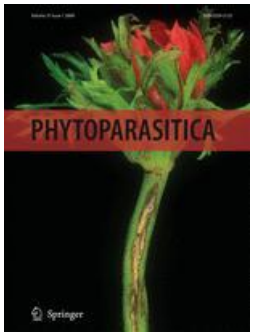

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Jasmonic and salicylic acid-induced resistance in sorghum against the stem borer Chilo partellus

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Jasmonic and salicylic Acid Induced Resistance in Sorghum against stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera; Pyralidae)

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Abstract

Induced resistance was studied in three sorghum genotypes (IS2205, ICSV1 and ICSV700) against *Chilo partellus* infestation and jasmonic acid (JA) and salicylic acid (SA) application. The activity of plant defensive enzymes [peroxidase (POD), polyphenol oxidase (PPO), superoxide dismutase (SOD), and catalase (CAT)], and the amounts of total phenols, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and proteins were recorded at six days after infestation. The induction of enzyme activities and the amounts of secondary metabolites varied among the genotypes and treatments. The genotype IS2205 showed stronger effect than those of ICSV1 and ICSV 700. Treatment with JA followed by insect infestation induced greater levels of enzymes and secondary metabolites. The results suggest that JA induces greater levels of resistance components in sorghum plants against insect pests. Thus, pretreatment of plants with elicitors including JA and SA could provide more opportunity for plant defense against herbivores.

Key words: Antioxidant enzymes; biotic stress; induced resistance; phytohormones; Sorghum, stem borer

Introduction

Herbivorous insects use diverse feeding strategies to obtain nutrients from their host plants. Rather than acting as passive victims in these interactions, plants respond to herbivory with the production of toxins and defensive proteins that target physiological processes in the insect (Zhao et al. 2009; Kawazu et al. 2012). This highly dynamic form of immunity is initiated by the recognition of insect oral secretions and signals from injured plant cells. Plants have developed a wide array of defense strategies against insect herbivory, which could be constitutive and/or induced (Scott et al. 2010; He et al. 2011; War et al., 2012). The constitutive resistance is always present in plants irrespective of the external stimuli, while as the induced resistance is occurs in response to the external stimuli. These initial cues are transmitted within the plant by signal transduction pathways that include calcium ion fluxes, phosphorylation cascades, and, in particular, the jasmonate pathway (Waling 2000; Shivaji et al. 2010; Scott et al. 2010; He et al. 2011). Jasmonic acid pathway plays a central and conserved role in promoting resistance to a broad spectrum of insects (Waling 2000; Shivaji et al. 2010). Although, constitutive resistance is the first and primary defense against insects, induced resistance is more reliable and effective. It reduces the reallocations costs as it is produced when in demand. This induced defense against insect herbivory can be direct or indirect. Indirect induced defenses attract natural enemies of herbivores, whereas direct induced defenses directly affect the performance and

preference of the attacking herbivore (Heng-Moss et al. 2004; Arimura et al. 2008; Scott et al. 2010). Chemical defense strategies involve secondary metabolites and proteins which may be present constitutively or induced by challenges such as herbivore wounding (Heng-Moss et al. 2004; War et al. 2011a,b,c, 2012). Direct and indirect defense mechanisms can function additively against the herbivore. Phytohormones are involved in plant defense against insect herbivores. These mediate plant signaling pathways, which lead to the production of various defensive secondary metabolites and proteins. The important phytohormones that play active roles in plant defense against various stresses are jasmonic acid (JA) and salicylic acid (SA; Zhao et al. 2009; Shivaji et al. 2010; Kawazu et al. 2012; War et al.2011b).

The important oxidative plant enzymes induced in plants in response to insect herbivory include peroxidases (POD), polyphenol oxidase (PPO), superoxide dismutase (SOD), phenylalanine ammonia lyase (PAL), lipoxygenase (LOX), catalase (CAT) and ascorbate peroxidase (APX) (Zhao et al., 2009; Scott et al. 2010; War et al. 2012). POD is an important antioxidative enzymes involved in plant defense against insect herbivory (He et al. 2011). It produces semiquinone free radicals and subsequently the quinines, which are highly toxic to insect pests (Barbehenn et al. 2010). The PPO is an antinutritional enzyme, and reduces the food quality of the plant tissues due to the oxidation of phenols to highly reactive and toxic quinines (Bhonwong et al. 2009; War et al. 2012). The SOD is an important antioxidative enzyme in plants involved in the conversion of toxic, highly reactive and unstable free radicals into less toxic and relatively stable H_2O_2 (Raychaudhuri and Deng 2000). CAT is an important enzyme in reactive oxygen species (ROS) scavenging systems (Khattab and Khattab 2005; Heidari 2009). Oxidation of phenols by results in the production of toxic quinones that affect the insect growth and development, while as some phenols are directly toxic to insect pests (Maffei et al. 2006; Howe and Jander 2008; War et al. 2013). H_2O_2 is an important stable ROS involved in plant defense against insect herbivory. It acts as a second messenger in signal transduction pathways, which lead to the production of toxic chemicals (Maffei et al. 2007). Malondialdehyde (MDA) is an important indicator of plant defense against insect pests (Gechev et al. 2002).

Sorghum, *Sorghum bicolor* Moench, is an essential food and cash crops for millions of people in Africa, Asia, USA, Australia and Latin America and is the fifth major cereal after wheat, rice, maize and barley. *Chilo partellus* (Swinhoe) is the most serious pest of sorghum and maize in Asia and Africa (Sharma et al., 2003). It is difficult to control, largely because of the cryptic and nocturnal habits of the adult moths. In addition, due to the protection provided to the immature stages by the stem of the host plant, the insecticidal film sprayed on the crop

does not reach to the target organism. The losses caused by this insect are to the tune of US$ one billion (ICRISAT 1992). It can potentially damage all the above ground parts of the plant from the second fortnight after seedling emergence till harvest of the crop. Young larvae feeding results into pinholes and followed by elongated lesions on the leaf whorls. When pest attacks at early stage it destroys the growing point commonly known as “dead heart” due to drying of two to three central leaves thus reducing plant vigor, reduce photosynthetic efficiency, delay in flowering and ultimately leads to the reduction in grain yield. The older larvae descend down inside the whorl leaves, bore inside the stem and cause stem tunneling that disrupts the nutrient supply to the above canopy, which leads to the chaffy panicles and ultimately reduction in fodder quality and yield. The present studies were carried out to understand the induced resistance in sorghum genotypes against *C. partellus* by exogenous application of JA and SA. The studies were focused on various antioxidative enzymes and secondary metabolites involved in plant resistance against insect pests.

Materials and Methods

Chemicals

The chemicals used in this study were of analytical grade. Ethylene diamine tetra acetic acid (EDTA), bovine serum albumin (BSA), guaiacol, polyvinylpyrolidone (PVP), proline, glucose, jasmonic acid, salicylic acid, jasmonic acid, salicylic acid, tannic acid, dithiothretol (DTT), disodium hydrogen phosphate, sodium dihydrogen phosphate, nitro-blue tetrazolium salt (NBT), methionine, L-phenylalanine, 4-chloronapthol, glucose, potassium iodide (KI), and sodium carbonate (Na_2CO_3) were obtained from Sigma Aldrich, USA. Catechol was obtained from Glaxo Laboratories, Mumbai, India. 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) was obtained from HiMedia Pvt. Ltd., Mumbai, India. Ammonium sulphate was obtained from Qualigens Fine Chemicals, Mumbai, India.

The spectrophotometer used for the estimation of biochemical parameters was Hitachi UV – 2900 (Hitachi, Japan).

Insects

Insects used for the studies were obtained from a well maintained insect rearing laboratory at International Crops for Semi-Arid Tropics and culture of *C. partellus* was maintained under controlled conditions, 16:8 h L: D regime at 25 ± 1 ºC and 65 ± 5 % RH on sorghum based artificial diet Taneja and Leuschner (1985). Aqueous sugar solution 10% was offered as food to the adults. The pupae were washed with 2% sodium hypochlorite solution and transferred to

plastic jars containing Vermiculite. Adults were transferred to wooden oviposition cages (30 x 30 x 30 cm), and provided with 10% of sucrose to study the oviposition preference.

Sorghum plants (*Sorghum bicolor* (L.) Moench)

Seeds of sorghum genotypes (IS2205, ICS1 and ICSV700) were sown in plastic pots measuring 30x 30 cm diameter in greenhouse (temperature 27 ± 3 °C, RH 65 ± 5 %) to study the effect of two signaling molecules (jasmonic and salicyclic acids) on induced resistance to *C. partellus.* Few days after germination, only three plants were allowed to grow in each plastic pot to provide a uniform plant stand for all the test genotypes. At stage V2 (five leaf stage), plants were sprayed with jasmonic acid (1mM) and salicylic acid (1mM) then infested with *C. partellus* (JA + IN and SA + IN, respectively) and another treatment was infested with third instar larva of *C. partellus* (IN) and separate unsprayed and uninfested control (UT) was set for all the genotypes. Before releasing the third instar larva, plants were enclosed by plastic jars to avoid the moment of larva from one plant to another plant. After six days of infestation, leaves were excised and collected from the infested and uninfested control plants to study the activity of various defensive enzymes including POD, PPO, and CAT, and the amounts of secondary metabolites such as phenols, tannins, and of H_2O_2, MDA and proteins.

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 16,000× g for 20 min and the supernatant was collected.

Peroxidase (POD) assay

Peroxidase activity was estimated as per the method of Shannon et al. (1966) with slight modification. The reaction mixture (2.9 ml) containing 0.1 M sodium phosphate buffer (pH 6.5), 0.8 mM H_2O_2 and 5 mM Guaiacol was taken in a test tube, to which 0.1 ml of enzyme source was added and the absorbance was read at 470 nm for 2 min at 15 sec intervals. Enzyme activity was expressed as ΔOD min^{-1}.

Polyphenol oxidase (PPO) assay

Polyphenol oxidase activity was estimated as per the method of Mayer and Harel (1979) with some modifications. To 2.9 ml of 0.1 M sodium phosphate buffer (pH 6.8), 0.1 ml of enzyme source and 0.1 ml of substrate (0.05M catechol) were added. Absorbance was read at 420 nm for 3 min at 30 sec interval. Enzyme activity was expressed as the enzyme activity was expressed as ΔOD min^{-1}.

Superoxide dismutase (SOD) assay

The activity of SOD was assayed by the method of Beauchamp and Fridovich (1971) with slight modifications. 3 ml of 0.05 M sodium phosphate buffer with 0.1% NaCl (pH 7.8) was taken in a test tube to which 0.3 ml of 0.1 mM EDTA, 0.3 ml of 0.13 mM methionine, 0.1 ml of 0.02 mM KCN, 0.3 ml of 0.75 mM NBT, 0.3 ml of 0.02 mM riboflavin and 0.1 ml of enzyme extract were added. The reaction mixture was illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes for 1h. Identical solutions that were kept under dark served as blanks. Absorbance was read at 560 nm against the blank and the activity was expressed as $\Delta OD\ min^{-1}$.

Catalase (CAT)

Catalase activity was assayed as described by Zhang et al. (2008). The reaction mixture consisted of 1 ml of Tris-HCl buffer (pH 7.0), 0.1 ml of partially purified enzyme extract and 0.2 ml of H_2O_2. Absorbance was read at 240 nm for 2 min and the enzyme activity was expressed as Units mg^{-1} protein.

Phenolic content

Leaves (0.5 g) were homogenized in 3 ml of 80% methanol and agitated for 15 min at 70 ºC. The homogenate was centrifuged at 10, 000 rpm for 10 min and the supernatant was collected, which was used for the estimation of total phenolsby the method of Zieslin and Ben-Zaken (1993) with some modifications. To 2 ml of 2% sodium carbonate (Na_2CO_3) taken in a test tube, 1 ml of methanol extract was added. The solution was incubated for 5 min at room temperature and 0.1 ml of 1 N Folin-Ciocalteau reagent was added. The solution was re-incubated for 10 min and absorbance of the blue color was measured at 760 nm. Phenolic concentration was expressed as mg Catechol Equivalents g^{-1} FW (mg GAE g^{-1} FW).

Hydrogen peroxide (H_2O_2) content

Hydrogen peroxide content was estimated by the method of Noreen and Ashraf (2009). Fresh leaf tissue (0.1 g) was homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA) in pestle and mortar and centrifuged at 12,000×g for 15 min. To the supernatant (0.5 ml), 0.5 ml of phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide (KI) were added. The absorbance was read at 390 nm. H_2O_2 concentration was expressed as µmol g^{-1} FW (extinction coefficient of H_2O_2 0.28 µM cm^{-1}).

Malondialdehyde (MDA) content

MDA content was determined by the method of Carmak and Horst (1991) with minor modification. Fresh leaf tissue (0.2 g) was homogenized in 3 ml 0.1% (w/v) trichloroacetic acid (TCA) at 4 ºC, centrifuged at 20,000 × g for 15

min. To 3 ml 0.5% (v/v) thiobarbituric acid (TBA) in 20% TCA, 0.5 ml of supernatant was added. The mixture was incubated at 95 ^{o}C in a shaking water bath for 50 min and the reaction was stopped by cooling the tubes in an ice water bath. Then samples were centrifuged at 10,000× g for 10 min and the absorbance of the supernatant was read at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using the absorption coefficient 155 $mmol^{-1}cm^{-1}$ and expressed as nanomol g^{-1} FW.

Protein content

Protein content was determined using the method of Lowery et al. (1951) using bovine serum albumin as standard.

Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using SPSS (Ver. 11.5). Tukey's HSD test was applied to separate the means.

RESULTS

POD activity

Across the treatments within the genotypes, JA treated plants showed significantly greater POD activity as compared to the plants treated with SA, infested and untreated control plants in IS2205 and ICSV700 (Fig. 1). No significant difference was observed in ICSV1 across the treatments. Among the genotypes, IS2205 plants showed significantly higher POD activity in all the treatments as compared to the corresponding treatments of ICSV1 and ICSV700**.**

PPO activity

The JA + IN treated plants in IS2205 and ICSV1 showed significantly greater PPO activity as compared to the SA + IN treated, IN and untreated control plants (Fig 2). However, in ICSV700, JA + IN and SA + IN treated plants showed significantly higher PPO activity than those of insect infested and untreated plants. Across the genotypes, IS2205 and ICSV1 showed significantly higher PPO activity in all the treatments as compared to that of the ICSV700.

SOD activity

The SOD activity of sorghum genotypes increased in various treatments (Fig 3). Among the treatments, JA + IN treated plants showed significantly greater SOD activity as compared to the plants treated with SA + IN, IN and

untreated control plants in all the tested genotypes. Across the genotypes, no significant difference as observed in SOD activity in JA + IN treated plants. However, SA + IN, IN treated and untreated control plants of ICSV1 showed significantly greater SOD activity than those of IS2205 and ICSV 700.

CAT activity

The SA + IN treated plants showed greater CAT activity among all the treatments in the tested sorghum genotypes followed by JA + IN treated and insect infested plants. However, the least activity was observed in the untreated control plants in all the genotypes (Fig 4). Among the genotypes, CAT activity was more in ICSV700 than the other genotypes (IS2205 and ICSV1) in JA and SA treated plants. Insect infested plants of IS2205 had significantly greater CAT activity than those of ICSV1 and ICSV700. Untreated control plants of ICSV1 had greater CAT activity than those of IS2205 and ICSV700.

Total phenols

Significant differences were found between the treated and untreated plants in the sorghum genotypes (Fig. 5). Among the treatments, JA + IN, SA+ IN treated and insect infested plants showed the increased levels of total phenols as compared untreated plants in all the genotypes. However, overall, the induction was significantly greater by JA treated and insect infested plants as compared to the SA + IN treated ones across the genotypes. Among the tested genotypes, IS2205 plants showed significantly greater phenolic content in all the treatments as compared to the corresponding treatments of ICSV1 and ICSV700.

H_2O_2 content

Plants treated with JA + IN and IN with insects showed greater levels of H_2O_2 as compared to the untreated plants in all the tested genotypes, however, JA + IN and SA + IN treated plants had more H_2O_2 than insect infested and untreated control plants (Fig 6). Across the genotypes, IS2205 and ICSV1 plants treated with JA + IN and SA + IN and infested with insects had greater levels of H_2O_2 than those of corresponding treatments of ICSV700. Untreated plants did not show any significant difference in H_2O_2 levels across the genotypes.

MDA content

Insect infested plants showed significantly greater MDA content than the plants treated with JA + IN, SA + IN and untreated plants in all the tested genotypes followed by the plants treated with SA and JA (Fig 7). Across the genotypes, IS2205 exhibited greater levels of MDA in all the treatments as compared to that of ISV1 and ICSV700.

Protein content

Protein content increased in plants treated with JA followed by infestation with *C. partellus* in all the sorghum genotypes as compared to the plants treated with SA + IN and infested and untreated control plants (Fig 8). Among the genotypes, ICSV700 and ICSV1 showed significantly higher protein content in plants pre-treated with JA and SA followed by insect infestation and the insect infested plants than that of IS2205.

Discussion

The ability of plants to recognize and respond defensively to insect attack constitutes a form of immunity that reduces herbivore survival, reproductive capacity, or preference for a plant. This is termed as "induced resistance". JA and SA are the important phytohormones involved in modulating plant defense against insect herbivory by mediating octadecanoid pathway and phenylpropanoid pathways, respectively (Shivaji et al. 2010; Scott et al. 2010). Exogenous application of JA and SA have been reported to enhance plant resistance against herbivores (Peng et al. 2004; Zhao et al. 2009; Scott et al. 2010; Shivaji et al. 2010; War et al. 2011a,b). Methyl jasmonate (MeJA) or *cis*-jasmone is a volatile derivative of JA and acts as a defense inducing agents in plants against the attacking herbivores (Bruinsma et al., 2009). The earlier and immediate response of plants to insect infestation results in the induced expression of plant metabolites and defensive enzymes. Induced resistance in plants is considered as a desirable crop protection strategy with relatively benign environmental impacts as it allows plants to be phenotypically plastic against different stresses. In this study we examined the defensive biochemical response of three sorghum genotypes to feeding by *C. partellus* and JA and SA treatments.

Our results revealed that pretreatment with JA and SA, followed by infestation with *C. partellus* resulted in greater POD activity in sorghum. However, a strong response was observed in IS2205 plants treated with JA and infested with insects than those treated with SA and infested with insects. This could be attributed to the higher accumulation of JA in plants infested with insects and because of the application of JA, and the strong ability of the IS2205 genotype to withstand the biotic stress. However, the lower POD activity in SA + IN plants than that of JA + IN and insect infested plants could be because of the cross talk between JA and SA (Cipollini et al. 2004; Koornneef and Pieterse 2008). Higher levels of POD activity in response to JA and SA application and/or insect attack will defend plants from the insects, pathogens and other stresses through cell lignifications, wound healing, and the production of secondary metabolites (Heng-Moss et al. 2004; Rangasamy et al. 2009). Our results correlate with several earlier results, where JA and insect infestation induced higher levels of POD and imparted resistance in plants against insect herbivory (Shivaji et al. 2010; War et al. 2011a, 2012).

Different genotype of sorghum showed differential induction of PPO in response to JA, SA and insect infestation. This might be due to the difference in sensitive up-regulation response of genotypes to the biotic stress. The PPO plays an important role in plant defense against insect herbivory as an antinutritional enzyme, and reduces the food quality (Bhonwong et al. 2009; War et al. 2012). The quinines formed from the oxidation of phenols interact with the nucleophilic side chain of amino acids and cause protein cross-linking, and thereby, reducing their availability to insect pests (Zhang et al. 2008; Bhonwong et al. 2009). PPO is also involved in the melanin formation that increases the cell wall resistance to insects and pathogens (Zhao et al. 2009).

Plants treated with JA and infested with insets showed significantly greater levels of SOD activity. The differential activity of SOD might be due to the difference in plant response across the treatments. The SOD is involved in the removal of highly toxic and unstable ROS (Raychaudhuri and Deng 2000). Saruhan et al. (2012) reported the induction of SOD activity by SA and its relation to the reduced oxidative damage. It has been further reported that *Helicoverpa zea* infestation produced higher levels of SOD activity in tomato and soybean (Felton et al. 1994; Bi and Felton 1995). It reduces the toxicity of ROS by converting them into less toxic and more stable components such as H_2O_2 and water (Khattab and Khattab 2005; Heidari 2009). Higher activity of CAT activity in plants plays a leading role in cell wall resistance, besides signals the expression of various plant defensive genes (Chen et al. 1993).

Phenols are the important plants secondary metabolites involved in defense against biotic and abiotic stresses. Total phenolic content was increased in plants treated with JA and infested with insect pests. Increase in total phenols is a common reaction of plants to herbivory (Karban and Baldwin 1997). Phenolic compounds directly affect the insect growth and development (Green et al. 2003; War et al. 2013). There are several reports showing the induction of phenols in plants in response to insect attack (Sharma et al. 2009; He et al. 2011; War et al. 2011a,b).

ROS production in plants in response to the oxidative stress by biotic and abiotic factors is common in plants (He et al. 2011; War et al. 2011a,b, 2012). ROS mediate various signaling pathways involved in plant defense against stresses (Maffei et al. 2007). Among all the ROS, H_2O_2 is regarded as the most important as it is highly stable and freely diffusible than all other ROS. It mediates the signal transduction pathways which lead to the expression of defense genes and thereby production of various defensive proteins in plants against insect herbivores (Maffei et al. 2007). In addition, H_2O_2 has been found to have direct toxicity against insects (Howe and Jander 2008; Meffai et al. 2007). It also defends plants against subsequent insect and pathogen invasion (Maffei et al. 2007). JA

and SA treatments followed by insect infestation showed greater H_2O_2 content in all the treatments. Our results correlate with earlier reports, where increase in the levels of H_2O_2 in plants after herbivore feeding and treatment with JA and SA has been observed (Walling 2000; Maffei et al. 2006; War et al. 2011a,b).

Plants infested with insects showed higher amounts of MDA in all the sorghum genotypes. The induction was more in IS2205 genotype as compared to that of ICSV1 and ICSV700. This might be attributed to the severe oxidative stress due to wounding by insects. It has been suggested that MDA levels accumulate in plants after herbivore attack and assists in the synthesis more complex defense compounds and activates antioxidative enzymes (Gechev et al. 2002; Zhang et al. 2008; War et al. 2011a,b). In addition, the emissions of green leaf volatiles, which are involved in indirect plant defense, are induced by lipid peroxidation after herbivore damage (Arimura et al. 2009). Our results are in line with earlier reports, where MDA levels were induced by insect damage (Huang et al. 2007; Zhang et al. 2008; War et al. 2011a,b).

In addition to secondary metabolites, which have been traditionally perceived as the major components of chemical defense strategies that regulate host plant utilization by insects (Sharma et al. 2009; War et al. 2011a, b, 2012, 2013), proteins are also an important contributor of the plant's chemical defense mechanism. Proteins are a major and the most common limiting nutrient for insect growth. These compounds can alter the physiology of herbivores by reducing their growth rate, adult size, and survival probability (Harvey et al., 2003). There was a significant increase in protein content in all the genotypes on various treatments. However, JA induced significantly greater protein content in plants than rest of the treatments. Increase in protein concentration might be endorsed to increased antioxidative enzyme activities after JA application and insect infestation. When under stress, plants produced various defense related enzymes and other protein based defensive compounds, thereby increasing the overall protein concentration (Lawrence and Koundal 2002; Chen et al. 2009; War et al. 2011,a,b,2012). There are several reports showing the elevation of protein concentration in response to insect attack and JA application (Chen et al. 2009; He et al. 2011; War et al. 2011a,b, 2012).

Conclusion

The sorghum genotypes responded differentially to the infestation by *C. partellus* and treatment with JA and SA in terms of the defensive enzyme activities such as POD, PPO, SOD, CAT and the total amounts of phenols, H_2O_2, and MDA. Since these enzymes and other defensive components are responsible for the plant defense against biotic and abiotic stresses, sorghum genotypes with higher activity of these enzymes and other defensive components could be

more resistant than the genotypes with low induced levels of these components. Alteration in digestibility and palatability of plant tissues by the induced compounds in response to insect attack affect insect growth and development adversely. The induced resistance could play an important role in pest management and defense mechanism against insect pests.

A detailed understanding of plant immunity to arthropod herbivores will provide new insights into basic mechanisms of chemical communication and plant-animal co-evolution and may also facilitate new approaches to crop protection and improvement.

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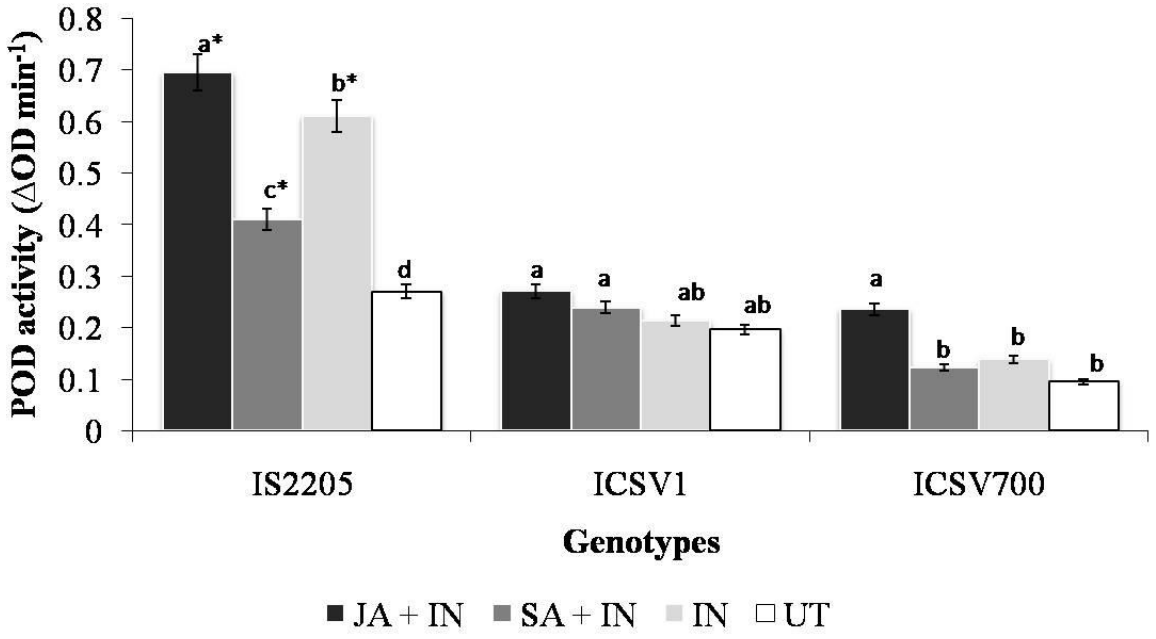

Fig. 1: Peroxidase (POD) activity (ΔOD min^{-1}) of sorghum genotypes after *Chilo partellus* infestation and jasmonic acid and salicylic acid application.

Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. JA+IN = Treatment with JA and infested with *C. partellus*; SA+IN = Treatment with JA and infested with *C. partellus*; IN = *C. partellus* infested plants; UT = Untreated control plants.

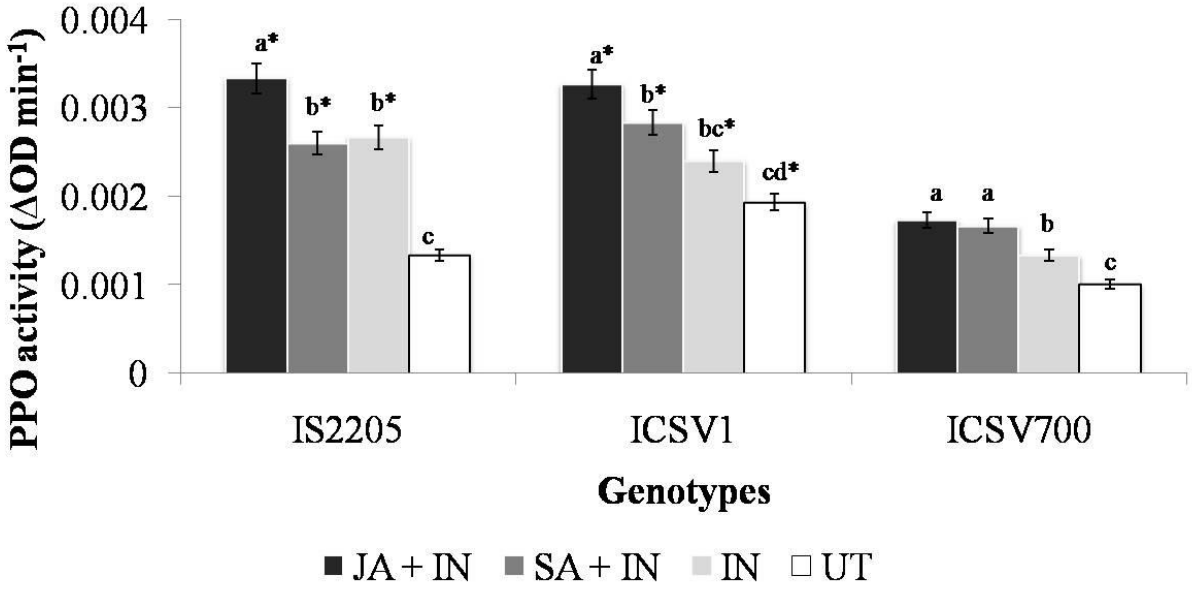

Fig. 2: Polyphenol oxidase (PPO) activity (ΔOD min^{-1}) of sorghum genotypes after *Chilo partellus* infestation and jasmonic acid and salicylic acid application.

Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. JA+IN = Treatment with JA and infested with *C. partellus*; SA+IN = Treatment with JA and infested with *C. partellus*; IN = *C. partellus* infested plants; UT = Untreated control plants.

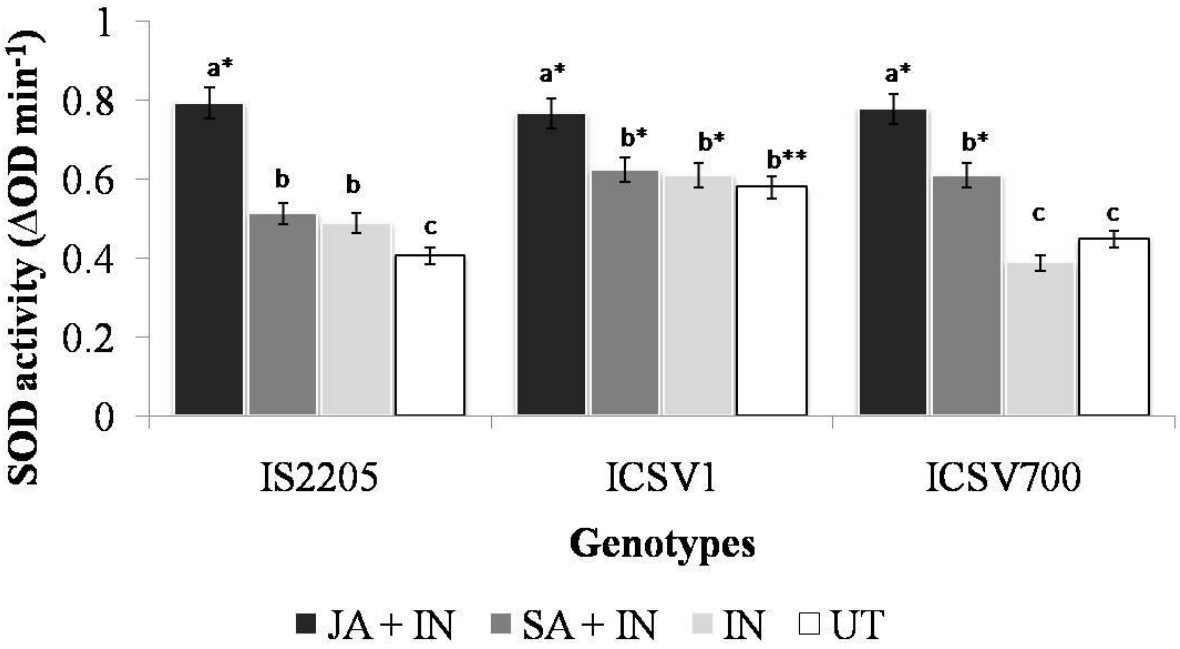

Fig. 3: Superoxide dismutase (SOD) activity (ΔOD min^{-1}) of sorghum genotypes after *Chilo partellus* infestation and jasmonic acid and salicylic acid application.

Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. JA+IN = Treatment with JA and infested with *C. partellus*; SA+IN = Treatment with JA and infested with *C. partellus*; IN = *C. partellus* infested plants; UT = Untreated control plants.

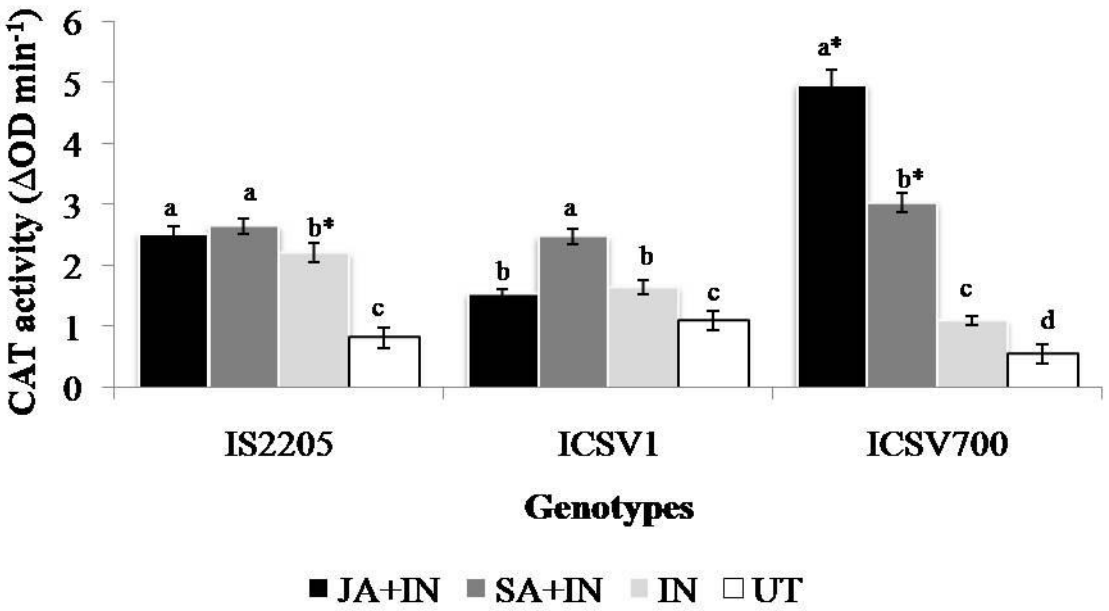

Fig. 4: Catalase activity (ΔOD min^{-1}) of sorghum genotypes after *Chilo partellus* infestation and jasmonic acid and salicylic acid application.

Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. JA+IN = Treatment with JA and infested with *C. partellus*; SA+IN = Treatment with JA and infested with *C. partellus*; IN = *C. partellus* infested plants; GAE = Gallic acid equivalents.

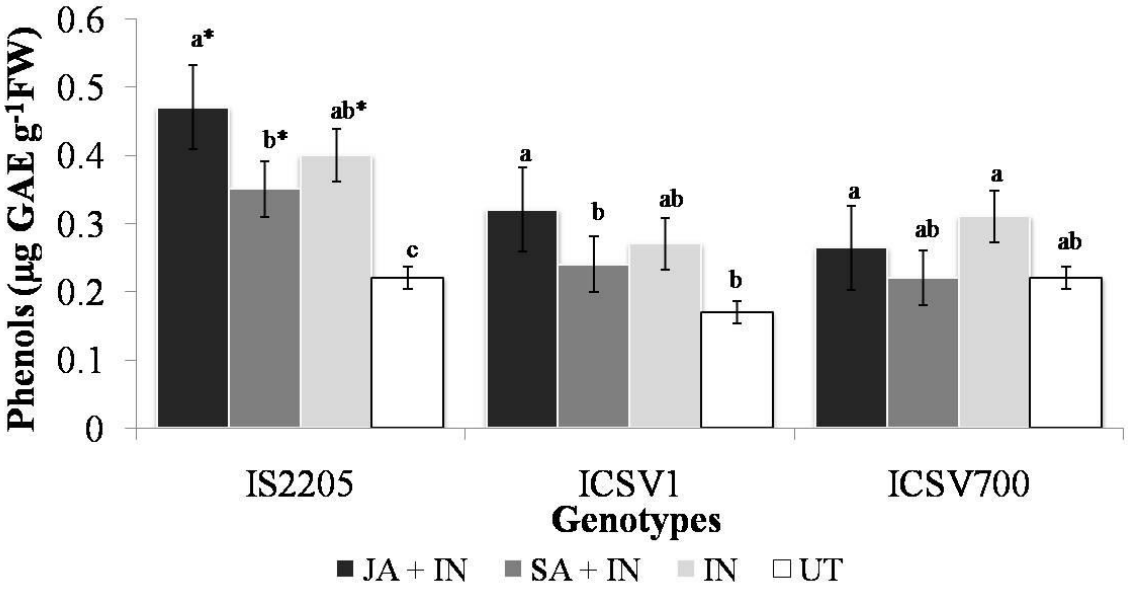

Fig. 5: Total phenols (µg GAE g^{-1} FW) of sorghum genotypes after *Chilo partellus* infestation and jasmonic acid and salicylic acid application.

Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. JA+IN = Treatment with JA and infested with *C. partellus*; SA+IN = Treatment with JA and infested with *C. partellus*; IN = *C. partellus* infested plants; UT = Untreated control plants; GAE = Gallic acid equivalents; FW = Fresh weight.

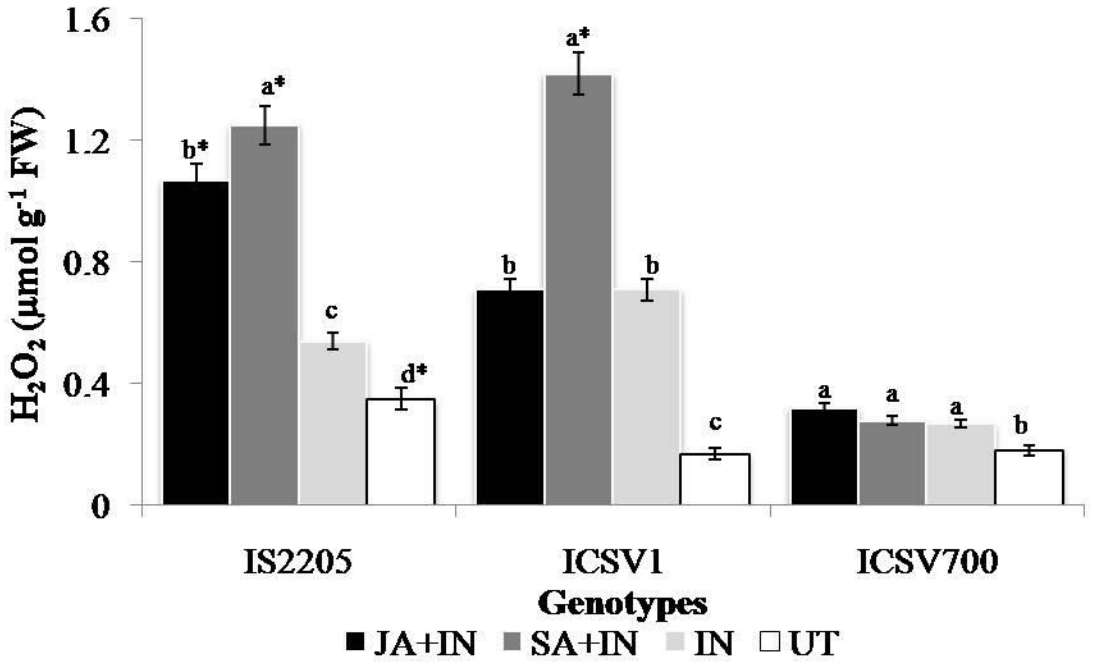

Fig. 6: H_2O_2 content (µmol g^{-1} FW) of sorghum genotypes after *Chilo partellus* infestation and jasmonic acid and salicylic acid application.
Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. JA+IN = Treatment with JA and infested with *C. partellus*; SA+IN = Treatment with JA and infested with *C. partellus*; IN = *C. partellus* infested plants; UT = Untreated control plants.

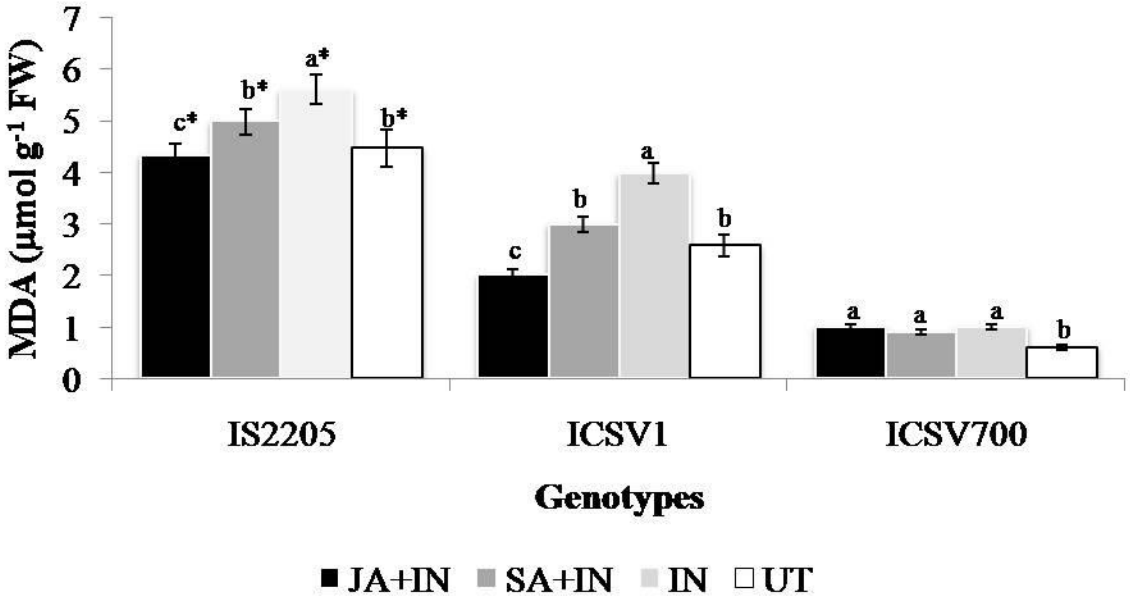

Fig. 7: Malondialdehyde (MDA) content (µmol g^{-1} FW) of sorghum genotypes after *Chilo partellus* infestation and jasmonic acid and salicylic acid application.
Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. JA+IN = Treatment with JA and infested with *C. partellus*; SA+IN = Treatment with JA and infested with *C. partellus*; IN = *C. partellus* infested plants; UT = Untreated control plants; FW = Fresh weight.

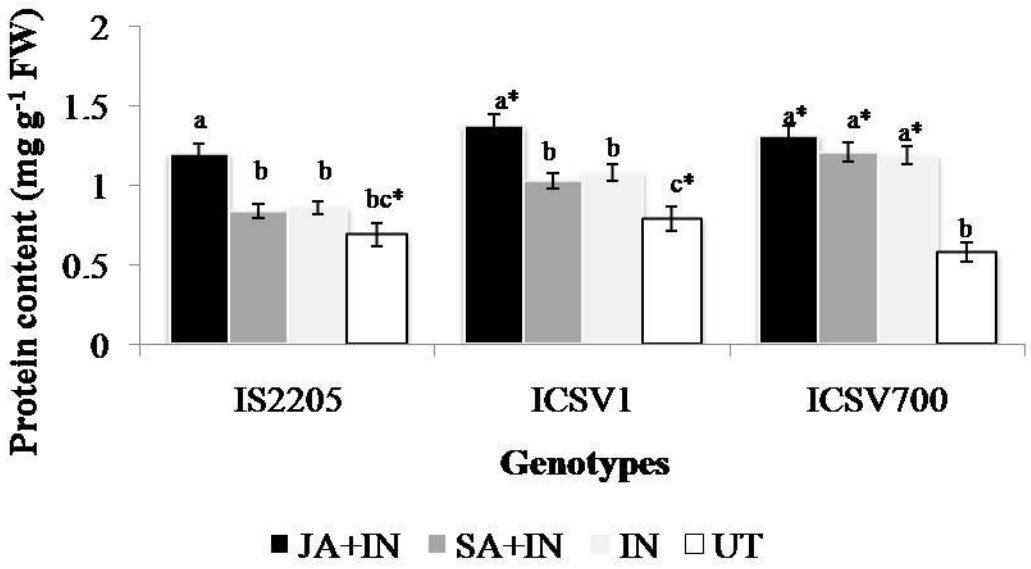

Fig. 8: Protein content (mg g^{-1} FW) of sorghum genotypes after *Chilo partellus* infestation and jasmonic acid and salicylic acid application.

Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at $P \leq 0.05$. JA+IN = Treatment with JA and infested with *C. partellus*; SA+IN = Treatment with JA and infested with *C. partellus*; IN = *C. partellus* infested plants; UT = Untreated control plants; FW = Fresh weight.

Table 1: Catalase (CAT) activity (ΔOD min^{-1}) of sorghum genotypes after *Chilo partellus* infestation and jasmonic acid and salicylic acid application.

Genotypes	Treatments			
	JA + IN	SA + IN	IN	UT
IS2205	2.52 ± 0.005^{a}	2.65 ± 0.004^{a}	$2.21 \pm 0.005^{ab*}$	0.82 ± 0.001^{c}
ICSV1	1.54 ± 0.005^{b}	2.48 ± 0.005^{a}	1.65 ± 0.005^{b}	$1.10 \pm 0.001^{bc*}$
ICSV700	$4.97 \pm 0.002^{a*}$	$3.04 \pm 0.003^{b*}$	1.10 ± 0.005^{c}	0.55 ± 0.001^{d}

Values (Mean ± SD) with similar letters in a row are not statistically different at $P \leq 0.05$.
*within a column shows significant difference across the genotypes within a treatment.
JA+IN = Treatment with JA and infested with *C. partellus*; SA+IN = Treatment with JA and infested with *C. partellus*; HIN = *C. partellus* infested plants; UT = Untreated control plants.