DOI: 10.1007/s11099-014-0059-0 PHOTOSYNTHETICA 52 (4): 519-528, 2014

Impact of elevated CO_2 on growth, physiology, yield, and quality of tomato (*Lycopersicon esculentum* Mill) cv. Arka Ashish

H. MAMATHA, N.K. SRINIVASA RAO+, R.H. LAXMAN, K.S. SHIVASHANKARA, R.M. BHATT, and K.C. PAVITHRA

Division of Plant Physiology and Biochemistry, Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bangalore, 560089, India

Abstract

Tomato meets the dietary nutrient and antioxidant requirements of diverse populations. Being a C_3 crop and an important vegetable, it is likely to be influenced by increased CO_2 concentrations under climate change situation. This study was conducted to investigate the effects of elevated CO_2 on overall physiology, water relations, growth, yield, and fruit quality of tomato (*Lycopersicon esculentum* Mill) cv. Arka Ashish. Plants were grown at elevated CO_2 [550 (EC_{550}) and 700 (EC_{700}) ppm of CO_2] in open top chambers. Increased assimilation rate, decreased stomatal conductance and transpiration rate were observed at elevated CO_2 (EC) concentrations. Reduced leaf osmotic potential and increased water potential were observed at EC compared with the control (380 ppm of CO_2) in flowering and fruiting stages. Lower total chlorophyll content was recorded at EC_{700}. Plant height was significantly higher at EC_{550} compared with EC_{700}. Higher number of branches was observed at EC_{700} as compared with plants grown at EC_{550} and the control. Leaf area was lower at EC_{700} compared with EC_{550} but specific leaf mass was higher at EC_{700}. Due to higher leaf dry mass and root dry mass, the plants grown at EC_{700} exhibited higher total dry mass compared to EC_{550} and the control. Increased number of flowers and fruits together with higher fruit set led to higher fruit yield at both EC concentrations. The highest yield increase was observed at EC_{700}. The fruits showed a lower content of phenols, flavonoids, ferric reducing antioxidant potential, total soluble solids, and titratable acidity in plants grown at EC as compared with the control. The ascorbic acid content was high at both EC_{700} and EC_{550}. Carotenoids and lycopene content was low at EC_{700} compared to higher content observed at EC_{550} and the control.

Additional key words: gas exchange; growth characteristics; leaf water status; pigments; yield characteristics.

Introduction

Carbon dioxide released through various human activities is the main greenhouse gas causing global warming. Its concentration in the atmosphere rose from the pre-industrial level of 280 ppm (IPCC 2007) to the present level of 395.15 ppm in August 2013 (www.esrl.noaa.gov/gmd/ccgg/trends/). Atmospheric CO_2 is expected to reach 700 ppm by the end of the century according to the Intergovernmental Panel on Climate Change (IPCC) under emission Scenario A1B (Carter *et al*. 2007). Increasing CO_2 concentration in the atmosphere focused scientists' interest on studying the response of crop plants to CO_2 enrichment. Studies under controlled environment have shown that CO_2 fertigation enhances photosynthetic rate (P_N) and the yield in both C_3 and C_4 crops (Kimball *et al*. 2002, Reddy *et al*. 2010). It is also observed that the increased intercellular CO_2 concentration leads to decrease in stomatal conductance (g_s), increased P_N, and water-use efficiency (WUE) at EC (Ainsworth and Long 2005, Leakey *et al*. 2009, Zhao *et al*. 2011, Li *et al*. 2013). Crop water use is a critical issue for crop production and increased WUE may represent one of the most significant plant responses to EC (Rogers *et al*. 1994). However, screening genotypes for high leaf transpiration (E) efficiency at projected, future CO_2 concentrations could be more efficiently accomplished under the EC concentrations (Bunce 2012). The studies suggest that many crops, notably C_3 crops, may respond positively to increase in atmospheric CO_2 in the absence of other stressful conditions (Long *et al*. 2004). It is evident that the steady increase in atmospheric CO_2 influences the

Received 4 July 2013, *accepted* 14 February 2014.
+Corresponding author; tel.: 091-9845647037, e-mail: nksraoiihr@gmail.com
Abbreviations: CO_2 – carbon dioxide; E – transpiration; FFM – fruit fresh mass; FM – fresh mass; FRAP – ferric reducing antioxidant potential; g_s – stomatal conductance; LDM – leaf dry mass; OTCs – open top chambers; P_N – photosynthetic rate; RDM – root dry mass; SDM – stem dry mass; SLM – specific leaf mass; TDM – total dry mass; TSS – total soluble solids; WUE – water-use efficiency; Ψ_s – osmotic potential; Ψ_w – water potential.

overall physiology, growth, and development of crops.

Tomato (*L. esculentum* Mill.) is one of the most consumed vegetables in the world and is rich in dietary nutrients and antioxidants. It is a good source of bioactive compounds, including carotenes (lycopene, β-carotene), ascorbic acid, and phenolic compounds. High amount of ascorbic acid has been reported by many workers (Leonardi *et al*. 2000, Stewart *et al*. 2000, Kaur *et al*. 2013). Being a C_3 plant, it has shown positive response to a range of EC concentrations. An increase in P_N was found in cv. Virosa grown under EC from 500 to 2,000 ppm of CO_2 (Nilsen *et al*. 1983). Significantly higher P_N, reduced g_s, and increased leaf area was observed in two species, 'Vedettos' (*L. esculentum*) and LA1028 (*L. chmielewskii*), grown at 900 ppm of CO_2 (Yelle *et al*. 1990). Lower *E* (Behboudian and Lai 1994) and the increased yield have been reported (Nilsen *et al*. 1983, Yelle *et al*. 1990, Reinert *et al.* 1997). The changes in physiology, phenology, growth, and the yield of crops lead to changes in quality of the produce. The EC effects on physiology and quality of fruits and vegetables have been summarized by Moretti *et al*. (2010). In general, high CO_2 has been reported to influence the fruit quality by affecting content of antioxidants, ascorbic acid, and sugars (Tajiri 1985, Islam *et al*. 1996, Idso *et al*. 2002, Wang *et al*. 2003). High ascorbic acid and sugar contents in tomato fruits were reported by Islam *et al*. (1996), when EC was used at different maturity stages. However, some of the studies reported that enhancements in antioxidant substance contents were very low in tomato under high CO_2 concentrations (Barbale 1970, Madsen 1971, 1975; Kimball and Michell 1981). As higher intake of flavonoids, ascorbic acid, and carotenoids have been reported to reduce the risks of many degenerative diseases (Agarwal and Rao 2000), it is pertinent to quantify the effect of EC concentrations on tomato fruit quality. Keeping in mind the overall effect of EC on physiology, growth, productivity, and quality of tomato, the present study was conducted primarily to quantify how EC at two different concentrations affects the gas-exchange characteristics, photosynthetic pigments, leaf water status, growth, dry mass, and the yield. As a secondary effect, the changes in fruit quality under two EC concentrations were also studied in fruits of tomato, cv. Arka Ashish.

Materials and methods

Experimental conditions and plant material: The study was conducted in open top chambers (OTCs) from mid-August to the end of December 2011 at the Indian Institute of Horticultural Research, Bangalore, India. Thirty-day-old seedlings were transplanted at a spacing of 50 cm between plants in four OTCs having a dimension of 3 × 3 × 2.4 m covered with polyvinylchloride sheet of 150 μm thickness with 95% light transmission. Five rows with five plants per row were maintained in each OTC. Plants were fertilized with a basal dose of 20.6 g of urea, 116.7 g of single super phosphate, and 11.1 g of KCl per square meter area. Another dose of top dressing urea (20.6 g) was applied 15 d after transplanting the seedlings. Mild irrigation was given once in three days through hose pipe. Two OTCs were maintained under ambient CO_2 of 380 ppm (control), one with EC of 550 ppm (EC_{550}), and another one with EC of 700 ppm (EC_{700}). The observations and samples were taken from one OTC maintained with each CO_2 concentrations. Pure CO_2 gas was diluted to 550 ppm and 700 ppm by mixing it with air before injecting it into the OTC. CO_2 concentrations within the OTCs were continuously monitored with *LAMBDA T* infrared CO_2 gas monitors (*ADC Bioscientific Ltd*., UK) through the inlet from OTC, which sampled air at a crop-canopy height. If the CO_2 concentrations inside the OTC dropped below the set concentration, solenoid valve opened to supply CO_2 from a gas cylinder and remained open until the set concentration was attained. As soon as CO_2 concentration crossed the set concentration, the valve closed. Thus, the desired CO_2 concentrations were maintained inside the OTC. EC mean concentrations of 550 ± 60 and 700 ± 70 ppm were monitored daily for seven hours from 09:00 to 16:00 h. Inside the OTCs, following parameters were observed during the experimental period: the mean maximum temperature of 27.3 ± 1.5°C, the mean minimum temperature of 17.4 ± 2.89°C, maximum relative humidity (RH) of 82.6 ± 6.5% (07:30 h), and minimum RH of 56.1 ± 7.7% (13:30 h).

Growth: Plant growth parameters were recorded in five plants from each CO_2 concentration. At peak flowering stage, plant height, number of branches, and leaf number were recorded. Leaf area was recorded using portable leaf area meter (*LI-3000C*, *LI-COR*, USA) and expressed in cm^2 per plant. Leaf (LDM), stem (SDM), root (RDM), and total dry mass (TDM) were recorded after drying the plant parts at 70°C for 60 h in an oven till constant dry mass was obtained. The specific leaf mass (SLM) was calculated as the ratio of leaf dry mass to leaf area (Radford 1967).

Gas-exchange parameters were recorded from the uppermost, fully expanded leaf from 09:30 to 11:30 h using a portable photosynthesis system (*LI-6400 XT*, *LI-COR*, USA) at fruiting stage (40 d after CO_2 treatments). A total of five measurements were taken from five plants of each treatment. Cuvette temperature, light intensity, and RH were set to mean atmospheric conditions, *i.e*., 32°C; 1,200 μmol(photon) m^{-2} s^{-1}, and 60%, respectively. Leaf was held in the chamber for 3 min to obtain stable readings. While recording gas-exchange characteristics,

the CO_2 concentrations in the leaf chamber were maintained at 700, 550, and 380 ppm in respective treatments. P_N, g_s, and E were calculated by the software operating in *LI-6400 XT*, using the von Caemmerer and Farquhar (1981) equations. P_N, g_s, and E were expressed as μmol(CO_2) m^{-2} s^{-1}, mol(H_2O) m^{-2} s^{-1}, and mmol(H_2O) m^{-2} s^{-1}, respectively.

Water and osmotic potential: Water potential (Ψ_w) and osmotic potential (Ψ_s) were recorded at both flowering and fruiting stages. Ψ_w was recorded from five fully expanded leaves from each treatment using a pressure chamber (*ARIMAD-3000*, *MRC,* Israel). Leaf Ψ_s was also measured from five fully expanded leaves using a vapour pressure osmometer (*VAPro*, *WESCOR*, USA). Ψ_s was measured after squeezing leaf cell sap manually onto filter paper and placing it in the osmometer sample chamber.

Chlorophyll (Chl) pigments: Chl was extracted from five fully expanded leaves in each treatment at flowering stage using dimethyl sulfoxide (DMSO) (Shoaf and Lium 1976). Fresh leaf tissue of 100 mg was cut into small pieces and incubated in 7.0 ml of DMSO at 65°C for 30 min. At the end of the incubation period, extract was taken and the volume was made up to 10 ml with DMSO. Absorbance of the extract was read at 645, 652, and 663 nm using DMSO as blank in spectrophotometer, (*UV-VIS, Systronics Ltd*., India.). Quantitative determinations were carried out as described by Mackinney (1941). The Chl content was expressed on leaf fresh mass (FM) in [mg g^{-1}(FM)].

Yield characteristics: Number of flowers per plant was recorded from five plants in each treatment at regular intervals starting from onset of flowering. In the same plants, the total fruit mass (g) per plant and number of fruits per plant were quantified by pooling the number of fruits and fruit mass harvested at regular intervals.

Quality parameters: Five ripened fruits were picked randomly from each treatment to determine the following quality parameters.

Total soluble solids (TSS) values were obtained by placing on prism a droplet of tomato juice squeezed from five ripened fruits, picked randomly from each treatment, using a digital refractometer (*DG-NXT*, *ARKO India Ltd.*, India). The results were reported as °Brix.

Titratable acidity: Titratable acidity was determined as per AOAC method (942.15) (AOAC 2000). Tomatoes were homogenized in a blender to a fine puree. Tomato puree of 10 g was mixed with distilled water, squeezed through a muslin cloth, and volume was made up to 50 ml. A known volume of the filtrate (10 ml) was titrated with 0.01N NaOH using phenolphthalein as indicator. Acidity was calculated as percentage of citric acid equivalents using citric acid standard curve.

Ascorbic acid content: Ascorbic acid content was determined by 2,6-dichlorophenol indophenol (DCPIP) AOAC method (967.21) (AOAC 2006). Ten grams of tomato puree were mixed thoroughly with 4% oxalic acid solution, squeezed through a muslin cloth, and the volume was made up to 50 ml. Ascorbic acid content was estimated by titrating a known quantity of the extract against DCPIP. Ascorbic acid content was calculated as mg of ascorbic acid equivalents per 100 g of fruit fresh mass (FFM) using a standard curve of L-ascorbic acid.

Total phenols: The content of fruit total phenols was extracted with 80% methanol and estimated following the Folin-Ciocalteu (FC) method (Singleton and Rossi 1965). Methanol extract was mixed with FC reagent and the colour was developed with 20% sodium carbonate reagent. The developed colour intensity was read at 700 nm using a spectrophotometer (*T80 + UV/VIS*, *PG Instruments Ltd.*, UK). Content was expressed as mg of gallic acid equivalents per 100 g of FFM.

Total flavonoids: Total flavonoids content was estimated according to Chun *et al*. (2003). Flavonoids content in the 80% methanol extract was estimated using 5% $NaNO_2$ and 10% $AlCl_3$. The absorbance of the pink mixture was read at 510 nm using a spectrophotometer (*T80 + UV/VIS*) and expressed as mg of catechin equivalents per 100 g of FFM.

Antioxidant capacity: It was determined as ferric reducing antioxidant potential (FRAP), using a modified method of Benzie and Strain (1996). Methanol extract (0.2 ml) was mixed with 1.8 ml of FRAP reagent. FRAP reagent was prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl, and 20 mM $FeCl_3$ in a 10:1:1 ratio. The intensity of the developed blue colour was measured at 593 nm using a spectrophotometer (*T80 + UV/VIS*). Antioxidant capacity was expressed as mg of ascorbic acid equivalents antioxidant capacity (AEAC) per 100 g of FFM.

Total carotenoids and lycopene content: Five grams of tomato sample was repeatedly extracted under low light conditions with acetone till the residue turned white. In a separating funnel, the acetone extract, 15 ml of hexane, and 100 ml of distilled water was added. The mixture was shaken well and allowed to stand for few minutes until two clear phases were formed. The upper hexane phase with carotenoids (Car) was collected and the lower aqueous phase was re-extracted with additional hexane, until the aqueous phase was colourless. Hexane fraction was dried using anhydrous Na_2SO_4 to remove the moisture. The volume was made up to 25 ml with hexane and absorbance was read at 470 nm for total Car and at

503 nm for lycopene using spectrophotometer (*T80 + UV/VIS*). Total Car and lycopene content were calculated using β-carotene and lycopene as standards. The values were expressed as mg per 100 g of FFM (Lichtenthaler 1987).

Statistical analysis: The data were analysed using one-way factorial analysis of variance (*ANOVA*) to test the significance of treatment. Computations were made with *ANOVA Package for Researcher's*, *version 7.01*, *1994*, *Pascal Intl Software Solutions*. The level of significance used in *t*-test was p=0.05. When *F*-test was significant, critical difference values were calculated at 5% probability level.

Results

Growth characteristics: Significant differences in plant height, number of branches, leaf number, and leaf area were observed in response to EC at the peak of the flowering stage. Plants grown at EC_{550} recorded significantly higher plant height (115.0 cm), which was 40.7 and 24.6% higher than those grown at EC_{700} (81.8 cm) and the control (92.3 cm), respectively (Table 1). The EC had a significant positive influence on number of branches and leaves per plant. Plants grown at EC_{700} showed higher number of branches (17.3) and leaves (88.3) compared with the plants grown at EC_{550} and the control (Table 1).

Leaf area per plant was the highest at EC_{550}, where it was 64.4 and 44.4% higher than in the control and EC_{700}, respectively (Table 1). However, LDM was significantly higher in the plants grown at EC_{700}; it was 13.6 and 31.2% higher than in the plants grown at EC_{550} and control, respectively (Fig. 1*A*). Significantly higher SDM was observed in the plants grown at EC_{550} as compared with the EC_{700} and the control plants (Fig. 1*B*). The higher LDM and RDM contributed to higher TDM in the plants grown at EC_{700}. (Fig. 1*D*). TDM in the plants grown at EC_{700} was 5.6 and 34.3% higher than in the plants grown at EC_{550} and the control plants, respectively.

Gas exchange: EC caused significant increase of P_N in tomato plants. The plants grown at EC_{700} recorded significantly higher P_N, which was 33.6 and 68.2% higher than that at EC_{550} and the control treatments, respectively (Fig. 2*A*). The g_s and *E* were lower at EC compared with the control plants (Fig. 2*B*,*C*). *E* decreased by 24.2 and 17.7% in the plants grown at EC_{700} and EC_{550}, respectively.

Chl pigments: During flowering, the plants grown at EC_{700} showed a lower Chl content compared with EC_{550} and the control plants. Total Chl at EC_{700} was 15 and 14.5% lower in comparison with the control and EC_{550} treatments, respectively (Fig. 3*D*). Chl *a* and *b* content showed also the same trend (Fig. 3*A*,*C*). There were no significant differences in the Chl *a*/*b* ratio; it was relatively higher at EC_{700} as compared with EC_{550} and the control plants (Fig. 3*B*).

Osmotic and water potential: The leaf Ψ_s decreased (more negative) under EC as compared with the control (Fig. 4*A*,*B*). The decrease was 11.0 and 6.3% at EC_{700} and EC_{550}, respectively, as compared with the control at the flowering stage. Similarly, at the fruiting stage, the percentage reduction in Ψ_s was 5.7 and 1.7 at EC_{700} and EC_{550}, respectively, as compared with the control. High osmotic adjustment was observed at EC_{700} (0.68, 0.78), followed by EC_{550} (0.53, 0.66), and the control (0.39, 0.58) both at the flowering and fruiting stages, respectively (data not shown).

Significantly higher Ψ_w (less negative) was recorded at EC compared with the control both at the flowering and fruiting stages (Fig. 4*C*,*D*). During flowering, plants grown at EC_{700} and EC_{550} showed 32 and 11.9% increase (less negative) in Ψ_w as compared with the control. Similarly, the Ψ_w increase at the fruiting stage was 28.9 and 11.5% at EC_{700} and EC_{550}, respectively, as compared with the control.

Yield characteristics: The maximum number of flowers and fruits per plant was recorded in plants grown at

Table 1. Effect of different concentrations of CO_2 on plant height (PHT), number of branches (NB), number of leaves (NL), leaf area (LA), and specific leaf mass (SLM) of tomato cv. Arka Ashish at peak flowering stage. Means in the column followed by the *same letter* do not differ significantly at p=0.05. CD – critical difference; SEM – standard error of mean (n = 5). * – significant at P<0.05.

CO_2 [ppm]	PHT [cm]	NB [number]	NL [number]	LA [cm^2 $plant^{-1}$]	SLM [mg cm^{-2}]
700	81.7^c	17.3^a	88.3^a	11,502^b	5.32^a
550	115.0^a	13.0^b	80.8^b	16,604^a	3.25^c
380 (control)	92.3^b	9.5^c	67.3^c	10,200^c	4.62^b
F-test	*	*	*	*	*
SEM	0.50	0.29	0.47	1.93	0.04
CD at 5%	1.50	0.87	1.40	5.80	0.11

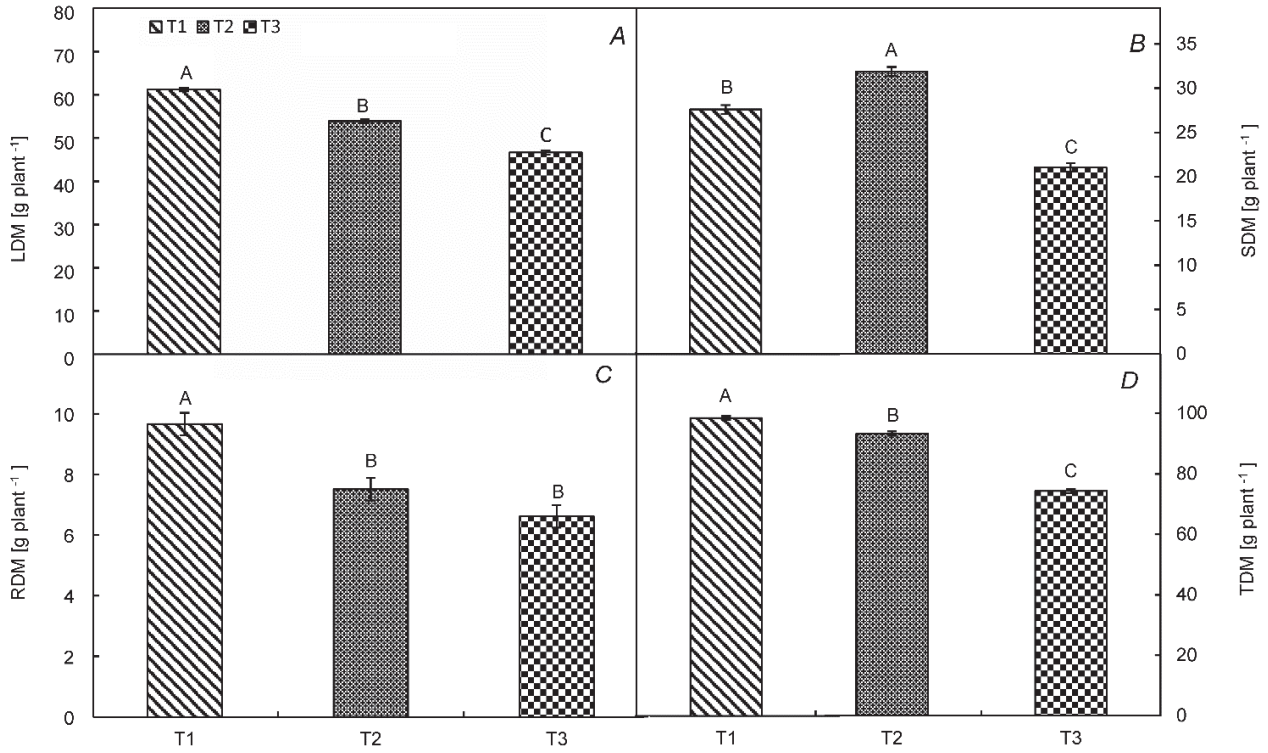

Fig. 1. Effect of different concentrations of CO_2 on leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM), and total dry mass (TDM) of tomato cv. Arka Ashish at peak flowering stage. Means in the column followed by the *same letter* do not differ significantly at p=0.05. T_1 – 700 ppm, T_2 – 550 ppm, T_3 – 380 ppm (control). The values are means ± SE (n = 5).

EC_{700}, followed by EC_{550}, and the control (Table 2). Percentage of fruit set (42.2%) and the fruit yield per plant (5.69 kg) was also higher in the plants grown at EC_{700} (Table 2). The plants grown at EC_{700} showed 46.1 and 125% increase in the fruit yield as compared with the plants grown at EC_{550} and the control, respectively. The plants grown at EC_{550} showed 54% higher fruit yield as compared with the control plants. However, higher average fruit mass (51 g) was recorded at EC_{550}.

Fruit quality: Quality analysis showed that lower phenols, flavonoids, FRAP, TSS, and titratable acidity values were recorded in the plants grown at the two EC concentrations as compared with the control plants (Table 3). Ascorbic acid content was 16.1 and 29.0% higher at EC_{700} and EC_{550}, respectively, as compared with the control plants. Car and lycopene contents were 9.1 and 9.3% lower at EC_{700}, but 2.6 and 1.9% higher at EC_{550}, respectively, as compared with the control plants.

Discussion

The enhanced P_N at EC influences growth and yield in both C_3 and C_4 crops (Kimball *et al*. 2002, Reddy *et al*. 2010). In the present study, we found the increased P_N at both EC concentrations selected. With increase in CO_2 concentrations, decreased g_s and E were observed. A wide range of studies proved that EC reduce E due to reduction in g_s (Tyree and Alexander 1993, Clark *et al*. 1999, Li *et al*. 2004). In tomato cv. Virosa, significant increase in P_N and reduction in E at two CO_2 concentrations (1,000 ppm and 700 ppm) were observed (Behboudian and Lai 1994). The reduction in E coupled with increased P_N, contributes to increase in WUE. A decrease in transpirational water loss is achieved under high CO_2; it results in improved plant water balance due to increased WUE in maize (Arena *et al*. 2011). This could be beneficial effect under climate change conditions. Analysis of 370 plant species through literature survey has shown a 33% increase in yields, 34% reduced E, and high WUE with a doubling of atmospheric CO_2 concentration (Kimball and Idso 1983). The decreased leaf Ψ_s at EC, both at the flowering and fruiting stages, could be due to increased carbohydrate and solute accumulation, resulting in high osmotic adjustment. The decreased Ψ_s at EC was reported by Sionit *et al.* (1981) in wheat plants grown under 1,000 ppm CO_2 under water stress; they recorded lower Ψ_s values with better osmoregulation as compared to the control plants. Increased Ψ_w at EC might be due to stomatal control and it is a probable reason for better plant growth under EC compared to ambient CO_2. A survey of the literature indicates that plants grown at EC maintain higher Ψ_w (Wullschleger *et al*. 2002).

The lower total Chl content recorded in the present study at EC_{700} is in agreement with lower Chl content in *Pinus ponderosa* seedlings grown at EC (Houpis *et al*.

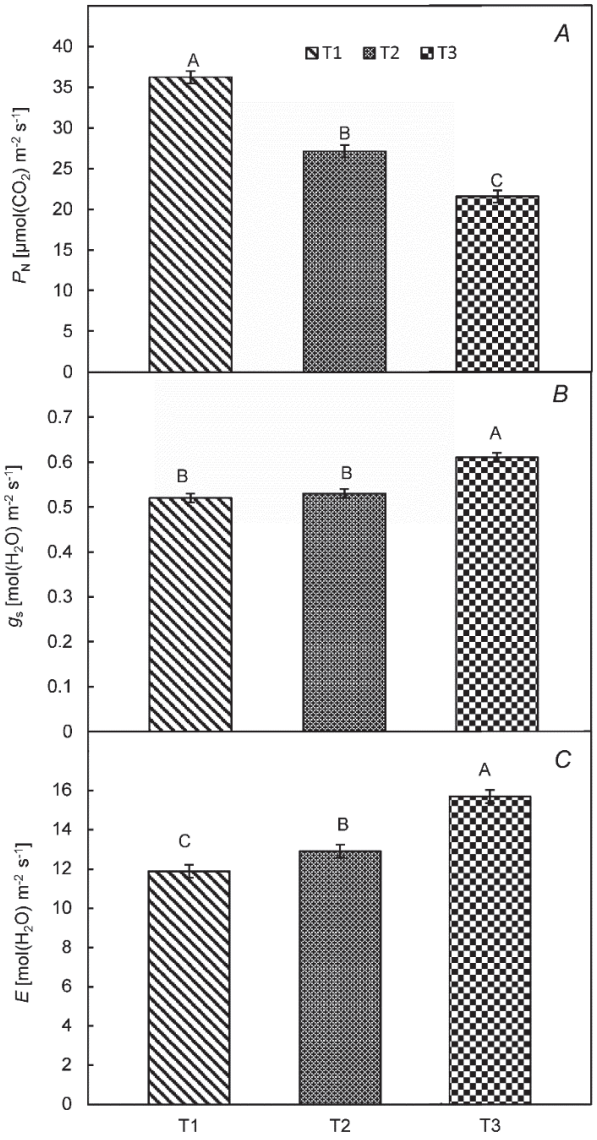

Fig. 2. Effect of different concentrations of CO_2 on net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) of tomato cv. Arka Ashish at fruiting stage. Means in the column followed by the *same letter* do not differ significantly at p=0.05. T_1 – 700 ppm, T_2 – 550 ppm, T_3 – 380 ppm (control). The values are means ± SE (n = 5).

1988). In mungbean (*Vigna radiata* L. Wilczek), Chl content at EC showed a fast decrease from the flowering stage onwards (Haque *et al*. 2005). Total Chl content was reduced by 27 and over 55% in yellow poplar and white oak seedlings grown at EC, respectively (Wullschlerger *et al*. 1992). The lower leaf Chl content could be due to low nitrogen content in leaf (Hocking and Meyer 1991). Leaf nitrogen content was significantly reduced in gladiolus plants grown at EC of 700 and 900 ppm (Kadam *et al*. 2012). Decreased Chl contents were correlated with decreased soluble protein and Rubisco protein in wheat flag leaves in response to EC treatment (Sicher and Bunce 1997).

In the present study, the plant height was significantly higher at EC_{550} compared with EC_{700} and the control plants (Table 1). Shwartz (2002) concluded that EC actually reduces plant growth when combined with higher temperatures, increased precipitation or increased nitrogen in the soil. Gladiolus plants grown at 700 ppm recorded maximum plant height as compared to plants grown at 900 ppm and the control (400 ppm) conditions (Kadam *et al*. 2012). This implies that increased CO_2 increases the plant height up to some extent; beyond that the plant height tends to decrease. The CO_2 concentration up to which the plant height increases may vary with crop. The study showed positive influence of EC on the number of branches and leaves. The increased number of branches and reduction in the plant height in the present study are in agreement with Conroy *et al*. (1990). They observed the decreased plant height with a higher branch number in *Pinus radiata* plants grown at EC, which was attributed to reduced apical dominance.

Concomitant to increase in the number of branches, leaf area per plant also increased (Table 1). The significantly higher leaf area was observed in plants grown at EC as compared with the control plants. The leaf area was lower at EC_{700} than at EC_{550}. However, higher SLM (thicker leaves) was observed at EC_{700} compared to EC_{550}. In tomato, SLM was always higher in CO_2 enriched plants, suggesting that assimilates were preferentially accumulated in the leaves as reserves rather than contributing to leaf expansion (Yelle *et al*. 1990). Gladiolus plants grown at 700 and 900 ppm of CO_2 showed significantly higher leaf area as compared with the control (400 ppm) plants. Between the treatments, leaf area at 700 ppm was higher than at 900 ppm (Kadam *et al*. 2012). Significant increase in leaf thickness was observed in three C_3 species (soybean, loblolly pine, and sweet gum) exposed to constantly monitored CO_2 concentrations of 340, 520, 718, and 910 ppm (Thomas and Harvey 1983). Thus, the CO_2 concentrations at which SLM increases may differ among species and cultivars.

With higher LDM and RDM, the plants grown at EC_{700} had higher TDM compared with EC_{550} and the control (Fig. 1). Tomato seedlings grown at 900 ± 100 ppm of CO_2 recorded higher SDM, RDM, and TDM as compared to seedlings grown at 350 ppm of CO_2 (Fierro *et al*. 1994). Tomato (*L. esculentum* L.) cv. Tiny Tim plants grown at 675 ppm of CO_2 showed increased biomass of 37, 53, 39, and 41% in leaf, stem, root, and total vegetative plant biomass, respectively (Reinert *et al*. 1997). Dry mass enhancement at EC results from increased P_N. The higher P_N at EC presumably contributed to higher biomass production in mungbean (Chowdhury *et al*. 2005). Increased production of biomass at EC was also associated with increased net assimilation rate and relative growth rate. (Mbikayi *et al*.1988). Both higher rates were recorded in tomato plants grown at EC (Yelle *et al*.1990).

The increase in the number of flowers and fruits per

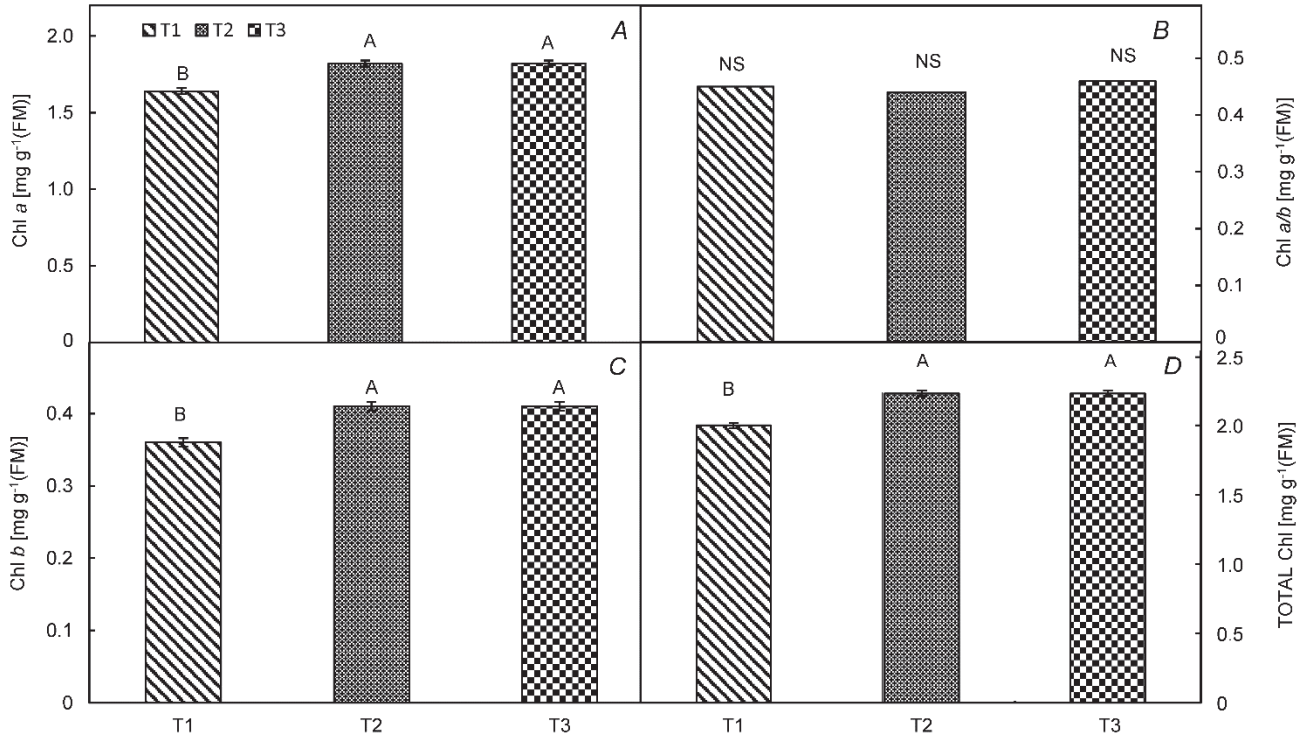

Fig. 3. Effect of different concentrations of CO_2 on chlorophyll (Chl) *a*, Chl *b*, Chl *a*/*b* ratio, and total Chl of tomato cv. Arka Ashish at flowering stage. Means in the column followed by the *same letter* do not differ significantly at p=0.05. T_1 – 700 ppm, T_2 – 550 ppm, T_3 – 380 ppm (control). The values are means ± SE (n = 5).

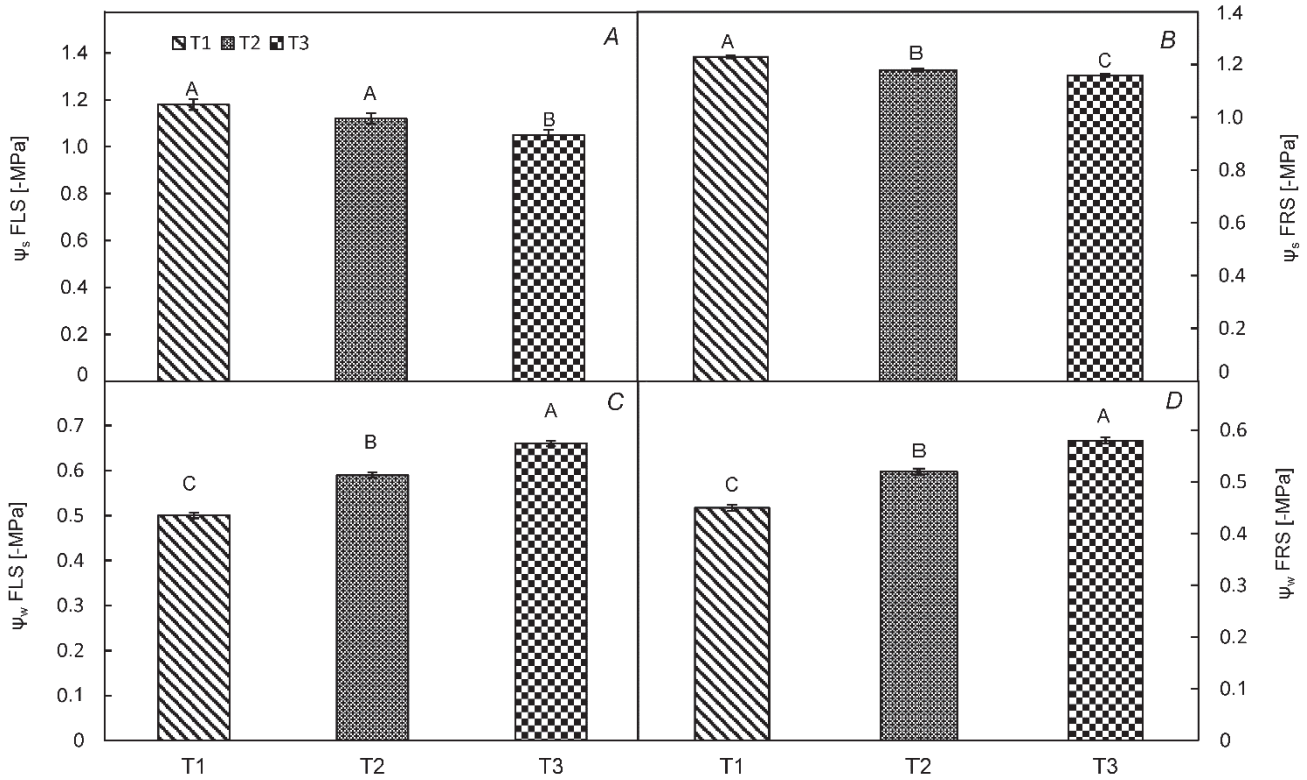

Fig. 4. Effect of different concentrations of CO_2 on osmotic potential (ψ_s) and water potential (ψ_w) of tomato cv. Arka Ashish at the flowering (FLS) and fruiting (FRS) stages. Means in the column followed by the *same letter* do not differ significantly at p=0.05. T_1 – 700 ppm, T_2 – 550 ppm, T_3 – 380 ppm (control). The values are means ± SE (n = 5).

Table 2. Effect of different concentrations of CO_2 on number of flowers per plant (NFLP), number of fruits per plant (NFRP), fruit set percentage (FRSP), average fruit mass (AFRM), and total fruit yield (TFY) of tomato cv. Arka Ashish. Means in the column followed by the *same letter* do not differ significantly at p=0.05. CD – critical difference; SEM – standard error of mean (n = 5). * – significant at P<0.05.

CO_2 [ppm]	NFLP [number]	NFRP [number]	FRSP [%]	AFRM [g]	TFY [kg plant^{-1}]
700	227^a	95.8^a	42.2^a	41.7^b	5.61^a
550	171^b	65.0^b	38.1^b	51.0^a	3.84^b
380 (control)	140^c	45.8^c	32.8^c	41.9^b	2.49^b
F-test	*	*	*	*	*
SEM	0.31	0.52	0.38	0.60	0.46
CD at 5%	0.94	1.56	1.13	1.81	1.38

Table 3. Effect of different concentrations of CO_2 on phenols, flavonoids, FRAP, ascorbic acid, carotenoids, lycopene (all expressed as [mg 100 g^{-1}(FFM)]), TSS, and acidity of tomato cv. Arka Ashish. Means in the column followed by the *same letter* do not differ significantly at p=0.05. CD – critical difference; SEM – standard error of mean (n = 5). * – significant at P<0.05.

CO_2 [ppm]	Phenols	Flavonoids	FRAP	Ascorbic acid	Carotenoids	Lycopene	TSS [°Brix]	Acidity [%]
700	12.4^b	2.30^b	14.8^b	14.4^b	21.1^b	13.7^b	3.10^b	0.32^b
550	8.2^c	1.79^c	11.9^c	16.0^a	23.8^a	15.4^a	3.45^a	0.30^b
380 (control)	14.6^a	2.48^a	15.5^a	12.4^c	23.2^a	15.1^a	3.50^a	0.44^a
F-test	*	*	*	*	*	*	*	*
SEM	0.128	0.018	0.088	0.077	0.328	0.220	0.031	0.011
CD at 5%	0.383	0.055	0.263	0.232	0.984	0.659	0.094	0.033

plant coupled with higher fruit set percentage resulted in the higher fruit yield per plant at both EC_{700} and EC_{550} concentrations (Table 2). However, lower average fruit mass at EC_{700} might be due to the higher number of fruits per plant. Peet *et al.* (1991) suggested that CO_2 enrichment increases sink strength more than source strength in tomatoes, and during the fruit development more carbohydrates may be partitioned to the fruits, leading to higher yields. Similarly, Yelle *et al*. (1990) recorded 80 and 21.5% increase in the early (first three weeks of harvest) and total yields, respectively, in tomato cv. Vedettos. Reinert *et al.* (1997) recorded 22–41% increase of the cumulative tomato yield at increasing CO_2 concentrations from 450–675 ppm. Islam *et al*. (2006) observed significantly larger fruits in tomato cvs. Momotaro, Lady first, and Minicarol grown at EC (850 ± 50 ppm) compared to ambient (350 ± 50 ppm) CO_2 concentration. Thus, EC under climate change conditions could lead to the higher yields in tomato.

Antioxidant compounds of fruits and vegetables have been widely studied and reported as a protection against many diseases, such as cancer, coronary heart disease, cataract, and arthritis (Shivashankara and Acharya 2010). Fruits grown under high CO_2 concentration showed higher radical scavenging ability (Shivashankara *et al*. 2013). The analysis of antioxidant parameters in our study showed the lower content of phenols, flavonoids, FRAP, TSS, and titratable acidity in plants grown at the two EC concentrations as compared with the control plants (Table 3). Decreased phenols and antioxidants activity at EC might be due to lower stress experienced by the plants grown at EC as observed by the higher Ψ_w (Fig. 4*C*,*D*) of these plants. This is in agreement with the earlier reports where higher stress levels have been reported to increase the phenol content (Estiarte *et al*. 1994, Petridis *et al*. 2012).

The ascorbic acid content was high at both EC_{700} and EC_{550} as compared to the control plants (Table 3). High ascorbic acid and sugar contents were recorded in tomato fruits by Islam *et al*. (1996) at EC and at different maturity degrees. The ascorbic acid content of sour orange (*Citrus aurantium* L.) was found to increase by 7% when trees were grown at 400 to 700 ppm of CO_2 in OTCs (Idso *et al*. 2002) and in strawberry under field conditions (Wang *et al*. 2003). Rao *et al*. (1995) observed that atmospheric CO_2 enrichment increases NADPH content and leads to the maintenance of higher activities of antioxidant enzymes, such as glutathione reductase. Consumption of ascorbic acid for maintaining the redox state of the cell *via* glutathione pathway is higher under stress conditions (Foyer *et al*. 1994). In our study, the plants experienced lower stress under EC conditions; this might result in maintenance of the higher ascorbic acid content. Car and lycopene content was lower at EC_{700} and high at EC_{550} and the control plants (Table 3). Significantly lower lycopene content was recorded in tomato (*L. lycopersicum*) fruits at 700 ppm of CO_2 (Helyes *et al*. 2011). Antioxidant contents were very low in tomato grown under high CO_2 concentrations (Barbale 1970, Madsen 1971, 1975; Kimball and Michell 1981).

The results of the present study suggest that EC concentrations had significant positive influence on P_N,

growth, and the yield characteristics combined with decreased g_s and E. Fruit quality also improved at EC up to 550 ppm with respect to ascorbic acid, Car, and lycopene content but decreased at EC_{700}. The antioxidant capacity, phenols, and flavonoids were lower at EC_{550} compared to EC_{700}.

References

Agarwal, S., Rao, V.A.: Tomato lycopene and its role in human health and chronic diseases. – Can. Med. Assoc. J. **163**: 739-744, 2000.

Ainsworth, E.A., Long, S.P.: What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analysis review of the response of photosynthesis, canopy properties and plant production to rising CO_2. – New Phytol. **165**: 351-371, 2005.

AOAC: Titratable acidity of fruit products. – In: Official Methods of Analysis (17th edn.). 942.15. AOAC International, Gaithersburg 2000.

AOAC: Ascorbic acid. – In: Official Methods of Analysis, 967.21, 45.1.14. AOAC International, Gaithersburg 2006.

Arena, C., Vitale L., Virzo De Santo, A.: Influence of irradiance on photosynthesis and PSII photochemical efficiency in maize during short-term exposure at high CO_2 concentration. – Photosynthetica **49**: 267-274, 2011.

Barbale, D.: The influence of the carbon dioxide on the yield and quality of cucumber and tomato in the covered areas. – Augsne un Raza (Riga). **16**: 66-73, 1970.

Behboudian, M.H., Lai, R.: Carbon dioxide enrichment in 'Virosa' tomato plant: Responses to enrichment duration and to temperature. – Hort. Sci. **29**: 1456-1459, 1994.

Benzie, I.F., Strain, J.J.: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. – Anal. Biochem. **239**: 70-76, 1996.

Bunce, J.A.: Leaf transpiration efficiency in four corn cultivars grown at elevated carbon dioxide. – Crop Sci. **52**: 2714-2717, 2012.

Carter, T.R., Jones, R.N., Lu, X. *et al.*: New assessment methods and the characterization of future conditions. – In: Parry, M.L., Canziani, O.F., Palutikof, J.P. *et al.* (ed.): Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Pp. 133-171. Cambridge University Press, Cambridge 2007.

Chowdhury, R.S., Karim, M.A., Haque, M.M. *et al.*: Effects of enhanced level of CO_2 on photosynthesis, nitrogen content and productivity of mungbean (*Vigna radiata* L. WILCZEK). – South Pacific Studies **25**: 97-103, 2005.

Chun, O.K., Kim, D.O., Moon, H.Y. *et al.*: Contribution of individual polyphenolics to total antioxidant capacity of plums. – J. Agr. Food Chem. **51**: 7240-7245, 2003.

Clark, H., Newton, P.C.D., Barker, D.J.: Physiological and morphological responses to elevated CO_2 and a soil moisture deficit of temperate pasture species growing in an established plant community. – J. Exp. Bot. **50**: 233-242, 1999.

Conroy, J.P., Milham, P.J., Mazur, M., Barlow, E.W.: Growth, dry weight partitioning and wood properties of *Pinus radiata* D. Don after 2 years of CO_2 enrichment. – Plant Cell Environ. **13**: 329-337, 1990.

Estiarte, M., Filella, I., Serra, J., Penuelas, J.: Effects of nutrient and water stress on leaf phenolic content of peppers and susceptibility to generalist herbivore *Helicoverpa armigera* (Hubner). – Oecologia **99**: 387-391, 1994.

Fierro, A., Tremblay, N., Gosselin, A.: Supplemental carbon dioxide and light improved tomato and pepper seedling growth and yield. – Hort. Sci. **29**: 152-154, 1994.

Foyer, C.H., Descourvieres, P., Kunert, K.J.: Protection against oxygen radicals: An important defense mechanism studied in transgenic plants. – Plant Cell Environ. **17**: 507-523. 1994.

Haque, M.S., Karim, M.A., Haque, M.M. *et al.*: Effect of elevated CO_2 concentration on growth, chlorophyll content and yield of mungbean (*Vigna radiata* L. Wilczek) genotypes. – Jap. J. Trop. Agr. **49**: 189-196, 2005.

Helyes, L., Lugasi, A., Peli, E., Pek, Z.: Effect of elevated CO_2 on lycopene content of tomato (*Lycopersicon lycopersicum* L. Karsten) fruits. – Acta Aliment. **40**: 80-86, 2011.

Hocking, P.J., Meyer, C.P.: Effect of CO_2 enrichment and nitrogen stress on growth, and partitioning of dry matter and nitrogen in wheat and maize. – Aust. J. Plant Physiol. **18**: 339-356, 1991.

Houpis, J.L.J., Surano, K.A., Cowles, S., Shinn, J.H.: Chlorophyll and carotenoid concentrations in two varieties of *Pinus ponderosa* seedlings subjected to long-term elevated carbon dioxide. – Tree Physiol. **4**: 187-193, 1988.

Idso, S.B., Kimball, B.A., Shaw, P.E. *et al.*: The effect of elevated atmospheric CO_2 on the vitamin C concentration of (sour) orange juice. – Agr. Ecosyst. Environ. **90**: 1-7, 2002.

IPCC: Summary for policymakers. – In: Solomon, S., Qin, D., Manning, M. *et al.* (ed.): Climate Change 2007: The physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Inter-governmental Panel on Climate Change. Cambridge University press, Cambridge, New York 2007.

Islam, S., Khan, S., Garner, J.O.: Elevated atmospheric CO_2 concentration enhances carbohydrate metabolism in developing *Lycopersicon esculentum* Mill. cultivars. – Int. J. Agr. Biol. **8**:157-161, 2006.

Islam, M.S., Matsui, T., Yoshida, Y.: Effect of carbon dioxide enrichment on physico-chemical and enzymatic changes in tomato fruits at various stages of maturity. – Sci. Hort. **65**: 137-149, 1996.

Kadam, G.B., Singh, K.P., Pal, M.: Effect of elevated carbon dioxide levels on morphological and physiological parameters in gladiolus. – Indian J. Hort. **69**: 379-384, 2012.

Kaur, C., Walia, S., Nagal, S. *et al.*: Functional quality and antioxidant composition of selected tomato (*Solanum lycopersicon* L.) cultivars grown in Northern India. – Food Sci. Technol. **50**: 139-145, 2013.

Kimball, B.A., Mitchell, S.T.: Effects of CO_2 enrichment, ventilation, and nutrient concentration on the flavour and vitamin C content of tomato fruit. – Hort. Sci. **16**: 665-666, 1981.

Kimball, B.A., Idso, S.B.: Increasing atmospheric CO_2: effects on crop yield, water use, and climate. – Agr. Water Manage. **7**: 55-72, 1983.

Kimball, B.A., Kobayashi, K., Bindi, M.: Response of agricultural crops to free air CO_2 enhancement. – Adv. Agron. **77**: 293-368, 2002.

Leakey, A.D.B., Xu, F., Gillespie, K.M. *et al.*: The genomic basis for stimulated respiratory carbon loss to the atmosphere by plants growing under elevated CO_2. – P. Natl. Acad. Sci. USA **106**: 3597-3602, 2009.

Leonardi, C., Ambrosino, P., Esposito, F., Fogliano, V.: Antioxidant activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. – J. Agr. Food Chem. **48**: 4723-4727, 2000.

Li, F.S., Kang, S.Z., Zhang, J.H.: Interactive effects of elevated CO_2, nitrogen and drought on leaf area, stomatal conductance, and evapotranspiration of wheat. – Agr. Water Manage. **67**: 221-233, 2004.

Li, Y., Zhang, Y., Zhang, X. *et al.*: Effects of elevated CO_2 and temperature on photosynthesis and leaf traits of an understory dwarf bamboo in subalpine forest zone, China. – Physiol. Plantarum **148**: 261-272, 2013.

Lichtenthaler, H.K.: Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. – Method. Enzymol. **148**: 350-382, 1987.

Long, S.P., Ainsworth, E.A., Rogers, A., Ort, D.R.: Rising atmospheric carbon dioxide: Plants FACE the future. – Ann. Rev. Plant Biol. **55**: 591-628, 2004.

Mackinney, G.: Absorption of light by chlorophyll solution. – J. Biol. Chem. **140**: 315-322, 1941.

Madsen, E.: Effect of CO_2 environment on growth, development, fruit production and fruit quality of tomato from a physiological viewpoint. – In: Chouard, P., de Bilderling, N. (ed.): Phytotronics in Agricultural and Horticultural Research. Pp. 318-330. Bordas, Paris 1975.

Madsen, E.: The influence of CO_2-concentration on the content of ascorbic acid in tomato leaves. – Ugeskr. Agron. **116**: 592-594, 1971.

Mbikayi, N.T., Hileman, D.R., Bhattacharya, N.C. *et al.*: Effects of CO_2 enrichment on the physiology and biomass production in cowpeas (V*igna ungiculata* L.) grown in open top chambers. – In: Proceedings of the International Congress of Plant Physiology 1. Pp. 640-645. India Society for Plant Physiology and Biochemistry, New Delhi 1988.

Moretti, C.L., Mattos, L.M., Calbo, A.G., Sargent, S.A.: Climate changes and potential impacts on postharvest quality of fruit and vegetable crops – a review. – Food Res. Int. **43**: 1824-1832, 2010.

Nilsen, S., Hovland, K., Dons, C., Sletten, S.P.: Effect of CO_2 enrichment on photosynthesis, growth and yield of tomato. – Sci. Hort. **20**: 1-14, 1983.

Peet, M.M., Willits, D.H., Tripp, K.E. *et al.*: CO_2 enrichment responses of chrysanthemum, cucumber and tomato: photosynthesis, growth, nutrient concentrations and yield. Impact of global climatic changes on photosynthesis and plant productivity. – In: Proceedings of the Indo-US workshop held on 8 – 12 January at New Delhi, India: Pp. 193-212, New Delhi 1991.

Petridis, A., Therios, I., Samouris, G. *et al.*: Effect of water deficit on leaf phenolic composition, gas exchange, oxidative damage and antioxidant activity of four Greek olive (*Olea europaea* L.) cultivars. – Plant Physiol. Biochem. **60**: 1-11, 2012.

Radford, P.J.: Growth analysis formulae: Their use and abuse. – Crop Sci. **8**: 171-175, 1967.

Rao, M.V., Hale, B.A., Ormrod, D.P.: Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide. Role of antioxidant enzymes. – Plant Physiol. **109**: 421-432, 1995.

Reddy, A.R., Rasineni, G.K., Raghavendra, A.S.: The impact of global elevated CO_2 concentration on photosynthesis and plant productivity. – Curr. Sci. **99**: 46-57, 2010.

Reinert, R.A., Eason, G., Barton, J.: Growth and fruiting of tomato as influenced by elevated carbon dioxide and ozone. – New Phytol. **137**: 411-420, 1997.

Rogers, H.H., Runion, G.B., Krupa, S.V.: Plant responses to atmospheric CO_2 enrichment with emphasis on roots and rhizosphere. – Environ. Pollut. **83**: 155-189, 1994.

Shivashankara, K.S., Acharya, S.N.: Bioavailability of dietary polyphenols and the cardiovascular diseases. – Open Nutraceuticals J. **3**: 227-241, 2010.

Shivashankara, K.S., Rao, N.K.S., Geetha, G.A.: Impact of climate change on fruit and vegetable quality. – In: Singh, H.P., Rao, N.K.S, Shivashankara, K.S. (ed.): Climate-resilient Horticulture: Adaptation and Mitigation Strategies. Pp. 237-244. Springer, New Delhi, Heidelberg, New York, Dordrecht, London 2013.

Shoaf, T.W., Lium, B.W.: Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. – Limnol. Oceanogr. **21**: 926-928, 1976.

Shwartz, M.: High carbon dioxide levels can retard plant growth, study reveals. – Stanford Report, December 11: 1-5, 2002.

Sicher, R.C., Bunce, J.A.: Relationship of photosynthetic acclimation to changes of Rubisco activity in field-grown winter wheat and barley during growth in elevated carbon dioxide. – Photosynth. Res. **52**: 27-38, 1997.

Singleton, V.J., Rossi, J.A.: Colorimetry of total phenolics with phospho-molybdicphosphotungstic acid reagents. – Am. J. Enol. Viticult. **16**: 144-158, 1965.

Sionit, N., Strain, B.R., Hellmers, H., Kramer, P.J.: Effects of atmospheric CO_2 concentrations and water stress on water relations of wheat. – Bot. Gaz. **142**: 191-196, 1981.

Stewart, A.J., Bozonnet, S., Mullen, W. *et al.*: Occurrence of flavonols in tomatoes and tomato-based products. – J. Agr. Food Chem. **48**: 2663-2669, 2000.

Tajiri, T.: Improvement of bean sprouts production by intermittent treatment with carbon dioxide. – Nippon Shokuhin Kogyo Gakkaishi **32**: 159-169, 1985.

Thomas, J.F., Harvey, C.N.: Leaf anatomy of four species grown under continuous CO_2 enrichment. – Bot. Gaz. **14**: 303-309, 1983.

Tyree, M.T., Alexander, J.D.: Plant water relations and the effects of elevated CO_2: a review and suggestions for future research. – Vegetatio **104/105**: 47-62, 1993.

von Caemmerer, S., Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. – Planta **153**: 376-387, 1981.

Wang, S.Y., Bunce, J.A., Maas, J.L.: Elevated carbon dioxide increases contents of antioxidant compounds in field grown strawberries. – J. Agr. Food. Chem. **51**: 4315-4320, 2003.

Wullschleger, S.D., Norby, R.J., Hendrix, D.L.: Carbon exchange rates, chlorophyll content, and carbohydrate status of two forest tree species exposed to carbon dioxide enrichment – Tree Physiol. **10**: 21-31, 1992.

Wullschleger, S.D., Tschaplinski, T.J., Norby, R.J.: Plant water relations at elevated CO_2 – implications for water-limited environments. – Plant Cell Environ. **25**: 319-331, 2002.

Recent monthly average Mauna Loa CO_2.– www.esrl.noaa.gov/gmd/ccgg/trends/: August 2013.

Yelle, S., Beeson Jr., R.C., Trudel, M.J., Gosselin, A.: Duration of CO_2 enrichment influences growth, yield, and gas exchange of two tomato species. – J. Am. Soc. Hort. Sci. **115**: 52-57, 1990.

Zhao, H., Xu, X., Zhang, Y., Korpelainen, H., Li, C.: Nitrogen deposition limits photosynthetic response to elevated CO_2 differentially in a dioecious species. – Oecologia **165**: 41-45, 2011.