Research Article

Physiological and Molecular Analyses of Drought Tolerance Responses in Amaranthus (*Amaranthus tricolor* L.) under Elevated Carbon Dioxide Environments

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Abstract

The level of CO_2 in the atmosphere is rising at an unprecedented rate. According to NOAA, 2014 global concentration of CO_2 has reached 400 ppm for the first time in recorded history and its concentration is expected to increase about 550 ppm within next 50-100 years. Carbon dioxide is the key substrate for plant growth as it represents the sole source for carbon (C), which is limited by present-day CO_2 concentrations (Webber *et al*., 1994). Increased CO_2 concentration has been found to increase water use efficiency in the majority of species studied. To understand the physiological and biochemical basis of varietal responses of amaranthus to water stress conditions and to study their modifications under CO_2 enriched environment, a pot culture experiment was conducted with three varieties of amaranthus i.e, Arun (V_1), CO -1 (V_2) and Renusree (V_3) in the Open Top Chambers (OTC) system. Plants were subjected to water stress during their critical stages of development and then were allowed to recover. Various biochemical and growth parameters like free amino acids, reducing sugar content, total dry matter content and antioxidants like ascorbic acid content were analysed to understand the effect of CO_2 enrichment on drought tolerance. Electrophoresis analysis of proteins was analysed using SDS PAGE. Elevated CO_2 was found to increase total dry matter production (0.99 g), reducing sugars (15.98 mg/g), free amino acids (1.19 mg/g) and ascorbic acid (116.31 mg/100g) content. Agriculture and allied sectors being the most vulnerable to climate change; it is an urgent imperative that adaptive strategies need to be developed for sustaining an enhancing agricultural production for achieving food security to an ever increasing population and to design improved production technologies with suitable varieties for a changing climatic scenario.

Keywords: Climate change, Global warming, Elevated CO_2, Water stress, Drought tolerance, Antioxidants, Reducing sugars, Free amini acid

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Introduction

The level of CO_2 in the atmosphere is rising at an unprecedented rate. According to NOAA, 2014 global concentration of CO_2 has reached 400 ppm for the first time in recorded history [1]. This rise, along with other trace gases in the atmosphere is widely thought to be a primary factor driving global climate change. Moreover the report of IPCC, 2012 [2] has reconfirmed the increasingly strong evidence of global climate change and projected that the globally averaged temperature of the air would rise by 1.8–6.4°C by the end of the century. 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.

Carbon dioxide is the key substrate for plant growth as it represents the sole source for carbon (C), which is limited by present-day CO_2 concentrations [4]. Increased CO_2 concentration has been found to ameliorate water stress in the majority of species studied. Under elevated CO_2 conditions, plants adopt many mechanisms to maintain high water potential and to resist water scarcity.

Different experimental techniques led to the conclusion that plants grown under elevated CO_2 possessed increased root surface and root volume due to increased allocation of carbon to root growth [5]. Such increase in the surface area of roots enables the plants grown under elevated CO_2 to exploit more water even from deep soil layers. However, the decrease in stomatal conductance may also be offset by increased leaf area in plants grown under elevated CO_2 and thus water use by the whole plant may not be proportional to stomatal conductance. CO_2 enrichment causes stimulation of photosynthesis, inhibition of photorespiration and increase in nitrogen use efficiency (NUE) and water use efficiency (WUE) [6,7], resulting in higher biomass production and changes in plant elemental composition. Amaranthus is the traditional leafy vegetable which has, over the centuries, provided rural communities with food and nutritional security. Considering the role of elevated CO_2 in the drought tolerance responses, the present investigation will help to understand the growth performance, productivity and water stress tolerance capacity of amaranthus under enriched CO_2 conditions.

Materials and methods

To understand the physiological and biochemical basis of varietal responses of amaranthus to water stress conditions and to study their modifications under CO_2 enriched environment, a pot culture experiment was conducted with three varieties of amaranthus i.e, Arun (V_1), CO -1 (V_2) and Renusree (V_3) in the Open Top Chambers (OTC) system at the Department of Plant Physiology, College of Agriculture, Vellayani, under Kerala Agricultural University. Two Open Top Chambers, one with CO_2 level of 600 ppm (T1) for assessing elevated CO_2 effect and a second control chamber for assessing chamber effect (T2) were used. 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. 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.

Open Top Chambers (OTC) are square type chambers constructed to maintain near natural conditions and elevated CO_2 conditions for experimental purposes. The basic structure of OTC was built of metal frame and covered with a 200 micron UV poly sheet. The chamber has $1m^2$ opening at the top to reduce the temperature build up. Two such chambers were built in the experimental field; one serves to impose CO_2 enrichment and the other serves as control chamber to study the chamber effects. CO_2 was released into the chamber from a CO_2 cylinder in a controlled manner. Microclimatic parameters (temperature, humidity and light) were measured within and outside the OTCs with the help of sensors on a real time basis (Figure 1).

The sum of root and shoot dry weights were taken as the total dry matter yield. The estimation of reducing sugars in plants was done following Dinirto Salicylic acid (DNS) method [8] and the total free amino acids were estimated by the Ninhydrin method [9]. Both of these parameters were expressed in terms of mg/g. The ascorbic acid content in plants was estimated volumetrically by the method explained by Sadasivam and Manickam [10] and expressed in terms of mg/100g. SDS - PAGE Electrophoresis separation of soluble protein and Rubisco in amaranthus leaves were carried out [11]. The results based on statistically analysed data pertaining to the experiment conducted during the course of investigation are presented below.

Figure 1 Various sensors and CO_2 cylinders used in open top chamber

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).

Reducing Sugars

Elevated CO_2 was found to have highly significant effect on reducing sugars content after stress. Significant increase in reducing sugars content was observed under elevated CO_2 (15.98 mg g^{-1}) followed by control chamber (13.60 mg g^{-1}) and open control (11.40 mg g^{-1}) (Table 1). Significantly higher reducing sugars content was observed for the variety Arun (14.96 mg g^{-1}) followed by CO-1 (13.48 mg g^{-1}) and Renusree (12.53 mg g^{-1}). After re-watering also, significantly increasing trend in reducing sugars content under elevated CO_2 was found continued. Significantly higher reducing sugars content was recorded under elevated CO_2 (20.01 mg g^{-1}) followed by control chamber (16.65 mg g^{-1}) and open control (12.21 mg g^{-1}). Among the varieties, reducing sugars content was observed significantly higher for the variety Arun (16.89 mg g^{-1}) followed by CO-1 (17.20 mg g^{-1}) and Renusree (14.77 mg g^{-1}) (Table 2).

Free Amino Acid

Significantly higher free amino acid content was observed under elevated CO_2 (1.19 mg g^{-1}) compared to control chamber (0.96 mg g^{-1}) and open control (0.89 mg g^{-1}) after stress. Among the varieties, highest free amino acid content was recorded for the variety CO-1 (1.13 mg g^{-1}) which was significantly higher compared to Renusree (0.83 mg g^{-1}). Significantly higher free amino acid content was observed under treatment T1 (1.28 mg g^{-1}) compared to treatment T2 (0.96 mg g^{-1}) and treatment T3 (1.09 mg g^{-1}) after re-watering . Variety CO-1 (1.26 mg g^{-1}) recorded significantly higher free amino acid content compared to Anagha (1.15 mg g^{-1}) and Renusree (0.92 mg g^{-1}).

Ascorbic Acid

Higher ascorbic acid content was observed under elevated CO_2 (116.31 mg g^{-1}) compared to open control (106.94 mg g^{-1}) which was significantly higher than control chamber (98.61 mg g^{-1}). Among the varieties, highest ascorbic acid content was recorded for the variety CO-1(134.72 mg g^{-1}) and it was significantly higher than Renusree (65.62 mg g^{-1}). After re-watering, highest ascorbic acid content was observed under treatment elevated CO_2 (28.24 mg g^{-1}) followed by treatment control chamber) (27.03 mg g^{-1}) and treatment T3 (open control) (23.03 mg g^{-1}). Renusree recorded highest ascorbic acid content among the varieties, which was significantly higher than Arun (16.15 mg g^{-1}).

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
Reducing sugars content (mg/g)	15.98	20.01	13.60	16.65	11.40	12.21	0.91	0.80
Free amino acid content (mg/g)	1.19	1.28	0.96	0.96	0.89	1.09	0.36	0.55
Ascorbic acid content (mg/100g)	116.31	28.24	98.61	27.03	106.94	23.03	1.04	2.36

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	0.75	0.76
Reducing sugars content (mg/g)	14.96	16.89	13.48	17.20	12.53	14.77	0.91	0.80
Free amino acid content (mg/g)	1.07	1.15	1.13	1.26	0.83	0.92	0.36	0.55
Ascorbic acid content (mg/100g)	121.52	13.88	134.72	12.26	65.62	10.88	1.04	2.36

Effect of Elevated CO_2 on Protein Profiling and RuBISCO in Amaranthus

In the present study, the electrophoresis analysis of proteins using SDS PAGE revealed that elevated CO_2 induced no changes in protein profiling and RuBISCO expression levels in amaranthus (Figure 2).

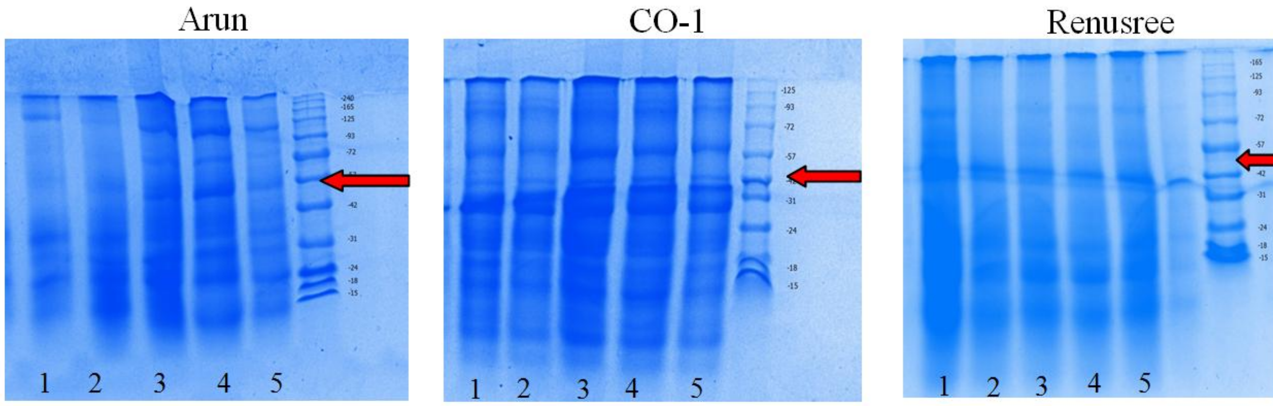

Figure 2 Protein profiling in Arun, CO-1 and Renusree varieties of amaranthus (1- EC Unstressed, 2- EC Stressed, 3- EC Unstressed, 4- EC Recovered, 5-Open control, RuBISCO (56 KDa))

Discussion

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 [12, 13]. Accumulation of carbohydrates in leaves is one of the most important responses observed in plants to elevated atmospheric CO_2 [14]. Elevated CO_2 conditions enhance the soluble sugar content of *Labisia pumila* [15]. In the current study on amaranthus, significant per cent rise of 28.66 and 38.9 was recorded in reducing sugars under elevated CO_2 in comparison with open control (Figure 3).

Several reports on effect of elevated CO_2 on carbohydrate accumulation were made by several workers found that leaf starch concentration was strongly enhanced by elevated CO_2 and influenced by water stress treatments in the cherry seedlings [16]. Increased carbohydrate content with carbon dioxide enrichment was reported in ginger and alfalfa [17, 15]. Increased starch content with CO_2 treatment was recorded in soybean [18]. High carbohydrate accumulation was reported in strawberry under elevated CO_2 condition [19]. Elevated CO_2 increases the accumulation of starch, total soluble sugars and reducing sugars in black gram during the flowering stage [20]. Elevated CO_2 conditions reportedly increased non-structural carbohydrate contents by 28% for clover and 16% for phalaris [21].

Carbon dioxide enrichment enhances the accumulation of both leaf starch and soluble carbohydrates [22, 23]. 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 [24]. 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 (Figure 4). Increase in soluble amino acid content under CO_2 enrichment has been reported in soybean and tobacco [25, 26].

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. In the present study on amaranthus, With CO_2 enrichment, a per cent increase of 8.05 (after stress) and 18.44 (after re-watering) in ascorbic acid content was observed under elevated CO_2 compared to control (Figure 5). Oxidative stresses do occur with water stress under elevated CO_2 conditions. 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 [27-29].

Under elevated CO_2, there can be imbalance in the supply and demand of carbohydrates resulting in their increased accumulation in the leaves. Carbohydrate accumulation in the leaves has been shown to down regulate the expression of photosynthetic genes in higher plants under elevated CO_2 [30]. In the present study, the electrophoresis analysis of proteins using SDS PAGE revealed that CO_2 enrichment did not modify the protein profile and expression levels of large or small sub units of RuBISCO in amaranthus.

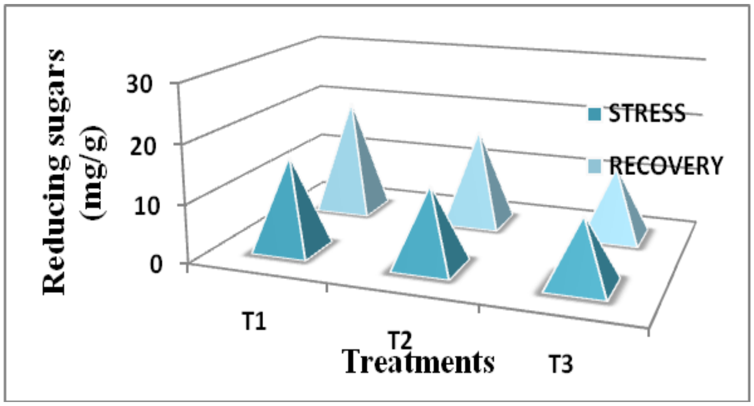

Figure 3 Effect of elevated CO_2 on reducing sugars (mg/g)

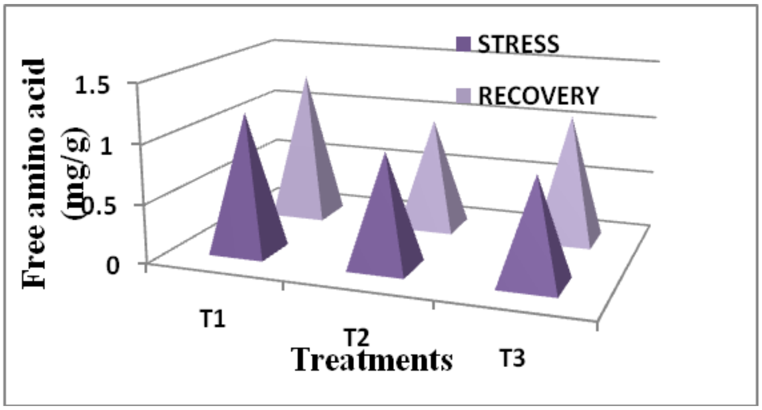

Figure 4 Effect of elevated CO_2 on free amino acid (mg/g)

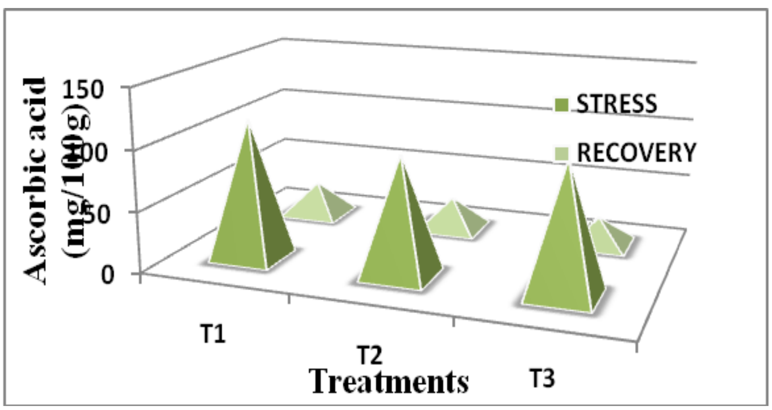

Figure 5 Effect of elevated CO_2 on ascorbic acid content (mg/100g) after stress and re watering in amaranthus

Conclusion

The increasing CO_2 concentrations in the atmosphere can have a fertilizing effect on plant metabolism, growth and development under favorable water and nutrient conditions. But such responses cannot be investigated under unpredictable weather patterns and abiotic stresses which are the characteristics features of changing climate. Selection and/or development of stress tolerant varieties is the judicial way of facing such a future. 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. Elevated CO_2 has also improved reducing sugars and free aminoacid content. 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 ascorbic acid 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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