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# Stress-induced defense in sorghum in response to attack by the spotted stem borer, *Chilo partellus* (Swinhoe)

**Hemant Kumar · Mukesh K. Dhillon · Tanmaya K. Bhoi**

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**Abstract** There is a complex interplay of biochemical interactions which determine the host plant reaction to herbivory. We studied the *Chilo partellus*-induced biochemical plant defense system in sorghum. Present studies found that the total protein, starch and total sugar contents were significantly lower, while antioxidant enzymes like ascorbate oxidase (AO), catalase (CAT), ascorbate peroxidase (APX), phenyl ammonia lyase (PAL), tyrosine ammonia lyase (TAL), and nonenzymatic antioxidants such as total phenols, total antioxidants and ferric ion reducing antioxidant power (FRAP) higher in the seedlings of all the test sorghum genotypes as compared to susceptible genotype, Swarna both under healthy and *C. partellus* damaged conditions. Further, the *C. partellus* damage resulted in significant increase in amounts of all the test constitutional, enzymatic and nonenzymatic biochemicals in the test genotypes, however their percent increase was highly variable across genotypes. These studies demonstrate that both constitutive and *C. partellus* damage induced enzymatic and nonenzymatic antioxidants like APX, AO, PAL, TAL, CAT, total phenols and FRAP were significantly greater in IS 2205, IS 18,551, ICSV 1, ICSV 700 and ICSV 93,046 than in susceptible check (Swarna) indicating their role in plant defense. Furthermore, these genotypes can be used in sorghum breeding for resistance against *C. partellus*.

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**Keywords** Sorghum · Borer · Induced defense · Enzymatic antioxidants · Nonenzymatic antioxidants

## Introduction

Sorghum, *Sorghum bicolor* (L.) Moench is one of the most important cereal crops feeding millions of people in the semi-arid tropics. It is damaged by more than 150 insect species including several borer species during different crop growth stages right from sowing till harvesting (Sharma, 1993). Among the borers, spotted stem borer, *Chilo partellus* (Swinhoe) is the most devastating pest of sorghum in Asia and Africa (Sharma et al., 2003; Huang et al., 2013). All the three mechanisms of plant resistance to insects are functional for *C. partellus* resistance in sorghum (Sharma et al., 2007). The insect herbivory stimulates the plants to produce many antinutritional compounds, toxic proteins and secondary metabolites which interfere in biological and biochemical functions of the insects (Smith & Clement, 2012).

In response to biotic stress, reactive oxygen species (ROS) acts as secondary messenger to signal defense reaction in the host plants (Asada, 2006). To prevent the oxidation, plant also develop ROS scavenging mechanism (Howe & Schilmiller, 2002). In this process, several naturally occurring plant cell antioxidants/enzymes like catalase (CAT), ascorbate peroxidase (APX), ascorbate oxidase (AO), phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL);

and plant defense compounds such as phenolics, flavonoids and tannins play important role in detoxification of ROS and maintaining redox balance (Gill & Tuteja, 2010; Huang et al., 2019).

Some of the constitutive biochemicals have been found to impart defense against *C. partellus* in maize and sorghum (Dhillon & Chaudhary, 2015; Dhillon & Kumar, 2017). It has also been shown that the oxidative state of the host plant produces ROS and toxic secondary metabolites, and is an important component for resistance against insects (Howe & Jander, 2008; Wu & Baldwin, 2010; He et al., 2011). An efficient antioxidant system could be useful to protect the sorghum plants against oxidative stress caused by *C. partellus*. However, the information regarding the relative levels of insect resistance and enzymatic and nonenzymatic anti-oxidative responses in sorghum under the insect pests attack is limited. Therefore, present studies were designed to elucidate the *C. partellus* damage-induced regulation system of certain biochemical constituents and effect on the antioxidative potential of diverse sorghum genotypes. The findings will also be useful for better understanding the metabolic pathways leading to synthesis of *C. partellus* defense compounds and their deployment in sorghum improvement program.

## Materials and methods

### Plant materials and insect culture

The experimental plant material consisted of six sorghum genotypes viz., ICSV 1, ICSV 700, ICSV 93,046, IS 18,551 and IS 2205 (resistant check), and Swarna (susceptible check). These genotypes except Swarna have been found to impart deleterious effects on the developmental biology of *C. partellus* (Kumar, 2018). The *C. partellus* culture maintained on artificial diet (Sharma et al., 1992) under laboratory conditions was used for inoculation on the test sorghum genotypes at ICAR-Indian Agricultural Research Institute, New Delhi.

### Collection of sorghum seedlings for biochemical analysis

The seedlings of test sorghum genotypes were raised on the potting mixture (2 red soil: 1farm yard manure) in the pots (12 L capacity). Ten seeds were sown in each pot. There were four pots for each sorghum genotype (two pots for infestation with *C. partellus* larvae and two pots un-infested as control). The pots were covered with nylon net to protect the seedlings from natural insect pest infestation. Fifteen-day old seedlings of each test genotype were inoculated with two third instar *C. partellus* larvae in the designated pots. At 3 days after exposure, the *C. partellus* infested and healthy seedlings were collected separately from the designated pots. The central whorls of test seedling samples were separately collected. One-gram frozen tissue of each test sample was immediately processed for making the sample extracts by following the protocol given by Hildebrand et al. (1986). The prepared sample extracts were frozen at -20ºC, and used for various biochemical estimations. There were three replications for each treatment in a completely randomized design.

### Constitutional biochemical content analysis

All the healthy and *C. partellus* damaged sorghum samples were analyzed for total protein, starch and total sugars. Total proteins were estimated by the method given by Bradford (1976), starch by the method given by Clegg (1956), and total sugars by concentrate sulphuric acid method given by Dubois et al. (1956). The values obtained for these biochemical constituents were expressed in mg/g of plant tissue.

### Enzymatic antioxidant analysis

All the healthy and *C. partellus* damaged test samples were analyzed for the activity of various antioxidant enzymes like APX (Ali et al., 2005), CAT (Aebi, 1984), PAL (Fritz et al., 1976), TAL (Thorpe & Beaudoin-Eagan, 1985) and AO (Diallinas et al., 1997) by following the methods given by various workers. The activities of these test antioxidant enzymes were expressed in respect to the change in absorbance/min/mg of protein.

### Nonenzymatic antioxidant analysis

All the healthy and *C. partellus* damaged sorghum samples were analyzed for total phenols, total antioxidants and ferric ion reducing antioxidant power (FRAP).

Total phenols were estimated using the method given by Singleton and Rossi (1965), total antioxidants by Prieto et al. (1999) and FRAP by Benzie and Strain (1999). The values obtained for these biochemical constituents were expressed in mg/g of plant tissue.

### Statistical analysis

The data on various biochemical constituents in the healthy and *C. partellus* damaged seedlings of test genotypes were subjected to analysis of variance (ANOVA) using factorial design. The significance of differences between the genotypes, treatments and genotype × treatment interactions were judged by *F*-test, and the differences among them were compared by least significant differences (LSD) at $P=0.05$ using the statistical software SAS® version 9.2.

## Results

### Constitutional biochemical contents

**Total protein** Total protein content varied from 2.49 to 5.10 mg/g in the healthy and 2.70 to 5.78 mg/g in *C. partellus* damaged seedlings of test genotypes (Table 1). The total protein content significantly varied in the seedlings of test genotypes ($F=28009.85$; df = 5,22; $P<0.001$) and under healthy and damaged conditions ($F=3798.09$; df = 1,22; $P<0.001$). It was significantly lower in the healthy and higher in *C. partellus* damaged seedlings across test genotypes (Table 1). The genotype × treatment interaction was also significant for the total protein content ($F=281.92$; df = 5,22; $P<0.001$). Both under healthy and *C. partellus* damaged conditions, the total protein was significantly lower in resistant check (IS 2205) followed by the test genotypes IS 18,551 and ICSV 700 in comparison to susceptible check (Swarna) (Fig. 1A).

**Starch** There was significant variation in starch content in the seedlings of test genotypes ($F=18971.5$, df = 5,22; $P<0.001$), under healthy and damaged conditions ($F=930.83$, df = 1,22; $P<0.001$), and for genotype × treatment interaction ($F=25.69$; df = 5,22; $P<0.001$). It varied from 1.37 to 6.64 mg/g in healthy and 1.67 to 7.21 mg/g in *C. partellus* damaged sorghum seedlings (Table 1). Across genotypes, starch content was significantly lower in the healthy than in *C. partellus* damaged sorghum seedlings (Table 1). Both under healthy and *C. partellus* damaged conditions, the starch content was significantly lower in resistant check (IS 2205) followed by IS 18,551 and ICSV 1 in comparison to susceptible check (Swarna) (Fig. 1B).

**Table 1** Amounts of various biochemical constituents in sorghum genotypes under healthy and *Chilo partellus* damaged conditions

| `Genotypes | Total protein (mg/g) | | | Starch (mg/g) | | | Total sugars (mg/g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Damaged | Healthy | Change over healthy | Damaged | Healthy | Change over healthy | Damaged | Healthy | Change over healthy |
| ICSV 1 | 4.91 | 4.61 | +0.30 | 3.32 | 3.12 | +0.20 | 11.14 | 9.62 | +1.52 |
| ICSV 93,046 | 4.42 | 4.05 | +0.37 | 4.45 | 4.16 | +0.29 | 9.85 | 9.02 | +0.83 |
| ICSV 700 | 3.71 | 3.48 | +0.30 | 5.29 | 5.02 | +0.27 | 11.74 | 10.76 | +0.98 |
| IS 18,551 | 3.31 | 3.24 | +0.07 | 2.63 | 2.12 | +0.51 | 12.45 | 11.15 | +1.30 |
| IS 2205 | 2.70 | 2.49 | +0.21 | 1.67 | 1.37 | +0.30 | 3.41 | 2.05 | +1.36 |
| Swarna | 5.78 | 5.10 | +0.68 | 7.21 | 6.64 | +0.57 | 14.66 | 12.88 | +1.78 |
| Mean | 4.14 | 3.83 | +0.32 | 4.10 | 3.74 | +0.36 | 10.54 | 9.25 | +1.30 |
| For comparing | LSD (P = 0.05) | | *P*-value | LSD (P = 0.05) | | *P*-value | LSD (P = 0.05) | | *P*-value |
| Genotype (G) | 0.01 | | < 0.001 | 0.04 | | < 0.001 | 0.08 | | < 0.001 |
| Treatment (T) | 0.01 | | < 0.001 | 0.02 | | < 0.001 | 0.04 | | < 0.001 |
| G × T | 0.02 | | < 0.001 | 0.05 | | < 0.001 | 0.11 | | < 0.001 |

The + sign represents increase in biochemical contents after damage by *C. partellus*

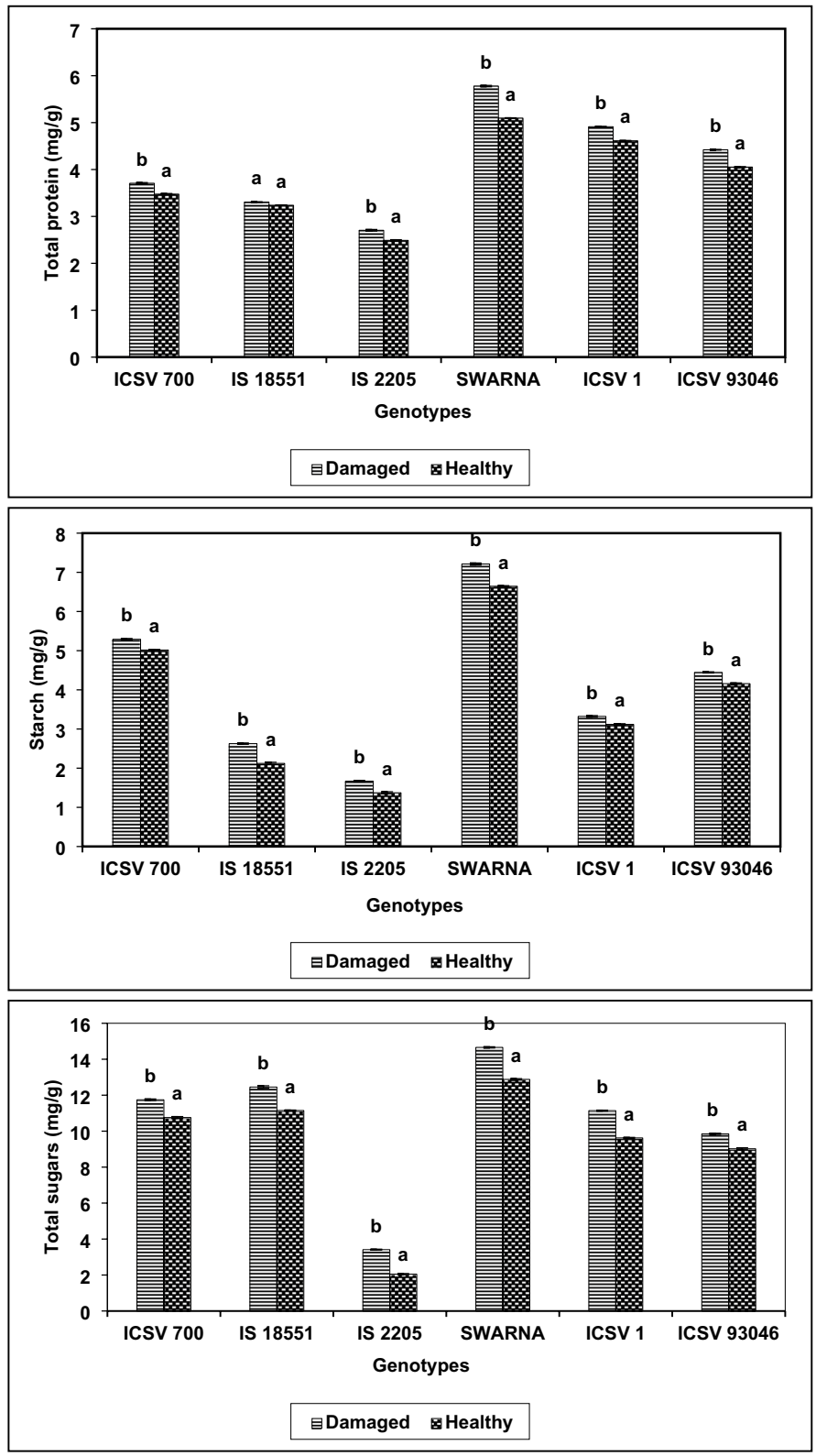


**Fig. 1** Amounts of total protein, starch and total sugars in healthy and *Chilo partellus* damaged seedlings of sorghum genotypes

**Total sugars** The total sugar content varied from 2.05 to 12.88 mg/g in healthy and 3.41 to 14.66 mg/g in *C. partellus* damaged seedlings of test genotypes (Table 1). Total sugar content significantly varied among the test genotypes ($F = 18606.48$; df = 5,22; $P < 0.001$) both under healthy and damaged conditions ($F = 3246.87$; df = 1,22; $P < 0.001$), and for genotype × treatment interactions ($F = 38.72$; df = 5,22; $P < 0.001$). Total sugar content was significantly lower in the healthy and higher in *C. partellus* damaged seedlings across test genotypes (Table 1). Both under healthy and *C. partellus* damaged conditions, the total sugar content was significantly lower in IS 2205 (resistant check) and higher in Swarna (susceptible check) as compared to other sorghum genotypes (Fig. 1C).

Enzymatic antioxidants

**Ascorbate peroxidase (APX)** The APX activity varied from 4.07 to 26.58 and 5.67 to 34.88 ΔOD/min/mg of protein in the healthy and *C. partellus* damaged seedlings of test genotypes, respectively (Table 2). There was significant variation for APX activity in the test genotypes ($F = 990.4$; df = 5,22; $P < 0.001$) both under healthy and *C. partellus* damaged ($F = 804.05$; df = 1,22; $P < 0.001$) conditions, and for genotype × treatment interactions ($F = 469.82$; df = 5,22; $P < 0.001$). The APX activity was significantly greater in *C. partellus* damaged than in healthy seedlings across test genotypes (Table 2). Both under healthy and *C. partellus* damaged conditions, the APX activity was significantly higher in IS 2205 and IS 18,551, and lower in Swarna (susceptible check) as compared to other test genotypes (Fig. 2A).

**Catalase (CAT)** The CAT activity varied from 0.55 to 10.00 ΔOD/min/mg of protein in healthy and 0.68 to 12.49 ΔOD/min/mg of protein in the *C. partellus* damaged seedlings of test genotypes (Table 2). There were significant differences in CAT activity in the sorghum genotypes ($F = 939.92$; df = 5,22; $P < 0.001$) both under healthy and *C. partellus* damaged ($F = 713.59$; df = 1,22; $P < 0.001$) conditions, and for genotype × treatment interactions ($F = 471.24$; df = 5,22; $P < 0.001$). The CAT activity was significantly greater in *C. partellus* damaged than in healthy seedlings across test genotypes (Table 2). Both under healthy and *C. partellus* damaged conditions, the CAT activity was significantly greater in IS 2205 (resistant check), ICSV 1, ICSV 93,046 and IS 18,551 than in Swarna (susceptible check) and ICSV 700 (Fig. 2B).

**Phenyl ammonia lyase (PAL)** The PAL activity varied from 0.56 to 8.54 and 1.54 to 10.92 ΔOD/min/mg of protein in the healthy and *C. partellus* damaged seedlings of the test genotypes, respectively (Table 2). There was significant variation in PAL activity in the seedlings of test genotypes ($F = 6151.08$, df = 5,22; $P < 0.001$) both under healthy and *C. partellus* damaged ($F = 5130.4$; df = 1,22; $P < 0.001$) conditions, and for genotype × treatment interactions ($F = 1048.3$; df = 5,22; $P < 0.001$). The PAL activity was significantly greater in *C. partellus* damaged than in healthy seedlings across test genotypes (Table 2). The PAL activity was significantly greater in IS 2205 (resistant check), IS 18,551, ICSV 700 and ICSV 93,046 than in Swarna (susceptible check) both under healthy and *C. partellus* damaged conditions (Fig. 2C).

**Tyrosine ammonia lyase (TAL)** The TAL activity varied from 0.68 to 6.46 and 2.56 to 8.23 ΔOD /min/mg of protein in the healthy and *C. partellus* damaged seedlings of test genotypes, respectively (Table 2). There was significant variation in TAL activity in the seedlings of test genotypes ($F = 994.22$; df = 5,22; $P < 0.001$) both under healthy and *C. partellus* damaged ($F = 1784.45$; df = 1,22; $P < 0.001$) conditions, and for genotype × treatment interactions ($F = 556.11$; df = 5,22; $P < 0.001$). The TAL activity was significantly greater in *C. partellus* damaged than in healthy seedlings across test genotypes (Table 2). The TAL activity was significantly greater in IS 2205, IS 18,551, ICSV 93,046 and ICSV 1 than in Swarna (susceptible check) under healthy as well as *C. partellus* damaged conditions (Fig. 2D).

**Ascorbate oxidase (AO)** The AO activity varied from 4.07 to 26.58 and 5.67 to 34.88 ΔOD/min/mg of protein in the healthy and *C. partellus* damaged seedlings of test genotypes, respectively (Table 2). There were significant differences in AO activity in the seedlings of test genotypes ($F = 990.4$; df = 5,22; $P < 0.001$) both under healthy and *C. partellus*

**Table 2** Activity of various enzymes in sorghum genotypes under healthy and *Chilo partellus* damaged conditions

| Genotypes | Ascorbate peroxidase (ΔOD/min/mg protein) | | | Catalase (ΔOD/min/mg protein) | | | Phenyl ammonia lyase (ΔOD/min/mg protein) | | | Tyrosine ammonia lyase (ΔOD/min/mg protein) | | | Ascorbate oxidase (ΔOD/min/mg protein) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D | H | Change over healthy | D | H | Change over healthy | D | H | Change over healthy | D | H | Change over healthy | D | H | Change over healthy |
| ICSV 1 | 9.04 | 6.90 | +2.14 | 5.67 | 4.37 | +1.30 | 2.55 | 1.54 | +1.01 | 4.72 | 2.03 | +2.69 | 16.50 | 12.74 | +3.76 |
| ICSV 93,046 | 10.29 | 4.79 | +5.50 | 5.08 | 4.89 | +0.19 | 3.58 | 1.43 | +2.15 | 5.22 | 3.05 | +2.17 | 14.98 | 13.38 | +1.60 |
| ICSV 700 | 6.45 | 4.80 | +1.65 | 2.39 | 1.50 | +0.89 | 3.42 | 1.44 | +1.98 | 3.37 | 2.27 | +1.10 | 10.44 | 10.38 | +0.06 |
| IS 18,551 | 15.00 | 6.40 | +8.60 | 11.25 | 0.88 | +10.37 | 7.64 | 0.75 | +6.89 | 7.49 | 1.23 | +6.26 | 31.60 | 7.23 | +24.37 |
| IS 2205 | 20.41 | 15.93 | +4.48 | 12.49 | 10.00 | +2.49 | 10.92 | 8.54 | +2.38 | 8.23 | 6.46 | +1.77 | 34.88 | 26.58 | +8.30 |
| Swarna | 1.96 | 1.21 | +0.75 | 0.68 | 0.55 | +0.13 | 2.05 | 0.56 | +1.49 | 3.47 | 0.68 | +2.79 | 5.67 | 4.07 | +1.60 |
| Mean | 10.53 | 6.67 | +3.85 | 6.26 | 3.70 | +2.56 | 5.03 | 2.38 | +2.65 | 5.42 | 2.62 | +2.80 | 19.01 | 12.40 | +6.62 |
| For comparing | LSD ($P=0.05$) | | *P*-value | LSD ($P=0.05$) | | *P*-value | LSD ($P=0.05$) | | *P*-value | LSD ($P=0.05$) | | *P*-value | LSD ($P=0.05$) | | *P*-value |
| Genotype (G) | 0.39 | | <0.001 | 3.12 | | <0.001 | 0.32 | | <0.001 | 0.16 | | <0.001 | 0.77 | | <0.001 |
| Treatment (T) | 0.22 | | <0.001 | 1.80 | | <0.001 | 0.19 | | <0.001 | 0.09 | | <0.001 | 0.44 | | <0.001 |
| G x T | 0.56 | | <0.001 | 4.41 | | 0.484 | 0.46 | | <0.001 | 0.23 | | <0.001 | 1.08 | | <0.001 |

D = Damaged maize seedlings. H = Healthy maize seedlings. The + sign represents increase in biochemical contents after damage by *C. partellus*

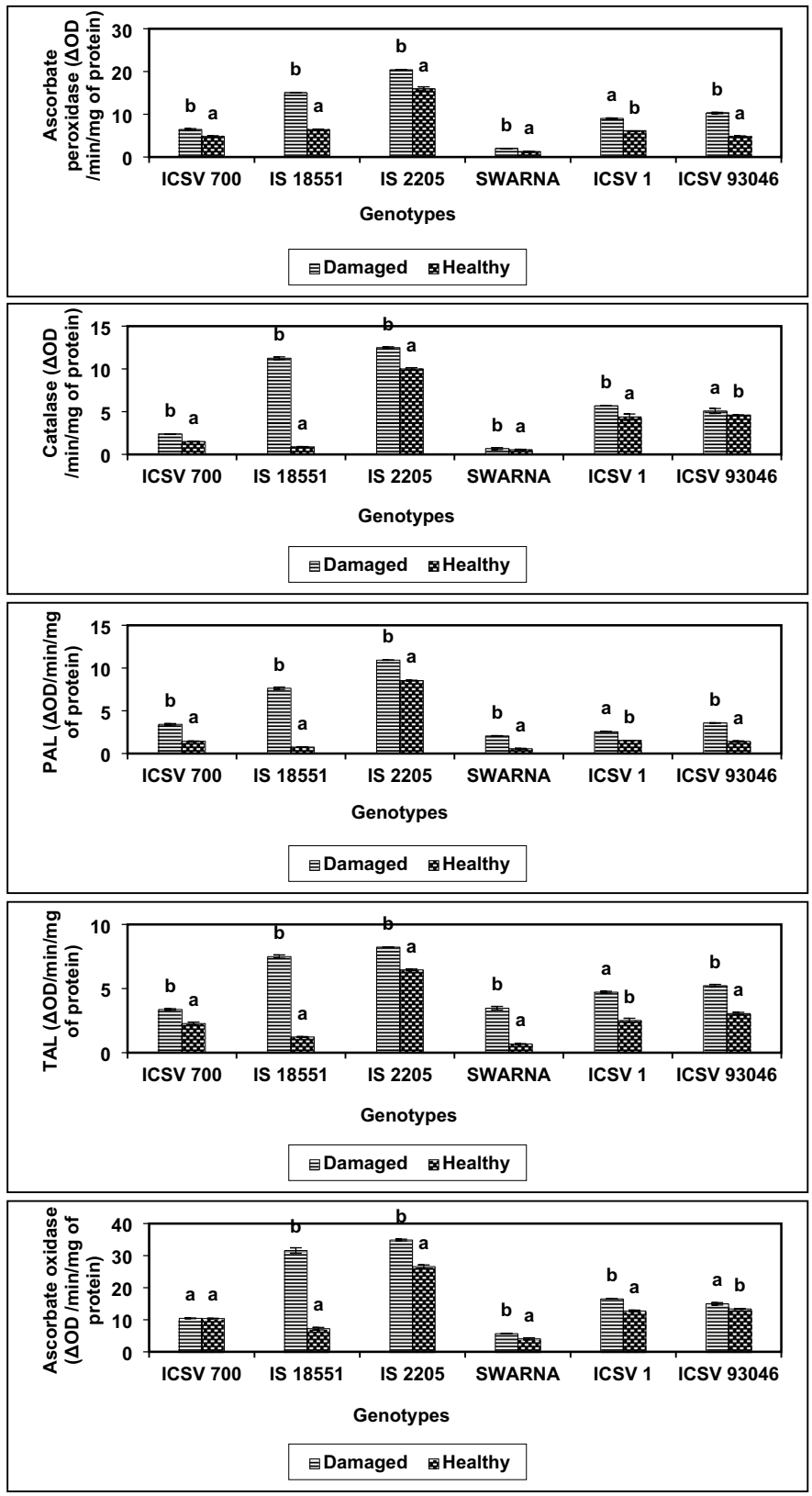


**Fig. 2** Activity of various enzymes viz., ascorbate peroxidase, catalase, phenyl ammonia lyase (PAL), tyrosine ammonia lyase (TAL) and ascorbate oxidase in healthy and *Chilo partellus* damaged seedlings of sorghum genotypes

damaged ($F=804.05$; df = 1,22; $P<0.001$) conditions, and for genotype × treatment interactions ($F=469.82$; df = 5,22; $P<0.001$). The AO activity was significantly greater in *C. partellus* damaged than in healthy seedlings across test genotypes (Table 2). Both under healthy and *C. partellus* damaged conditions, the AO activity was significantly greater in IS 2205 (resistant check), ICSV 1, ICSV 93,046 and IS 18,551 as compared to susceptible check (Swarna) (Fig. 2E).

Non-enzymatic antioxidants

**Total phenols** Total phenol content varied from 0.20 to 0.58 mg/g and 0.27 to 0.61 mg/g in the healthy and *C. partellus* damaged seedlings, respectively (Table 3). There was significant variation in total phenols in the seedlings of different sorghum genotypes ($F=26462.42$; df = 5,22; $P<0.001$) both under healthy and *C. partellus* damaged ($F=3230.58$; df = 1,22; $P<0.001$) conditions, and for the genotype × treatment interactions ($F=99.68$; df = 5,22; $P<0.001$). Total phenol content was significantly higher in the *C. partellus* damaged than in healthy seedlings of the test genotypes (Table 3). Both under healthy and *C. partellus* damaged conditions, the total phenol content was significantly higher in IS 2205 (resistant check), ICSV 1, ICSV 700, ICSV 93,046 and IS 18,551 as compared to Swarna (susceptible check) (Fig. 3A).

**Total antioxidants** Total antioxidants varied from to 3.99 to 12.64 mg/g in healthy and 4.65 to 15.15 mg/g in *C. partellus* damaged seedlings of test genotypes (Table 3). There were significant differences in total antioxidants in the seedlings of test genotypes ($F=35462.76$; df = 5,22; $P<0.001$) both under healthy and *C. partellus* damaged ($F=7709.14$; df = 1,22; $P<0.001$) conditions, and for the genotype × treatment interactions ($F=393.32$; df = 5,22; $P<0.001$). Further, the total antioxidants were significantly higher in *C. partellus* damaged than in healthy seedlings of the test genotypes (Table 3). Both under healthy and *C. partellus* damaged conditions, the total antioxidants were significantly higher in IS 2205 (resistant check), ICSV 1, ICSV 700, ICSV 93,046 and IS 18,551 than in Swarna (susceptible check) (Fig. 3B).

**Ferric ion reducing antioxidant power (FRAP)** The FRAP content varied from to 0.42 to 1.49 and 0.52 to 1.60 mg/g in healthy and *C. partellus* damaged seedlings of test genotypes, respectively (Table 3). There was significant variation in FRAP content in the seedlings of test genotypes ($F=18055.27$;

**Table 3** Amounts of various non-enzymatic antioxidants in sorghum genotypes under healthy and *Chilo partellus* damaged conditions

| Genotypes | Total phenols (mg/g) | | | Total antioxidants (mg/g) | | | FRAP (mg/g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Damaged | Healthy | Change over healthy | Damaged | Healthy | Change over healthy | Damaged | Healthy | Change over healthy |
| ICSV 1 | 0.49 | 0.46 | +0.03 | 7.38 | 6.63 | +0.75 | 0.70 | 0.58 | +0.12 |
| ICSV 93,046 | 0.31 | 0.27 | +0.04 | 10.92 | 9.53 | +1.39 | 1.34 | 1.18 | +0.16 |
| ICSV 700 | 0.39 | 0.36 | +0.03 | 8.18 | 7.29 | +0.89 | 0.88 | 0.75 | +0.13 |
| IS 18,551 | 0.44 | 0.41 | +0.03 | 9.04 | 7.85 | +1.19 | 1.23 | 1.16 | +0.07 |
| IS 2205 | 0.61 | 0.58 | +0.03 | 15.15 | 12.64 | +2.51 | 1.60 | 1.49 | +0.11 |
| Swarna | 0.27 | 0.20 | +0.07 | 4.65 | 3.99 | +0.66 | 0.52 | 0.42 | +0.10 |
| Mean | 0.42 | 0.38 | +0.04 | 9.22 | 7.99 | +1.23 | 1.05 | 0.93 | +0.12 |
| For comparing | LSD ($P=0.05$) | | *P*-value | LSD ($P=0.05$) | | *P*-value | LSD ($P=0.05$) | | *P*-value |
| Genotype (G) | 0.002 | | <0.001 | 0.05 | | <0.001 | 0.008 | | <0.001 |
| Treatment (T) | 0.001 | | <0.001 | 0.02 | | <0.001 | 0.005 | | <0.001 |
| G x T | 0.003 | | <0.001 | 0.07 | | <0.001 | 0.012 | | <0.001 |

The + sign represents increase in biochemical contents after damage by *C. partellus*

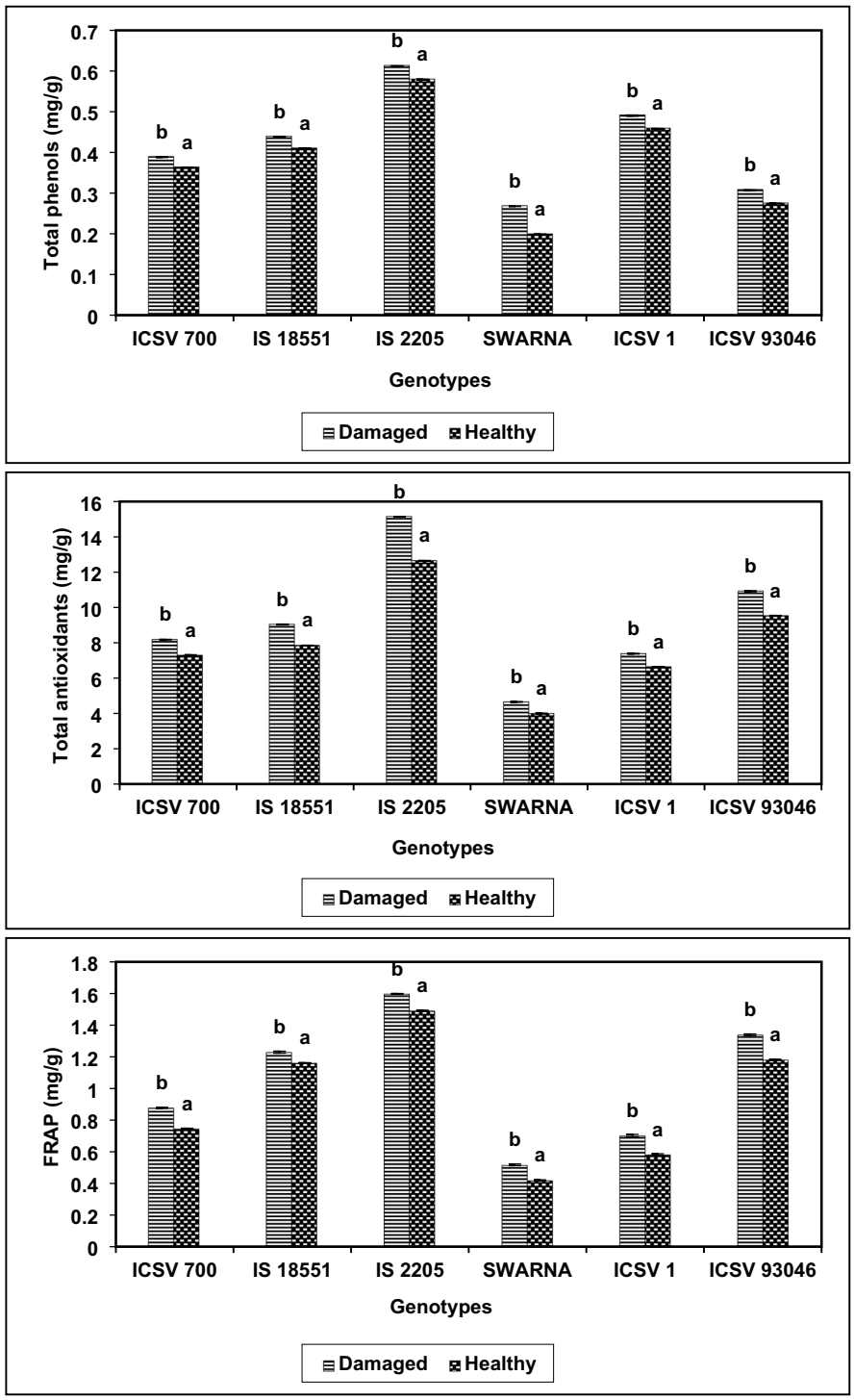


**Fig. 3** Amounts of total phenols, total antioxidants and ferric ion reducing antioxidant power (FRAP) in healthy and *Chilo partellus* damaged seedlings of sorghum genotypes

df = 5,22; $P < 0.001$) both under healthy and *C. partellus* damaged ($F = 2064.2$; df = 1,22; $P < 0.001$) conditions, and for genotype × treatment interactions ($F = 25.34$; df = 5,22; $P < 0.001$). The FRAP content was significantly higher in *C. partellus* damaged than in healthy seedlings of the test genotypes (Table 3). Both under healthy and *C. partellus* damaged conditions, the FRAP content was significantly higher in

IS 2205 (resistant check), IS 18,551, ICSV 93,046 and ICSV 700 than in Swarna (susceptible check) and ICSV 1 (Fig. 3C).

## Discussion

Both constitutive and herbivory induced host plant biochemicals determine the biological performance and resistance against insect pests (Howe & Jander, 2008; Wu & Baldwin, 2010; Smith & Clement, 2012; Dhillon & Chaudhary, 2015; Dhillon & Kumar, 2017; Bhoi et al., 2021). The present studies revealed significant increase in total protein, starch and total sugars in all the test sorghum seedlings due to damage by *C. partellus*, however the percent increase in these biochemical constituents was highly variable across the test genotypes (Fig. 4). Furthermore, the total protein, starch and total sugar contents were significantly greater in both *C. partellus* damaged and healthy seedlings of Swarna (susceptible check) and lower in IS 2205 (resistant check) and IS 18,551 (except total sugars) in comparison to other sorghum genotypes. The rise in total protein content due to *C. partellus* damage may be attributed to synthesis of antinutritional proteins, while increase in starch and total sugars to help withstand the plants from herbivory stress. Similar findings have also been reported due to *C. partellus* damage in maize (Bhoi et al., 2021). Furthermore, the greater amounts of total protein and total sugar contents in sorghum seedlings have been reported to impart susceptibility to *C. partellus* (Kabre & Ghorpade, 1999; Dhillon & Chaudhary, 2018). Similarly, the sugar content in the *Bauhinia brevipes* Vogel leaves has also been reported to be influenced by insect herbivory (Cornelissen and Fernandes 2001). The stress induced accumulation of ROS is related to increase in soluble sugars and is thought to be a stress-adaptive response (Roitsch, 1999).

The increased oxidative state could be responsible for herbivory resistance in the host through generation of ROS and their consequent removal by antioxidant system of the plant (Felton et al., 1992). The production of ROS is an early response to biotic stress, cues to induce plant defense against the stress (Maffei et al., 2007). Antioxidant enzymes including catalase, peroxidase, and superoxide dismutase show increased activity in response to ROS production (Libik-Konieczny et al., 2011). In the present studies, the activity of APX, CAT, PAL, TAL and AO were significantly higher in IS 2205 (resistant check) and lower in Swarna (susceptible check) in comparison to other test sorghum genotypes both under healthy and *C. partellus* damaged conditions. Earlier studies

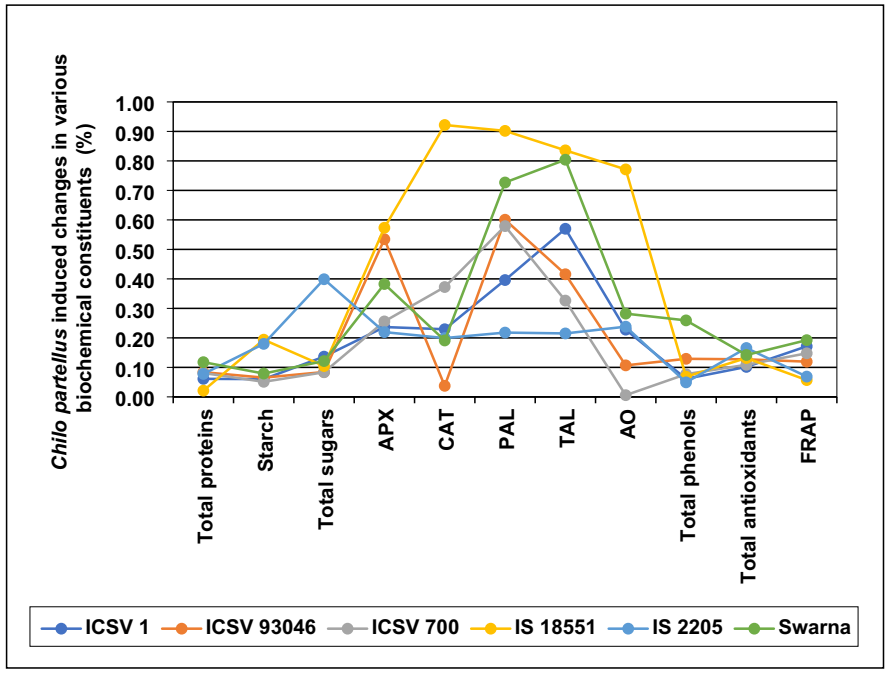


**Fig. 4** Changes in biochemical constituents in response to damage by *Chilo partellus* in the seedlings of different sorghum genotypes

also reported greater activity of peroxidase, polyphenol oxidase, PAL and TAL in resistant as compared to susceptible seedlings of sorghum (Vashisth et al., 2022). Moreover, the damage by *C. partellus* resulted in significant increase in APX, CAT, PAL, TAL and AO activity, wherein highest percent increase in these enzymatic antioxidants was recorded in IS 18,551 (Fig. 4). Earlier studies have also found that the herbivory result in increased activity of CAT and PAL in the host plants (Bi & Felton, 1995; Sharma et al., 2016). Increased CAT activity due to biotic stress serves as a local signal to activate protective genes and boost host resistance (Chen et al., 2009). Since APX is part of peroxide detoxification process, its increased activity has been shown to assist in protection of host plants against biotic stress (Tománková et al., 2006).

The enzyme PAL generate a number of phenylpropanoids and phenolics, several of which are essential for plant defense system (Asada, 1992). Increased PAL activity due to herbivory allows phenolics to be oxidised to quinones through the shikimic acid pathway, causing herbivore toxicity (Zhao et al., 2009; Rani & Jyothsna, 2010). In the present studies, the *C. partellus* damage resulted in greater and/or on par percent increase in levels of APX, CAT, PAL, TAL and AO in susceptible check (Swarna) in comparison to test genotypes, indicating the role of these antioxidant enzymes in shielding plants from biotic stresses. The greater percent increase in most of these antioxidant enzymes due to *C. partellus* damage have also been reported in the susceptible maize genotypes (Bhoi et al., 2021). Similarly, Sau et al. (2022) also reported greater amounts of constitutive and induced defense biochemicals in borer-resistant maize genotypes.

Phenols play an important role in the reduction of ROS, which then initiate several reactions that stimulate defence enzymes (Maffei et al., 2007). In the present studies, the total phenols, antioxidants and FRAP were significantly lower in Swarna (susceptible check) than in other test sorghum genotypes under healthy and as well as borer-infestation conditions. However, the damage by *C. partellus* resulted in significantly greater increase in total phenols and FRAP in Swarna (susceptible check), whereas lower increase in the resistant genotypes IS 2205 and IS 18,551 as compared to other genotypes (Fig. 4). These findings suggest that the greater constitutive contents of nonenzymatic antioxidants like total phenols and FRAP could be playing an important role in *C. partellus* defense in sorghum. Vashisth et al. (2022) also found greater amounts of phenols in resistant than in susceptible sorghum genotypes.

The present studies demonstrated that both constitutive and insect damage induced enzymatic and nonenzymatic antioxidants like APX, AO, PAL, TAL, CAT, total phenols and FRAP were significantly greater in IS 2205, IS 18,551, ICSV 1, ICSV 700 and ICSV 93,046 (except in a few cases) in comparison to susceptible check (Swarna), suggesting that these biochemical constituents play important role in plant defense, and thus can be deployed in *C. partellus*-resistance breeding program.

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**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Consent for publication** Not applicable.

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

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