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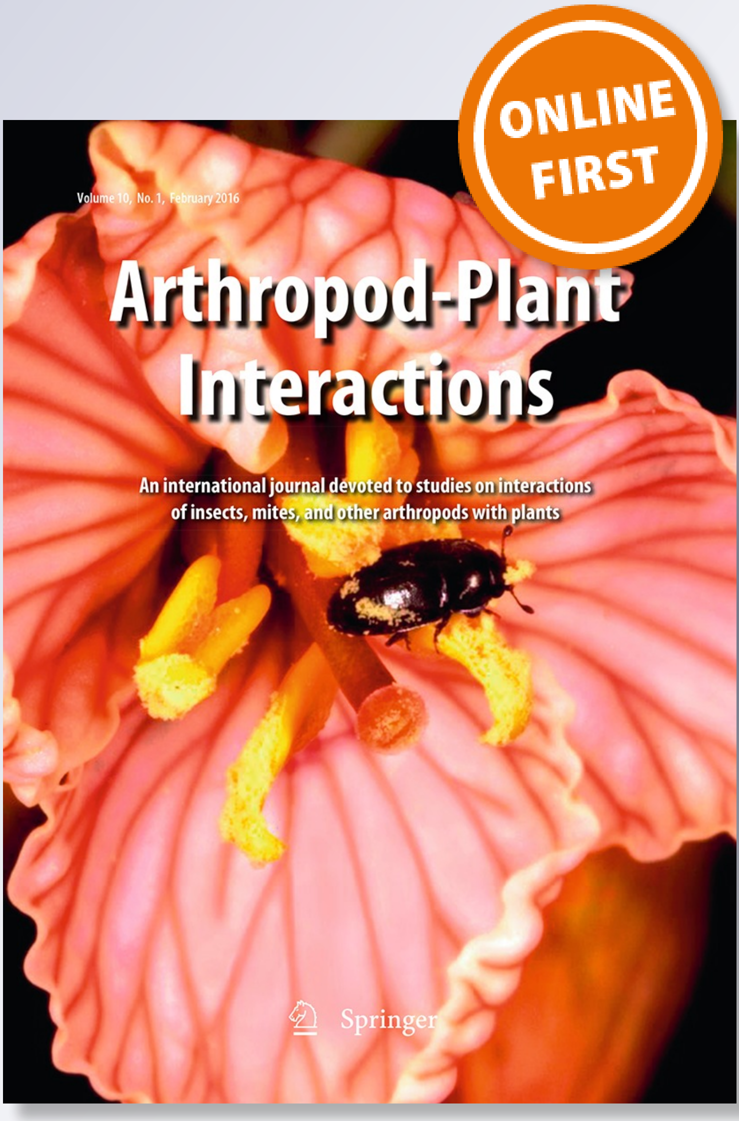

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ORIGINAL PAPER

Elevated CO_2 influences host plant defense response in chickpea against *Helicoverpa armigera*

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Abstract Global atmospheric concentration of CO_2 is likely to increase from 350 to 750 ppm over the next 100 years. The present studies were undertaken to understand the effects of elevated CO_2 on enzymatic activity and secondary metabolites in chickpea in relation to expression of resistance to pod borer, *Helicoverpa armigera*. Fifteen-day-old chickpea plants [ICCL 86111—resistant and JG 11—commercial cultivar] grown in the greenhouse were transferred to open-top chambers (OTC) and kept under 350, 550 and 750 ppm of CO_2. Twenty neonates of *H. armigera* were released on each plant at 7 days after shifting the pots to the OTCs. Un-infested plants were maintained as controls. After 7 days of infestation, the activities of defensive enzymes [peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL)] and amounts of total phenols and condensed tannins increased with an increase in CO_2 concentration in chickpea. The nitrogen balance index was greater in plants kept at 350 ppm CO_2 than in plants kept under ambient conditions. The *H. armigera*-infested plants had higher H_2O_2 content; amounts of oxalic and malic acids were greater at 750 ppm CO_2 than at 350 ppm CO_2. Plant damage was greater at 350 ppm than at 550 and 750 ppm CO_2. This information will be useful for understanding effects of increased levels of CO_2 on expression of resistance to insect pests to develop strategies to mitigate the effects of climate change.

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Keywords Climate change · Elevated CO_2 · Secondary metabolites · Plant defense · Chickpea · *Helicoverpa armigera*

Introduction

Elevated atmospheric carbon dioxide (CO_2) profoundly influences the primary productivity by affecting plant growth, physiology, primary and secondary metabolism, photosynthesis, resource allocation and gene regulation (Lindroth 2010; Robinson et al. 2012). The changes in biochemical and morphological traits related to plant defense have a major bearing on expression of host plant resistance to insect pests (Robinson et al. 2012; War et al. 2012, 2013). Further, climatic factors including elevated CO_2 also affect crop production by inducing changes in distribution and abundance of the herbivores and their natural enemies (Sharma 2014). Over the past 250 years, CO_2 concentration has increased from 280 to 390 ppm (Intergovernmental Panel on Climate Change 2007) and has been predicted to increase up to 750 ppm by the end of this century (Falkowski et al. 2000). The CO_2 levels in the environment determine the rate of photosynthesis, which influences both physiology and the biochemical composition of the plants, carbon (C)/ nitrogen (N) ratio and allocation of resources to different plant parts (Long et al. 2004). Increased CO_2 will increase the rate of photosynthesis and water use efficiency of the plants. However, the increase in crop yield might not be in proportion to the increase in CO_2, because increased vegetative growth may not result in increased grain yields, and altered plant–insect dynamics will affect crop production (DeLucia et al. 2012). The reduction in N and increase in C:N ratio in response to elevated CO_2 will alter the amounts of secondary metabolites such as flavonoids, alkaloids and terpenoids (Sun et al. 2013), which will affect the nutritional quality of the host plants and, thus, their palatability to insect herbivores (Bhonwong et al. 2009). Furthermore, N is the limiting component in insect's food, and reduced amounts of N will impair the nutritional quality of the food for the herbivores (Guo et al. 2014). To compensate for reduced N under elevated CO_2, the insects will consume more foliage and, thus, result in increased damage to crop plants (Bezemer and Jones 1998).

Elevated CO_2 will also change physiological responses of the host plant that will alter the activity of oxidative enzymes such as peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), superoxide dismutase and catalase (Badiani et al. 1993; Polle et al. 1997).

Most of the studies on the effects of elevated CO_2 have focused on biochemical composition of plants, and very few studies have been carried out about the effects of elevated CO_2 on insect–host plant interactions (Rao et al. 2006; Zavala et al. 2013). An understanding of these interactions is important in predicting the effects of climate change on expression and stability of host plant resistance to insect pests. Therefore, we studied the effects of elevated CO_2 on secondary metabolites and enzymatic activity in chickpea, C*icer arietinum* (L.), and their counter effects on survival and growth of the pod borer, *Helicoverpa armigera* (Hubner), which is the most important constraint to increased crop production in the semiarid tropics.

Materials and methods

Open-top chambers (OTCs)

The experimental site is located at the Centre of Excellence, Climate Change Research on Plant Protection (CoE, CCRPP) facility ICRISAT (17°30′N and 78°16′E and 1881 feet), Patancheru, Hyderabad, India. It consisted of three open-top chambers (OTC), each of 64 m^3 (4 × 4 × 4 m). The OTC frame was made of iron rods and covered with transparent Plexiglas (polyvinyl chloride). The top of the chamber (frustum) was kept open to allow free air exchange to reduce the temperature and humidity effects in the chamber. One OTC was kept under ambient conditions (without external supply of CO_2), while the other two OTCs were maintained at 550 and 750 ppm of CO_2 by connecting these to CO_2 cylinders. The plenum at the base (0.3 m) was provided for circulation of carbon dioxide within the OTCs. CO_2 gas of commercial grade (100 %) was used to enrich CO_2 within the chambers. The CO_2 was distributed from the cylinders to the OTCs by polyurethane tubing and regulated through solenoid valves to ensure uniform distribution of CO_2 within the chamber. The actual concentration of carbon dioxide within the OTCs was monitored by a portable CO_2 analyzer and controlled by computer-supported regulation of inlet valves (Uprety et al. 2006; Vanaja et al. 2006). A CO_2 analyzer was also placed in the open area outside the OTCs for comparing the CO_2 levels inside and outside the OTCs. Each chamber was also fitted with sensors to measure temperature and relative humidity continuously. Data acquisition system was used to manage CO_2 concentrations and data collection (Genesis Technologies, Mumbai, India).

Chickpea plants

Two chickpea genotypes (ICCL 86111—resistant and JG 11—commercial cultivar) were grown under greenhouse conditions (27 ± 5 °C and 65–95 % RH). The plants were raised in plastic pots (30 cm diameter and 40 cm deep) containing a mixture of loam soil, sand and farmyard manure (2:1:1). Five seedlings were retained in each pot at 5 days after seedling emergence. Fifteen days after germination, plants were transferred to three OTCs maintained at three different concentrations of CO_2 (350, 550 and 750 ppm) and an open field of the same area (under ambient conditions, 380 ppm CO_2). Each chamber had 27 pots of each chickpea genotype. A set of pots (nine) were infested to study survival and development of insect larvae, and a set of pots (nine) were infested to perform biochemical analysis of insect-infested plants, and another set (nine) was remained un-infested, considering three pots as one replication, and all the replications were kept within one OTC. Similar scheme was followed for other two OTCs and in the open area outside the OTCs.

H. armigera infestation

Newly emerged larvae of *H. armigera* were obtained from the stock culture maintained on artificial diet under laboratory conditions (26 ± 1 °C, 15 h photoperiod and 75 ± 5 % relative humidity) at ICRISAT (Chitti Babu et al. 2014). Twenty larvae were released on each plant at 7 days after transferring the plants to the OTCs by using a camel-hair brush. Un-infested plants were maintained as a control.

Enzyme extraction

Seven days after infestation, chickpea leaves were collected on ice packs and immediately kept at −80 °C till use. Leaf tissue (0.1 g) was grounded using a pestle and mortar in 1.5 ml of ice-cold 0.1 M sodium phosphate buffer (pH 7.5) containing 5 mM 2-mercaptoethanol, 1 % polyvinylpyrrolidone (PVP), 1 mM DTT and 0.5 mM

EDTA. The homogenate was centrifuged at 14,000 rpm for 20 min, and the supernatant was used as an enzyme source.

Estimation of enzyme activities

POD and PPO activity was estimated in fresh leaves as per the method of Shannon et al. (1966) and Mayer and Harel (1979), respectively. The PAL and TAL activity was estimated as described by Campos-Vergas and Saltveit (2002) and Khan et al. (2003), respectively. All the enzyme activities were expressed as OD/min/g FW.

Estimation of secondary metabolites

The amounts of total phenols were determined according to Zieslin and Ben-Zaken (1993) using the standard curve prepared with gallic acid and expressed as µg gallic acid equivalents g^{-1} FW (µgGAEg^{-1} FW) (Zieslin and Ben-Zaken 1993). The condensed tannin content was estimated using the vanillin–hydrochloride method and expressed as µg catechin equivalents g^{-1} FW (µgCEg^{-1} FW) (Robert 1971).

H_2O_2 content

H_2O_2 concentration was determined in fresh leaf tissue according to Noreen and Ashraf (2009) and expressed as µmol g^{-1} FW.

Nutritional components

Carbohydrate content was estimated by following the anthrone reagent method using glucose as a standard (Sadasivam and Manickam 1996). The total protein content was estimated by Lowry's method (Lowry et al. 1951) using bovine serum albumin as a standard.

Chlorophyll content and nitrogen basal index (NBI)

Amounts of chlorophyll and NBI were recorded using phenol meter (Force A Paris, France). Chlorophyll content was expressed as µg cm^{-2}. Fully expanded leaf was placed between the two parts of the phenol meter, and ten observations were recorded from each plant and three plants for each treatment.

Estimation of organic acids by HPLC

HPLC system of Waters Series having separation module (2695) equipped with photodiode array detector (2996) and an Atlantis dC_{18} column (4.6 × 250 mm; Atlantis, Ireland). Water washings of the chickpea leaf surface extracts were filtered through a polyvinyl difluoride filter (PVDF; Millipore, Millex-GV, filter 0.22 µm dia.). The samples were eluted using isocratic solvent system at a flow rate of 0.8 ml/min, run time was 20 min, and the compounds were detected at 210 nm with 20 µl injected volume of the extract. The mobile phase was 25 mM potassium phosphate prepared in MilliQ water and pH adjusted to 2.5 by orthophosphoric acid. Standard samples of known organic acids (oxalic, malic, citric, fumaric and acetic acids) were used to spike the HPLC peaks to identify different acids. After identification of peaks corresponding to different organic acids, a range of concentrations for each organic acid were run through the HPLC to obtain a normal curve. The amounts of different organic acids present in the leaves of chickpea genotypes were estimated from normal curves based on peak areas.

Larval survival and larval weights

Seven days after infestation, the plants were visually rated for injury by *H. armigera* on a scale of 1 to 9, where $1 \leq 10\ \%$ damage and $9 \geq 80\ \%$ damage (Sharma et al. 2003). The larvae were collected after 7 days of infestation from the plants, and data were recorded on larval survival and larval weights.

Statistical analysis

The data were subjected to analysis of variance, and correlations between leaf injury and larval weights with enzyme activity and secondary metabolites were computed by using SPSS (11.5 ver.). The significance of differences between the treatments was judged by Tukey's "*t*" test and the Dunnett's *T* test.

Results

Effect of CO_2 on the activity of POD and PPO

Chickpea plants kept at 750 ppm CO_2 had significantly greater POD activity [ICCL 86111 (3.1) and JG 11 (2.1) OD/min/gFW] than the plants at 350 ppm CO_2 [ICCl 86111 (2.0) and JG 11 (1.2) OD/min/gFW)] ($F_{(3,11)} = 5.9$, $p = 0.041$) (Fig. 1a, b). Higher PPO activity was observed at 750 ppm [ICCL 86111 (0.3) and JG 11 (0.19), OD/min/gFW], than at 350 ppm CO_2 [ICCL 86111 (0.18) and JG 11 (0.11), OD/min/gFW), $F_{(3,11)} = 3.8$, $p = 0.035$] (Fig. 2a, b). The insect-infested plants had significantly greater POD and PPO activities [ICCL 86111 (3.8 and 0.31 OD/min/gFW) and JG11 (2.5 and 0.22 OD/min/gFW)] than the uninfested control plants [ICCL 86111 (3.1 and 0.3 OD/min/gFW) and JG11 (2.1 and 0.19 OD/min/gFW), respectively] across the CO_2 regimes. However, the POD and PPO

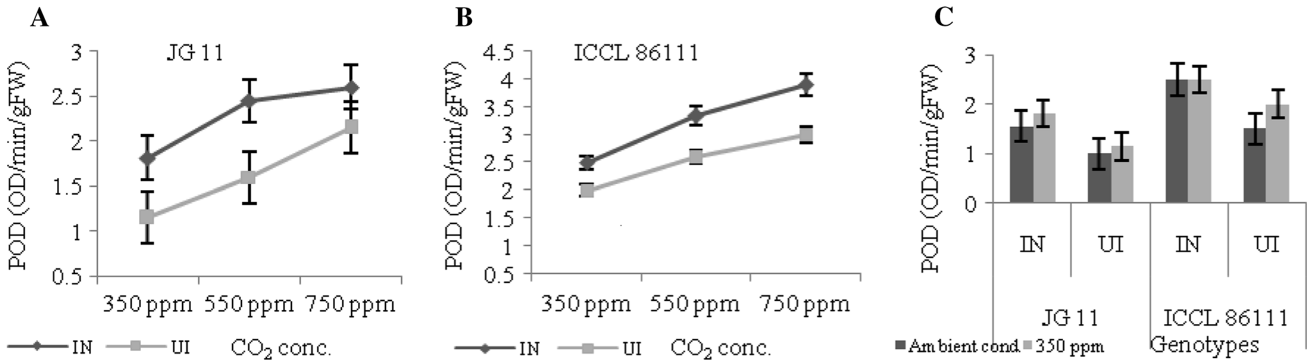

Fig. 1 POD activity of *H. armigera*-infested and un-infested chickpea plants raised under different CO_2 levels [A = JG 11 (**a**), ICCL 8611 (**b**)] and under ambient conditions and at 350 ppm CO_2 (**c**)

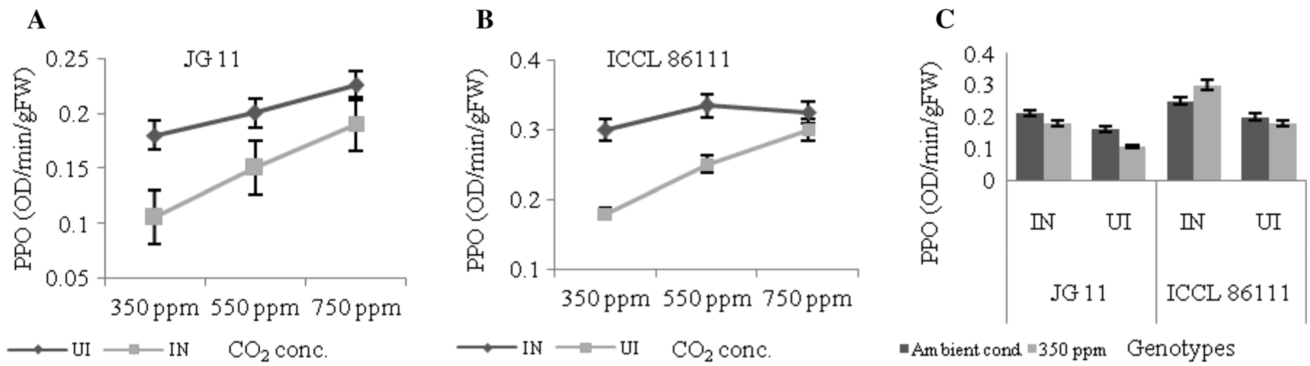

Fig. 2 PPO activity of *H. armigera*-infested and un-infested chickpea plants at different CO_2 levels [JG 11 (**a**), ICCL 8611 (**b**)] and under ambient conditions and at 350 ppm CO_2 (**c**)

activities did not differ significantly between the plants under ambient conditions and the plants kept at 350 ppm CO_2 in the OTC [POD ($F_{(1,5)} = 2.4$ and 2.9, $p = 0.174$), PPO ($F_{(1,5)} = 4.7$ and 3.5, $p = 0.174$), for ICCL 86111 and JG 11, respectively, $p = 0.083$] (Figs. 1c, 2c).

Effect of CO_2 on activity of PAL and TAL

The mean PAL activity of insect-infested plants of both genotypes was significantly greater than that of un-infested plants [ICCL 86111 (4.54 and 3.76 OD/min/gFW, respectively, $F_{(3,11)} = 25.7$, $p = 0.001$) and JG 11 (3.93 and 3.27 OD/min/gFW), respectively, $F_{(3,11)} = 18.8$, $p = 0.009$] (Fig. 3a, b). No significant differences were observed between infested and un-infested control plants under ambient conditions and at 350 ppm inside OTC in both genotypes; however, ICCL 86111 had comparatively greater PAL activity than JG 11 (Fig. 3c).

There were no significant differences in TAL activity across CO_2 regimes in both genotypes ($F_{(3,11)} = 3.2$ and 2.9 for ICCL 86111 and JG 11, respectively, $p = 0.066$) (Fig. 4a, b). However, a marginal increase in TAL activity was recorded in plants kept under 550 ppm CO_2 than those under 350 and 750 ppm. The JG 11 plants kept at 350 ppm CO_2 showed greater TAL activity than the plants kept under ambient conditions (2.5 and 3.2 OD/min/gFW, respectively). However, in ICCL 86111, no significant differences were observed in TAL activity between plants under ambient conditions and 350 ppm CO_2 ($F_{(1,5)} = 5.1$, $p = 0.096$) (Fig. 4c).

Effect of CO_2 on phenols and tannins

The mean phenol content of the insect-infested plants was significantly greater than the un-infested plants in both the genotypes across the CO_2 regimes [ICCL 86111 (18.83 and 16.35 mg TAE/gFW), ($F_{(3,11)} = 10.8$, $p = 0.028$), JG 11 (15.82 and 14.32 mg TAE/gFW, ($F_{(3,11)} = 7.3$, $p = 0.031$), in infested and un-infested plants, respectively] (Table 1). The tannin content of ICCL 86111 insect-infested plants was significantly greater (12.60 mg CE/gFW) than the corresponding un-infested plants (10.40 CE/gFW) at 550 ppm (Table 1). In JG 11, insect-infested plants had significantly greater tannin content at 550 and 750 ppm (11.82 and 11.23 CE/gFW, respectively) than the

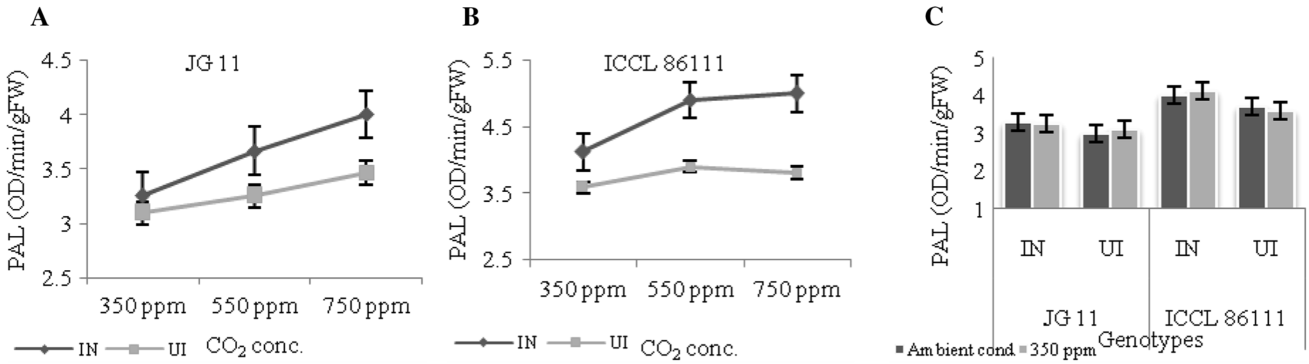

Fig. 3 PAL activity of *H. armigera*-infested and un-infested chickpea plants at different CO_2 levels, JG 11 (**a**), ICCL 8611 (**b**), under ambient condition and at 350 ppm CO_2 (**c**)

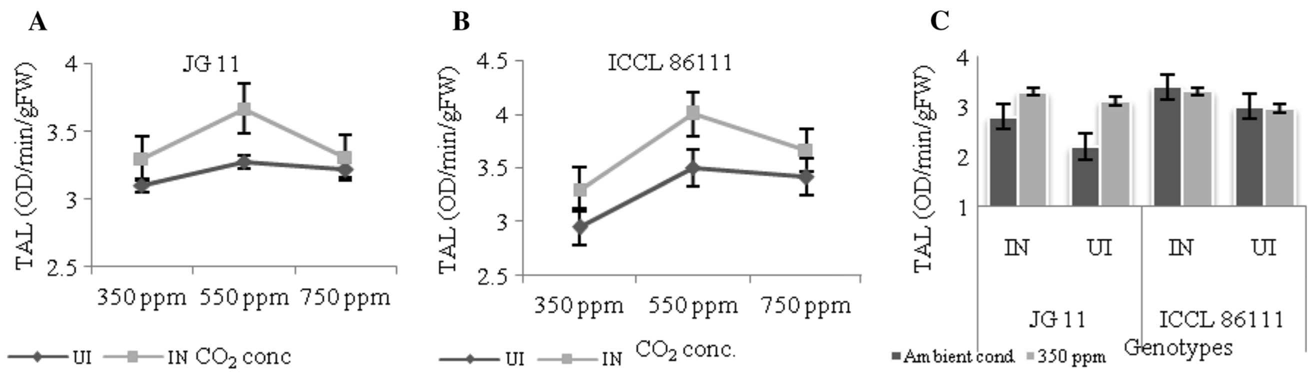

Fig. 4 TAL activity of *H. armigera*-infested and un-infested chickpea plants at different CO_2 levels [JG 11 (**a**), ICCL 8611 (**b**)] and under ambient conditions and at 350 ppm CO_2 (**c**)

corresponding un-infested plants (10.40 and 9.78 CE/gFW, respectively).

Effect of CO_2 on NBI and chlorophyll content

The NBI generally decreased with an increase in CO_2 levels. The insect-infested plants generally had significantly lower NBI as compared to the un-infested control plants ($p \leq 0.01$) (Table 1). The mean NBI of ICCL 86111 was significantly greater (32.94 and 20.43) than JG 11(25.08 and 18.18), respectively, for un-infested and infested plants. Plants kept at 350 ppm CO_2 in the OTC had significantly greater NBI in both genotypes [ICCL 86111 (45.47 and 28.35) and JG11 (40.72 and 33.05)], than those kept under ambient conditions [ICCL 86111 (30.95 and 16.45) and JG 11 (19.9 and 23.6)], in un-infested and insect-infested plants, respectively ($F_{(3,11)} = 23.9$ and 38.1, respectively, for ICCL 86111 and JG 11, $p = 0.001$).

The chlorophyll content of plants grown at 350 ppm CO_2 and under ambient conditions was significantly greater than those at higher levels of CO_2 (Table 1). Both the genotypes showed significant differences in chlorophyll content between the insect-infested and control plants across CO_2 levels. ICCL 86111 insect-infested plants had relatively lower chlorophyll content at 550 and 750 ppm of CO_2 (23.3 and 21.65 µg cm^{-2}, respectively) than in plants kept at 350 ppm of CO_2 (28.15 µg cm^{-2}).

Effect of CO_2 on H_2O_2

The *H. armigera*-infested plants had significantly greater levels of H_2O_2 as compared to the un-infested control plants at 350 and 550 ppm CO_2 in both genotypes (Fig. 5a, b). At 350 ppm CO_2, the insect-infested plants of JG 11 had greater H_2O_2 content (17.5 µmol g^{-1} FW) than ICCL 86111 (13.7 µmol g^{-1} FW). However, at 750 ppm of CO_2, there were no significant differences in H_2O_2 content between the infested and un-infested control plants in both genotypes ($F_{(1,5)} = 4.5$ and 3.4 for ICCL 86111 and JG 11, respectively, $p = 0.38$). As compared to the plants kept at 350 ppm, plants under ambient conditions had significantly lower H_2O_2 content [ambient (21.04 and 20.25 µmol g^{-1} FW) and 350 ppm (16.46 and 12.0 µmol g^{-1} FW), respectively, in JG 11 and ICCL 86111] (Fig. 5c).

Table 1 Amounts of phenols (mg TAE/g FW), tannins (mg CE/g FW), NBI and chlorophyll content (μg cm^{-2}) of chickpea plants infested with *Helicoverpa armigera* under different CO_2 regimes

CO_2 (ppm)	ICCL 86111		JG 11	
	Un-infested	Infested	Un-infested	Infested
Total phenols				
350 ppm	15.8 ± 0.05[a]	17.85 ± 0.08[a]	12.67 ± 0.07[a]	13.84 ± 0.12[a]
550 ppm	16.2 ± 0.71[a]	19.31 ± 0.07[a]*	14.47 ± 0.10[ab]	15.92 ± 0.14[ab]
750 ppm	16.9 ± 0.27[a]	19.70 ± 0.08[a]*	14.78 ± 0.20[ab]	16.03 ± 0.14[ab]
Ambient conditions	16.5 ± 0.67[a]	18.49 ± 0.67[a]	15.35 ± 0.45[b]	17.50 ± 0.91[b]
Mean	16.35	18.83	14.32	15.82
Tannins				
350 ppm	9.41 ± 0.15[a]	11.85 ± 0.48[a]	9.41 ± 0.98[a]	10.60 ± 0.52[a]
550 ppm	10.40 ± 0.17[a]	12.60 ± 0.74[a]*	10.40 ± 0.30[a]	11.82 ± 0.74[a]*
750 ppm	10.47 ± 0.99[a]	11.70 ± 0.58[a]	9.78 ± 0.29[a]	11.23 ± 0.84[a]*
Ambient conditions	9.90 ± 0.97[a]	12.11 ± 0.92[a]*	8.4 ± 0.34[a]	10.2 ± 0.79[a]
Mean	10.05	12.07	9.49	10.96
NBI				
350 ppm	45.47 ± 2.03[c]	28.35 ± 1.38[b]*	40.72 ± 3.17[d]	30.05 ± 1.22[c]*
550 ppm	29.52 ± 1.07[ab]	18.05 ± 1.80[a]*	28.95 ± 1.20[b]	16.07 ± 1.10[a]*
750 ppm	25.85 ± 1.51[a]	18.87 ± 1.28[a]*	10.77 ± 0.99[a]	10.0 ± 1.14[a]*
Ambient conditions	30.95 ± 2.21[b]	16.45 ± 1.05[a]*	19.9 ± 1.35[b]	16.6 ± 1.29[b]*
Mean	32.94	20.43	25.08	18.18
Chlorophyll content				
350 ppm	28.6 ± 1.15[b]	28.15 ± 2.41[ab]	17.20 ± 1.91[ab]	30.22 ± 2.55[c]
550 ppm	27.83 ± 1.70[b]	23.30 ± 1.97[a]*	30.17 ± 2.30[c]	19.65 ± 1.71[a]
750 ppm	17.35 ± 1.39[a]	21.65 ± 1.58[a]*	14.95 ± 1.20[a]	17.78 ± 1.08[a]
Ambient conditions	29.53 ± 2.09[b]	29.53 ± 2.09[ab]	21.85 ± 1.94[b]	29.53 ± 2.13[b]
Mean	25.82	25.66	21.04	24.29

Values (mean ± SE) with the same letter within a column are not significantly different at $p \leq 0.05$

* Values within a row across the infested and un-infested plants are significantly different at $p \leq 0.05$

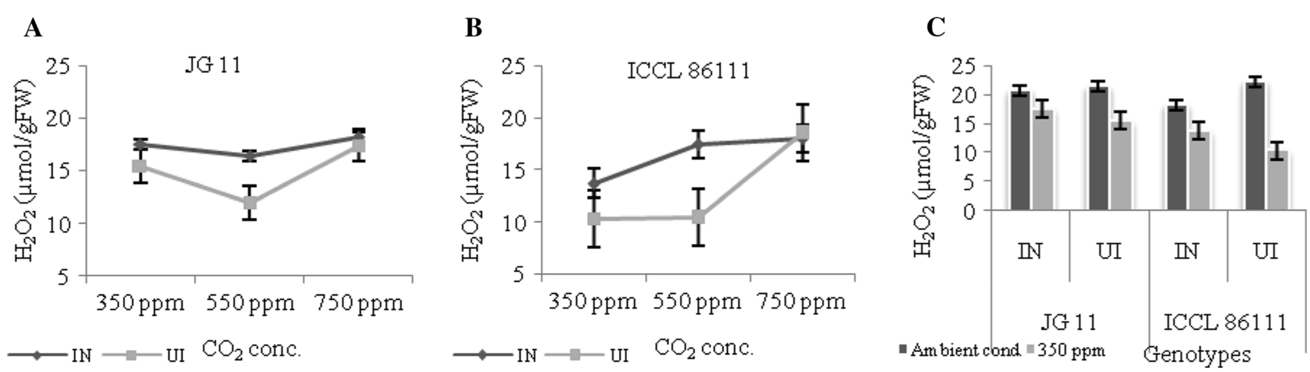

Fig. 5 H_2O_2 content (μmol/g FW) of *H. armigera*-infested and un-infested chickpea plants at different CO_2 levels [JG 11 (**a**), ICCL 8611 (**b**)] and under ambient conditions and at 350 ppm CO_2 (**c**)

Effect of CO_2 on carbohydrate and protein contents

Carbohydrate content increased with an increase in CO_2 in un-infested control plants, but decreased in insect-infested plants in both genotypes (Table 2). The insect-infested plants had significantly reduced carbohydrate content than the un-infested plants both under ambient conditions (20.8 and 19.6 mg/gFW for ICCL 86111 and JG 11, respectively) and at 350 ppm CO_2 (34.4 and 35.2 mg/gFW for ICCL 86111 and JG 11, respectively, $F_{(1,5)} = 19.9$,

Table 2 Carbohydrate and protein content of chickpea plants infested with *H. armigera* under different CO_2 regimes

CO_2 (ppm)	ICCL 86111		JG 11	
	Un-infested	Infested	Un-infested	Infested
Carbohydrate content (mg/0.1 g leaf tissue)				
350 ppm	33.6 ± 3.12[a]	34.4 ± 2.50[b]*	33.6 ± 1.45[a]	35.2 ± 1.56[a]*
550 ppm	44.0 ± 2.00[b]	27.2 ± 1.25[ab]*	40.0 ± 2.00[b]	27.2 ± 1.23[a]*
750 ppm	48.0 ± 1.50[b]	24.0 ± 2.20[a]*	44.8 ± 2.22[bc]	24.8 ± 1.56[a]*
Ambient conditions	33.6 ± 2.52[a]	20.8 ± 1.09[a]*	36.8 ± 1.20[a]	19.6 ± 1.72[a]*
Mean	39.80	26.6	38.8	27.0
Protein content (mg/g leaf tissue)				
350 ppm	49.5 ± 1.48[b]	33.0 ± 1.50[a]*	48.0 ± 1.82[c]	30.0 ± 2.00[a]*
550 ppm	45.0 ± 1.80[b]	39.0 ± 2.97[ab]*	42.0 ± 1.24[b]	32.0 ± 2.12[a]*
750 ppm	33.0 ± 1.50[a]	40.0 ± 3.83[b]*	33.0 ± 1.50[a]	36.0 ± 2.12[a]
Ambient conditions	46.0 ± 2.56[b]	42.1 ± 2.56[b]	40.0 ± 2.70[b]	42.0 ± 2.10[b]
Mean	43.38	38.53	40.75	35.0

Values (mean ± SE) with the same letter within a column are not significantly different at $p \leq 0.05$

* Values within a row across the infested and un-infested plants are significantly different at $p \leq 0.05$

$p = 0.008$). Significant differences were observed in carbohydrate content between ICCL 86111 and JG 11 infested and un-infested plants.

Protein content decreased with an increase in CO_2 in the un-infested plants of both the genotypes, but increased with an increase in CO_2 in the insect-infested plants (Table 2). The mean protein content of insect-infested plants was significantly greater than the un-infested control plants across all CO_2 regimes [ICCL 86111 (43.38 and 38.53 mg/g leaf tissue) and JG 11 (40.75 and 35.0 mg/g leaf tissue), respectively].

Effect of CO_2 on organic acids on the leaf surface

The *H. armigera*-infested plants had significantly greater amounts of oxalic and malic acids at 750 ppm than at 550 ppm (Table 3). The mean amounts of oxalic acid were significantly greater in insect-infested plants of JG 11 than the un-infested control plants (2.39 and 1.39 mg/gFW, respectively). The insect-infested plants also had significantly higher mean amounts of malic acid across CO_2 levels than the un-infested control plants [ICCL 86111 (3.02 and 1.52) mg/gFW and JG 11 (4.78 and 0.72) mg/gFW, respectively)].

Effect of CO_2 on leaf damage, larval survival and larval weights

Leaf damage by *H. armigera* differed significantly across CO_2 concentrations and the genotypes ($p \leq 0.05$). The interaction effects were nonsignificant. Significantly greater leaf damage was observed in plants grown at 350 ppm CO_2 (DR 4.1) as compared to those grown at 550 and 750 ppm (DR 3.5 and 3.6, respectively) (Table 4). Larval survival decreased with an increase in CO_2 in both the genotypes (Table 4). Larval survival was significantly greater in insects fed on plants kept under ambient conditions than in plants kept at 350 ppm in the OTC in both genotypes [ICCL 86111 (74 and 69 %) and JG 11 (78 and 72 %), respectively]. Larval weights did not differ significantly across the CO_2 regimes in both the genotypes (Table 4).

Discussion

Increased concentrations of CO_2 and high temperatures alter the nutritional composition of food, which may render the host plant unpalatable or nutritionally better for the herbivores (Sterner and Elser 2002).

The POD and PPO form an important component of plant antioxidative defense against biotic and abiotic stresses (Barbehenn et al. 2010; War et al. 2012, 2013). They act as scavengers of reactive oxygen species (ROS) and anti-nutritional components, respectively. The activities of POD and PPO increased under elevated CO_2; however, the differences were not statistically significant. Insect-damaged plants of both genotypes had higher activities of both the enzymes as compared to un-infested control plants, suggesting their role in providing protection to the plants against ROS-induced damage due to biotic stress in chickpea than the abiotic stress. POD is involved in phenol oxidation, cross-linking of polysaccharides and monomers, lignification and suberization, which are the main components of host plant resistance against insect pests (Barbehenn et al. 2010) and also the production of

Table 3 Oxalic and malic acids (mg/g FW) of chickpea plants infested with *H. armigera* under different CO_2 regimes

CO_2 (ppm)	ICCL 86111		JG 11	
	Un-infested	Infested	Un-infested	Infested
Oxalic acid				
350 ppm	1.74 ± 0.009[a]	2.34 ± 0.05[ab]*	1.37 ± 0.04[a]	2.89 ± 0.06[ab]*
550 ppm	1.67 ± 0.008[a]	1.72 ± 0.07[a]	1.55 ± 0.09[a]	1.91 ± 0.04[a]
750 ppm	2.04 ± 0.01[ab]	2.66 ± 0.03[b]	1.54 ± 0.09[a]	3.32 ± 0.05[b]*
Ambient conditions	1.95 ± 0.05[ab]	1.68 ± 0.09[a]*	1.11 ± 0.02[a]	1.47 ± 0.09[a]
Mean	1.85	2.10	1.39	2.39
Malic acid				
350 ppm	0.53 ± 0.006[a]	2.81 ± 0.05[b]*	0.57 ± 0.01[a]	6.05 ± 0.90[c]*
550 ppm	1.44 ± 0.08[b]	1.29 ± 0.03[a]	0.77 ± 0.008[a]	2.77 ± 0.02[a]*
750 ppm	1.06 ± 0.05[b]	6.19 ± 0.12[c]*	0.60 ± 0.005[a]	6.01 ± 0.01[c]*
Ambient conditions	3.07 ± 0.09[c]	1.79 ± 0.05[ab]*	0.96 ± 0.007[a]	7.27 ± 0.31[c]*
Mean	1.52	3.02	0.72	4.78

Values (mean ± SE) with the same letter within a column are not significantly different at $p \leq 0.05$

* Values within a row across the infested and un-infested plants are significantly different at $p \leq 0.05$

Table 4 Plant damage, larval survival and larval weights of *H. armigera* after feeding on chickpea plants raised under different CO_2 regimes

CO_2 (ppm)	DR[#]			Larval survival (%)		Larval weight (mg/larva)	
	ICCL 86111	JG 11	Mean DR	ICCL 86111	JG 11	ICCL 86111	JG 11
350	3.7 ± 0.58[a]	4.5 ± 0.19[a]	4.1 ± 0.38	69 ± 9.89[b]	72 ± 3.78[a]	1.81 ± 0.65[a]	2.32 ± 0.65[ab]
550	3.1 ± 0.54[a]	3.9 ± 0.56[a]	3.5 ± 0.55	53 ± 4.93[a]	73 ± 7.99[a]	2.25 ± 0.44[ab]	1.75 ± 0.55[a]
750	3.2 ± 0.38[a]	4.1 ± 0.91[a]	3.6 ± 0.19	59 ± 6.81[a]	70 ± 9.81[a]	1.74 ± 0.12[a]	1.82 ± 0.28[a]
Ambient conditions	4.9 ± 0.91[ab]	5.3 ± 0.11[b]	5.1 ± 0.64	74 ± 6.78[b]	78 ± 5.33[ab]	2.88 ± 0.78[b]	2.80 ± 0.11[b]
Mean	3.73	4.45		63.75	73.25	2.17	2.17

Values (mean ± SE) with the same letter within a column are not significantly different at $p \leq 0.05$

* Values within a row across the infested and un-infested plants are significantly different at $p \leq 0.05$

[#] DR = *Helicoverpa* damage rating on a scale of 1–9 (1 ≤ 10 %, 2 = 10–20 %, 3 = 20–30 %, 4 = 30–40 %, 5 = 40–50 %, 6 = 50–60 %, 7 = 60–70 %, 8 = 70-80 % and 9 ≥ 80 % leaf area damaged) 7 days after infestation

anti-nutritive compounds (War et al. 2012). PPO reduces food quality of plant tissues by oxidizing phenols into highly reactive and toxic quinines, which cross-link with proteins leading to their unavailability to insect pests (Bhonwong et al. 2009; War et al. 2012).

The plants raised under elevated CO_2 possess high activity of PAL and TAL, resulting in increased production and accumulation of secondary metabolites (Muzika 1993). Thus, the increased activities of these enzymes in response to CO_2 affect the expression of plant resistance to insect pests. Plants also respond to insect damage by reducing nitrogen availability in plant tissues (Winger et al. 2006), thereby making them less palatable to insect pests. Elevated CO_2 resulted in greater activity of PAL and TAL in chickpea which might be due to greater availability of phenylalanine and tyrosine, in response to elevated CO_2 and insect damage (Meyer et al. 2006).

The CO_2 enrichment increases the plant defensive secondary metabolites and antioxidative activity (Stutte et al. 2008; Robinson et al. 2012). Chickpea plants showed increased levels of phenols under elevated CO_2, and the insect-damaged plants had greater amounts than the respective un-infested plants. The insect-resistant genotype ICCL 86111 had greater amounts of phenols than that in JG 11. Plants respond to insect damage by reducing the N levels in tissues and increased C:N ratio due to increased C-based secondary metabolites that defend plants against insect herbivory (Winger et al. 2006). Elevated CO_2 levels did not significantly affect the levels of tannins in the genotypes; however, the insect-infested plants at 550 and 750 ppm had greater levels of tannins than the corresponding un-infested plants. This corresponds to the increased levels of phenols at elevated CO_2 levels. Tannins bind to proteins in insect gut and thereby inhibit the protein hydrolysis, thus reducing the nutritional quality of the plant tissues (Barbehenn and Constabel 2011).

There were significant differences in chlorophyll content of plants under different regimes of CO_2 and between insect-infested and un-infested plants. Reduced chlorophyll content at higher levels of CO_2 could be attributed to the low N content in the leaves (Uprety and Mahalaximi 2000; Himanen et al. 2008). NBI, an important indicator of plant resistance to herbivores, decreased under elevated CO_2. The insect-infested plants had lower NBI than the corresponding un-infested plants, which may be due to low N content and increased levels of flavonoids.

H_2O_2 content of chickpea plants was higher at 750 ppm of CO_2 in both genotypes. Although at lower levels of CO_2 (350 and 550 ppm), the H_2O_2 levels showed significant differences between infested and un-infested plants, but at 750 ppm, both infested and un-infested plants had almost similar H_2O_2 content. The H_2O_2 is involved in plant defense against insect pests through signaling plant defensive pathways and by causing oxidative damage to insect midgut (Maffei et al. 2007).

Carbohydrate content of chickpea plants increased with an increase in CO_2 in un-infested plants and showed an opposite trend in *H. armigera*-infested plants, but the reverse was seen in case of protein content. The increased carbohydrate levels in un-infested plants might be due to increased photosynthesis at higher levels of CO_2. However, the reduced carbohydrate content in the insect-infested plants could be attributed to the reduced chlorophyll levels due to foliage damage and production of C-based defensive secondary metabolites. The reduced protein content in insect-infested plants under higher levels of CO_2 is largely due to reduction in N content (Chen et al. 2005). Under elevated CO_2, there is greater carbon uptake as the leaf gas exchange increases (Ibrahim and Jaafar 2011). Further, increased carbohydrate content leads to suppression of RuBISCO and some of the photosynthetic proteins genes under elevated CO_2 (Sallas et al. 2003), which might result in reduced protein content.

Oxalic acid decreased, but the malic acid increased with an increase in CO_2. Furthermore, insect-infested plants had greater levels of organic acids than the un-infested plants. Oxalic and malic acid are important components of host plant resistance to *H. armigera* in chickpea (Devi et al. 2013). In artificial diet, oxalic acid inhibits the growth and development of *H. armigera* larvae (Sarmah et al. 2012). Oxalic acid also induces resistance in plants against biotic stresses by stimulating the production of phenolics, peroxidase and pathogenesis-related proteins (Jayaraj et al. 2010).

There were no significant differences in plant injury in chickpea across CO_2 regimes. The results observed here are in contrast to the earlier reports, wherein increased CO_2 increased the foliage feeding (Chen et al. 2005). Reduced damage by insect pests in plants under elevated CO_2 has been attributed to production of secondary metabolites and the nutritional characteristics of plant tissues (Karowe and Grubb 2011). Plants grown under elevated CO_2 showed significant effects on survival and weights of *H. armigera* larvae. This reduction in larval survival and weights at elevated CO_2 can be attributed to the decreased food quality of the plant tissues due to reduced N availability and the greater amounts of secondary metabolites. Organic acids such as oxalic and malic acids also contribute to reduced larval survival and weights of *H. armigera* in chickpea (Devi et al. 2013).

Conclusion

Increased activities of PAL and TAL, under high CO_2 concentration, might result in increased production of C-based secondary metabolites, but lower levels of N-based compounds. Carbohydrate levels and biomass increase under elevated CO_2, but the reduction in nitrogen and the protein contents affect the nutritional quality of the host plant. There is a need for in-depth studies to elucidate the effects of elevated CO_2 on insect–plant interactions, to overcome the possible counter-adaptations that insect pests may develop to biochemical changes in the host plants due to climate change.

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