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Biochemical defense in maize against *Chilo partellus* (Swinhoe) through activation of enzymatic and nonenzymatic antioxidants

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Biochemical defense against herbivores is one of the most important components of plant resistance to insects. Here, we studied the constitutive and induced biochemical defense through activation of enzymatic and nonenzymatic antioxidants in response to damage by the spotted stem borer, *Chilo partellus* (Swinhoe) in six maize genotypes including resistance and susceptible checks. The levels of total sugars, total soluble protein and starch were significantly lower, while total phenol and total antioxidant higher in resistant than susceptible maize genotypes both under damaged and healthy plant conditions. The activity of antioxidant enzymes like AO, CAT, APX, PAL and TAL were significantly higher in resistant than susceptible genotype, Basi Local, which further increased in response to damage by *C. partellus*. The nonenzymatic antioxidant scavenging activity of FRAP was also significantly higher in resistant maize genotypes, which further increased upon damage by *C. partellus*. Total antioxidant activity increased from 22.2 to 96.3% across test maize genotypes in response to damage by *C. partellus*, wherein maximum increase was recorded in CML 345. These findings clearly demonstrate that both constitutive and induced biochemical compounds through activation of enzymatic and nonenzymatic antioxidant defense systems impart resistance against *C. partellus* in CPM 8, CPM 13, CPM 15, CPM 18 and CML 345, thus could be used in insect resistance breeding program. These studies could also be useful for detailed understanding on metabolic pathways regulating biochemical defense and up- and down-regulation of associated genes in plant defense against biotic stresses.

Keywords: Antioxidants, Biotic stress, Corn, Induced defense, Insect resistance breeding, Maize, Sweet corn, *Zea mays*

Maize (*Zea mays* L.) is one of the most important cereal crops for food, feed, green cobs, popcorn, baby corn, sweet corn, fodder, starch and several industrial products, depending on the region and socioeconomic conditions[1]. It is damaged by 139 insect species at different growth stages, of which spotted stem borer, *Chilo partellus* (Swinhoe) is one of the most important pests and poses a great challenge causing 18 to 25% yield loss in maize under different agro-climatic conditions in Asia and Africa[2,3]. Several morphological, anatomical and biochemical plant traits have been reported to confer resistance to *C. partellus*[4,5]. Further, the plant resistance to herbivores is a complex trait which also depends on the interplay of several other factors like absence or insufficient amount of essential nutrients, nutrient imbalances, and presence of toxic substances, anti-metabolites and enzymes which adversely affect food digestion and utilization[6,7].

The production of reactive oxygen species (ROS) in response to biotic and abiotic stresses act as secondary messenger to signal defense reaction in plants[8,9]. Because of high metabolic cost, most defense mechanisms are induced, which enhance phenotypic plasticity in the host plant to limit chances of adaptation by the herbivore[10]. To provide tight regulation of ROS levels in the living cells, plants have evolved a wide battery of enzymatic and nonenzymatic antioxidants responsible for maintaining the redox balance[11]. In this series there are several naturally occurring plant cell antioxidants/enzymes like catalase (CAT), ascorbate peroxidase (APX), ascorbate oxidase (AO), phenyl ammonia lyase (PAL) and tyrosine ammonia lyase (TAL); plant defense compounds such as phenolics, flavonoids and tannins which play an important role in detoxification of ROS[12].

Plant resistance to *C. partellus* depends on interplay of several components including biochemicals, which finally sum up in the expression of resistance[13,14]. Poor understanding of biochemical mechanisms of insect-plant interactions has been the biggest impediment in development of resistant varieties. Although there is adequate information on contribution of constitutional biochemical compounds

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in host plant defense against *C. partellus*[14-16], little is known about antioxidant defense and regulation of defense compounds in response to damage by *C. partellus*. Therefore, in the present investigation, we tried to understand the regulation system of certain biochemical constituents and plant defense in the form of activation of enzymatic and nonenzymatic antioxidants in response to damage by *C. partellus* in diverse maize genotypes.

Materials and Methods

Plant materials and insect rearing

Six maize genotypes *viz.*, CPM 8, CPM 18 (yellow kernel), CPM 13, CPM 15 (white kernel) reported resistant to *C. partellus*[17,18], CML 345 (resistant check), and Basi Local (susceptible check) were used in the present studies. The *C. partellus* culture was maintained on artificial diet[19] under laboratory conditions at 27 ± 2ºC, 65 ± 5% relative humidity, and 12L:12D at the Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi.

Collection of maize seedlings for biochemical analysis

The seedlings of above-mentioned maize genotypes were raised in the pots (12 L capacity) on the potting mixture consisted of red soil and farm yard manure (2:1). Ten seeds were sown in each pot and there were 4 pots (2 pots each for *C. partellus* inoculation and un-inoculated control) for each maize genotype and covered with nylon net to protect from damage by insect pests and mimicking natural environmental conditions. The plants were watered as and when needed. The 15-day old seedlings in designated pots were inoculated with third instar *C. partellus* larvae in the central whorl of each test maize genotype. After 72 h of exposure, healthy and *C. partellus* infested seedlings were collected separately. The central whorls of damaged and counterpart healthy seedlings were taken from these samples and processed immediately for estimation of different biochemical constituents. For extract formation, 1.0 g frozen tissues were homogenized at 4 ºC in an ice-chilled mortar with liquid N_2 in 10 mL phosphate buffer (50 mM, pH 7.5, 1.0 mM, EDTA)[20] with 50 mg PVP per g of tissue. Crude homogenates were centrifuged at 15000 rpm for 15 min at 4ºC, and the supernatant fractions were frozen at –20ºC and used for estimation of biochemical constituents. In total there were six test genotypes, two treatments (healthy and *C. partellus* damaged), and all the test samples were prepared and analyzed for biochemical contents in three replications.

Constitutional biochemical content analysis

Total soluble protein

Protein content was determined by Bradford method using BSA as standard[21], and expressed in mg/g of plant tissue.

Total sugars

Total sugar content in the test plant samples was estimated by concentrate sulphuric acid method[22] using glucose as standard, andexpressed in mg/g of plant tissue.

Total starch content

Total starch content was estimated by perchloric acid digestion method[23] using glucose as standard, and expressed in mg/g of plant tissue.

Enzymatic antioxidant analysis

Ascorbate peroxidase

The APX activity was determined as described by Asada[24], with slight modifications. Reaction was started with 100 mM tris-acetate buffer (pH 7.0), 2 mM ascorbic acid and 20 µL enzyme extract in 1.0 mL of reaction mixture with 2 mM of H_2O_2 as substrate to initiate the reaction. The reaction was allowed to run for 3 min at 25ºC and the decrease in absorbance was monitored at 290 nm. The APX activity was calculated using molar extinction coefficient (2.5 $mM^{-1}cm^{-1}$) and expressed in enzyme units/mL. One unit of enzyme determines the amount necessary to decompose 1.0 µM of substrate consumed/min at 25ºC.

Catalase

The CAT activity was determined as described by Aebi[25]. The disappearance of H_2O_2 was monitored by measuring decrease in absorbance at 240 nm. Reaction was carried in 1.0 mL final volume reaction mixture, containing potassium phosphate buffer (pH 7.0) and 50 µL of enzyme extract added with 60 mM H_2O_2 to initiate the reaction. The reaction was monitored at 25ºC for 3 min. Activity was calculated using molar extinction coefficient (0.036 $mM^{-1}cm^{-1}$) and expressed in enzyme units/mL. One unit of enzyme determines the amount necessary to decompose 1.0 µM of H_2O_2 per min at 25ºC.

Phenyl ammonia lyase and Tyrosine ammonia lyase

The PAL and TAL activities in cell free extracts were measured as described earlier by Abell & Shen[26]. The PAL assay was initiated by adding the crude enzyme extract to a solution of Tris–HCl

(50 mM, pH 8.5) buffer containing l-phenylalanine (1.0 mM). The reaction was followed by monitoring the *trans*-cinnamic acid production at 290 nm and activity was calculated using a molar extinction coefficient (9 $mM^{-1}cm^{-1}$). One unit of activity indicated deamination of 1.0 mol of phenylalanine to *trans*-cinnamic acid per min. The TAL activity was similarly measured using tyrosine as the substrate and *p*-coumaric acid production was monitored at 315 nm. The activity was determined using an extinction coefficient (10 mM^{-1} cm^{-1}) for *p*-coumaric acid. One unit of activity indicated deamination of 1.0 mol of tyrosine to *p*-coumaric acid per min. The PAL and TAL activities were expressed in enzyme units/mL.

Ascorbate oxidase

The AO activity was measured as described earlier by Diallinas *et al*.[27]. The reaction mixture contained 2.5 mM ascorbic acid in 50 mM phosphate buffer (pH 7.0) with 50 µL crude enzyme extract. The decrease in absorbance was observed for 3 min at 265 nm due to ascorbate oxidation. The AO activity was calculated using molar extinction coefficient (10 $mM^{-1}cm^{-1}$) and expressed in enzyme units/mL.

Non-enzymatic antioxidant analysis

Total phenols

Total phenols were estimated using Folin-Ciocalteu reagent method[28], and expressed in mg/g of plant tissue.

Total antioxidant

Total antioxidant content in the test plant samples was estimated by total antioxidant reagent method using ascorbic acid as standard[29], and expressed in mg/g of plant tissue.

Ferric reducing antioxidant power (FRAP)

The FRAP activity is based on ability of the sample to reduce Fe^{+3} to Fe^{+2} ions in the presence of TPTZ, and was measured as described earlier by Benzie & Strain[30]. FRAP contents were expressed in mg/g of plant tissue.

Statistical analysis

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

Results

Constitutional biochemical contents

Total soluble protein

Total soluble protein content varied between 2.62 to 6.80 mg/g and 2.81 to 8.03 mg/g in the healthy and *C. partellus* damaged seedlings of test maize genotypes, respectively. There was significant variability in amount of total soluble protein in the healthy ($F = 205100$; df = 5,22; $P < 0.001$) and *C. partellus* damaged seedlings ($F = 20002$; df = 1,22; $P < 0.001$) of test maize genotypes (Table 1). Across genotypes, total soluble protein was significantly lower in healthy, and higher in *C. partellus* damaged maize seedlings (Fig. 1). Furthermore, genotype × treatment interaction for total soluble protein also differed significantly in the seedlings of test genotypes ($F = 2549$; df = 5,22; $P < 0.001$). The total soluble protein content both under healthy and

Table 1 — Amounts of various constitutional biochemical constituents in the seedlings of different maize genotypes under healthy and *Chilo partellus* damaged conditions

`Genotypes	Total soluble protein (mg/g)			Starch content (mg/g)			Total sugars (mg/g)		
	Damaged	Healthy	Change over healthy	Damaged	Healthy	Change over healthy	Damaged	Healthy	Change over healthy
CPM 13	6.54	6.25	+0.29	6.61	6.30	+0.31	16.23	14.66	+1.57
CPM 15	4.12	3.89	+0.23	5.60	4.86	+0.74	12.79	11.55	+1.24
CPM 18	3.96	3.48	+0.48	4.76	4.36	+0.40	11.63	9.84	+1.79
CPM 8	4.65	4.36	+0.29	5.86	5.63	+0.23	14.67	13.21	+1.46
Basi Local	8.03	6.80	+1.23	7.85	7.54	+0.31	17.79	15.16	+2.63
CML 345	2.81	2.62	+0.19	2.06	1.69	+0.37	3.96	2.33	+1.63
Mean	5.02	4.57	+0.45	5.46	5.06	+0.40	12.85	11.13	+1.72
LSD 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	0.07		<0.001	0.02		<0.001
Treatment (T)	0.01		<0.001	0.04		<0.001	0.01		<0.001
G × T	0.02		<0.001	0.10		<0.001	0.02		<0.001

[The + sign represent increase in the content of the biochemical parameter after damage by *C. partellus*]

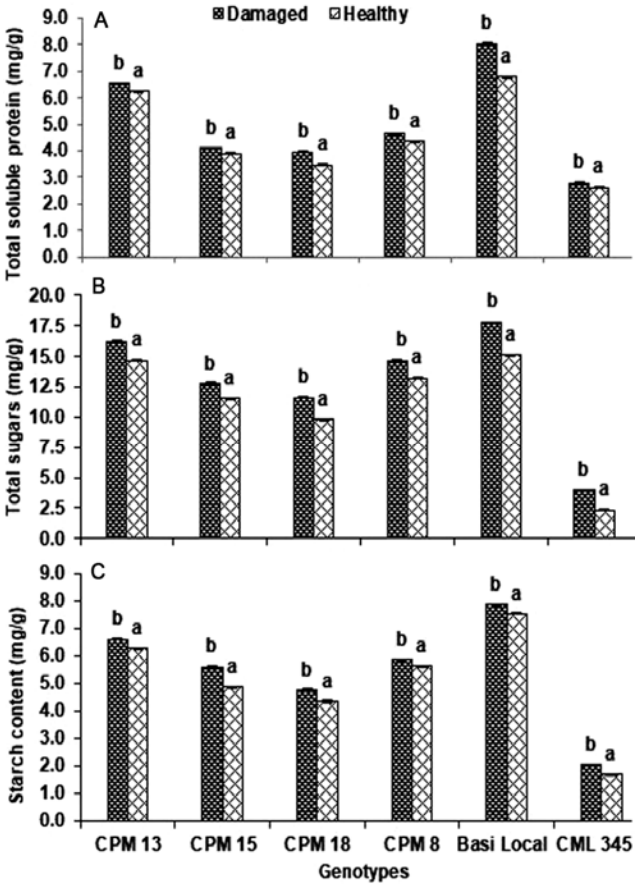

Fig. 1 — Amounts of various nutritional biochemical constituents (total soluble protein, starch content and total sugars) in healthy and *Chilo partellus* damaged seedlings of maize genotypes.

C. partellus damaged conditions was significantly higher in susceptible check, Basi Local and lower in resistant check, CML 345 as compared to other test maize genotypes (Table 1).

Total sugars

There were significant differences in amount of total sugar in the seedlings of test maize genotypes (F = 861500; df = 5,22; P <0.001) both under healthy and *C. partellus* damaged (F = 166300; df = 1,22; P <0.001) conditions. The genotype × treatment interaction was also significant for total sugar content in the seedlings of test maize genotypes (F = 2170; df = 5,22; P <0.001). The total sugar content was significantly higher in susceptible check, Basi Local and lower in resistant check, CML 345 as compared to other test maize genotypes, both under healthy and *C. partellus* damaged conditions (Table 1). Across maize genotypes, total sugar content was significantly lower in healthy than in *C. partellus* damaged seedlings (Fig. 1).

Starch content

The starch content varied between 1.69 to 7.54 mg/g in the healthy and 2.06 to 7.85 mg/g in *C. partellus* infested seedlings of test maize genotypes (Table 1). There were significant differences in starch content in the seedlings of test maize genotypes (F = 7174.16, df = 5,22, P <0.001) both under healthy and *C. partellus* damaged (F = 428.42, df = 1,22, P <0.001) conditions, and the genotype × treatment interaction was also significant (F = 14.93; df = 5,22; P <0.001). Starch content both under healthy and *C. partellus* damaged conditions was significantly higher in susceptible (Basi Local) and lower in resistant (CML 345) genotypes in comparison to other genotypes (Table 1). Across genotypes, starch content was significantly lower in healthy than in *C. partellus* damaged maize seedlings (Fig. 1).

Enzymatic antioxidants

Ascorbate peroxidase

There was significant variation in activity of APX in the seedlings of test maize genotypes (F = 1684.64; df = 5,22; P <0.001) both under healthy and *C. partellus* damaged (F = 313.13; df = 1,22; P <0.001) conditions. The APX activity was significantly higher in CPM 13, CPM 15, CPM 18 and CPM 8 as compared to susceptible genotype, Basi Local, while lower than in resistant genotype, CML 345, both under healthy and *C. partellus* damaged conditions (Table 2 and Fig. 2). The genotype × treatment interaction was also significant for APX activity in the seedlings of test maize genotypes (F = 5.98; df = 5,22; P <0.001).

Catalase

There were significant differences in CAT activity in the seedlings of test maize genotypes (F = 1936.50; df = 5,22; P <0.001) both under healthy and *C. partellus* damaged (F = 165.03; df = 1,22; P<0.001) conditions. However, genotype × treatment interaction for the CAT activity in test maize seedlings was non-significant (F = 0.93; df = 5,22; P = 0.484). Across genotypes, the CAT activity was significantly higher in *C. partellus* damaged than in healthy maize seedlings (Fig. 2). Among the genotypes, CAT activity was significantly higher in CPM 15 and CML 345 as compared to other test maize genotypes both under healthy and *C. partellus* damaged conditions (Table 2).

Phenyl ammonia lyase

There was significant variation in activity of PAL in the seedlings of test maize genotypes (F = 3163.26;

Table 2 — Activity of various enzymes in response to damage by *Chilo partellus* in different maize genotypes

Genotypes	Ascorbate peroxidase (U/mL)			Catalase (U/mL)			Phenyl ammonia lyase (U/mL)			Tyrosine ammonia lyase (U/mL)			Ascorbate oxidase (U/mL)		
	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
CPM 13	1494.0	1166.7	+327.3	38.1	25.7	+12.4	180.0	105.2	+74.8	182.0	149.3	+32.7	474.7	422.4	+52.3
CPM 15	1535.7	1321.4	+214.3	102.7	92.9	+9.8	169.6	119.3	+50.3	304.0	258.0	+46.0	617.5	537.6	+79.9
CPM 18	2595.2	2160.7	+434.5	85.4	70.9	+14.5	210.4	185.2	+25.2	374.0	322.0	+52.0	443.9	399.4	+44.5
CPM 8	1833.3	1494.0	+339.3	41.3	30.5	+10.8	214.1	136.3	+77.8	227.3	205.3	+22.0	447.0	394.8	+52.2
Basi Local	1006.0	577.4	+428.6	24.2	15.4	+8.8	141.5	64.4	+77.1	126.0	88.0	+38.0	391.7	247.3	+144.4
CML 345	4238.1	3631.0	+607.1	145.9	135.4	+10.5	634.8	531.1	+103.7	455.3	390.7	+64.6	892.5	843.3	+49.2
Mean	2117.1	1725.2	+391.9	72.9	61.8	+11.1	258.4	190.2	+68.2	278.1	235.6	+42.5	544.5	474.1	+70.4
LSD comparing	for 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)	79.50		<0.001	3.12		<0.001	9.31		<0.001	7.62		<0.001	20.48		<0.001
Treatment (T)	45.90		<0.001	1.80		<0.001	5.38		<0.001	4.40		<0.001	11.82		<0.001
G x T	112.50		<0.001	4.41		0.484	13.17		<0.001	10.77		<0.001	28.96		<0.001

[D, Damaged maize seedlings; H, Healthy maize seedlings. The + sign represent increase in the content of the biochemical parameter after damage by *C. partellus*]

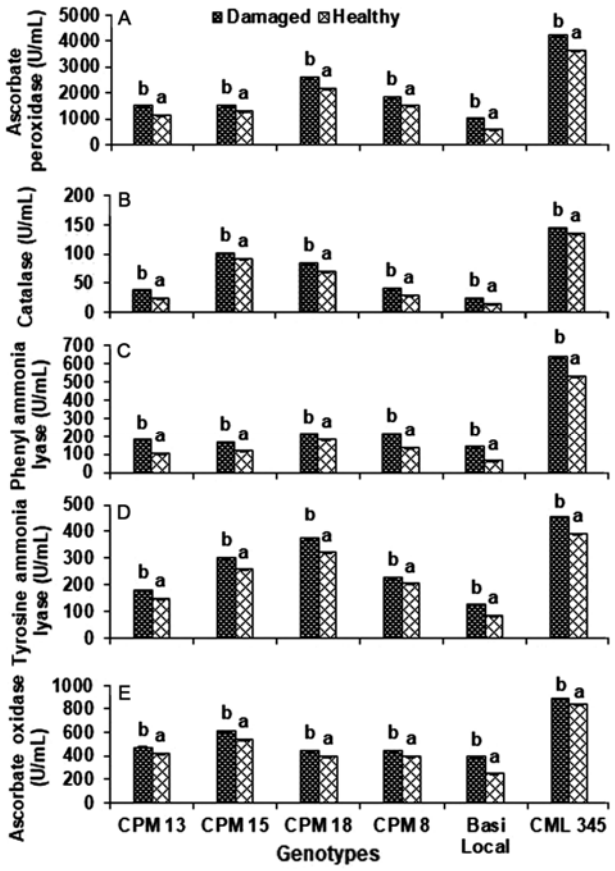

Fig. 2 — Amounts of various enzymes (ascorbate peroxidase, catalase, phenyl ammonia lyase, tyrosine ammonia lyase and ascorbate oxidase) in healthy and *Chilo partellus* damaged seedlings of maize genotypes.

df = 5,22; *P* <0.001) both under healthy and *C. partellus* damaged (F = 690.65; df = 1,22; *P* <0.001) conditions, and for genotype × treatment interaction (F = 18.06; df = 5,22; *P* <0.001). Across maize genotypes, the PAL activity was significantly higher in *C. partellus* damaged than in healthy maize seedlings, except CPM 18 (Table 2). Among the test genotypes, PAL activity was significantly higher in CPM 18 than in CPM 13, CPM 15 and CPM 8 both under healthy and *C. partellus* damaged conditions (Fig. 2).

Tyrosine ammonia lyase

There were significant differences in TAL activity in the seedlings of test maize genotypes (F = 2042.49; df = 5,22; *P* <0.001). However, the differences for TAL activity among healthy and *C. partellus* damage treatments (F = 402.59; df = 1,22; *P* = 0.24) and genotype × treatment interactions (F = 8.38; df = 5,22; *P* = 0.167) were non-significant (Table 2). Among test genotypes, the TAL activity was significantly lower in CPM 13 than in CPM 15, CPM 18 and CPM 8 both under healthy and *C. partellus* damaged conditions (Fig. 2).

Ascorbate oxidase

There were significant differences in activity of ascorbate oxidase enzyme in the seedlings of different maize genotypes (F = 772.94; df = 5,22; *P* <0.001) under healthy and *C. partellus* damaged (F = 152.47; df = 1,22; *P* <0.001) conditions, and for genotype × treatment interaction (F = 7.53; df = 5,22; *P* <0.001). Across genotypes, ascorbate oxidase activity was significantly lower in healthy than in *C. partellus* damaged maize seedlings. The ascorbate oxidase activity was significantly higher in CPM 13, CPM 15, CPM 18 and CPM 8 than in susceptible (Basi Local),

while lower than in resistant (CML 345) genotypes, both under healthy and *C. partellus* damaged conditions (Table 2).

Overall, the activity of enzymatic antioxidants *viz.*, APX, AO, CAT, PAL and TAL was significantly higher in resistant (CML 345) and lower in susceptible (Basi Local) checks in comparison to other test genotypes, both under healthy and *C. partellus* damaged conditions (Table 2). Further, the incremental increase in the activity of APX, PAL and TAL in response to damage to by *C. partellus* was higher in resistant, CML 345, while increase in AO was higher in susceptible, Basi Local checks as compared to other genotypes.

Non-enzymatic antioxidants

Total phenol

The total phenol content varied significantly from 0.75 to 7.09 mg/g in healthy (F = 100300; df = 5,22; P <0.001) and 1.48 to 4.05 mg/g in the *C. partellus* damaged seedlings (F = 8959.91; df = 1,22; P <0.001) (Table 3). Total phenol content was significantly higher in *C. partellus* damaged as compared to healthy maize seedlings (Fig. 3). Furthermore, the genotype × treatment interaction was also found significant for total phenol content in the seedlings of test maize genotypes (F = 588.6; df = 5,22; P <0.001).

Total antioxidant

The total antioxidant content varied from to 2.51 to 11.67 mg/g in healthy and 3.32 to 14.55 mg/g in *C. partellus* damaged seedlings of test maize genotypes (Table 3). There was significant variability in amount of total antioxidant in the seedlings of test maize genotypes (F = 164600; df = 5,22; P <0.001) both under healthy and *C. partellus* damaged (F = 31605.3; df = 1,22; P <0.001) conditions. The genotype × treatment interaction was also significant for total antioxidant content in the seedlings of test maize genotypes (F = 2187; df = 5,22; P <0.001). Across genotypes, the total antioxidant content was significantly higher in *C. partellus* damaged than in healthy maize seedlings (Fig. 3).

Ferric reducing antioxidant power.

The FRAP content varied from to 0.51 to 1.65 mg/g in healthy and 0.68 to 2.07 mg/g in *C. partellus*

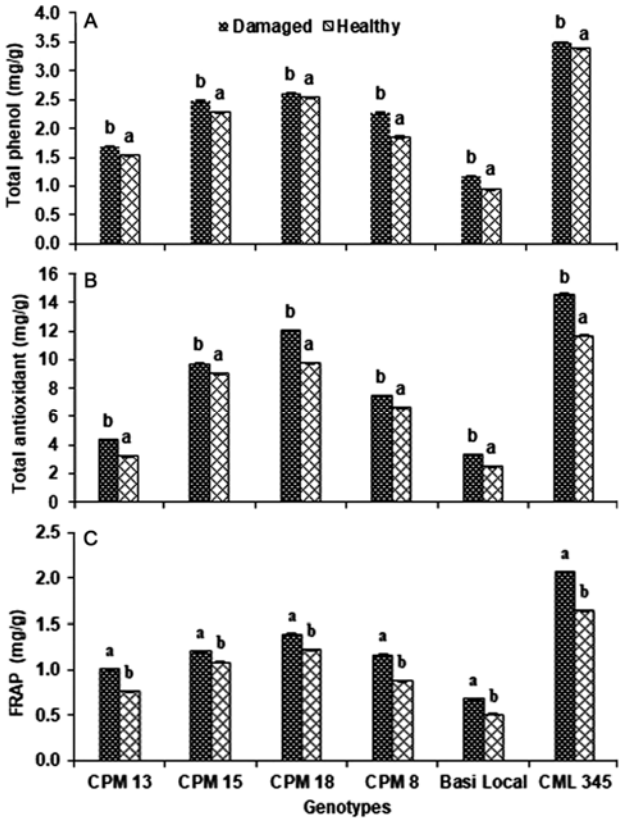

Fig. 3 — Amounts of total phenols, total antioxidants and FRAP in healthy and *Chilo partellus* damaged seedlings of maize genotypes.

Table 3 — Expression of various nonenzymatic antioxidants in response to damage by *Chilo partellus* in different maize genotypes

Genotypes	Total phenol (mg/g)			Total antioxidant (mg/g)			FRAP (mg/g)		
	Damaged	Healthy	Change over healthy	Damaged	Healthy	Change over healthy	Damaged	Healthy	Change over healthy
CPM 13	1.69	1.53	+0.16	4.36	3.24	+1.12	1.01	0.77	+0.24
CPM 15	2.48	2.28	+0.20	9.72	9.05	+0.67	1.20	1.08	+0.12
CPM 18	2.62	2.53	+0.09	12.05	9.75	+2.30	1.39	1.22	+0.17
CPM 8	2.28	1.84	+0.44	7.49	6.64	+0.85	1.17	0.88	+0.29
Basi Local	1.18	0.96	+0.22	3.32	2.51	+0.81	0.68	0.51	+0.17
CML 345	3.50	3.40	+0.10	14.56	11.67	+2.89	2.07	1.65	+0.42
Mean	2.29	2.09	+0.20	8.58	7.14	+1.44	1.25	1.02	+0.23
LSD for comparing	LSD (P = 0.05)		P-value	LSD (P = 0.05)		P-value	LSD (P = 0.05)		P-value
Genotype (G)	0.008		<0.001	0.03		<0.001	0.002		<0.001
Treatment (T)	0.004		<0.001	0.02		<0.001	0.001		<0.001
G × T	0.011		<0.001	0.04		<0.001	0.003		<0.001

[The + sign represent increase in the content of the biochemical parameter, respectively after damage by *C. partellus*]

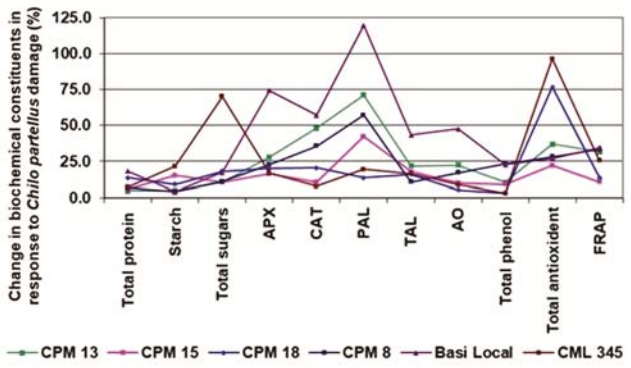

Fig. 4 — Changes in biochemical constituents and antioxidant enzymes in seedlings of different maize genotypes in response to damage by *Chilo partellus*.

damaged seedlings of test maize genotypes (Table 3). There was significant variability in content of FRAP in the seedlings of different maize genotypes ($F = 271700$; df = 5,22; $P < 0.001$) both under healthy and *C. partellus* damaged ($F = 122900$; df = 1,22; $P < 0.001$) conditions. The genotype × treatment interaction was also significant for FRAP content in the seedlings of test maize genotypes ($F = 444.8$; df = 5,22; $P < 0.001$). Across genotypes, FRAP content was significantly higher in *C. partellus* damaged than in healthy maize seedlings (Fig. 3).

All genotypes showed increase in antioxidant enzymatic activity after induction of stress by *C. partellus*. However, CML 345 maintained higher CAT, APX, AO, PAL and TAL activity, while Basi Local (57.1%) showed remarkable increase in enzymatic activity under stress (Fig. 4).

Discussion

Plants in response to herbivory realize significant shift in oxidative status due to increased production of ROS, the major regulatory signaling molecules in plants[31]. This increased ROS activate the antioxidative enzymes to further increase the levels of primary compounds and secondary metabolites to induce resistance against insect damage[32,33]. Similar trend in increase in amounts of total soluble protein, starch and sugars was also recorded in the test maize seedlings in response to damage by *C. partellus* in the present study. However, the percent increase in protein content was highly variable among the genotypes. Percent increase in protein was the highest in Basi Local (susceptible genotype) suggesting upregulation of antioxidant defense genes to fight the stress induced by *C. partellus*. This increase in total protein content in response to *C. partellus* damage could be due to increased proportion of antinutritional proteins and activation of defense enzymes. The increased production of starch and sugars also help the plant to sustain herbivory stress by providing energy to the plant to activate secondary defense machinery. Accumulation of ROS in response to stress is associated with soluble sugar accumulation, which is considered to be an adaptive response to stress conditions[34]. Although there was increase in amount of total sugars across maize genotypes in response to damage by *C. partellus*, the highest percent increase was recorded in susceptible check Basi Local, which could be due to genetic makeup of this genotype.

Enhanced activities of antioxidant enzymes, such as catalase, non-specific peroxidase and superoxide dismutase are frequently observed as response to ROS generation[35]. The increase in levels of CAT activity is known to act as local signal to activate defense genes and involve in increasing the cell wall resistance[36]. Furthermore, CAT and APX are found to be notably most distinguished enzymes in abiotic stress conditions since the former mainly occurs in peroxisomes and does not require a reductant for catalyzing dismutation reaction. The higher activity of APX has also been found to help in defending the host plants from biotic stress as this enzyme belongs to the detoxification mechanism of peroxide[37]. However, present studies showed a significant increase in levels of AO and APX activity in susceptible genotype (Basi Local) under *C. partellus* stress conditions, suggesting that these two antioxidant enzymes also play role in defending the plants from biotic stresses.

Nonenzymatic antioxidants under study were found to follow similar trend of increase in their concentration in resistant (CML 345) as compared to susceptible (Basi Local) genotype under healthy and stressed conditions. Percent change in total phenol content in response to damage was higher in susceptible (22.1 %) than resistant (3.1 %) genotype, suggesting that susceptible genotype face more physical damage which leads to accumulation of more phenol (Fig. 4). Phenols play an important role in cyclic reduction of ROS such as superoxide anion and hydroxide radicals, H_2O_2 and singlet oxygen, which in turn activate a cascade of reactions leading to activation of defense enzymes[38,39]. FRAP content change was also more in response to *C. partellus* damage in susceptible (33.4%), while less increase in

resistant (25.6%) genotype. Total antioxidant content increase was highest in resistant genotype CML 345 (96.0%) after *C. partellus* damage (Fig. 4). These findings indicate that resistant plants produce high titer of other nonenzymatic antioxidants like ascorbic acid, tocopherol and glutathione, which further increase upon exposure to biotic stress.

PAL is the entry-point enzyme into the phenylpropanoid pathway responsible for the synthesis of plant phenylpropanoids or phenolics, many of which play important role in plant defense under stress conditions[9]. Increase in PAL activity in response to insect damage leads to oxidation of phenolics to quinone through shikimic acid pathway and enhanced production of quinones cause toxicity to herbivores[40-42]. Present study recorded increased activity of PAL and TAL in test maize genotypes, which further increased in response to damage by *C. partellus*. Although highest activity was observed in resistant genotype (CML 345) both under healthy and damaged conditions, percent increase in PAL (119.7%) and TAL (43.2%) activity was the highest in susceptible genotype (Basi Local) in response to damage by *C. partellus*. Earlier studies have also reported increased CAT and PAL activity in host plants in response to feeding by insect pests[32,42,43]. The higher increase in PAL activity in resistant genotypes under damage conditions as compared to healthy counterparts might be responsible for lower leaf damage, as PAL catalyses the elimination of amino-group of L-phenylalanine to generate trans-cinnamate, and by the action of cinnamate-4-hydroxylase (C4H), p-coumaric acid is produced that further protects the host plant from secondary infection[44]. The p-coumaric acid has also been reported negatively associated with pupal period of *C. partellus* in maize[14].

The present study demonstrated furthermore increase in activity of defense enzymes in susceptible genotype (Basi Local). Higher levels of antioxidant activity decrease the availability of ascorbate in plant tissues, which in turn increase the oxidative stress leading to reduced insect growth and development[45,46]. The phenolic compounds possess wide range of functions, like structural support, pigmentation, signaling and defense against abiotic and biotic stresses in plants[47,48], thus increase in phenolic compounds in response to herbivory is a common defense phenomenon[46,49]. The further increase in levels of nonenzymatic antioxidants in response to *C. partellus* damage in the present studies could be due to generation of high free radicals and production of oxidative molecules. Involvement of polyphenols in constitutive defense of roots of grapevine, *Vitis* spp. in response to attack by grape phylloxera, *Daktulosphaira vitifoliae* Fitch has also been demonstrated recently[50]. Present studies also revealed that the nonenzymatic antioxidant scavenging activity of FRAP was significantly higher in resistant maize genotypes, which in response to damage by *C. partellus* larvae further increased over the healthy plants. The higher concentration of antioxidative enzymes, total antioxidant, FRAP activity and phenols in resistant than susceptible genotypes in response to *C. partellus* might be due to their differential ability to acclimate and induce antioxidant enzymes and nonenzymatic antioxidants in the form of secondary metabolites due to variation in their genetic makeup.

Conclusion

The results have shown that the amounts of constitutional biochemical compounds were significantly higher in susceptible than resistant maize genotypes both under damaged and healthy plant conditions. However, the activity of enzymatic and nonenzymatic antioxidants were significantly higher in resistant maize genotypes, which further increased upon damage by *C. partellus*. These studies, thus conclude that both constitutive and induced biochemical compounds through activation of enzymatic and nonenzymatic antioxidant defense systems impart resistance against *C. partellus* in CPM 8, CPM 13, CPM 15, CPM 18 and CML 345, which could be used in insect resistance breeding program. These findings could also be useful for detailed understanding on metabolic pathways regulating biochemical defense and up- and down-regulation of associated genes in plant defense against biotic stresses.

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Conflict of interest

The authors declare no conflict of interest

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