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Research Article

Carbon Dioxide Enrichment Induced Drought Tolerance Responses in Amaranthus (*Amaranthus tricolor* L.)

Dheeraj Chatti*, R.V. Manju

Department of Plant Physiology, College of Agriculture, Vellayani, Kerala Agricultural University, Thiruvananthapuram, Kerala, India

Abstract

Based on reports by the IPCC (Intergovernmental Panel on Climate Change) atmospheric CO_2 concentration is rising. Elevated CO_2 concentration has been found to ameliorate water stress in the majority of species studied. Physiological and biochemical basis of varietal responses of amaranthus to water stress conditions and their modifications under elevated CO_2 environment was studied by conducting a pot culture experiment conducted with three varieties of amaranthus i.e, Arun (V_1), CO -1 (V_2) and Renusree (V_3). Open Top Chambers (OTC) system was used for subjecting the plants to elevated CO_2 environments. During their critical stages of development, plants were subjected to water stress and then were allowed to recover. Various biochemical parameters like protein content, starch content and antioxidants like Phenol content, Superoxide dismutase (SOD) activity along with total dry matter content were analysed to understand the effect of CO_2 enrichment on drought tolerance. Elevated CO_2 was found to increase total dry matter production (0.99 g), phenol content (25.46 mg/g), SOD activity (1.65 $g^{-1}minute^{-1}$) and decrease starch (3.22 mg/g) and protein (13.90 mg/g) content.

The challenges extended by the changing climate situations along with the progressively reducing water availability, studies on drought tolerance responses as modified by elevated CO_2 environments is highly significant. The results of this study will also help in designing improved production technologies with suitable varieties for a changing climatic scenario.

Keywords: Climate change, Global warming, Elevated CO_2, water stress, Drought tolerance, antioxidants, oxidative stress

***Correspondence**
Author: Dheeraj Chatti
Email: chattidheeraj@gmail.com

Introduction

Agricultural productivity is decreasing worldwide due to detrimental effects of various biotic and abiotic stresses. Drought, which is the most important environmental stress, severely impairs plant growth and development, limits plant production and the performance of crop plants more than any other environmental factor. According to the Intergovernmental Panel on Climate Change (IPCC), by the year 2050, the current atmospheric CO_2 level of 384 μmol l^{-1} (800 Gt) is predicted to rise to 1000 Gt. Human-caused increases in atmospheric CO_2 concentration are thought to be largely responsible for recent increases in global mean surface temperatures and are expected to increase by 1.4 to over 5ºC by 2100 [1, 2]. Increase in global average temperatures would further result in drastic shifts in the annual precipitation with a 20% reduction per year and about 20% loss in soil moisture [3] and can increase potential evapotranspiration, leading to a more severe water deficit in arid and semiarid areas.

CO_2 is the ‘food’ that sustains essentially all plants on the face of the earth as well as those in the sea. Carbon dioxide being a primary substrate for photosynthesis, a rising concentration will have a direct effect on plant growth by enhancing the production of assimilates although not proportional. The indirect effects of rising carbon dioxide concentration include changes induced by other environmental variables which occur as a result of the effect of increased CO_2 on global climate.

Under elevated CO_2 conditions, plants adapt many mechanisms to cope up with the stress factors. Plant growth is nearly always stimulated by elevation of CO_2. Photosynthesis increases, more plant biomass accumulates per unit of water consumed, and economic yield is enhanced. Interactive studies on water availability and elevated CO_2 show that there will be a partial closure of stomata due to increased CO_2 concentration in the substomatal cavity decreasing partial pressure of CO_2 in the leaf and this CO_2 - dependent amplification of stomatal response could improve water use efficiency at the leaf and whole plant level.

Materials and methods

A pot culture experiment was conducted at the Department of Plant Physiology, College of Agriculture, Vellayani, under Kerala Agricultural University with three varieties of amaranthus i.e, Arun (V_1), CO -1 (V_2) and Renusree (V_3). The Open Top Chamber (OTC) system was used as a technology for CO_2 enrichment. One Open Top Chamber with CO_2 level of 600 ppm (T1) was used for CO_2 enrichment and a second control chamber for assessing chamber effect (T2) (Figure 1). A set of experimental plants was maintained in the open field as control (T3). One month old potted plants of amaranthus were shifted to the CO_2 treatment conditions inside OTC. Plants were maintained under well irrigated conditions for one week. Water stress conditions were imposed by withdrawing irrigation for two days after shifting and stress observations were taken. Thereafter plants were re-watered and on the 5^{th} day of re-watering, recovery observations were taken. The experiment was laid out in CRD with three treatments three replications and two stress levels.

Elevated CO_2 environment was created by using Open Top Chambers (OTC). Open Top Chambers (OTC) are square type chambers constructed to maintain near natural conditions and elevated CO_2 conditions for experimental purposes. OTCs were made with metal frame and covered with a 200 micron UV poly sheet with $1m^2$ opening at the top. Two such chambers were built in the experimental field; one serves to create CO_2 enrichment and the other serves as control chamber to study the chamber effects. Elevated CO_2 was released into the chamber from a CO_2 cylinder in a controlled manner.

The sum of root and shoot dry weights were taken as the total dry matter yield. The total soluble protein content of leaf samples was estimated using simple protein dye binding assay [4] using bovine serum albumin (BSA) as the standard. The protein content was expressed as mg/g FW. The estimation of starch in plants was done following the Anthrone method [5]. Estimation of phenols was done by Folin-Ciocalteau method [6] and the Superoxide dismutase (SOD) activity was quantified following the method described by Kakkar *et al*.[7] and expressed in terms of g^{-1} $activity^{-1}$.

Figure 1 Open Top Chamber for CO_2 enrichment

Table 1 Effect of elevated CO_2 on various parameters after stress and re-watering in amaranthus

Parameter	Elevated CO_2(T1)		Ambient CO_2(T2)		Open control(T3)		CD(0.05)	
	Stress	Recovery	Stress	Recovery	Stress	Recovery	Stress	Recovery
Dry matter production (g)	0.99	0.97	0.85	0.87	0.29	0.80	32.05	32.5
Protein content (mg/g)	13.90	16.40	14.45	21.60	15.73	25.56	5.80	3.98
Starch content (mg/g)	3.22	2.78	2.28	1.97	3.54	2.53	1.47	0.88
Phenol content (mg/g)	25.46	7.36	7.10	2.75	1.49	5.28	2.86	6.92
SOD activity ($g^{-1}minute^{-1}$)	1.65	2.05	0.93	2.59	0.84	1.94	0.12	0.06

Results

Dry Matter Production

Dry matter production was found significantly higher under elevated CO_2 (0.99 g) compared to control chamber (0.85 g) and open control (0.29 g) after stress (Table 1). Among the varieties, highest mean value for dry matter production was recorded for varieties CO-1 (0.76 g) and Renusree (0.75 g) (Table 2). After re-watering, highest dry matter production was recorded under treatment elevated CO_2 (0.97 g), which was found significantly higher compared to treatment open control (0.80 g) (Table 1). Among the varieties, highest dry matter production was recorded for the variety CO-1 (1.10 g), which was found significantly higher, compared to Arun (0.78 g) and Renusree (0.76 g) (Table 2).

Table 2 Effect of elevated CO_2 on various parameters after stress and re-watering in three varieties of amaranthus

Parameter	Arun (V1)		CO-1(V2)		Renusree (V3)		CD(0.05)	
	Stress	Recovery	Stress	Recovery	Stress	Recovery	Stress	Recovery
Dry matter production (g)	0.62	0.78	0.76	1.10	0.75	0.76	32.05	32.5
Protein content (mg/g)	12.55	17.87	15.75	27.90	15.79	17.78	5.80	3.98
Starch content (mg/g)	.3.39	2.80	2.98	2.21	2.67	2.27	1.47	0.88
Phenol content (mg/g)	10.94	4.81	14.05	5.24	9.07	5.33	2.86	6.92
SOD activity ($g^{-1}minute^{-1}$)	1.28	2.05	1.33	2.59	0.82	1.94	0.12	0.06

Total Soluble Protein

Reduction in total soluble protein content was observed under elevated CO_2 after stress. Protein content was observed lower under treatment T1 (13.90 mg g^{-1}) followed by treatment T2 (14.45 mg g^{-1}) and treatment T3 (15.73 mg g^{-1}) (Table 1). Among the varieties, highest protein content was recorded for the variety Renusree (15.79 mg g^{-1}) which was significantly higher than Arun (12.55 mg g^{-1}) (Table 2). After re-watering, significant reduction in protein content under treatment T1 (16.40 mg g^{-1}) was observed followed by treatment T2 (21.60 mg g^{-1}) and treatment T3 (25.56 mg g^{-1}) (Table 1). Among the varieties, significantly higher total soluble protein content was recorded for CO-1 (27.90 mg g^{-1}) compared to Anagha and Renusree (Table 2).

Starch

Starch content under elevated CO_2 (3.22 mg g^{-1}) was observed lower compared to open control (3.54 mg g^{-1}) and significantly higher compared to control chamber (2.28 mg g^{-1}) after stress (Table 1). Highest starch content among varieties was recorded for Arun (3.39 mg g^{-1}) followed by CO-1(2.98 mg g^{-1}) and Renusree (2.67 mg g^{-1}) (Table 2). After re-watering, highest starch content was recorded under elevated CO_2 (2.78 mg g^{-1}) which was significantly higher than control chamber (1.97 mg g^{-1}) (Table 1). Among the varieties, significantly higher starch content was recorded for the variety Arun (2.80 mg g^{-1}) compared to CO-1 and Renusree (Table 2).

Phenol Content

Elevated CO_2 was found to have highly significant effect on phenol content after stress. Significant increase in phenol content was observed under elevated CO_2 (25.46 mg g^{-1}) followed by control chamber (7.10 mg g^{-1}) and open control (1.49 mg g^{-1}) (Table 1). Among the varieties, highest phenol content was recorded for the variety CO-1(14.05 mg g^{-1}) followed by Arun (10.94 mg g^{-1}) and Renusree (9.07 mg g^{-1}) (Table 2). After re-watering, highest phenol content was observed under elevated CO_2 (7.36 mg g^{-1}), which was significantly higher compared to control chamber(2.75 mg g^{-1}) (Table 1). Among the varieties highest phenol content was recorded for Renusree (5.33 mg g^{-1}) (Table 2).

SOD

Elevated CO_2 was found to have positive and significant influence on SOD activity after stress. Significantly higher SOD activity was recorded under treatment T1 (1.65 $g^{-1}minute^{-1}$) than treatment T2 (0.93 $g^{-1}minute^{-1}$) and treatment T3 (0.84 $g^{-1}minute^{-1}$) (Table 1). CO-1 recorded highest SOD activity (1.33 $g^{-1}minute^{-1}$) among the varieties and it was significantly higher than Renusree (0.82 $g^{-1}minute^{-1}$) (Table 2). SOD activity under T1 (2.05 $g^{-1}minute^{-1}$) was observed higher compared to T3 (1.94 $g^{-1}minute^{-1}$) and lower compared to treatment T2 (2.59 $g^{-1}minute^{-1}$) after re-watering (Table 1). Among the varieties, highest SOD was recorded for the variety CO-1(2.59 $g^{-1}minute^{-1}$) which was significantly higher than Renusree (1.94 $g^{-1}minute^{-1}$) (Table 2).

Discussion

Exposure of plants to elevated CO_2 conditions influences both primary and secondary metabolites [8]. Elevated CO_2 decreased soluble protein content in spring wheat cultivars [9] decline in soluble protein contents could be largely due to a reduction in ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBISCO) protein. The reduction in protein contents in plants grown under doubled CO_2 were delayed after stress compared to control which suggested that drought-induced oxidative damage to protein had been significantly reduced by doubled CO_2, possibly by protecting the Rubisco protein from oxidative damage. Protein accumulation was found to be lowest in barley leaves [10] enriched with high CO_2 concentration. In the present experiment soluble protein content was found decreasing under elevated CO_2 after stress and re-watering compared to control. In amaranthus, per cent reduction in total soluble protein content under enriched CO_2 chamber was recorded as 11.63 and 35.80 after stress and re-watering respectively in comparison with open control. These results are in complete agreement with research done in sun flower and maize [11, 12], where they found reduction in protein content with CO_2 enrichment.

Under elevated CO_2 condition, carbohydrates accumulation in plant tissues is most pronounced since their intensity of usage is lower than their production under these conditions [13, 14]. Accumulation of carbohydrates in leaves is one of the most important responses observed in plants to elevated atmospheric CO_2 [15]. Elevated CO_2 conditions enhance the soluble sugar content of *Labisia pumila* [16]. In the current study on amaranthus, under enriched CO_2 treatment, there observed a decline by 9.03% in starch content after stress but after re-watering, 7.19% increment was observed compared to open control. Several reports on effect of elevated CO_2 on carbohydrate accumulation were made by several workers. Elevated CO_2 (800 µmol mol^{-1} CO_2) increased carbohydrates accumulation in tomato plants [17] increased starch content with CO_2 treatment was reported in soybean [18]. High carbohydrate accumulation was reported in strawberry under elevated CO_2 condition [19].

Since the metabolism of carbohydrates is essential for the synthesis of amino acids, it is reasonable to assume that the effects of CO_2 enrichment can be similar for free amino acids also [20]. Ample carbon was available to support amino acid synthesis and to increase in soluble amino acids under CO_2 enrichment.

In the present study on amaranthus, significant increment of 25.21% and 14.84% free amino acid content was recorded under elevated CO_2 in comparison with open control after stress and re-watering respectively. Increase in soluble amino acid content under CO_2 enrichment has been reported in soybean and tobacco [21, 22].

Phenolics are aromatic benzene ring compounds produced by plants mainly to defend stress. These secondary metabolites play important roles in plant development, particularly in lignin and pigment biosynthesis. Elevated CO_2 leads to increased concentration of soluble phenolic compounds in leaves [23]. Elevated CO_2 induced increase in the total phenol content in wheat leaves [24, 25]. Elevated CO_2 was shown to have significant impact on phenol content in the current study. In amaranthus, a rise in phenol content by 94.14% after stress and 28.2% after re-watering was observed (Figure 2).

Various abiotic stresses can lead to the over production of Reactive oxygen species (ROS) in plants which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress. Antioxidants are the substances that protect cell from the oxidative damage. The antioxidant studied in this experiment is superoxide dismutase (SOD). Elevated CO_2 was shown to have positive and significant influence on antioxidant production and activity. In the present study on amaranthus, 49.39% (after stress) and 5.36% (after re-watering) rise in SOD activity was recorded under elevated CO_2 compared to open control (Figure 3). Oxidative stresses do occur with water stress. The enhanced rates of photosynthesis and carbohydrate production resulting from atmospheric CO_2 enrichment can enable plants to defend with such stresses by providing more of the raw materials needed for antioxidant enzyme synthesis. This may be the reason for higher production of antioxidants under such a situation. The results were in accordance with earlier findings [26]. Several contradicting results were also reported. SOD activity declined significantly after water stress for 10 days in two spring wheat cultivars (*Triticum aestivum* L. Longchun 292 and Longchun 8139) regardless of ambient or doubled CO_2 [9]. Activities of superoxide dismutase, catalase and ascorbate peroxidase were declined under elevated CO_2 in *Catharanthus roseus* [27].

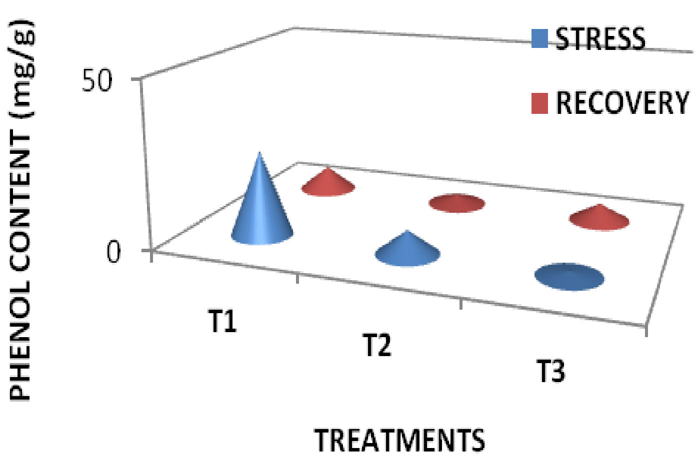

Figure 2 Effect of elevated CO_2 on phenol content (mg/g)

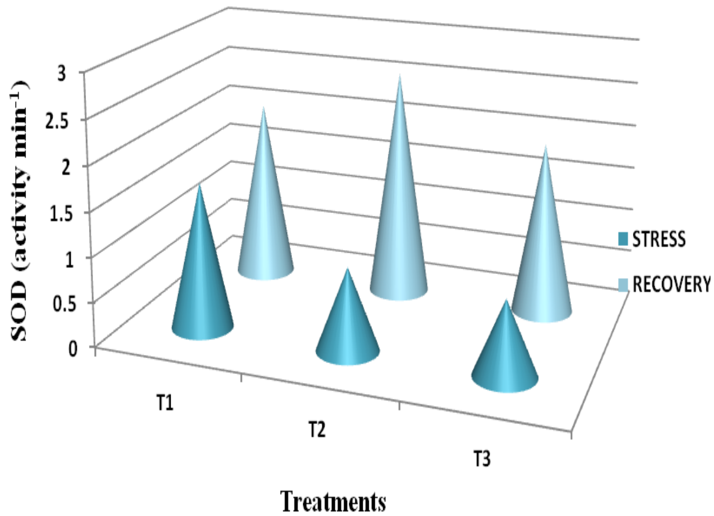

Figure 3 Effect of elevated CO_2 on SOD content after stress and rewatering in amaranthus

Conclusion

Considering all biochemical studies conducted, it can be concluded that carbon dioxide enrichment has a positive role in improving water stress tolerance and recovery responses in the case of amaranthus. It was achieved mainly due to better activation of defense mechanisms. High total dry matter in amaranthus for the variety CO-1 was achieved in elevated CO_2 under water stress conditions because of activation of drought tolerance mechanisms like accumulation of more antioxidants like SOD, phenol which helps to fight against oxidative stress induced by drought. Varietal variation was found existing in Carbon dioxide enrichment induced drought tolerance responses which gives better scope for the selection of suitable varieties for a changing climatic scenario.

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