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Biochemical mechanisms of induced resistance to *Chilo partellus* in sorghum

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ABSTRACT

Host plant resistance is an important component of pest management, and information on contribution of different mechanisms of resistance is important for developing cultivars with resistance to the target pests. Therefore, induced resistance was studied in five sorghum genotypes against *Chilo partellus* by using infested and non-infested plants under greenhouse conditions. The activity of plant defensive enzymes and the secondary metabolites were recorded at 7 days after infestation and their induction varied among the genotypes and treatments. The resistant sorghum genotypes ICSV 700, IS 2205 and ICSV 93046 suffered lower leaf damage by the neonate larvae of *C. partellus* (damage rating (DR) 2.8–3.7) as compared to the susceptible checks, ICSV 1 and *Swarna* (DR 6.4 and 7.0, respectively). ICSV 700, IS 2205 and ICSV 93046 exhibited greater enzymatic activity [peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL)] and had more amounts of phenols than the susceptible check, *Swarna*. This information will be useful for developing sorghum genotypes with resistance to *C. partellus* for sustainable crop production.

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KEYWORDS
Enzyme; host plant resistance; pest management; secondary metabolite; spotted stem borer

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop, and is an important source of food for people living in the semi-arid tropics (SAT) in Asia and Africa, where it is mainly produced and consumed by rural poor. It is cultivated on 40.07 million ha, with an annual production of 57.89 million metric tonnes during 2019 (FAO 2021). In India, it is cultivated on 4.09 million ha with an annual production of 3.47 million tonnes during 2019 (FAO 2021). However, the average productivity is quite low (0.09 t/ha), despite the potential productivity of over 50 q/ha (Kumara Charyulu et al., 2016). Despite remarkable achievements in increasing sorghum productivity and production, there is a major challenge to make sorghum production profitable under rainfed subsistence farming conditions in the SAT.

Insect pests are one of the major yield reducing-factors in sorghum, and result in losses of over $1000 million in grain and forage yield worldwide (ICRISAT 1992, 2007). Over 150 species of insect pests damage sorghum worldwide, of which spotted stem borer, *Chilo partellus* (Swinhoe) is the most damaging pest in Asia and Africa (Sharma, 1993; Sharma et al., 2003). It damages all the above ground parts of the plant from the second fortnight after seedling emergence until crop harvest. Feeding by the young larvae results in pinholes in the leaves, followed by elongated lesions on the leaf whorls. Stem borer damage at the early stage destroys the growing point resulting in the drying of central 1–2 leaves, commonly known as 'deadheart', which reduces plant vigor and photosynthetic efficiency, delaying flowering, and ultimately leads to the reduction in fodder and grain yield. The third instar larvae descend down to the base of the stem, bore inside and result in stem tunneling, which disrupts the nutrient supply to the plant. It may also lead to the production of chaffy panicles, and ultimately to reduce the fodder quality and grain yield. Therefore, it is difficult to control this pest because of nocturnal habit of the adult moths, and the cryptic feeding behavior of the larvae inside the leaf whorls and the stem. Insecticidal sprays are ineffective as they do not reach the larvae inside the plant. A number of sorghum genotypes with resistance to *C. partellus* have been identified; however, the levels of resistance are low to moderate (Sharma 1993, 1997; Sharma

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et al., 2003), but high levels of resistance have been reported in the wild relatives of sorghum (Kamala et al., 2012). Several morphological and biochemical factors contribute to expression of resistance to stem borer, *C. partellus* in sorghum (Sharma and Nwanze, 1997; Sharma et al., 2007; Dhillon and Chaudhary 2018). A number of factors have earlier been reported to be associated with resistance to spotted stem borer in sorghum such as – erectness of leaves, orientation of the leaf hairs, tightness of the leaf sheath and midrib, diameter of the leaf whorl, and large internode length (Woodhead and Taneja 1987; Taneja and Woodhead 1989; Kishore 1991a), which influence the dispersal of neonate larvae resulting in low deadheart formation. The larval duration on the sorghum stem has been reported to be positively correlated with plant height and nodes plant^{-1}, but negatively correlated with peduncle length (Singh and Rana 1984). Early panicle initiation and rapid internode elongation (Taneja and Woodhead 1989), and stem tunneling (Kishore 1991b) have earlier been reported to be the major plant characters associated with resistance to stem borer. Stem tunneling rather than leaf feeding and deadhearts is the primary cause of yield loss (Alghali 1986). However, these are not the only damage parameters responsible for yield reduction in sorghum (Singh et al. 1983; Pathak and Olela 1983; Taneja and Leuschner 1985). Fast-growing sorghum genotypes with long and thin stems, but with fewer and longer internodes, short peduncles, and yellowish–green leaves with high trichome density have also been reported to be associated with resistance to *C. partellus* (Singh et al. 1983; Patel and Sukhani 1990). However, wild relatives sorghum cannot be used successfully due to reproductive barriers (sexual incompatibility and narrow genetic variability). The host plants respond to herbivore damage through the production of secondary plant metabolites and defensive proteins that target physiological processes in the insect pests (Kawazu et al., 2012; Zhao et al., 2009; War et al., 2012). Induced resistance is highly dynamic in nature, and 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 herbivores, which could be constitutive and/or induced (He et al., 2011; Scott et al., 2010; War et al., 2012). A chemical defense strategy involves secondary metabolites and proteins, which may be present constitutively or induced by challenges such as herbivore wounding (Heng-Moss et al., 2004).

Induction of oxidative enzymes in response to insect herbivores includes peroxidases (POD), polyphenol oxidase (PPO) and catalase (CAT), and increased amounts of phenols, hydrogen peroxide (H_2O_2), and proteins (Scott et al., 2010; Zhao et al., 2009). POD is an important anti-oxidative enzyme involved in plant defense against insect herbivores (He et al. 2011). It produces semi-quinone free radicals and subsequently the quinines, which are highly toxic to insect pests (Barbehenn et al., 2010). PPO is an anti-nutritional 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). Oxidation of phenols resulting in the production of toxic quinones those affect the insect growth and development, while some phenols are directly toxic to insect pests (Howe and Jander 2008). Phenylalanine-ammonia lyase (PAL) is a key enzyme of the pathway for the biosynthesis of phenolic compounds in plants and it is at the interface between primary and secondary metabolism (Naoumkina et al. 2010). CAT is an important enzyme in reactive oxygen species (ROS) scavenging systems (Heidari 2009; Khattab and Khattab 2005). H_2O_2 is an important stable ROS involved in plant defense against insect herbivores. It acts as a second messenger in signal transduction pathways, which leads to production of toxic chemicals (Maffei et al., 2007).

Therefore, the present studies were carried out to understand the biochemical mechanisms of induced resistance in sorghum against the *C. partellus* to develop strategies for breeding sorghum genotypes with resistance to this pest for sustainable crop production.

2. Material and methods

2.1. Insects

The insects used for the studies were obtained from the insect rearing laboratory at the International Crops for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana State, India. The *C. partellus* culture was maintained under controlled conditions, 16 h:8h L:D regime at 25 ± 1 °C and 65 ± 5% r.h. on a sorghum-based artificial diet (Taneja and Leuschner 1985). Aqueous sugar solution (10%) was offered as a food to the adults. The pupae were washed with 2% sodium hypochlorite solution, and transferred to plastic jars containing vermiculite. Adults were transferred to iron oviposition cages (30 × 30 × 30 cm), and provided with butter paper for oviposition.

2.2. Sorghum plants

Five genotypes of sorghum were evaluated for resistance to insects under field conditions, including

three genotypes earlier known to be resistant to *C. partellus* (ICSV 700, IS 2205, ICSV 93046) and a susceptible check (ICSV 1 and *Swarna*) (Sharma et al., 2003). The genotypes used in the present experiment are not commercial and they are available with ICRISAT genebank used for breeding purposes. Seeds of sorghum genotypes were sown in plastic pots measuring 30 deep and 30 cm dia. in the greenhouse (27 ± 3 °C, 65 ± 5% RH). Seven days after germination, only three plants were retained in each plastic pot to have a uniform plant stand for all the test genotypes with three number of replications. At five-leaf stage (V2 stage), each plant was infested with 10 number of third instar larvae of *C. partellus*. A set of uninfested control (UT) plants was also grown similarly for all the genotypes. Before releasing the third instar larvae, the plants were enclosed in plastic jars including control to avoid the movement of larvae from one plant to another.

2.3. Leaf damage rating

Leaf damage was evaluated on a 1–9 damage rating scale after seven days of infestation (1 = < 10% leaf area damages, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, and 9 = > 80% leaf area damaged (Sharma et al. 1997). Larvae were recovered from the plants, counted and weighed to record data on larval survival (%) and larval weights (mg/larva).

2.4. Chemicals

Ethylene diamine tetra acetic acid (EDTA), bovine serum albumin (BSA), guaiacol, polyvinylpyrrolidone (PVP), proline, glucose, tannic acid, dithiothretol (DTT), disodium hydrogen phosphate, sodium dihydrogen phosphate, nitro-blue tetrazolium salt (NBT), methionine, l-phenylalanine, 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–Ciocalteu reagent were obtained from Merck, Mumbai, India. Thiobarbituric acid (TBA) was obtained from HiMedia Pvt. Ltd., Mumbai, India. Ammonium sulphate was obtained from Qualigens Gine Chemicals, Mumbai, India. The chemicals used in this study were of analytical grade. The spectrophotometer used for the estimation of biochemical parameters was Hitachi UV – 2900 (Hitachi, Japan).

2.5. Enzyme extraction

Seven days after infestation, the leaf samples were collected from the infested and uninfested plants. 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 for estimating enzymatic activity. For all the test genotypes enzyme extraction, three numbers of replications were maintained.

2.5.1. Peroxidase (POD) assay

Peroxidase activity was estimated according to the method of Shannon et al. (1966), with a 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-s intervals. Enzyme activity was expressed as OD $min^{-1}g^{-1}$ FW.

2.5.2. Polyphenol oxidase (PPO) assay

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

2.5.3. Phenylalanine ammonia lyase (PAL) assay

PAL activity was determined by the method of Campos-Vergas and Saltveit (2002) with slight modifications. To 0.4 ml of 50 mM l-phenylalanine (dissolved in 20 mM potassium phosphate buffer (pH 8.8), 0.2 ml of enzyme extract and 0.4 ml of 50 mM potassium phosphate buffer (pH 8.8) were added. The reaction mixture was incubated at 40°C for 30 min. Change in absorbance was measured at 290 nm and the activity expressed as OD/min/g FW.

2.5.4. Tyrosine ammonia lyase (TAL) assay

TAL activity was determined by the method of Khan et al., (2003) with slight modifications. To 0.5 ml of 0.05 M tyrosine in 0.1 M tris HCl (pH 9.5), 0.4 ml 50 mM potassium phosphate buffer (pH 8.8) and 0.1 ml of enzyme source were added. The reaction mixture was incubated at 37°C for 1 hour. The reaction was stopped by adding 0.05 ml of 5 N HCl, and therefore the absorbance was measured at 333 nm and the activity expressed as OD/min/g FW.

2.5.5. 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 1 min and the enzyme activity was expressed as OD/min.

2.6. Estimation of secondary metabolites

2.6.1. Phenolic content

Leaves (0.5 g) were homogenized in 3 ml of 80% methanol and agitated for 15 min at 70 °C. The homogenate was centrifuged at 10,000 rpm for 10 min and the supernatant was collected, which was used for the estimation of total phenols by 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-Ciocalteu 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/FW).

2.6.2. 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) trichoroacetic 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}).

2.6.3. Protein content

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

2.7. Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using Genstat Version 14.0 (VSN International Ltd; www.vsni.co.uk). The data on leaf damage were analyzed by factorial analysis with genotypes as the main treatment, and the infestation levels as the sub-treatment. The data for biochemical profile were analyzed using randomized complete block design. Tukey's HSD test was used to separate the means, when the treatment effects were statistically significant ($P \leq 0.05$).

3. Results

3.1. Evaluation of sorghum genotypes resistance to C. partellus under greenhouse conditions

Leaf damage The leaf damage rating due to *C. partellus* was significantly lower in ICSV 700 (2.67), IS 2205 (3.67), ICSV 93046 (4.00) than in ICSV 1 (6.00) and *Swarna* (7.00) ($F(4,14) = 45.3$, $P \leq 0.05$). Among the genotypes, larval weight (mg/larva) was significantly low of insects fed on ICSV 93046, IS 2205 and ICSV 700 (resistant) than those fed on ICSV 1 and *Swarna* (susceptible). However, larval survival (%) was significantly lower in ICSV 93046 (26.67), IS 2205 (43.33) and ICSV 700 (26.67) than the susceptible checks ICSV 1 (60.00) and *Swarna* (70.00) (Figure 1).

3.2. Biochemical profile of the sorghum plants grown under greenhouse conditions

Activity of the enzymes POD, PPO, PAL, TAL and CAT, and the amounts of secondary metabolites such as total phenols, H_2O_2 and total proteins of sorghum genotypes showed considerable variability across the test genotypes. Greater activity of POD, PPO, PAL and TAL (Figures 2–5) was observed in *C. partellus* infested genotypes (such as ICSV 700, IS 2205, and ICSV 93046 [$F(4,14) = 0.88, 1.77, 7.79$ and 2.65, respectively, at $P < 0.05$] than in the susceptible checks, ICSV 1 and *Swarna*. IS 2205 and ICSV 93046 had significantly greater CAT activities (Figure 6) in *C. partellus* infested plants [$F(4,14) = 12.03$, $p < 0.05$] than ICSV 700, ICSV 1 and *Swarna*. Amounts of total phenols (Figure 7) were also significantly greater in ICSV 700, IS 2205, and ICSV 93046 as compared to the susceptible checks, ICSV 1 and *Swarna* [$F(4,14) = 0.28$, $p < 0.05$]. There was a significant and negative correlation between leaf damage rating and the amounts of total phenols ($r = -0.87^*$, $p < 0.05$). The H_2O_2 content (Figure 8) was significantly greater in ICSV 1 [$F(4,14) = 0.31$, $p < 0.05$] than in other genotypes tested. There was a positive correlation between leaf damage rating and H_2O_2 content ($r = 0.75^*$, $p < 0.05$) of infested sorghum plants, suggesting that greater leaf damage resulted in increased activity of H_2O_2. Protein content (Figure 9) was significantly higher in *C. partellus* infested plants of ICSV 700, IS 2205, ICSV 1, ICSV 93046 and *Swarna* [$F(4,14) = 0.79$, $p < 0.05$]. A strong negative correlation was observed between protein

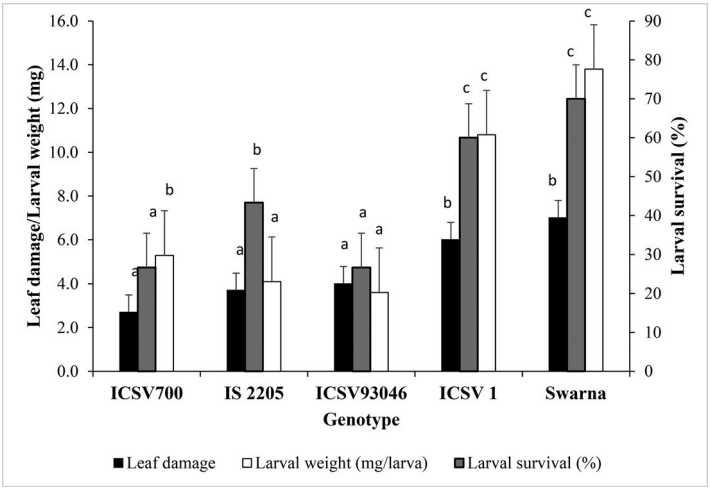

Figure 1. Expression of resistance to spotted stem borer, *C. partellus* in terms of leaf damage rating (scale 1–9); larval survival (%); and larval weight (mg/larva). Among genotypes, bars (mean ± SD) of the same color with a common letter do not differ statistically at $p \leq 0.05$ (Tukey's HSD test). IN = *C. partellus* – infested plants; UT = untreated control plants.

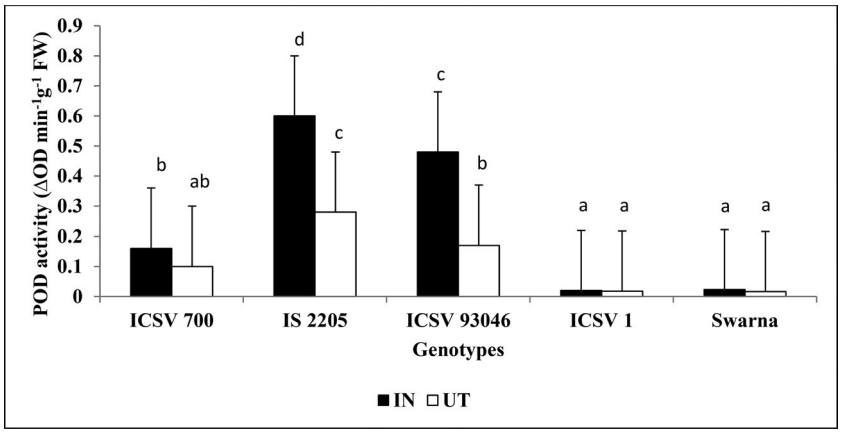

Figure 2. Peroxidase (POD) activity (ΔOD $min^{-1}g^{-1}$ FW) in *C. partellus* infested and uninfested plants of different sorghum genotypes. Among genotypes, bars (mean ± SD) of the same color with a common letter do not differ statistically at $p \leq 0.05$ (Tukey's HSD test). IN = *C. partellus* – infested plants; UT, untreated control plants.

content and leaf damage by *C. partellus* ($r = -0.57^*$, $p < 0.05$).

C. partellus infestation induced higher POD, PPO, PAL, CAT activity in the tested sorghum genotypes. Infested resistant genotypes of ICSV 93046 and IS 2205 exhibited nearly two fold increase in POD, PPO and CAT activity than that of uninfested control plants in comparison to susceptible genotypes. Significant differences were found in phenolic content between control and infested plants of all the five genotypes (Figure 7). However, resistant genotypes (ICSV 700, IS 2205 and ICSV 93046) showed higher increase in phenolic content in infested plants than their respective control plants in comparison to susceptible genotypes (ICSV 1 and *Swarna*). Plants infested with *C. partellus* had higher H_2O_2 content than the uninfested control plants (Figure 8), although this increase was higher in susceptible genotypes than the resistant ones. Significant difference was recorded in protein content between control and infested plants of all the genotypes (Figure 9).

4. Discussion

Our study originated from the observations that the three genotypes of *S. bicolor,* ICSV 700, ICSV 93046 and IS 2205 are resistant to *C. partellus* while the genotypes ICSV 1 and *Swarna* are susceptible (Sharma et al., 2003; Bantilan et al., 2004, International Board for Plant Genetic Resources 1993). Dhillon and Chaudhary (2018) also reared *C. partellus* on germplasm genotypes IS 2205 which significantly reduced larval and pupal weights, prolonged growth period, larval survival and adult emergence and resulted lower deadhearts followed by varieties ICSV 700, ICSV 93046 in comparison to susceptible check, *Swarna,* indicating variable level

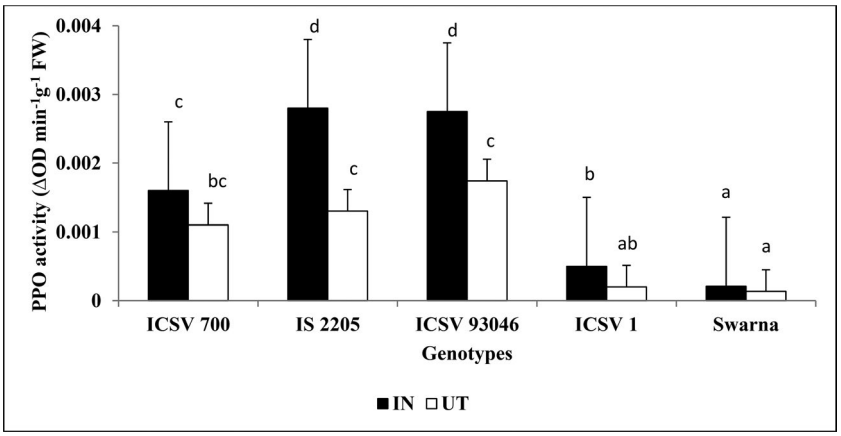

Figure 3. Polyphenol oxidase (PPO) activity (ΔOD $min^{-1}g^{-1}$ FW) in *C. partellus* infested and uninfested plants of different sorghum genotypes. Among genotypes, bars (mean ± SD) of the same color with a common letter do not differ statistically at $p \leq 0.05$ (Tukey's HSD test). IN = *C. partellus* – infested plants; UT, untreated control plants.

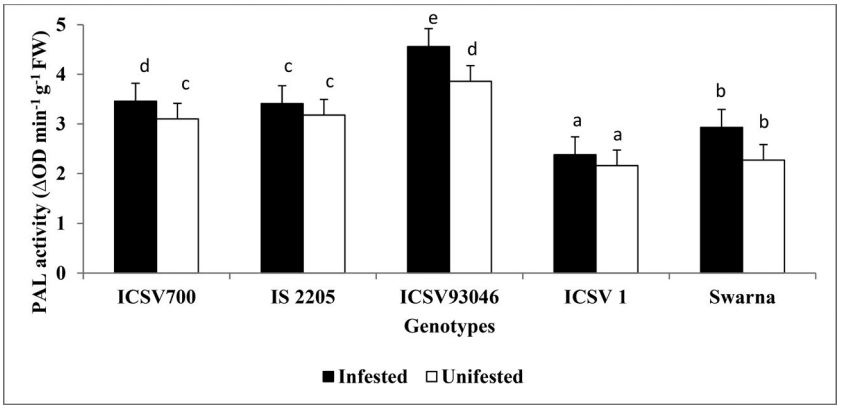

Figure 4. Phenylalanine ammonia lyase (PAL) activity (ΔOD $min^{-1}g^{-1}$ FW) in *C. partellus* infested and uninfested plants of different sorghum genotypes. Among genotypes, bars (mean ± SD) of the same color with a common letter do not differ statistically at $p \leq 0.05$ (Tukey's HSD test). IN = *C. partellus* – infested plants; UT, untreated control plants.

of antibiosis in these groups against spotted stem borer. Earlier studies have also reported these genotypes as source of resistance (Sharma et al., 2005) having antibiosis as a predominant mechanism of resistance to *C. partellus* (Kumar et al., 2006; Dhillon and Kumar 2017). The most effective, economic and environment friendly strategy for pest management (Sharma 2007) is the host plant resistance, which is exhibited through morphological, physiological and biochemical features of the host plant (Howe and Jander 2008; Sharma et al., 2009; War et al., 2012). This capability of plants to recognize and counter the herbivore attack constitutes a form of immune response that reduces herbivore survival, reproductive capacity, or preference for a plant, which is classified as 'induced resistance'. In this study, we examined the induced biochemical response of five sorghum genotypes to feeding by *C. partellus* under greenhouse conditions. Leaf damage by *C. partellus* was lower in the stem borer resistant genotypes ICSV 700, IS 2205 and ICSV 93046 as compared to ICSV 1 and *Swarna*, as has been observed earlier by Sharma et al., (2003).

Greater activity of POD, PPO, PAL and TAL was recorded in the stem borer resistant genotypes ICSV 700, IS 2205 and ICSV 93046 than in the susceptible checks, ICSV 1 and *Swarna*. Greater activity of POD in response to insect damage defends the plants from biotic and other stresses through cell wall lignification, wound healing, and the production of secondary metabolites (Heng-Moss et al., 2004; Rangasamy et al., 2009). The insect-resistant genotypes possess higher levels of anti-oxidative enzymes and secondary metabolites, as they respond strongly to different stresses (Heng-Moss et al., 2004; Chen et al., 2009; Gulsen et al., 2010; War et al., 2012). Similar response was observed in the present studies, wherein, insect infestation induced greater activity of POD, and conferred resistance in plants against insect herbivores (Shivaji et al., 2010; War et al., 2012).

Different genotypes of sorghum showed differential induction of PPO in response to insect

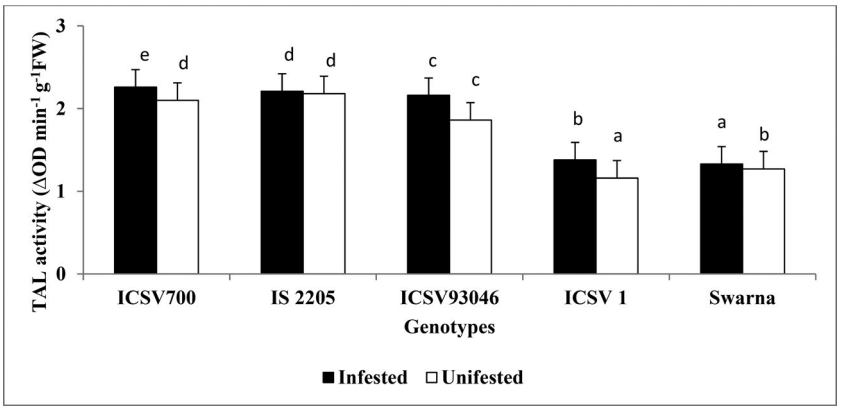

Figure 5. Tyrosine ammonia lyase (TAL) activity (ΔOD $min^{-1}g^{-1}$ FW) in *C. partellus* infested and uninfested plants of different sorghum genotypes. Among genotypes, bars (mean ± SD) of the same color with a common letter do not differ statistically at $p \leq 0.05$ (Tukey's HSD test). IN = *C. partellus* – infested plants; UT, untreated control plants.

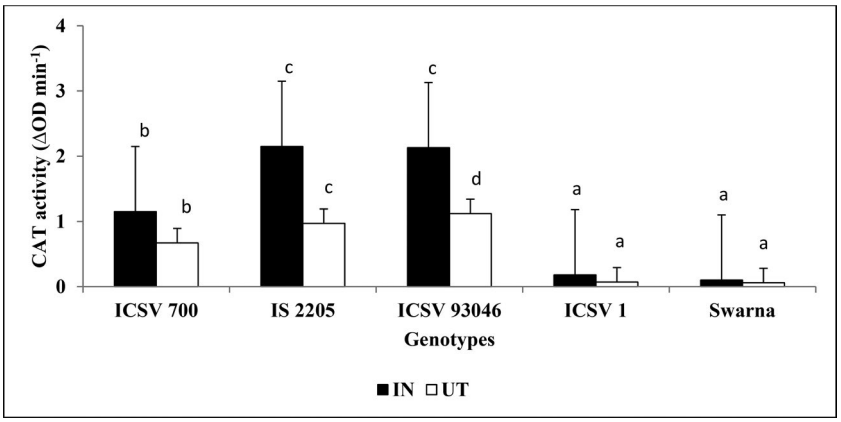

Figure 6. Catalase activity (ΔOD min^{-1}) in *C. partellus* infested and uninfested plants of different sorghum genotypes. Among genotypes, bars (mean ± SD) of the same color with a common letter do not differ statistically at $p \leq 0.05$ (Tukey's HSD test). IN = *C. partellus* – infested plants; UT, untreated control plants.

infestation. This might be due to the differences in sensitivity of different genotypes in up-regulation of this enzyme in response to the biotic stresses. The PPO plays an important role in host plant defense against insect herbivores as an anti-nutritional enzyme, and reduces food quality (Bhonwong et al., 2009; War et al., 2012). PPO is also involved in the melanin formation that enhances the cell wall resistance to insect pests and pathogens (Zhao et al., 2009). Simultaneously, the quinones formed as a result of oxidation of phenols interact with the nucleophilic side chain of amino acids and cause protein cross-linking and, thereby, reducing their availability to the herbivores (Bhonwong et al., 2009; Zhang et al., 2008). Activity of CAT was higher in resistant genotypes than in susceptible ones and simultaneously increased under the infestation of *C. partellus*. Greater activity of CAT in plants enhances cell wall resistance, besides signaling the expression of several plant defensive genes (Chen et al., 1993).

Phenols, H_2O_2, and total proteins were also significantly greater in insect-resistant genotypes than in the susceptible check, *Swarna*. Phenols are important plant secondary metabolites involved in plant defense against biotic and abiotic stresses. Total phenolic content was greater in plants infested with *C. partellus*, and this is a common reaction of plants to damage by the herbivores (Karban and Baldwin 1997). Phenolic compounds directly affect the insect growth and development (Green et al., 2003; War et al., 2013), and several reports have shown induction of phenols in plants in response to insect attack (He et al., 2011; Sharma et al., 2009). Phenolic compounds are either directly toxic to insect pests or activate the production of various toxic secondary metabolites by mediating the transduction pathways, and by activating various defensive enzymes (Walling 2000; Maffei et al., 2007; Bhonwong et al., 2009). Oxidation of phenols produces toxic quinones, which covalently bind to leaf proteins, thereby inhibiting, protein digestion in herbivores (Bhonwong et al., 2009).

To compensate nutritional requirements for growth, development and life processes, insects increase the rate and quantity of food intake, thus leading to accumulation of desired amount of

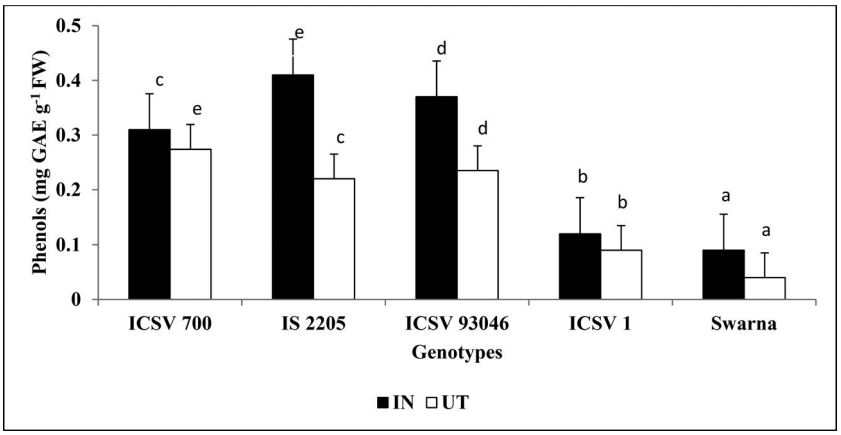

Figure 7. Total phenols (mg GAE g^{-1} FW) in *C. partellus* infested and uninfested plants of different sorghum genotypes. Among genotypes, bars (mean ± SD) of the same color with a common letter do not differ statistically at $p \leq 0.05$ (Tukey's HSD test). IN = *C. partellus* – infested plants; UT, untreated control plants.

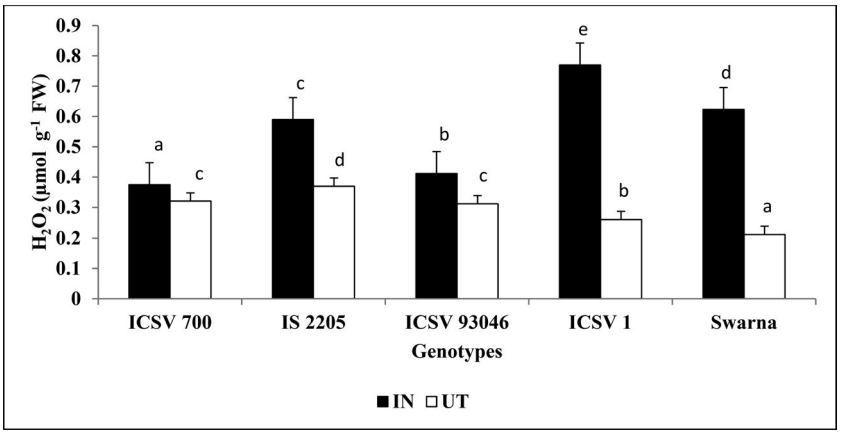

Figure 8. H_2O_2 content (µmol g^{-1} FW) in *C. partellus* infested and uninfested plants of different sorghum genotypes. Among genotypes, bars (mean ± SD) of the same color with a common letter do not differ statistically at $p \leq 0.05$ (Tukey's HSD test). IN = *C. partellus* – infested plants; UT, untreated control plants.

proteins, amino acids, lipophilic metabolites and other nutritional compounds (Dhillon and Kumar 2017; Dhillon et al., 2014; Kumar and Dhillon 2015). The phenolics are known to provide structural support, pigmentation, signaling and defense against biotic and abiotic stresses in plants (Dixon and Paiva 1995). However the origin of hydrolysed ferulic and p-coumaric acids is mainly the phenolic acid-carbohydrate complex of cell wall (Fincher and Stone 1986).The amounts of ferulic and p-coumaric acids were highly variable in the IS 2205, ICSV 700 and *Swarna*, although these variations were not found in accordance to their levels of antibiosis, indicating genotype-specific role of these phenolic acids in sorghum defense against *C. partellus* (Dhillon and Chaudhary 2018).

In general, insects need primarily ten amino acids for overall growth and development (Parra 2012). Earlier studies in cereal crops have shown that amounts of Alanine, Histidine, and Threonine are associated with resistance to aphids (Weibull 1988; Kazemi and Van Emden 1992). Insects increase the rate and quantity of the amount of amino acids required for growth and development to compensate nutritional requirements (Chapman 1998; Dhillon et al. 2014). Studies by Dhillon and Kumar (2017) on amino acid profiling of *S. bicolor* vis-à-vis *C. partellus* for biochemical interactions and plant resistance revealed that Cystine and Aspartic acid were negatively correlated with different biological parameters indicating its contribution in sorghum defense against *C. partellus*. Likewise, concentrations of Asparagine, Aspartic acid, and Glutamic acid have also been reported to be responsible for antibiosis against *Myzus persicae* (Sulzer) and white fly, *Bemisia tabaci* (Gennadius) (Dixit et al. 2013). The *C. partellus* acquired lower amounts of cyclic and aliphatic amino acids from the resistant and moderately resistant genotypes, than from the susceptible genotypes. However, the uptake of hydroxyl or sulfur-containing amino acids was greater in *C. partellus* larvae fed on moderately resistant varieties as compared to that

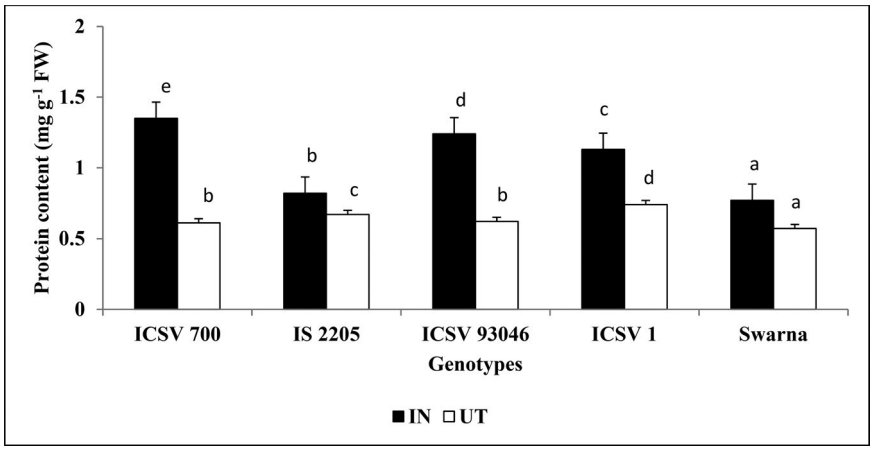

Figure 9. Protein content (mg g^{-1} FW) in *C. partellus* infested and uninfested plants of different sorghum genotypes. Among genotypes, bars (mean ± SD) of the same color with a common letter do not differ statistically at $p \leq 0.05$ (Tukey's HSD test). IN = *C. partellus* – infested plants; UT, untreated control plants.

from the susceptible genotypes (Dhillon and Kumar 2017). Higher amounts of certain amino acids in the insect larvae could be because of increased production and/or accumulation in response to stress as a result of enzyme inhibition by secondary metabolites or poor utilization of amino acids in protein synthesis in the insect as a result of adverse effects of host plant resistance (Dhillon and Kumar 2017).

ROS production in plants in response to the oxidative stress by biotic and abiotic factors is a common phenomenon in plants (He et al., 2011; War et al., 2012). ROS mediate various signaling pathways involved in plant defense against biotic and abiotic stresses (Maffei et al., 2007). Among all the ROS, H_2O_2 is the most important component, as is highly stable and more freely diffusible than all other ROS. It mediates the signal transduction pathways, which lead to the expression of defensive genes, and thereby, production of various defensive proteins in plants against insect herbivores (Maffei et al., 2007).In addition, H_2O_2 has also been found to have direct toxicity against insect pests (Howe and Jander 2008; Maffei et al., 2007). It also defends the plants against subsequent insect and pathogen attack (Maffei et al., 2007). Infestation by the third instar larvae of *C. partellus* resulted in an increase in H_2O_2 content in all the genotypes. Similar increase in levels of H_2O_2 in plants herbivore damaged plants has been observed by Walling (2000). The H_2O_2 is one of the most important reactive oxygen species involved in plant defense against insect pests. It acts as a secondary messenger, and mediates various transduction pathways, which produces various plant defensive compounds (Maffei et al., 2007; Howe and Jander 2008). In addition, H_2O_2 also causes oxidative damage to insect midgut (Maffei et al., 2007). In addition to secondary metabolites, which have traditionally been perceived as the major components of chemical defense strategies that regulate host plant utilization by insects (Sharma et al., 2009; War et al., 2012, 2013), proteins are also an important contributor to the plant's chemical defense mechanism. Proteins are one of the most common limiting nutrient for insect nutrition and growth, which can alter the physiology of herbivores by reducing their growth rate, adult size, and survivability (Harvey et al., 2003). The higher amounts of proteins could be attributed to the greater activity of plant defensive enzymes, and the production of other plant defensive proteins. There was a significant increase in protein content in all the genotypes as a result of insect damage. Increase in protein content might be due to increased anti-oxidative enzyme activities after insect infestation. When under stress, plants produce various defense-related enzymes and other protein-based defensive compounds, thereby, increasing the overall protein concentration (Chen et al., 2009; Lawrence and Koundal 2002; War et al., 2012). The insect-resistant genotypes have been reported to possess higher levels of antioxidative enzymes and secondary metabolites, and they respond strongly to different stresses (Heng-Moss et al. 2004; Chen et al. 2009; Gulsen et al. 2010; War et al. 2011). Several reports have earlier documented increase in protein concentration in response to insect attack (Chen et al., 2009; He et al., 2011; War et al., 2012).

Variations for biochemical constituents in the sorghum genotypes irrespective of their levels of resistance suggests that the interaction among different biochemical compounds and the morphological traits, rather than a particular biochemical constituent play a greater role in host plant defense against *C. partellus*. Genotypic resistance in sorghum to *C. partellus* was largely due to activities of enzymes such as POD, PPO, PAL, TAL and CAT, which

influence the production and accumulation of secondary metabolites, and the amounts of total phenols, H_2O_2 and protein content, and hence, these could be used as biochemical markers to select sorghum genotypes with resistance to *C. partellus* for integrated pest management and sustainable crop production.

5. Conclusions

The sorghum genotypes responded differentially to infestation by *C. partellus* in terms of the activity of defensive enzyme such as POD, PPO, PAL, TAL, CAT, and the amounts of total phenols, H_2O_2, and proteins. Since increased activity of these enzymes and production and accumulation of defensive components are responsible for host plant defense against biotic and abiotic stresses, sorghum genotypes with greater activity of these enzymes, and greater amounts of secondary metabolites have higher levels of resistance to *C. partellus* than the susceptible genotypes, which have a limited capacity for production of secondary metabolites. Alteration in digestibility and palatability of plant tissues by the induced production of secondary metabolites in response to insect damage affect insect growth and development adversely. Therefore, induced resistance can play an important role in host plant resistance, and development of integrated pest management in sorghum. A detailed understanding of induced resistance to herbivores will provide new insights into basic mechanisms of chemical communication and insect – host plant co-evolution to facilitate new approaches in crop protection.

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There are no relevant financial or non-financial competing interests to report.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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