

## Research Article

# Growth Parameters Contributing to Increased Drought Tolerance Responses in Amaranthus (*Amaranthus tricolor* L.) under Elevated Carbon Dioxide

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## Abstract

The threat of global warming and the demands of an increasing world population will increase water scarcity, resulting in a growing demand for water use efficient and drought tolerant crop plants. Under elevated CO<sub>2</sub> conditions, plants adapt many mechanisms to cope up with the stress factors. Plant growth is nearly always stimulated by elevation of CO<sub>2</sub>. A pot culture experiment was conducted with three varieties of amaranthus i.e, Arun (V<sub>1</sub>), CO -1 (V<sub>2</sub>) and Renusree (V<sub>3</sub>) with the objective to study the physiological basis of varietal responses of amaranthus to water stress conditions and to study their modifications under elevated CO<sub>2</sub> environment. The technology used for subjecting the plants to elevated CO<sub>2</sub> environments is the Open Top Chambers (OTC) system. Various growth parameters like number of leaves, root weight, shoot weight, root shoot ratio, specific leaf area, total dry matter content were analysed to understand the effect of CO<sub>2</sub> enrichment on drought tolerance. Elevated CO<sub>2</sub> was found to increase growth parameters like root weight, shoot weight, specific leaf area and total dry matter production. Significantly higher values were recorded for root weight (0.92 g), shoot weight (6.88 g), total dry matter production (5.74 g) under elevated CO<sub>2</sub>.

It has become imperative to elucidate the responses and adaptation of crops to water scarce conditions under changing climatic scenario and take actions to improve the drought tolerance ability of crop plants and to ensure higher crop yields against unfavorable environmental stresses which help to design improved production technologies with suitable varieties for a changing climatic scenario.

**Keywords:** Climate change, Global warming, Elevated CO<sub>2</sub>, Water stress, Growth parameters, Drought tolerance

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## Introduction

From the past 150 years, atmospheric CO<sub>2</sub> concentration has increased from about 280 ppm to current levels of 390 ppm and its concentration is expected to increase about 550 ppm within next 50-100 years. This has led to so many changes like global warming and increase in water scarcity for agricultural practices.

Disproportions in plant's normal metabolic machinery due to various environmental setbacks affect its overall physiology leading to limited productivity in crops. Drought is one such environmental setback which is continually posing to be the most deleterious abiotic stress factor causing considerable loss in crop yield worldwide. Prediction of long lasting droughts in future under the present changing climate scenario by Intergovernmental Panel on Climate Change (IPCC) [1] has further intensified the importance of drought among other abiotic stresses.

It is predicted that the globally averaged surface temperature will be 1.1 to 6.4°C warmer by the end of the 21st century compared to that in 1980-1999 leading to more extreme climatic events like increased potential evapotranspiration, leading to a more severe water deficit in arid and semiarid areas, enhanced ecosystem vulnerability as well as exaggerated severe aridification and desertification.

Increased CO<sub>2</sub> concentration has been found to ameliorate water stress in the majority of species studied. Under elevated CO<sub>2</sub> conditions, plants adopt many mechanisms to maintain high water potential and to resist water scarcity. The results of many studies indicate that lower evaporative flux density associated with high CO<sub>2</sub> induced stomatal closure results in increased net photosynthesis and better water use efficiency. Under elevated CO<sub>2</sub> conditions, it has also been found that plants maintain higher total water potentials to increase biomass production, have larger root shoot ratios and to be generally more drought tolerant. Changes in photosynthate allocation pattern phytochemical profiles were also observed under elevated CO<sub>2</sub> conditions. Interactive studies on water availability and elevated CO<sub>2</sub> show that there will be a partial closure of stomata due to increased CO<sub>2</sub> concentration in the substomatal cavity

decreasing partial pressure of CO<sub>2</sub> in the leaf and this CO<sub>2</sub> - dependent amplification of stomatal response could improve water use efficiency at the leaf and whole plant level.

Amaranthus is the traditional leafy vegetable which has, over the centuries, provided rural communities with food and nutritional security. The results of this study will also help to design improved production technologies with suitable varieties of amaranthus for a changing climatic scenario.

## Materials and methods

A pot culture experiment was conducted with three varieties of amaranthus i.e, Arun (V<sub>1</sub>), CO -1 (V<sub>2</sub>) and Renusree (V<sub>3</sub>) at the Department of Plant Physiology, College of Agriculture, Vellayani, under Kerala Agricultural University. The technology used for CO<sub>2</sub> enrichment was Open Top Chamber (OTC) system (Figure 1). Two Open Top Chambers were used, one with CO<sub>2</sub> level of 600 ppm (T1) and a second control chamber with control chamber level for assessing chamber effect (T2). 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<sub>2</sub> 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<sup>th</sup> day of re-watering, recovery observations were taken. The experiment was laid out in CRD with three treatments three replications and two stress levels.

Technology used for creating CO<sub>2</sub> enriched environment is Open Top Chambers (OTC). Open Top Chambers (OTC) are square type chambers constructed to maintain near natural conditions and elevated CO<sub>2</sub> conditions for experimental purposes. The basic structure of OTC was built of metal frame and installed in the experimental field. OTCs were covered with a 200 micron UV poly sheet. The chamber was constructed with 3 x 3 x 3 dimension, 45° slope and 1m<sup>2</sup> opening at the top. Two such chambers were built in the experimental field; one serves to impose CO<sub>2</sub> enrichment and the other serves as control chamber to study the chamber effects. Elevated CO<sub>2</sub> was released into the chamber from a CO<sub>2</sub> cylinder in a controlled manner. Measurements of microclimatic parameters (temperature, humidity and light) were done within and outside the OTCs with the help of sensors on a real time basis. On an average basis, mean temperature of 46.15°C relative humidity of 65.96% were recorded inside the chambers during the experimental period. Potted plants were kept within these chambers for a period of two months and observations were taken.

For calculating specific leaf area, fully expanded third leaf (from main stem apex) was collected from each plant. Leaflets were separated, petioles were discarded and area was measured. Leaflets were oven dried at 80°C for 2 days and the dry weight was taken. SLA was calculated using the formula;

$$SLA(cm^2 / g) = \frac{\text{leaf area}}{\text{dry weight}}$$

The roots of plants were cut at the base level and washed free of adhering soil with water. The roots were then oven dried and dry weight was recorded. Shoot weight was measured by weighing the above ground part of the plants in a weighing balance. Ratio of weights of dried roots and shoots of sample plants were calculated and mean values were calculated. The sum of root and shoot dry weights were taken as the total dry matter yield.



**Figure 1** Open Top Chamber for CO<sub>2</sub> enrichment

## Results

### Specific Leaf Area

Higher specific leaf area was recorded under treatment T1 ( $193.36 \text{ cm}^2 \text{ g}^{-1}$ ) compared to treatment T2 ( $180.82 \text{ cm}^2 \text{ g}^{-1}$ ) and treatment T3 ( $171.81 \text{ cm}^2 \text{ g}^{-1}$ ) after stress (Table 1). Among the varieties, highest specific leaf area was recorded for the variety CO-1 ( $234.23 \text{ cm}^2 \text{ g}^{-1}$ ) (Table 2). After re-watering, decreasing trend of specific leaf area was observed under treatment T1 ( $187.16 \text{ cm}^2 \text{ g}^{-1}$ ) compared to treatment T2 ( $297.30 \text{ cm}^2 \text{ g}^{-1}$ ) and treatment T3 ( $202.08 \text{ cm}^2 \text{ g}^{-1}$ ) (Table 1). Among the varieties, variety CO-1 recorded highest specific leaf area ( $297.3 \text{ cm}^2 \text{ g}^{-1}$ ) (Table 2).

### Root Weight

Significantly higher root weight was observed under elevated  $\text{CO}_2$  (0.92 g) compared to control chamber (0.69g) and open control (0.53 g) after stress (Table 1). Among the varieties, highest root weight was recorded for the variety CO-1 (0.83 g), which was found significantly higher than variety Renusree (0.57 g) (Table 2). After re-watering, significantly higher root weight was observed under elevated  $\text{CO}_2$  (0.22 g) compared to open control (0.16g) (Table 1) and among the varieties, highest root weight was recorded for the variety CO-1 (0.22 g), which was found significantly higher than variety Renusree (0.16 g) (Table 2).

### Shoot Weight

After stress, shoot weight was found significantly higher under treatment T1 (6.88 g) than treatment T2 (5.31 g) and treatment T3 (4.45 g) (Table 1). Significantly higher shoot weight was recorded for the variety CO-1 (7.69 g) after re-watering compared to Arun and Renusree (Table 2). After re-watering, significantly higher shoot weight was observed under treatment T1 (0.75 g) compared to treatment T3 (0.63 g) (Table 1). Significantly higher shoot weight was recorded for the variety CO-1 (0.88 g), compared to Arun (0.58 g) and Renusree (0.59 g) (Table 2).

### Root Shoot Ratio

Significant reduction of root shoot ratio was observed under elevated  $\text{CO}_2$  (0.25) than open control (0.76) after stress and increment was observed after re-watering (Table 1). Highest root shoot ratio was observed for the variety Arun after stress (0.49) and re-watering (0.36) (Table 2).

**Table 1** Effect of elevated  $\text{CO}_2$  on growth parameters after stress and re-watering in amaranthus

Parameter	Elevated $\text{CO}_2$ (T1)		Ambient $\text{CO}_2$ (T2)		Open control(T3)		CD(0.05)	
	Stress	Recovery	Stress	Recovery	Stress	Recovery	Stress	Recovery
Specific leaf area ( $\text{cm}^2 \text{ g}^{-1}$ )	193.36	187.16	180.82	297.30	171.81	202.08	32.05	32.5
Root weight (g)	0.92	0.22	0.69	0.20	0.53	0.16	0.21	0.06
Shoot weight (g)	6.88	0.75	5.31	0.67	4.45	0.63	0.98	0.11
Root shoot ratio	0.25	0.31	0.22	0.31	0.76	0.28	0.29	0.10
Dry matter production (g)	0.99	0.97	0.85	0.87	0.29	0.80	0.13	0.12

**Table 2** varietal variation observed with elevated  $\text{CO}_2$  in various parameters after stress and re-watering among three varieties of amaranthus

Parameter	Arun		CO-1		Renusree		CD(0.05)	
	Stress	Recovery	Stress	Recovery	Stress	Recovery	Stress	Recovery
Specific leaf area ( $\text{cm}^2 \text{ g}^{-1}$ )	158.49	187.16	234.23	297.30	153.27	202.08	32.05	32.5
Root weight (g)	0.74	0.20	0.83	0.22	0.57	0.16	0.21	0.06
Shoot weight (g)	4.65	0.58	7.69	0.88	4.29	0.59	0.98	0.11
Root shoot ratio	0.49	0.36	0.39	0.26	0.33	0.28	0.29	0.10
Dry matter production (g)	0.62	0.78	0.76	1.10	0.75	0.76	0.13	0.12

### **Dry Matter Production**

Dry matter production was found significantly higher under elevated CO<sub>2</sub> (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<sub>2</sub> (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).

### **Discussion**

Plant development and morphogenesis is governed by the effects of several environmental conditions super imposed upon genetic constraints. Thus genetically identical plants can exhibit very different structural features when subjected to different environmental conditions. Plant growth is nearly always stimulated by elevation of CO<sub>2</sub>. With CO<sub>2</sub> enrichment, Photosynthesis increases, plant biomass accumulated per unit of water consumed increases, and economic yield also gets enhanced.

Specific leaf area (SLA) is an indicator of leaf thickness. Exposure to elevated CO<sub>2</sub> can cause an increase in leaf thickness due to increased number of palisade cells, which contributed to leaf thickness [2]. The reduction in specific leaf area under elevated CO<sub>2</sub> can also be due to the high accumulation of starch and lower rate of leaf expansion. In amaranthus, a rise by 11.14% and 1.58% in specific leaf area was recorded under elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub> and open control after stress and recovery which was in accordance with the study conducted in norway spruce [3].

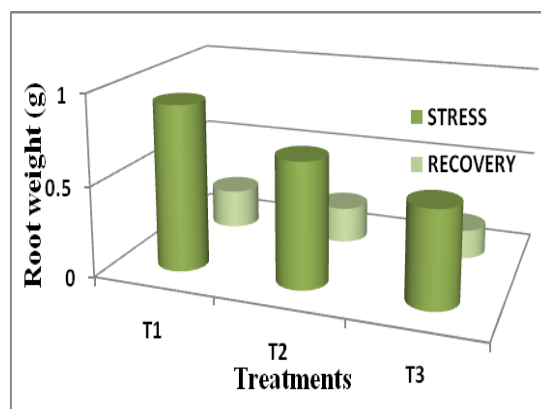
An extensive root system is advantageous to support plant growth during the early crop growth stage and extract water from shallow soil layers that is otherwise easily lost by evaporation. CO<sub>2</sub> enrichment can affect root physiology and morphology. Previous studies have shown that elevated CO<sub>2</sub> increased the density of roots by influencing both mass and unit root length per volume of soil and this is most evident in roots located in the upper layers of soil [4].

In the present study on amaranthus, highest root weight was maintained under elevated CO<sub>2</sub> than open control after stress and re-watering. it was recorded as 42.39% and 27.27% increase in root weight under elevated CO<sub>2</sub> compared to open control for amaranthus after stress and re-watering respectively (Figure 2). This is in agreement with many studies conducted in winter wheat [5] and many annual plant species [6].

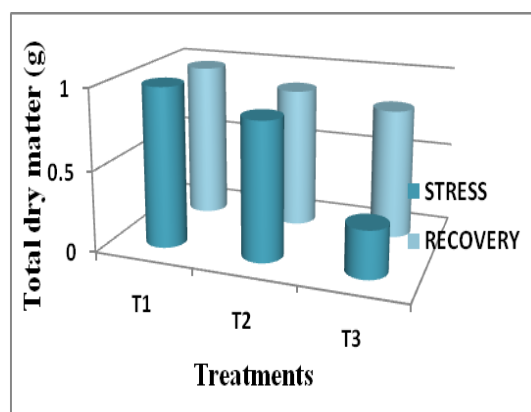
Shoot growth can be stimulated by exposure of plant canopies to high CO<sub>2</sub> concentration. The general consensus is that photosynthesis and Carbon allocation to plant shoots increases as atmospheric CO<sub>2</sub> rises which leads to an increase in above plant biomass.

In the present study, increase in shoot weight was recorded for all the varieties of amaranthus under carbon dioxide enriched treatment compared to open control both after stress and re-watering. In amaranthus 35% and 16% increase in shoot weight after stress and recovery was observed respectively under elevated CO<sub>2</sub>. This result was in agreement with former reports in *Fagus sylvatica* [7] and in *Larrea tridentate* [8]. Root/shoot ratio is the simple calculation of the ratio of root dry mass to shoot dry mass and should serve as a measure of the preferential allocation of C to roots or shoots [9]. In this experiment on amaranthus 67% reduction in Root/shoot ratio was observed after stress and a little increment by 5% was observed after re-watering which were in accordance with the works done in [8].

Elevated CO<sub>2</sub> stimulates photosynthesis in various intensities during different phenological phases [9] and its direct consequence is increased dry matter production [10, 11]. In present study, water stress induced reduction in dry matter production under elevated CO<sub>2</sub> was found to be less compared to open control. Dry matter production for amaranthus 70.7% and 17.52% increase in dry matter production was observed after stress and recovery respectively under elevated CO<sub>2</sub> compared to open control (Figure 3). This was in agreement with findings in soybean, dry bean, peanut and in cowpea [12-15].



**Figure 2** Effect of elevated CO<sub>2</sub> on root weight (g) after stress and rewatering in amaranthus



**Figure 3** Effect of elevated CO<sub>2</sub> on total dry matter (g) after stress and rewatering in amaranthus

## Conclusion

The present investigation was carried out with the objective to study the physiological basis of varietal responses of amaranthus to water stress conditions and to study their modifications under elevated CO<sub>2</sub> environments. Considering all the growth parameters, 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. High total dry matter content for the variety CO-1 in amaranthus was achieved in elevated CO<sub>2</sub> under water stress conditions because of activation of drought tolerance mechanisms like maintaining high root weight which helps in efficient water absorption. 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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