance in different Groundnut germplasm lines to Asian armyworm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Acta Physiol Plant* **34**:343–352 (2012).

19. Grayer RJ, Kimmins FM, Padgham DE, Harborne JB and Ranga Rao DV, Condensed tannin levels and resistance in groundnuts (*Arachis hypogoea* (L.) against *Aphis craccivora* (Koch). *Phytochemistry* **31**:3795-3799 (1992).

20. Rao RVS, Sridhar R, Singh U and Ranga Rao GV, Bio-chemical basis in groundnut (*Arachis hypogaea*) resistant to leafminer (*Aproaerema modicella*). *Indian J Agri Sci* 68(2):104-109 (1998).

21. Sharma HC, Pampathy G, Dwivedi SL and Reddy LJ, Mechanism and diversity of resistance to insect pests in wild relatives of groundnut. *J Econ Entomol* **96(6):**1886-1897 (2003).

22. Armes NJ, Bond GS and Cooter RJ, The laboratory culture and development of *Helicoverpa armigera.* Bulletin No. 57. Natural Resource Institute, Chatham, UK, pp. 21 (1992).

23. Shannon LM, Kay E and Lew JY, Peroxidase isozymes from horse radish roots. Isolation and physical properties. *The J Bio Chem* **241**:2166-2172 (1966).

24. Mayer AM and Harel E, Polyphenol oxidases in plant. *Phytochemistry* **18**:193–215 (1979).

25. Hildebrand D and Hymowitz T, Lipoxygenase activities in developing and germinating soybean seeds with and without lipoxygenase-1. *Bot Gazzette* **144**:212-216 (1983).

26. Beauchamp C and Fridovich I, Superoxide dismutase: improve assay and an assay applicable to acrylamide gels. *Annal Biochem* **44**:276–287 (1971).

27. Campos-Vergas R and Saltveit ME, Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. *Physiol Plant* **114**:73-84 (2002).

28. Zhang SZ, Hau BZ and Zhang F, Induction of the activities of antioxidative enzymes and the levels of malondialdehyde in cucumber seedlings as a consequence of *Bemisia tabaci* (Hemiptera: Aleyrodidae) infestation. *Arthropod-Plant Interact* **2:**209-213 (2008).

29. Asada K and Takahashi M, Production and scavenging of active oxygen in photosynthesis. In *Photoinhibition.* Edited by Kyle DJ, Osmond CB, Arntzen CJ: Elsevier, Amsterdam, pp. 227–287 (1987).

30. Kakade ML, Rackis JJ, McGhee JE and Puski G, Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. *Cereal Chem* **51**:376-382 (1974).

31. Zieslin N, and Ben-Zaken R, Peroxidase activity and presence of phenolic substances in penduncles of rose flowers. *Plant Physiol Biochem* **31**:333-339 (1993).

32. Robert EB, Method for estimation of tannin in grain sorghum. *Agronomy J* **63**:511-512 (1971).

33. Woisky R and Salatino A, Analysis of propolis: some parameters and procedures for chemical quality control. *J Api Res* **37**:99-105 (1998).

34. Noreen Z and Ashraf M, Change in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.). *Environ Exp Bot* **67**:395-402 (2009).

35. Carmak I and Horst JH, Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean. (*Glycine max*). *Physiol Plant* **83**:463-468 (1991).

36. Lowery OH, Rosebrough NI, Farr AL and Randall RJ, Protein measurement with the folin phenol reagent. *J Biol Chem* **193**:265-275 (1951).

37. Shivaji R, Camas A, Ankala A, Engelberth J, Tumlinson JH, Williams WP, *et al*, Plants on constant alert: elevated levels of jasmonic acid and jasmonate-induced transcripts in caterpillar-resistant maize. *J Chem Ecol* **36**:179-191 (2010).

38. Ludwig AA, Romeis T and Jones JD, CDPK-mediated signaling pathways: specificity and cross-talk. *J Exp Bot* **55**:181-188 (2004).

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

40. Rangasamy M, Rathinasabapathi B, McAuslane HJ, Cherry RH, and Nagata RT, Oxidative response of St. Augustinegrasses to feeding of southern chinch bug, *Blissus insularis* Barber. *J Chem Ecol* **35**:796-805 (2009).

41. Chen Y, Ni X and Buntin GD, Physiological, nutritional and biochemical bases of corn resistance to foliage-feeding fall armyworm. *J Chem Ecol* **35**:297-306 (2009).

42. Bhonwong A, Stout MJ, Attajarusit J and Tantasawat P, Defensive role of tomato Polyphenol oxidase against cotton bollworm (*Helicoverpa armigera*) and Beet armyworm (*Spodoptera exigua*). *J Chem Ecol* **35**:28-38 (2009).

43. Ritter H and Schulz G, Structural basis for the entrance into the phenylpropanoid metabolism catalyzed by phenylalanine ammonia-lyase. *Plant Cell* **16**:3426-3436 (2004).

44. Mao YB, Cai WJ, Wang JW, Hong GJ, Tao XY, Wang LJ, *et al*, Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nature Biotech* **25**:1307-1313 (2007).

45. Bruinsma M, Posthumus MA, Mumm R, Mueller MJ, van Loon JJA and Dicke M, Jasmonic acid-induced volatiles of *Brassica oleracea* attract parasitoids: Effects of time and dose, and comparison with induction by herbivores. *J Exp Bot* **60**:2575–2587 (2009).

46. Felton GW, Bi J, Summers CB, Mueller AJ and Duffey SS, Potential role of lipoxygenases in defense against insect herbivory. *J Chem Ecol* **20**:651-666 (1994a).

47. Felton GW, and Summers CB, Potential role of ascorbate oxidase as a plant defense protein against insect herbivory. *J Chem Ecol* **19:**1553-1568 (1993).

48. Usha Rani P and Jyothsna Y, Biochemical and enzymatic changes in rice as a mechanism of defense. *Acta Physiol Plant* **32**:695-701 (2010).

49. Bi J and Felton GW, Foliar oxidative stress and insect herbivory: Primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. *J Chem Ecol* **1**:1511-1530 (1995).

50. Raychaudhuri S and Deng XW, The role of superoxide dismutase in combating stress in higher plants. *Bot Review* **66**: 89-98 (2000).

51. Saruhan N, Saglam A and Kadioglu A, Salicylic acid pretreatment induces drought tolerance and delays leaf rolling by inducing antioxidant systems in maize genotypes. *Acta Physiol Plant* 34:97–106 (2012).

52. Felton GW, Summers CB and Mueller AJ, Oxidative responses in soybean foliage to herbivory by bean leaf beetle and three-corned alfalfa leafhopper. *J Chem Ecol* **20**:639-650 (1994b).

53. Parde VD, Sharma HC and Kachole MS, *In vivo* inhibition of *Helicoverpa armigera* gut pro-proteinase activation by non-host plant protease inhibitors. *J Insect Physiol* **56**:1315–1324 (2010).

54. Wu JS, Wang L and Baldwin IT, Methyl jasmonate-elicited herbivore resistance: does MeJA function as a signal without being hydrolyzed to JA? *Planta* **227**:1161–1168 (2008).

55. Johnson ET and Dowd PF, Differentially enhanced insect resistance, at a cost, in *Arabidopsis thaliana* constitutively expressing a transcription factor of defensive metabolites. *J Agri Food Chem* **52**:5135-5138 (2004).

56. Piubelli GC, Hoffmann-Campo CB, de Arruda IC, Franchini JC and Lara FM, Flavonoid increase in soybean as a response to *Nezara viridula* injury and its effect on insect-feeding preference. *J Chem Ecol* **29**:1223–1233 (2003).

57. Orozco-Cárdenas ML, Narvaez-Vasquez J and Ryan CA, Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin and methyl jasmonate. *Plant Cell* **13**:179-191 (2001).

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

59. Hao Z, Wang L, He Y, Liang J and Tao R, Expression of defense genes and activities of antioxidant enzymes in rice resistance to rice stripe virus and small brown planthopper. *Plant Physiol Biochem* **49**:744-751 (2011).

60. Howe GA, Lightner J, Browse J and Ryan CA, An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *Plant Cell* **8**: 2067–2077 (1996).

61. Thaler JS, Farag MA, Pare PW and Dicke M, Jasmonate-deficient plants have reduced direct and indirect defenses against herbivores. *Ecology Lett* **5**:764–774 (2002).

FIGURE CAPTIONS

Fig. 1: Enzyme activities of groundnut plants pre- and/or simultaneously treated with JA and SA and infested with *H. armigera*

(A) Peroxidase (POD) activity (IU g^{-1} FW); (B) Polyphenol oxidase (PPO) activity (IU g^{-1} FW); (C) Phenylalanine ammonia lyase (PAL) activity (µmol cinnamic acid min^{-1} mg^{-1} protein); (D) Lipoxygenase (LOX) activity (IU g^{-1} FW)

Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. * on same color bars shows the significance across the genotypes within a treatment, ***, **,* = significant at $P \leq$ 0.001, 0.01 and 0.05, respectively. PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN = Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

Fig. 2: Fig. 2: Enzyme activities of groundnut plants pre- and/or simultaneously treated with JA and SA and infested with *H. armigera*

(A) Superoxide dismutase (SOD) activity (IU g^{-1} FW); (B) Ascorbate peroxidase (APX) activity (IU mg^{-1} protein); (C) Catalase (CAT) activity (µmol min^{-1} mg^{-1} protein); (D) The *in vitro* protease inhibitor (PI) activity (%)

Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. * on same color bars shows the significance across the genotypes within a treatment, ***, **,* = significant at $P \leq 0.001$, 0.01 and 0.05, respectively. PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN = Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

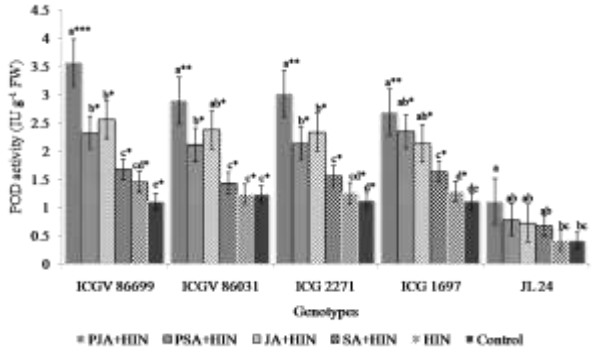
Fig. 3: Amounts of plant secondary metabolites and other components of groundnut plants pre- and/or simultaneously treated with JA and SA and infested with *H. armigera*

(A) Total phenols (µg GAE g^{-1} FW); (B) Flavonoid content (µg CE g^{-1} FW); (C) Condensed tannins (µg CE g^{-1} FW); (D) H_2O_2 content (µmol g^{-1} FW)

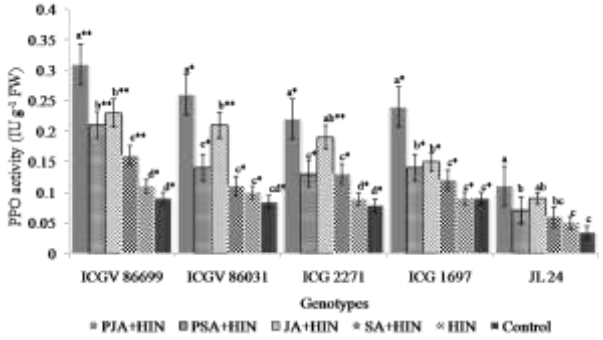
Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. * On same color bars shows the significance across the genotypes within a treatment, ***, **,* = significant at $P \leq 0.001$, 0.01 and 0.05, respectively. PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN = Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants; GAE = Gallic acid equivalents; CE = Catechin equivalents.

Fig. 4: Malondialdehyde (MDA) (µmol g^{-1} FW) (4A) and protein contents (mg g^{-1} FW) (4B) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.

Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. * on same color bars shows the significance across the genotypes within a treatment, ***, **,* = significant at $P \leq 0.001$, 0.01 and 0.05, respectively. PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN = Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.



A



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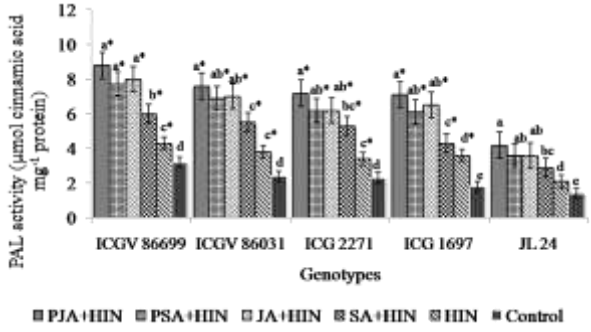

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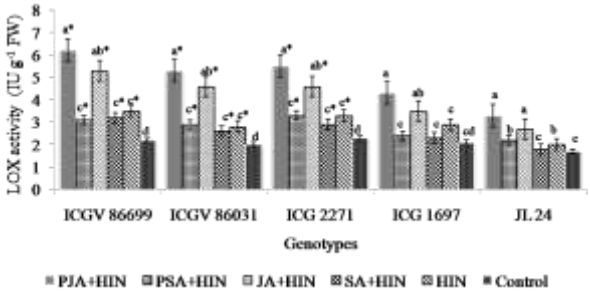
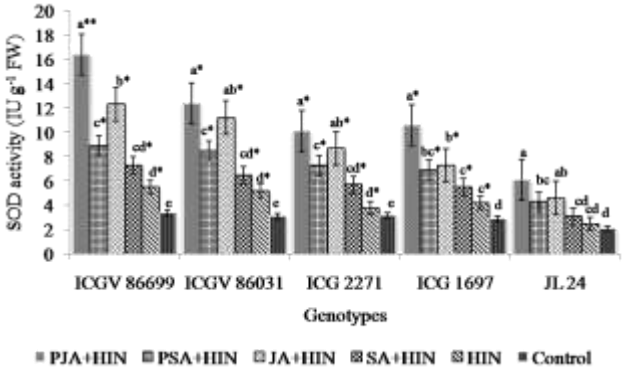
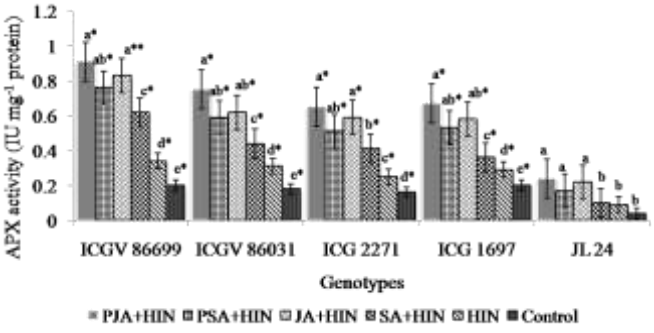


D

Fig. 1A,B,C,D



A



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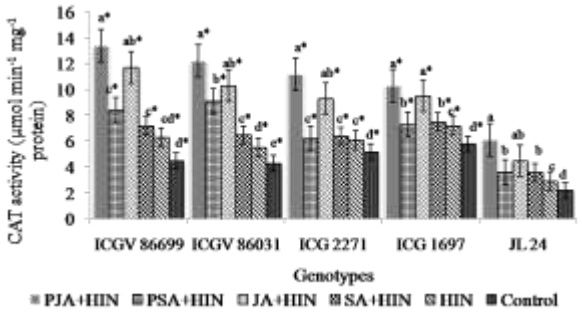

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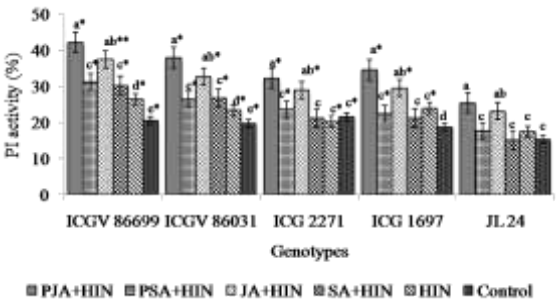
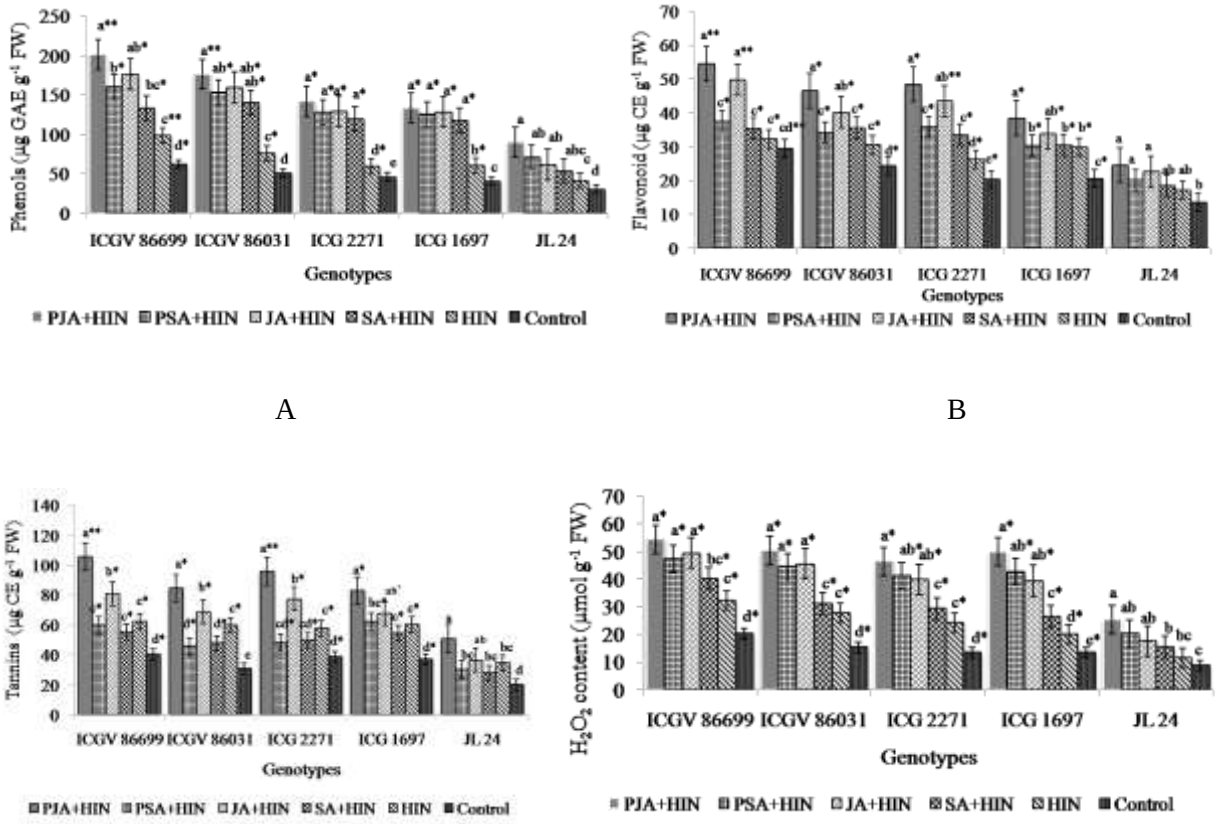


D

Fig. 2 A,B,C,D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Flavonoid (µg CE g-1 FW)
Tannins (µg CE g-1 FW)
H2O2 content (µmol g-1 FW)
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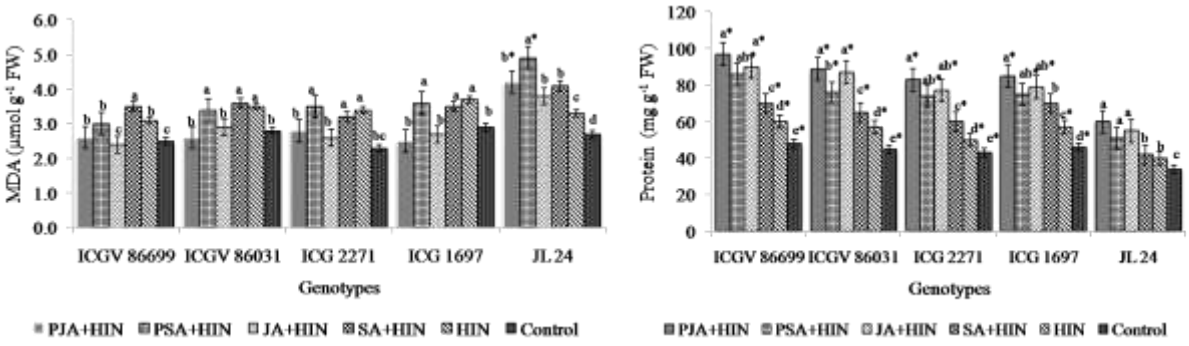
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotypes
PJA+HIN
PSA+HIN
JA+HIN
SA+HIN
HIN
Control
A
B
C
D

Fig. 3 A,B,C,D


MDA (µmol g-1 FW)
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5.0
4.0
3.0
2.0
1.0
0.0
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotypes
PJA+HIN
PSA+HIN
JA+HIN
SA+HIN
HIN
Control
Protein (mg g-1 FW)
120
100
80
60
40
20
0
ICGV 86699
ICGV 86031
ICG 2271
ICG 1697
JL 24
Genotypes
PJA+HIN
PSA+HIN
JA+HIN
SA+HIN
HIN
Control

A **B**

Fig. 4 A,B

Table 1: Plant damage and *Helicoverpa armigera* larval survival on plants treated with jasmonic acid and salicylic acid.

Genotypes	Plant damage rating (DR)[x]					Survival (%)				
	PJA+HIN	PSA+HIN	JA+HIN	SA+HIN	HIN	PJA+HIN	PSA+HIN	JA+HIN	SA+HIN	HIN
ICGV 86699	2.0 ± 0.9^{c*}	2.6 ± 0.5^{b}	2.4 ± 0.9^{bc*}	2.7 ± 0.4^{b}	3.2 ± 0.7^{b}	20.4 ± 2.1^{c*}	32.3 ± 2.3^{bc}	30.2 ± 4.6^{c}	36.5 ± 3.4^{bc}	41.2 ± 3.1^{c}
ICGV 86031	2.5 ± 0.8^{bc*}	3.0 ± 0.3^{b}	2.6 ± 0.8^{b*}	3.2 ± 0.6^{b}	3.5 ± 0.3^{b}	26.6 ± 2.1^{bc*}	34.3 ± 2.2^{bc}	35.5 ± 3.3^{c}	39.6 ± 4.4^{bc}	47.4 ± 2.1^{b}
ICG 2271	3.2 ± 0.9^{b*}	3.5 ± 0.3^{b*}	3.1 ± 0.6^{b*}	3.5 ± 0.7^{b*}	4.0 ± 0.6^{b}	32.4 ± 1.4^{b*}	40.5 ± 3.8^{b}	40.4 ± 2.1^{b}	44.5 ± 2.1^{b}	48.9 ± 3.1^{b}
ICG 1697	3.0 ± 0.7^{b*}	3.4 ± 0.6^{b}	3.0 ± 0.4^{b*}	3.6 ± 0.9^{b}	3.9 ± 0.7^{b}	35.7 ± 3.2^{b*}	44.8 ± 2.6^{b}	48.2 ± 3.2^{b}	50.5 ± 3.6^{b}	54.4 ± 4.7^{b}
JL 24	5.5 ± 1.1^{a*}	6.4 ± 1.1^{a}	6.2 ± 1.2^{a}	7.0 ± 0.6^{a}	7.5 ± 1.3^{a}	58.3 ± 2.1^{a*}	69.4 ± 3.8^{a}	75.9 ± 2.3^{a}	79.6 ± 4.1^{a}	81.4 ± 6.6^{a}

Values (Mean ± SD) carrying same letter(s) within a column are not significantly different at $P \leq 0.05$ (Tukey's HSD test).
* in a row shows significant difference in plant damage and larval survival across the treatments within a genotype.
[x] DR = *Helicoverpa* damage rating to a scale 1-9 ($1 \leq 10$ % and $9 \geq 80$ %) 6 days after infestation.
PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

Table 2: Weight (mg)* of *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.

Genotypes	Treatments				
	PJA+HIN	**PSA+HIN**	**JA+HIN**	**SA+HIN**	**HIN**
ICGV 86699	$37.5 \pm 3.1^{d*}$	48.6 ± 5.3^{d}	47.5 ± 5.6^{d}	59.7 ± 3.5^{e}	69.6 ± 3.6^{d}
ICGV 86031	$44.5 \pm 2.8^{bc*}$	60.6 ± 3.7^{c}	75.5 ± 7.7^{bc}	74.4 ± 3.7^{de}	97.7 ± 5.3^{c}
ICG 2271	$55.4 \pm 3.2^{b*}$	65.6 ± 5.3^{c}	87.6 ± 3.4^{b}	98.8 ± 4.7^{bc}	110.3 ± 8.8^{bc}
ICG 1697	$59.6 \pm 2.7^{b*}$	80.6 ± 6.4^{b}	95.5 ± 4.3^{b}	114.4 ± 6.3^{ab}	127.5 ± 7.3^{b}
JL 24	$73.6 \pm 4.3^{a*}$	102.4 ± 7.6^{a}	120.3 ± 8.7^{a}	129.5 ± 9.5^{a}	159.5 ± 10.0^{a}

Values (Mean ± SD) carrying same letter(s) within a column are not significantly different at $P \leq 0.05$ (Tukey's HSD test).
* in a row shows significant difference in larval weight across the treatments within a genotype. PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.