Elevated CO2 influences host plant defense response in chickpea against Helicoverpa armigera

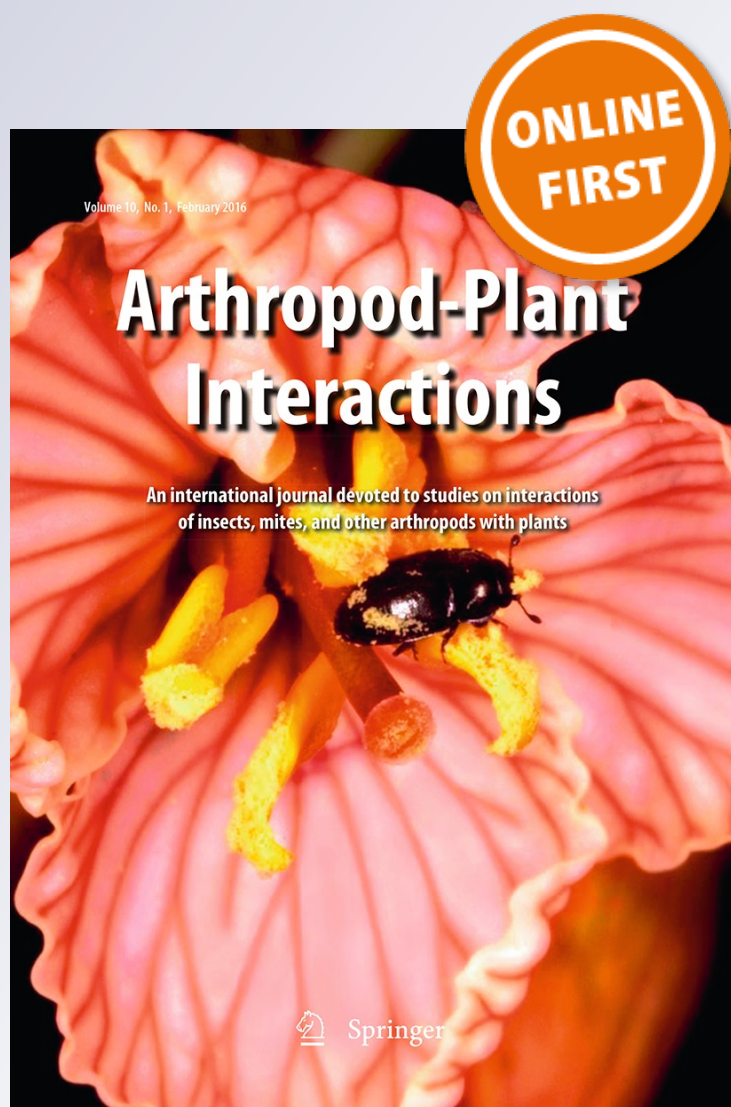## Hari C. Sharma, Abdul Rashid War, Mandeep Pathania, Suraj P Sharma, S. MD. Akbar & Rajendra S Munghate

ONLINE FIRST

Volume 10, No. 1, February 2016

Arthropod-Plant Interactions

An international journal devoted to studies on interactions
of insects, mites, and other arthropods with plants

Springer

Springer

Springer

CrossMark

ORIGINAL PAPER

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

Hari C. Sharma[1] · Abdul Rashid War[1] · Mandeep Pathania[1] · Suraj P Sharma[1] · S. MD. Akbar[1] · Rajendra S Munghate[1]

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.

Published online: 11 March 2016

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

Springer

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 ($4 \times 4 \times 4$ 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 ($\mu gGAEg^{-1}$ 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 ($\mu gCEg^{-1}$ 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 $\mu mol\ g^{-1}$ 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 $\mu g\ cm^{-2}$. 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 un-infested 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
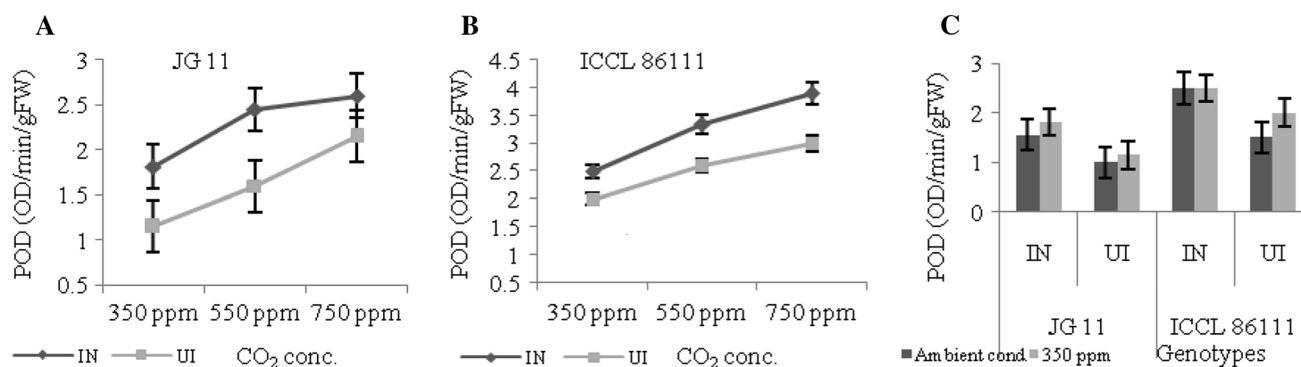
Fig. 1 POD activity of *H. armigera*-infested and un-infested chickpea plants raised under different CO_2 levels [A = JG 11 (**a**), ICCL 8611 (**b**)] and under ambient conditions and at 350 ppm CO_2 (**c**)
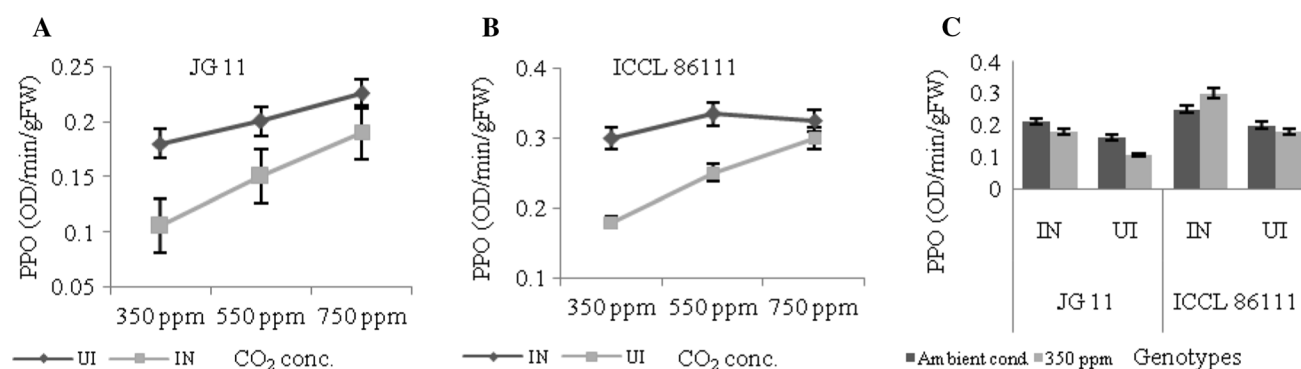
Fig. 2 PPO activity of *H. armigera*-infested and un-infested chickpea plants at different CO_2 levels [JG 11 (**a**), ICCL 8611 (**b**)] and under ambient conditions and at 350 ppm CO_2 (**c**)

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
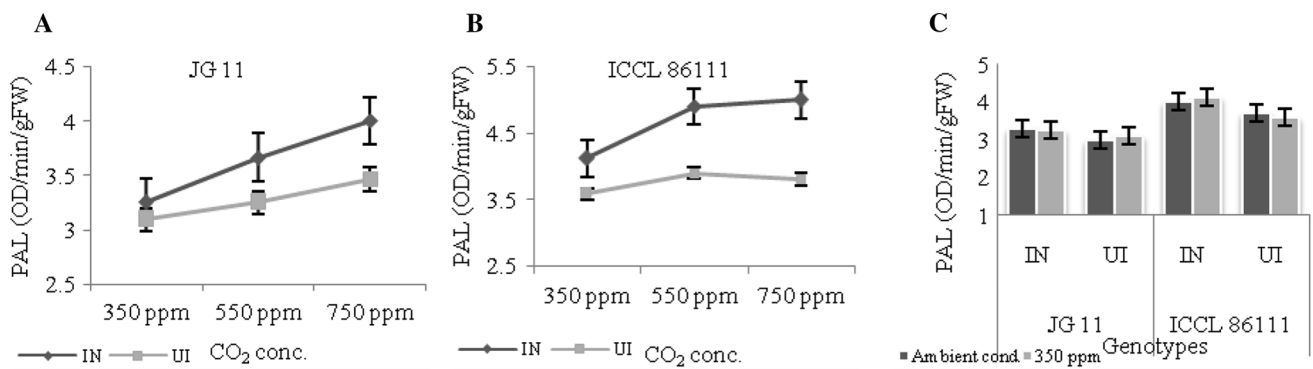
Fig. 3 PAL activity of *H. armigera*-infested and un-infested chickpea plants at different CO_2 levels, JG 11 (**a**), ICCL 8611 (**b**), under ambient condition and at 350 ppm CO_2 (**c**)
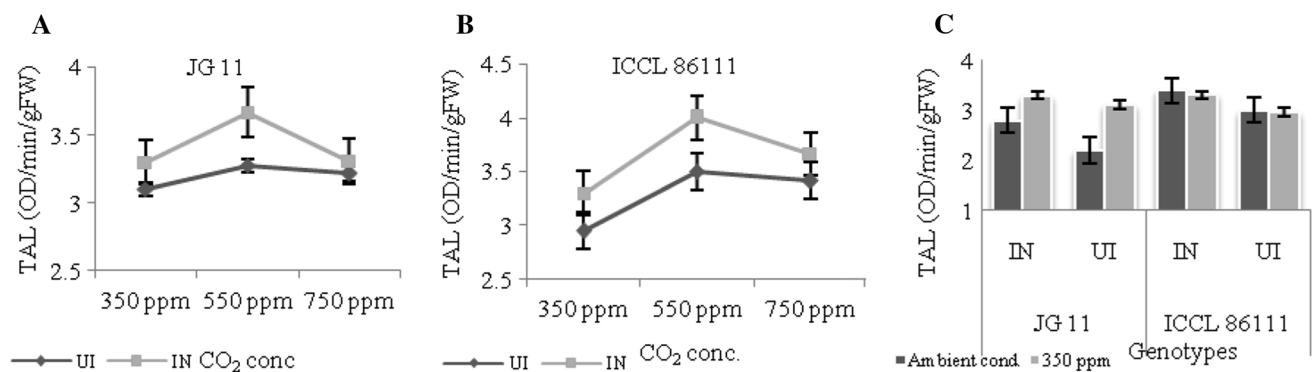
Fig. 4 TAL activity of *H. armigera*-infested and un-infested chickpea plants at different CO_2 levels [JG 11 (**a**), ICCL 8611 (**b**)] and under ambient conditions and at 350 ppm CO_2 (**c**)

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 ($\mu g\ cm^{-2}$) 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	$19.31 \pm 0.07^{a*}$	14.47 ± 0.10^{ab}	15.92 ± 0.14^{ab}
750 ppm	16.9 ± 0.27^a	$19.70 \pm 0.08^{a*}$	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	$12.60 \pm 0.74^{a*}$	10.40 ± 0.30^a	$11.82 \pm 0.74^{a*}$
750 ppm	10.47 ± 0.99^a	11.70 ± 0.58^a	9.78 ± 0.29^a	$11.23 \pm 0.84^{a*}$
Ambient conditions	9.90 ± 0.97^a	$12.11 \pm 0.92^{a*}$	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	$28.35 \pm 1.38^{b*}$	40.72 ± 3.17^d	$30.05 \pm 1.22^{c*}$
550 ppm	29.52 ± 1.07^{ab}	$18.05 \pm 1.80^{a*}$	28.95 ± 1.20^b	$16.07 \pm 1.10^{a*}$
750 ppm	25.85 ± 1.51^a	$18.87 \pm 1.28^{a*}$	10.77 ± 0.99^a	$10.0 \pm 1.14^{a*}$
Ambient conditions	30.95 ± 2.21^b	$16.45 \pm 1.05^{a*}$	19.9 ± 1.35^b	$16.6 \pm 1.29^{b*}$
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	$23.30 \pm 1.97^{a*}$	30.17 ± 2.30^c	19.65 ± 1.71^a
750 ppm	17.35 ± 1.39^a	$21.65 \pm 1.58^{a*}$	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 $\pm$ 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 $p \leq 0.05$
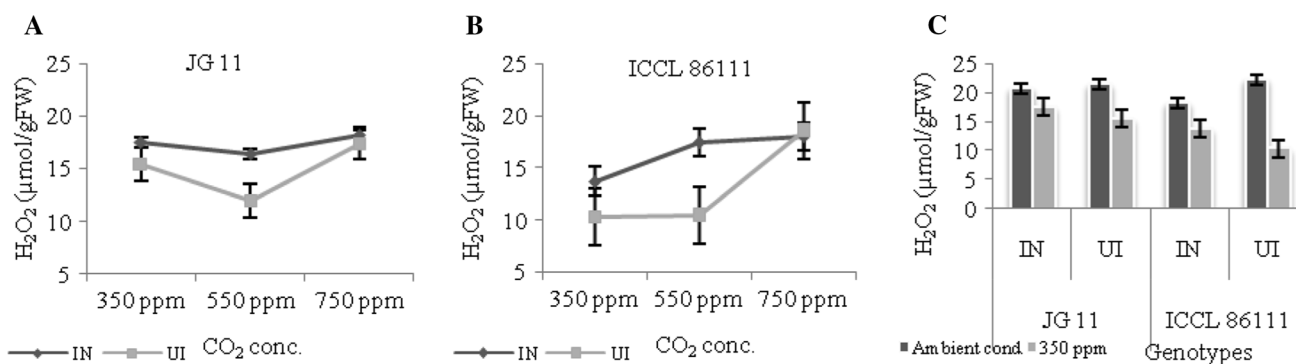
Fig. 5 H_2O_2 content ($\mu mol/g$ FW) of *H. armigera*-infested and un-infested chickpea plants at different CO_2 levels [JG 11 (**a**), ICCL 8611 (**b**)] and under ambient conditions and at 350 ppm CO_2 (**c**)

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	$34.4 \pm 2.50^{b*}$	33.6 ± 1.45^a	$35.2 \pm 1.56^{a*}$
550 ppm	44.0 ± 2.00^b	$27.2 \pm 1.25^{ab*}$	40.0 ± 2.00^b	$27.2 \pm 1.23^{a*}$
750 ppm	48.0 ± 1.50^b	$24.0 \pm 2.20^{a*}$	44.8 ± 2.22^{bc}	$24.8 \pm 1.56^{a*}$
Ambient conditions	33.6 ± 2.52^a	$20.8 \pm 1.09^{a*}$	36.8 ± 1.20^a	$19.6 \pm 1.72^{a*}$
Mean	39.80	26.6	38.8	27.0
Protein content (mg/g leaf tissue)				
350 ppm	49.5 ± 1.48^b	$33.0 \pm 1.50^{a*}$	48.0 ± 1.82^c	$30.0 \pm 2.00^{a*}$
550 ppm	45.0 ± 1.80^b	$39.0 \pm 2.97^{ab*}$	42.0 ± 1.24^b	$32.0 \pm 2.12^{a*}$
750 ppm	33.0 ± 1.50^a	$40.0 \pm 3.83^{b*}$	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 $\pm$ 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	$2.34 \pm 0.05^{ab*}$	1.37 ± 0.04^a	$2.89 \pm 0.06^{ab*}$
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	$3.32 \pm 0.05^{b*}$
Ambient conditions	1.95 ± 0.05^{ab}	$1.68 \pm 0.09^{a*}$	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	$2.81 \pm 0.05^{b*}$	0.57 ± 0.01^a	$6.05 \pm 0.90^{c*}$
550 ppm	1.44 ± 0.08^b	1.29 ± 0.03^a	0.77 ± 0.008^a	$2.77 \pm 0.02^{a*}$
750 ppm	1.06 ± 0.05^b	$6.19 \pm 0.12^{c*}$	0.60 ± 0.005^a	$6.01 \pm 0.01^{c*}$
Ambient conditions	3.07 ± 0.09^c	$1.79 \pm 0.05^{ab*}$	0.96 ± 0.007^a	$7.27 \pm 0.31^{c*}$
Mean	1.52	3.02	0.72	4.78

Values (mean $\pm$ 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 $\pm$ 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 ($1 \leq 10$ %, 2 = 10–20 %, 3 = 20–30 %, 4 = 30–40 %, 5 = 40–50 %, 6 = 50–60 %, 7 = 60–70 %, 8 = 70-80 % and $9 \geq 80$ % 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$_2$O$_2$ 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$_2$O$_2$ levels showed significant differences between infested and un-infested plants, but at 750 ppm, both infested and un-infested plants had almost similar H$_2$O$_2$ content. The H$_2$O$_2$ 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.

References

Badiani M, D'Annibale A, Paolacci AR, Miglietta F, Rashchi A (1993) The antioxidant status of soybean (*Glycine max*) leaves grown under nature CO$_2$ enrichment in the field. Aust J Plant Physiol 20:275–284

Barbehenn RV, Constabel CP (2011) Tannins in plant herbivore interactions. Phytochemistry 72:1551–1565

Barbehenn R, Dukatz C, Holt C, Reese A, Martiskainen O, Salminenm JP, Yip L, Constabel CP (2010) Feeding on poplar leaves by caterpillars potentiates foliar peroxidase action in their guts and increases plant resistance. Oecologia 164:993–1004

Bezemer TM, Jones TH (1998) Plant-insect herbivore interactions in elevated atmospheric CO$_2$: quantitative analyses and guild effects. Oikos 82:212–222

Bhonwong A, Stout MJ, Attajarusit J, Tantasawat P (2009) Defensive role of tomato polyphenol oxidases against cotton bollworm (*Helicoverpa armigera*) and beet armyworm (*Spodoptera exigua*). J Chem Ecol 35:28–38

Campos-Vergas R, Saltveit ME (2002) Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. Physiol Plant 114:73–84

Chen F, Wu G, Ge F, Parajulee MN, Shrestha RB (2005) Effects of elevated CO$_2$ and transgenic *Bt* cotton on plant chemistry,

performance, and feeding of an insect herbivore, the cotton bollworm. Entomol Expert Appl 115:341–350

Chitti Babu G, Sharma HC, Madhumati T, Raghavaiah G, Krishna Murthy KVM, Rao VS (2014) A semi-synthetic chickpea flour based diet for long-term maintenance of laboratory culture of *Helicoverpa armigera*. Indian J Entomol 76(4):336–340

DeLucia EH, Nabity PD, Zavala JA, Barenbaum MR (2012) Climate change: resetting plant–insect interactions. Plant Physiol 160:1677–1685

Devi VS, Sharma HC, Rao PA (2013) Influence of oxalic and malic acids in chickpea leaf exudates on the biological activity of CryIAc towards *Helicoverpa armigera*. J Insect Physiol 59(4):394–399

Falkowski PG, Scholes RJ, Boyle E, Canadell J, Canfield D, Elser J, Gruber N, Hibbard K, Hogberg P, Linder S, Mackenzie FT, Moore B, Pedersen T, Rosenthal Y, Seitzinger S, Smetacek V, Steffen W (2000) The global carbon cycle: a test of our knowledge of earth as a system. Science 290(5490):291–296

Guo H, Sun Y, Li Y, Liu X, Zhang W, Ge F (2014) Elevated CO_2 decreases the response of the ethylene signaling pathway in *Medicago truncatula* and increases the abundance of the pea aphid. New Phytol 201:279–291

Himanen SJ, Nissinen A, Dong W-X, Nerg A-M, Stewart JRCN, Poppy GM, Holopainen JK (2008) Interactions of elevated carbon dioxide and temperature with aphid feeding on transgenic oilseed rape: are *Bacillus thuringiensis* (Bt) plants more susceptible to nontarget herbivores in future climate. Glob Change Biol 14:1–18

Ibrahim MH, Jaafar HZ (2011) Enhancement of leaf gas exchange and primary metabolites under carbon dioxide enrichment up-regulates the production of secondary metabolites in *Labisia pumila* seedlings. Molecules 16:3761–3777

Intergovernmental Panel on Climate Change (2007) IPCC fourth assessment report: climate change 2007. IPCC, Geneva

Jayaraj J, Bhuvaneswari R, Rabindran R, Muthukrishnan S, Velazhahan R (2010) Oxalic acid-induced resistance to *Rhizoctonia solani* in rice is associated with induction of phenolics, peroxidase and pathogenesis-related proteins. J Plant Interact 5(2):147–157

Karowe DN, Grubb C (2011) Elevated CO_2 increases constitutive phenolics and trichomes, but decreases inducibility of phenolics in *Brassica rapa* (Brassicaceae). J Chem Ecol 37:1332–1340

Khan W, Prithiviraj B, Smith DL (2003) Chitosan and chitin oligomers increase phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in soybean leaves. J Plant Physiol 160:859–863

Lindroth RL (2010) Impacts of elevated atmospheric CO_2 and O_3 on forests: phytochemistry, trophic interactions, and ecosystem dynamics. J Chem Ecol 36:2–21

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants face the future. Ann Rev Plant Biol 55:591–628

Lowry H, Rosebrough NI, Far AL, Ranall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265–275

Maffei ME, Mithofer A, Boland W (2007) Insects feeding on plants: rapid signals and responses preceding the induction of photo-chemical release. Phytochemistry 68:2946–2959

Mayer AM, Harel E (1979) Polyphenol oxidases in plant. Phytochemistry 18:193–215

Meyer S, Cerovic ZG, Goulas Y, Montpied P, Demotes S, Bidel LPR, Moya I, Dreyer E (2006) Relationship between assessed polyphenols and chlorophyll contents and leaf mass per area ratio in woody plants. Plant Cell Environ 29:1338–1348

Muzika RM (1993) Terpenes and phenolics in response to nitrogen fertilization: a test of the carbon/nutrient balance hypothesis. Chemoecology 4:3–7

Noreen Z, Ashraf M (2009) Change in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.). Environ Exp Bot 67:395–402

Polle A, Eiblmeier M, Sbeppard L, Murray M (1997) Responses of antioxidative enzymes to elevated CO_2 in leaves of beech (*Fagus sylvatica* L.) seedlings grown under a range of nutrient regimes. Plant Cell Environ 20:1317–1321

Rao MS, Khan MAM, Srinivas K, Vanaja M, Rao GGSN, Ramakrishna YS (2006) Effects of elevated carbon dioxide and temperature on insect-plant interactions—a review. Agric Rev 27(3):200–207

Robert EB (1971) Method for estimation of tannin in grain sorghum. Agrochem J 63:511–512

Robinson EA, Ryan GD, Newman JA (2012) A meta-analytical review of the effects of elevated CO_2 on plant-arthropod interactions highlights the importance of interacting environmental and biological variables. New Phytol 194:321–336

Sadasivam S, Manickam A (1996) Biochemical methods. New Age International Limited and Tamil Nadu Agricultural University, Coimbatore

Sallas L, Luomala EM, Utriainen J, Kainulainen P, Holopainen JK (2003) Contrasting effects of elevated carbon dioxide concentration and temperature on Rubisco activity, chlorophyll fluorescence, needle ultrastructure and secondary metabolites in conifer seedlings. Tree Physiol 23:97–108

Sarmah BK, Acharjee S, Sharma HC (2012) Chickpea: crop improvement under changing environment conditions. In: Tuteja N, Gill SS, Tuteja R (eds) Improving crop productivity in sustainable agriculture. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 361–381

Shannon LM, Kay E, Lew JY (1966) Peroxidase isozymes from horse radish roots. Isolation and physical properties. J Biol Chem 241:2166–2172

Sharma HC (2014) Climate change effects on insects: implications for crop protection and food security. J Crop Improv 29(2):229–259

Sharma HC, Pampathy G, Dwivedi SL, Reddy LJ (2003) Mechanism and diversity of resistance to insect pests in wild relatives of groundnut. J Econ Entomol 96(6):1886–1897

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, USA, p 439

Stutte GW, Eraso I, Rimando AM (2008) Carbon dioxide enrichment enhances growth and flavonoid content of two *Scutellaria* species. J Am Soc Hortic Sci 133(5):631–638

Sun Y, Guo H, Zhu-Salzman K, Kang L, Ge F (2013) Elevated CO_2 increases the abundance of the peach aphid on Arabidopsis by reducing jasmonic acid defenses. Plant Sci 210:128–140

Uprety DC, Mahalaximi V (2000) Effect of elevated CO_2 and nitrogen nutrition on photosynthesis, growth and carbon–nitrogen balance in *Brassica juncea*. J Agron Crop Sci 184:271–276

Uprety DC, Garg SC, Bisht BS, Maini HK, Dwivedi N, Paswan G, Raj A, Saxena DC (2006) Carbon dioxide enrichment technologies for crop response studies. J Sci Ind Res 65:859–866

Vanaja M, Maheswari M, Ratnakumar P, Ramakrishna YS (2006) Monitoring and controlling of CO_2 concentrations in open top chambers for better understanding of plants response to elevated CO_2 levels. Indian J Radio Space 35:193–197

War AR, Paulraj MG, Tariq Ahmad, Buhroo AA, Hussain B, Ignacimuthu S, Sharma HC (2012) Mechanisms of plant defense against insect herbivores. Plant Signal Behav 7(10):1306–1320

War AR, Hussain B, Sharma HC (2013) Induced resistance in groundnut by jasmonic acid and salicylic acid through alteration

of trichome density and oviposition by *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). AOB Plants. doi:10.1093/aobpla/plt053

Winger A, Purdy S, Maclean A, Pourtau N (2006) The role of sugars in integrating environmental signals during the regulation of leaf senescence. New Phytol 161:781–789

Zavala JA, Nabity PD, DeLucia EH (2013) An emerging understanding of mechanisms governing insect herbivory under elevated CO$_2$. Annu Rev Entomol 58:79–97

Zieslin N, Ben-Zaken R (1993) Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiol Biochem 31:333–339