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Elevated CO_2 and temperature effect on growth and physiology of two *Physalis* species

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ABSTRACT

Physalis minima L. and *Physalis peruviana* L. are the two important species in India which grow under wild, weedy or cultivated conditions. Fruits of these species are edible with high nutritional values and may be suitable for stuff for the production of new functional foods and drinks. Climate change has been reported to influences almost every aspects of agriculture. Rise in atmospheric CO_2 and temperature have been reckoned the two most significant variables involved in climate change. A study was conducted in open top chambers (OTCs) to understand the effect of elevated temperature (ambient + 2±0.5 °C) and elevated CO_2 (550±50 ppm) individually and in combination on *P. minima* and *P. peruviana*. Study showed that elevated temperature as well as elevated CO_2 individually and in combination had positive effect on growth and development, rate of photosynthesis, and water use efficiency of both the *Physalis* species. Rate of transpiration and stomatal conductance increased marginally in plants grown at elevated temperature, but a marked decrease was evident at elevated CO_2 individually and in combination with elevated temperature as compared that in plants grown in ambient conditions in both the species. No significant changes were observed in relative water content and relative stress injury under any of the treatments in two species. Treatments- and species- specific changes were evident with respect to the activity of antioxidant enzymes and nitrate reductase, and peptide banding pattern using SDS-PAGE.

INTRODUCTION

Physalis minima is an abundant weed species in India commonly found in crops (*viz*. rice and soybean) as well as in non-cropped areas. The plant is an annual and can grow well in most soil types. It has broad leaves and grows rapidly on disturbed area, which makes it difficult to control. *Physalis peruviana* L. commonly known as 'goldenberry' or 'rasbhari' is considered mainly as weed but also being grown by some farmers on marginal lands. Utilization of wild/weedy species which are nutritionally sound, environmentally well adapted and acceptable to local population may go a long way in alleviating malnutrition and ensuring food security with dietary diversification (Pagare *et al.* 2016). *Physalis* species are nutrient-rich food sources traditionally grown and consumed by local communities in India and many other parts of Asia and Africa. Fruits of *Physalis* species are known to contain carbohydrates, lipids, minerals, vitamins, and phytosterols and â-carotene (Puente *et al.* 2011) and also have been reported to contain several active ingredients for curing diseases like cancer, leukemia, malaria, asthma, hepatitis, dermatitis and rheumatism (Joshi and Joshi 2015).

Climate change influences not only the performance of individual plant species but also can impact interactions with other species at different growth stages (Sarathambal *et al*. 2016). Rise in atmospheric CO_2 and temperature are two most important drivers involved in climate change (Ziska and George 2004). In addition to a thorough assessment of yield potential and nutritional aspects, domestication of a weedy/wild species also demands in depth studies of adaptive potential of such species towards stress and climatic conditions. During the present study, an effort has been made to assess the effect of climate change (elevated temperature and elevated CO_2) on two *Physalis* species (*P. minima* and *P. peruviana*).

MATERIALS AND METHODS

This study was conducted in open top chambers (OTCs) at ICAR- Directorate of Weed Research, Jabalpur (23°102 N 79°562 E) Madhya Pradesh, India. Plants of the *Physalis* species (*P. minima* L. and *P. peruviana* L.) were grown in pots having 6 kg of soil and vermi-compost mixture (3:1 w/w). Treatments included ambient, elevated temperature (ambient + 2 °C), elevated CO_2 (550 ± 50 ppm), and elevated temperature + elevated CO_2. Elevated temperature was achieved through infrared heaters fitted inside the OTC chambers and precisely maintained with automatic control device through on/off mechanism by taking into account ambient temperature as reference at a given time. The desired temperature was maintained round the clock throughout the experiment. Elevated CO_2 treatment was maintained only during sunshine hours only. Different treatments were imposed from 10 days after sowing (DAS) till the end of the experiment. Sampling for different growth parameters was done at 30 and 60 days after treatments (DAT), while physiological and biochemical observations were taken at 60 DAT only. All the observations were made at least three times and analyzed using completely randomized design.

Leaves of plant were separated and total leaf area was measured using area meter (LI-3100C^R, Lincoln, Nebraska, USA). Shoot: root ratio was calculated from the data on dry weight of shoot and root. Growth rate was calculated as RGR (mg/plant/day) = $(W_2 - W_1)/(t_2 - t_1)$, where W_1 and W_2 are dry weights of aboveground parts of plant at time t_1 (30 DAT) and t_2 (60 DAT). Rates of photosynthesis, transpiration and stomatal conductance were measured using integrated portable photosynthesis system (LI-COR, LI-6400, Lincoln, NE, USA). All the observations were taken between 10.00 to 11.30 AM in second leaf from top.

For relative stress injury (RSI), leaf piece (5 cm^2) was washed with distilled water and then placed in a tube containing deionized water and kept at 27 °C for 6 h in diffused light. Then electrical conductivity (EC1) was recorded. Further, the same sample of leaf was again kept in a tube containing deionized water and autoclaved for 15 min to kill sample. After taking out the leaf, electrical conductivity (EC2) was measured at room temperature and RSI was calculated using the formula:

$$RSI = \frac{EC1}{EC2} \times 100$$

For relative water content (RWC), a piece (5 cm^2) was cut and weighed to obtain the fresh weight (FW). The turgid weight (TW) was recorded after keeping the leaves in deionized water for 4 h. Finally, samples were oven-dried at 60°C until constant weight was obtained and then the dry weight (DW) was recorded. The relative water content (RWC) was calculated using the following formula:

$$RWC = \frac{(FW-DW)}{(TW-DW)} \times 100$$

For biochemical observations, leaf samples were taken from second leaf from top and snap frozen in liquid nitrogen, and stored at -80 °C till further use. A 0.1 g of leaves were harvested and ground to a fine powder in liquid nitrogen. Ground powder was homogenized in 1.5 ml of cold phosphate buffer (100 mM, pH 7.0) containing 1% polyvinylpyrrolidone (PVP) and 1 mM EDTA and then centrifuged at 4 °C for 15 min at 10000g. The supernatant was separated and stored on ice till the assay of enzyme activity. This extract was used for the enzyme assay with spectrophotometer of all the enzymes except APX for which extraction buffer was supplemented with 2 mM L-ascorbate. Protein content of extract was determined using dye binding method.

Activity of catalase (EC 1.11.1.6) was determined by monitoring H_2O_2 removal as the decrease in absorbance at 240 nm as suggested by Aebi (1983). Enzyme extract (0.1 ml) was taken and to it, 1.0 ml of potassium phosphate buffer (50 mM, pH 7.0) was added. Reaction was initiated by adding 0.1 ml of 100 mM H_2O_2. The change in absorbance was recorded at 240 nm for 2 min. The enzyme activity was expressed as units/mg protein/min and a change of 0.1 absorbance corresponds to one unit of enzyme activity.

Ascorbate peroxidase (EC 1.11.1.11]) activity was determined by monitoring the oxidation of ascorbate according to the method suggested by Nakano and Asada (1981) with slight modification using reaction mixture consisting of enzyme extract (0.1 ml), 1.0 ml of potassium phosphate buffer (50 mM, pH 7.0) containing ascorbic acid (0.5 mM). The reaction was initiated by the addition of 0.1 ml of H_2O_2 (1 mM) and the decrease in absorbance was recorded at 290 nm for 2 min. The change of 0.1 absorbance corresponds to one unit of enzyme activity and enzyme activity was expressed as units/mg protein/min.

Guaiacol peroxidase (EC 1.11.1.7) activity was measured as suggested by Rao *et al*. (1996) using reaction mixture consisting of 0.1 ml enzyme extract, 1.0 ml 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, and 0.1 ml of 10 mM guaiacol. Reaction was started by adding and 0.1 ml of 10 mM H_2O_2. The increase in absorbance was recorded for 2 min at 470 nm and change of 0.1 absorbance has been taken as one unit and enzyme activity was expressed as units/mg protein/min.

Superoxide dismutase (EC 1.15.1.1) activity was estimated using xanthine-xanthine oxidase system as suggested by Beyer and Fridovich (1987). Enzyme extract (0.1 ml) was taken and to it 0.8 ml of 50 mM potassium phosphate buffer (pH 7.8), 0.05 ml NBT (2.24 mM), 0.1 unit of catalase (Sigma) and 0.1 unit of xanthine oxidase (Sigma) were added. Reaction was initiated by adding 0.05 ml of xanthine (2.36 mM) and change in absorbance was followed upto 2 min at 560 nm. A blank reaction was run using all the components but without sample extract to get the maximum intensity of colour. The enzyme activity was calculated as units (amountof enzyme required to inhibit NBT reduction by 50%) and expressed as units/mg protein/min.

Glutathione reductase (EC 1.11.1.9) activity was estimated by the method as suggested by Smith *et al*. (1988) in a reaction mixture consisting of 1.0ml 100 mM potassium phosphate buffer (pH 7.6), 0.1 ml enzyme extract, 0.05 ml 6 mM 5,5'-dithio-bis (2-nitrobenzoic acid) and 0.1 ml 0.2 mM oxidized glutathione. Reaction was initiated by addition of 0.05 ml of 5 mM NADPH. Change in absorbance at 412 nm was notedupto 2 min. The change of 0.1 absorbance is taken as one unit and enzyme activity was expressed as units/mg protein/min.

The *in vivo* assay of nitrate reductase (EC 1.7.1.2) in leaf was done according to the procedure of Jaworski (1971) with slight modifications as suggested by Ahmad *et al*. (2010). Fresh leaf tissue was cut into 1.0 cm^2 pieces and placed in ice-cold incubation medium containing 2.0 ml of 0.05M potassium phosphate buffer (pH-7.8) and 2.0 ml of 0.4M KNO_3 solution. The tubes were evacuated with a vacuum pump and then incubated in water bath at 28 ºC for 60 min under dark conditions. At the end of incubation period, tubes were kept in boiling water bath for 5 min to stop the enzyme activity and to allow complete leaching of the nitrite in the medium. Nitrite was estimated by the method of Evans and Nason (1953). An aliquot 0.1 ml of the reaction mixture was taken and 1.0 ml each of 1.0% sulphanilamide in 1N-HCl and 0.025% aqueous solution of N-(1-Napthyl)-ethylene di-ammonium dichloride were added. After 30 min, intensity of pink colour was measured by taking absorbance at 540 nm. Amount of nitrite was calculated using standard curve prepared using potassium nitrite solution. The enzyme activity was expressed as ìmole NO_2/mg protein/ min.

Changes in peptide profile of the leaves were determined by SDS-PAGE using discontinuous buffer system.

RESULTS AND DISCUSSION

Effect of elevated temperature and elevated CO_2 on growth and development

Growth and development of both the species (*P. minima* and *P. peruviana*) were affected in treatments and species-specific manner. Elevated temperature (ET) as well as elevated CO_2 (EC) individually and in combination (ET + EC) had positive effect on growth of both the species as compared that plants grown in ambient conditions (**Plate 1**).

Different growth parameters, *viz*. leaf area per plant, plant dry weight, shoot: root ratio and relative growth rate were assessed in two *Physalis* species at 30 and 60 DAT. Leaf area per plant increased marginally in plants grown under elevated temperature, elevated CO_2 individually and in combination as compared that in plants grown in ambient conditions in both the species and at both the

Plate 1. Effect of elevated temperature and elevated CO_2 on growth and development of two *Physalis* species at 60 DAT

sampling stages, however, difference was found to be non-significant. Significantly higher leaf area was noted in *P. peruviana* as compared to *P. minima* under ambient conditions as well under climate change conditions irrespective of sampling stages (**Table 1**). Similarly, plant dry weight also increased to some extent in plants grown under elevated temperature, elevated CO_2 individually and in combination as compared that in plants grown in ambient conditions in both the species and at both the sampling stages, however, again difference among the treatments was non-significant. Higher plant dry weight (almost 1.5 times) was recorded in *P. peruviana* as compared to *P. minima* under ambient conditions as well under climate change conditions at both the sampling stages (**Table 1**). Decrease in shoot: root ratio was evident in *P. minima* under elevated CO_2 alone or in combination of elevated temperature as compared to that under ambient conditions, however, no significant difference was observed at elevated temperature alone. On the other hand, no significant difference was observed in shoot: root ratio among the treatments in *P. peruviana*. Higher shoot: root ratio was observed in *P. minima* as compared to *P. peruviana* under ambient conditions as well under climate change conditions **(Table 1).** Relative growth rate (RGR) was calculated from the dry weights of above ground plants parts between 30 to 60 DAT. Only marginal differences in RGR was observed in plants grown under elevated temperature, elevated CO_2 individually and in combination as compared that in plants grown in ambient conditions. Significantly, higher RGR was recorded in *P. peruviana* as compared to *P. minima* under ambient conditions as well under climate change conditions (**Table 1**).

Enrichment of atmospheric CO_2 has been reported to increase growth of cucumber, tomato and lettuce. Plant growth is the direct effect of CO_2, which is beneficial for plants (Jablonski *et al.* 2002). It is expected that prolonged exposure of plants at increased atmospheric CO_2 concentration will enhance biomass (Poorter and Perez-Soba 2002). Yelle *et al.* (1990) observed that two weeks exposure of high CO_2 (900 ppm) resulted in 55 and 33% increase in leaf area and specific leaf weight, respectively, in tomato plants. Ziska (2003) studied the effect of elevated CO_2 on growth of six species namely *Cirsium arvense*, *Convolvulus arvensis*, *Euphorbia esula*, *Sonchus arvensis*, *Centaurea maculosa* and *Centaurea solstitialis*. Stimulation of plant biomass among species in response elevated CO_2 averaged 46%, with the largest response (+72%) observed for *Cirsium arvense*. Franzaring *et al*. **(**2008) conducted an experiment in *Brassica napus* in FACE system at elevated CO_2 and found that CO_2 enrichment had significantly increased plant height, shoot weight and dry weight of reproductive organs, indicating that plant development and the reallocation from vegetative to generative organs were sped up. It was also suggested that the plant phenology was generally more affected by elevated CO_2 during all growth stages (Lee 2011).

Table 1. Effect of elevated temperature and CO_2 on leaf area, plant dry weight, shoot: root ratio and relative growth rate (RGR) of *P. minima* and *P. peruviana*

Treatment	Leaf area (cm^2)		Plant dry weight (g/plant)		Shoot: Root		RGR (mg dry wt/plant/day)
	30 DAT	60 DAT	30 DAT	60 DAT	30 DAT	60 DAT	
Species							
P. minima	762^{b}	1674^{b}	10.4^{b}	22.2^{b}	16.0^{a}	14.8^{a}	391^{b}
P. peruviana	1482^{a}	3617^{a}	15.5^{a}	30.9^{a}	7.1^{b}	7.2^{b}	513^{a}
Climate change							
Control	1095^{a}	2579^{b}	12.0^{c}	25.4^{c}	12.6^{a}	12.1^{a}	447^{a}
Elevated temperature	1109^{a}	2651^{ab}	12.7^{b}	26.3^{b}	12.4^{a}	11.6^{a}	454^{a}
Elevated CO_2	1136^{a}	2668^{a}	13.2^{b}	26.9^{a}	10.7^{b}	10.1^{b}	459^{a}
Elevated temperature + elevated CO_2	1148^{a}	2684^{a}	13.9^{a}	27.4^{a}	10.8^{b}	10.0^{b}	450^{a}
Combinations							
P. minima × control	736^{b}	1575^{b}	9.6^{c}	21.0^{d}	18.1^{a}	17.0^{a}	378^{b}
P. minima × elevated temperature	748^{b}	1692^{b}	10.2^{c}	21.9^{cd}	17.5^{ab}	16.0^{a}	393^{b}
P. minima × elevated CO_2	781^{b}	1710^{b}	10.8^{c}	22.7^{c}	14.4^{bc}	13.1^{b}	398^{b}
P. minima × (elevated temperature + elevated CO_2)	785^{b}	1718^{b}	11.0c	22.9^{c}	14.2^{c}	13.0^{b}	397^{b}
P. peruviana × control	1455^{a}	3583^{a}	14.3^{b}	29.8^{b}	6.9^{d}	7.3^{c}	516^{a}
P. peruviana × elevated temperature	1471^{a}	3611^{a}	15.2^{b}	30.7^{ab}	7.1^{d}	7.2^{c}	516^{a}
P. peruviana × elevated CO_2	1492^{a}	3626^{a}	15.6^{ab}	31.2^{a}	7.0^{d}	7.1^{c}	519^{a}
P. peruviana × (elevated temperature + elevated CO_2)	1521^{a}	3650^{a}	16.8^{a}	31.9^{a}	7.3^{d}	7.0^{c}	502^{a}

DAT- Days after treatment; the values with same letter cases were not significantly different at $p \leq 0.05$ level.

The impact of these climate events has been documented in many crop species. Temperature is a major determinant in phonological development including flowering (Bahuguna and Jagadish 2015). Temperature and CO_2 together are expected to have significant impacts on key processes involved in physiology and phenology of plants. The beneficial effects of elevated CO_2 have been reported for many crops, however, it is also suggested that elevated temperature would counterbalance the beneficial effects of CO_2. Hence, the response of individual species to combinations of CO_2 and high temperatures is a critical research issue in order to work out impact of future climate change. Our results showed a slightly positive effect of elevated temperature and elevated CO_2 individually and in combination on different aspects of growth and development in two *Physalis* species.

Effect of elevated temperature and elevated CO_2 on physiological aspects

Rates of photosynthesis increased marginally in plants grown at elevated temperature and significantly at elevated CO_2 individually and in combination with elevated temperature as compared that in plants grown in ambient conditions in both the *Physalis* species. Significantly higher rates of photosynthesis were noticed in *P. peruviana* as compared to *P. minima* under ambient conditions as well under climate change conditions. However, increase in photosynthesis rates was more in *P. minima* as compared to that in *P. peruviana* under elevated CO_2 individually and in combination with elevated temperature (**Table 2**). Stomatal conductance is a measure of opening of stomata through which gas exchange takes place. In *P. minima*, a significant increase in stomatal conductance was noticed at elevated temperature; however, it decreased significantly at elevated CO_2 as compared to that under ambient conditions. A marginal increase in stomatal conductance was noticed in combination treatment (elevated temperature + elevated CO_2). Similarly, in *P. peruviana*, slight increase in stomatal conductance was observed at elevated temperature, while a significant decrease in stomatal conductance was evident at elevated CO_2 and elevated temperature + elevated CO_2 as compared to that under ambient conditions. In terms of absolute values, *P. peruviana* exhibited significantly higher stomatal conductance as compared to *P. minima* irrespective of treatments (**Table 2**). Rates of transpiration increased marginally in plants grown at elevated temperature, but a marked decrease in the rate of transpiration was evident at elevated CO_2 individually and in combination with elevated temperature as compared that in plants grown in ambient conditions in both the species. Decrease in transpiration rates was more in *P. minima* as compared to that in *P. peruviana* under elevated CO_2, however, in terms of absolute values; significantly higher rates of transpiration were recorded in *P. peruviana* as compared to *P. minima* under ambient conditions as well under climate change conditions (**Table 2**). Relative stress injury (RSI) is an indicator of membrane damage. Significantly higher values of RSI were noticed in *P. minima* in comparison to *P. peruviana*, however, no

Table 2. Effect of elevated temperature and CO_2 on photosynthesis, stomatal conductance, transpiration, RSI and RWC of *P. minima* and *P. peruviana*

Treatment	Photosynthesis (μmoles/m/s)	Stomatal conductance (m moles/m^2/s)	Transpiration (mmoles/m^2/s)	RSI (%)	RWC (%)
Species					
P. minima	14.8^{b}	115^{b}	4.6^{b}	18.4^{a}	81.5^{a}
P. peruviana	25.4^{a}	157^{a}	5.5^{a}	13.3^{b}	81.9^{a}
Climate change					
Control	16.8^{d}	140^{b}	5.6^{a}	15.6^{b}	82.1^{a}
Elevated temperature	18.6^{c}	154^{a}	5.8^{a}	17.1^{a}	81.1^{a}
Elevated CO_2	22.0^{b}	120^{d}	4.2^{c}	14.9^{b}	81.2^{a}
Elevated temperature + elevated CO_2	23.0^{a}	132^{c}	4.7^{b}	15.7^{ab}	82.3^{a}
Combinations					
P. minima × control	12.1^{d}	115^{c}	5.2^{cd}	18.0^{a}	81.7^{a}
P. minima × elevated temperature	13.8^{d}	130^{bc}	5.4^{bc}	19.5^{a}	80.7^{a}
P. minima × elevated CO_2	16.6^{c}	97^{d}	3.6^{e}	17.7^{ab}	82.4^{a}
P. minima × (elevated temperature + elevated CO_2)	16.8^{c}	119^{c}	4.4^{d}	18.3^{a}	81.3^{a}
P. peruviana × control	21.5^{b}	165^{a}	6.0^{ab}	13.4^{c}	82.6^{a}
P. peruviana × elevated temperature	23.4^{b}	177^{a}	6.2^{a}	14.6^{bc}	81.6^{a}
P. peruviana × elevated CO_2	27.4^{a}	143^{b}	4.1^{cd}	12.1^{c}	80.0^{a}
P. peruviana × (elevated temperature + elevated CO_2)	29.4^{a}	144^{b}	4.8^{cd}	13.0^{c}	83.4^{a}

The values with same letter cases are not significantly different at $p \leq 0.05$ level.

significant difference in RSI was observed in any of the treatments (**Table 2**). No significant difference in relative water content (RWC) of two *Physalis* species at 60 DAT was observed with respect to treatments and species.

Carbon dioxide, being a substrate for photosynthesis, may have direct effect on photosynthesis and other related gas exchange parameters. Exposure of C_3 plants to elevated CO_2 frequently results in an immediate increase in the rate of CO_2 assimilation (Leishman *et al.* 1999). In another study, Besford *et al.* (1990) found that photosynthesis of tomato leaves exposed to ambient and elevated CO_2 reached the same maximum value during leaf development, however, leaves of plants grown at elevated CO_2 developed more rapidly and exhibited maximum photosynthesis sooner. It was emphasized that increase and decrease in photosynthetic capacity depend on the stage of leaf development in tomato.

In agreement to our findings, decrease in stomatal conductance and transpiration has been reported by Morrison (1985) in both C_3 and C_4 species at elevated CO_2 and contention was further strengthened from significant increase in the transpiration efficiency (CO_2 assimilated per unit of H_2O transpired). Carlson and Bazaz (1980) reported that doubling the CO_2 concentration from 300 to 660 ppm resulted in an increase in water use efficiency in different species to a variable extent (5% in sunflower, 54% in corn, 48% in soybean). Oliva *et al.* (2002) studied the effect of elevated CO_2 (720 ppm) on plants of *Solanum curtilobum* and *S. tuberosum* grown in open top chambers and observed 55 to 59% reduction in stomatal conductance, however such a reduction did not limit the net photosynthetic rate, which was in fact increased by 53%. The transpiration rate was reduced by 16% in both the species while instantaneous water use efficiency increased by 80% in *S. tuberosum* and 90% in *S. curtilobum.* Plants grown under elevated CO_2 also showed 36 and 66% increment in total dry biomass, whereas yields (dry mass of tubers) were increased by 40 and 85 % in *S. tuberosum* and *S. curtilobum*, respectively. Two well documented responses of plants to elevated CO_2 are an increase in the rate of photosynthesis and a decrease in stomatal conductance (Unsworth and Hogsett 1996), and our results are in agreement to above report.

Effect of elevated temperature and elevated CO_2 on activity of enzymes

Effect of elevated temperature and elevated CO_2 individually and in combination was studied on the activity of important enzymes involved in antioxidant defence (catalase, ascorbate peroxidase, guaiacol peroxidase, superoxide dismutase and glutathione reductase) and nitrate reductase in the leaves of two *Physalis* species (*P. minima* and *P. peruviana*) at 60 DAT. In *P. minima*, a significant increase in catalase activity was recorded in plants grown at elevated temperature alone and in combination with elevated CO_2 as compared that in plants grown in ambient conditions, however, at elevated CO_2 alone, no significant deference was observed in catalase activity. In *P. peruviana*, increase in the activity of catalase was also evident at elevated temperature and in combination treatment (elevated temperature + elevated CO_2), while a decrease was observed at elevated CO_2. In terms of absolute values, higher activity of catalase was observed in *P. minima* as compared *P. peruviana* under climate change conditions, however under ambient conditions, higher activity was recorded in *P. peruviana* (**Table 3**).

Ascorbate peroxidase is another important enzyme of antioxidant defence pathway. In *P. minima*, ascorbate peroxidase activity decreased significantly in plants grown at elevated temperature and elevated CO_2 as compared to that in plants grown under ambient conditions. In *P. peruviana*, significant decrease in ascorbate peroxidase activity was noticed only at elevated CO_2. In terms of absolute values, not much difference was recorded in the activity of ascorbate peroxidase in two species. Guaiacol peroxidase activity decreased significantly in plants grown at elevated CO_2 and combination treatment (elevated temperature + elevated CO_2) as compared that in plants grown in ambient conditions in *P. minima*, however, in *P. peruviana*, significant decrease in guaiacol peroxidase activity was noticed only at elevated CO_2. No significant change in guaiacol peroxidase activity was observed at elevated temperature in either species. In terms of absolute values, higher activity of guaiacol peroxidase was recorded in *P. minima* as compared to *P. peruviana* under ambient conditions as well under climate change conditions.

Superoxide dismutase mediates the dismutation of superoxide radicals. Activity of superoxide dismutase increased significantly in plants grown at elevated temperature and combination treatment (elevated temperature + elevated CO_2) as compared that in plants grown in ambient conditions in *P. minima*. On the other hand, no significant change was observed in superoxide dismutase activity at different treatments in *P. peruviana*. In terms of

absolute values, higher activity of superoxide dismutase was recorded in *P. peruviana* as compared to *P. minima* under ambient conditions as well under climate change conditions except at elevated temperature where activity of superoxide dismutase was almost similar in two species. No significant change in the activity of glutathione reductase was observed due to treatments in both the *Physalis* species, however, in terms of absolute values; higher activity of glutathione reductase was recorded in *P. peruviana* as compared to *P. minima* irrespective of treatments (**Table 3**).

Effects of elevated CO_2 concentrations on the antioxidant capacity and flavonoid content in strawberry fruits were studied under field conditions. Elevated CO_2 (300 and 600 ppm above ambient) concentrations resulted an increase in ascorbic acid, glutathione in strawberry. To determine whether elevated CO_2 reduces or exacerbates the detrimental effects of O_3 on aspen (*Populustremuloides* Michx.), aspen clones 216 and 271 (O_3 tolerant), and 259 (O_3 sensitive) were exposed to ambient levels of CO_2 and O_3 or elevated levels of CO_2, O_3 or CO_2+O_3 in the FACE facility and physiological and molecular responses were monitored and compared (Wustman *et al*. 2001). Antioxidant activities and phenylalanine ammonialyase (PAL) and 1-aminocyclopropane-1-carboxylic acid (ACC)-oxidase transcript levels showed a general increase in all O_3 treated clones, while remained low in CO_2 and CO_2+O_3 plants, which indicate that the ascorbate-glutathione and phenylpropanoid pathways were activated under ozone stress and suppressed during exposure to elevated CO_2. Our results also in agreement to the above findings as activity of most of the enzymes involved in antioxidants pathways decreased under elevated CO_2. McKee *et al*. (1995) suggested a protective role of elevated CO_2 against O_3 stress in matured flag leaves of wheat and advocated that protective effect of elevated CO_2 is mediated through decrease in stomatal conductance, which reduces O_3 influx received by the plant. High temperature-induced oxidative stress has been reported by many researchers (Larkindale and Knight 2002). In the present study, changes in different component of antioxidant defence pathway were evident under elevated temperature alone or in combination of elevated CO_2.

Nitrate reductase (NR) is a key enzyme in the nitrogen assimilation process. In *P. minima*, a significant increase in the activity of nitrate reductase was noticed in plants grown at elevated temperature, elevated CO_2 and combination treatment (elevated temperature + elevated CO_2) as compared to that in plants grown in ambient conditions. In *P. peruviana*, no significant change was observed in nitrate reductase activity at elevated temperature; however, significant increase in nitrate reductase activity was observed at elevated CO_2 and combination treatment as compared to that under ambient conditions. In terms of absolute values, higher activity of nitrate reductase was noticed in *P. peruviana* as compared to *P. minima* irrespective of treatments (**Table 3**). Geiger *et al*. (1999) suggested that higher rates of

Table 3. Effect of elevated temperature and CO_2 on activity of catalase, ascorbate peroxidase, guaiacol peroxidase, superoxide dismutase, glutathione reductase and nitrate reductase (units/mg protein/min) of *P. minima* and *P. peruviana*

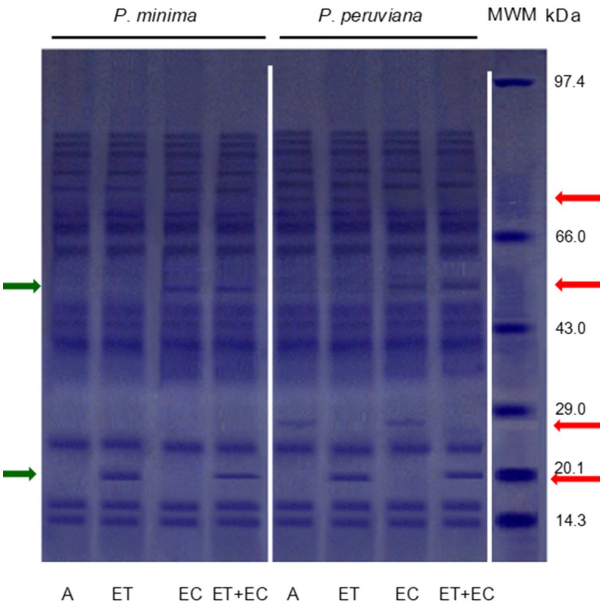
Treatment	Catalase	Ascorbate peroxidase	Guaiacol peroxidase	Superoxide dismutase	Glutathione reductase	Nitrate reductase
Species						
P. minima	4.5^{a}	28.6^{b}	13.1^{a}	4.2^{b}	5.5^{b}	5.8^{b}
P. peruviana	4.2^{b}	31.0^{a}	6.7^{b}	5.4^{a}	6.4^{a}	16.4^{a}
Climate change						
Control	3.7^{c}	31.4^{a}	11.3^{a}	4.4^{c}	6.0^{a}	8.2^{d}
Elevated temperature	5.3^{a}	30.0^{b}	11.5^{a}	5.6^{a}	6.3^{a}	10.3^{c}
Elevated CO_2	3.7^{c}	27.5^{c}	7.7^{c}	4.4^{c}	5.3^{b}	12.9^{b}
Elevated temperature + elevated CO_2	4.6^{b}	30.3^{b}	9.2^{b}	4.9^{b}	6.0^{a}	13.0^{a}
Combinations						
P. minima × control	3.5^{e}	30.8^{ab}	14.9^{a}	3.3^{d}	5.5^{cde}	1.6^{d}
P. minima × elevated temperature	5.7^{a}	28.1^{c}	15.0^{a}	5.4^{a}	5.9^{abcd}	7.0^{c}
P. minima × elevated CO_2	3.8^{e}	25.7^{d}	10.2^{bc}	3.6^{cd}	5.0^{e}	7.2^{c}
P. minima × (elevated temperature + elevated CO_2)	4.9^{b}	29.9^{bc}	12.4^{b}	4.4^{bc}	5.4^{de}	7.5^{c}
P. peruviana × control	4.0^{cd}	31.9^{a}	7.7^{d}	5.5^{a}	6.5^{abc}	14.8^{b}
P. peruviana × elevated temperature	4.8^{b}	31.9^{a}	7.9^{cd}	5.7^{a}	6.8^{a}	13.6^{b}
P. peruviana × elevated CO_2	3.7^{de}	29.4^{bc}	5.2^{e}	5.2^{ab}	5.7^{bcde}	18.7^{a}
P. peruviana × (elevated temperature + elevated CO_2)	4.1^{c}	30.7^{ab}	6.0^{de}	5.3^{a}	6.6^{ab}	18.5^{a}

The values with same letter cases are not significantly different at $p \leq 0.05$ level

nitrate assimilation are required to support faster growth under elevated carbon dioxide. Similarly, increase in nitrate reductase activity under elevated CO_2 was observed in *Vigna radiata* (Sharma and Sengupta 1990). In agreement, our results also showed increase in nitrate reductase activity under elevated CO_2, temperature and combination of these two only in *P. minima*, while in *P. peruviana*, no such change was evident.

Effect of elevated temperature and elevated CO_2 on peptide profile (SDS-PAGE)

Peptide profiling using SDS-PAGE was performed in the leaves of two *Physalis* species grown under ambient condition, elevated temperature, elevated CO_2 and elevated temperature + elevated CO_2 at 60 DAT. A total of 16 bands could be visualized in *P. minima*, while in *P. peruviana*, 18 bands could be resolved. In of *P. minima*, 9th band from top appeared only at elevated CO_2, and elevated temperature + elevated CO_2 and seems to be specific to elevated CO_2. Similarly, 14th band from top appeared only at elevated temperature and elevated temperature + elevated $CO_2$and can considered specific to elevated temperature. In *P. peruviana*, 6th band from top appeared under control conditions and at elevated temperature, but absent in other treatments indicating that this band disappeared whenever plants were exposed to elevated CO_2. Band number 10 from top appeared only at elevated CO_2, and elevated temperature + elevated CO_2 indicating that this band is specific to elevated CO_2. On the other hands, band number 16 from top appeared only at elevated temperature and elevated temperature + elevated CO_2indicating its specificity to elevated temperature. Band number 6 and 14 in *P. peruviana* seems to be species-specific (**Figure 1**).



MWM: molecular weight markers, A: ambient, ET: elevated temperature (ambient + 2 ºC), EC- Elevated CO_2 (550 ppm), ET + EC: elevated temperature + Elevated CO_2

Figure 1. Changes in peptide profile (SDS-PAGE) in two *Physalis* species (*P. minima* and *P. peruviana*) under elevated temperature and elevated CO_2. Arrows indicate position of differentially expressed peptides.

Protein is important macromolecule for any living organism. Till date, not much work has been done which can conclusively explain the extent and nature of the effects of elevated CO_2 on protein metabolism in plants. Meta-analysis techniques were used to examine the effect of elevated atmospheric CO_2 on the protein concentration of major food crops. Bokhari *et al*. (2007) studied proteomic response of rice seedling leaves to elevated CO_2 levels using 2-dimensional electrophoresis (2-DE). It was found that 57 spots showing differential expression patterns. Further analysis using MALDI-TOF/TOF-MS revealed that most of the proteins belonged to photosynthesis, carbon metabolism, and energy pathways. Several molecular chaperones and ascorbate peroxidase were also found to respond to higher CO_2 levels. Concomitant with the down regulation of photosynthesis and stomatal conductance, the levels of enzymes of the regeneration phase of the Calvin cycle were also decreased. Effects of elevated CO_2 (double of ambient) on soluble protein content and 2-dimensional electrophoretic pattern were studied in rice leaves grown in CO_2 controlled chambers (Fukayama *et al.* 2009). Soluble protein contents were slightly decreased in leaves grown under elevated CO_2, whereas the polypeptide profiles of soluble protein analyzed by 2-DE using the same amount of protein were totally unchanged between ambient and elevated CO_2.

In conclusion, *P. peruviana* was found to possess more resiliency against changes in climatic factors as compared to *P. minima*. Such attribute can be ascribed to higher relative growth rate and better inherent antioxidant potential under climate change regime.

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