Physiological Basis of Varietal Responses of Amaranthus (Amaranthus tricolor L.) to Water Stress Conditions and their Modifications under Elevated CO2 Environments

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Abstract
Increased CO2 concentration has been found to ameliorate water stress in the majority of species studied. Physiological basis of varietal responses of amaranthus to water stress conditions and their modifications under elevated CO2 environment was studied by conducting a pot culture experiment with three varieties of amaranthus i.e, Arun (V1), CO -1 (V2) and Renusree (V3). For subjecting the plants to elevated CO2 environments, Open Top Chambers (OTC) system was used. water stress conditions were imposed on plants during their critical stages of development and then were allowed to recover. Various physiological parameters like relative water content, stomatal frequency, transpiration rate, membrane integrity (% leakage) and photosynthesis rate along with total dry matter production were analysed to understand the effect of CO2 enrichment on drought tolerance. Elevated CO2 was found to increase total dry matter (0.99 g), photosynthesis rate (16.89 mmol CO2m-2 s-1) while decreasing the stomatal frequency (606.63no cm-2), per cent leakage (6.12%) and transpiration rate (1.61 mmol water m-2 s-1) where as no change was observed in relative water content.

In the threat of global warming, water scarcity and demands of an increasing world population, Site specific CO2 enrichment studies with respect to specific crops is highly significant for designing improved production technologies with suitable varieties for a changing climatic scenario.

Keywords: Climate change, Global warming, Elevated CO2, water stress, Drought tolerance
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Introduction
According to the Intergovernmental Panel on Climate Change (IPCC), by the year 2050, the current atmospheric CO2 level of 384 μmol l-1 (800 Gt) is predicted to rise to 1000 Gt. This time only humans are the drivers of these changes and not glacial-interglacial cycles. Human-caused increases in atmospheric CO2 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.

Drought is a major limiting factor for plant productivity in large areas of the world, where it affects growth of both agricultural and forest species and also influences distribution and composition of vegetation. Increased CO2 concentration has been found to ameliorate water stress in the majority of species studied. Under elevated CO2 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 CO2 induced stomatal closure results in increased net photosynthesis and better water use efficiency.

Under elevated CO2 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 photosynthe allocation pattern phytochemical profiles were also observed under elevated CO2 conditions. Interactive studies on water availability and elevated CO2 show that there will be a partial closure of stomata due to increased CO2 concentration in the substomatal cavity decreasing partial pressure of CO2 in the leaf and this CO2 -dependent amplification of stomatal response could improve water use efficiency at the leaf and whole plant level.

Global warming and the demands of an increasing world population will increase water scarcity, which results in increased demand for water use efficient and drought tolerant crop plants. 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. This investigation will help to design improved production technologies with suitable varieties for a changing climatic scenario.

Materials and methods

Three varieties of amaranthus i.e, Arun (V₁), CO -1 (V₂) and Renusree (V₃) were used to conduct a pot culture experiment at the Department of Plant Physiology, College of Agriculture, Vellayani, under Kerala Agricultural University. CO₂ enrichment was achieved by using Open Top Chamber (OTC) technology (Figure 1). One Open Top Chambers with CO₂ level of 600 ppm (T1) was used for elevated CO₂ treatment and a second control chamber with ambient CO₂ level for assessing chamber effect (T2). A set of plants was maintained in the open field control (T3). Pots planted with one month old amaranthus 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 5th day of re-watering, recovery observations were taken. The experiment was laid out in CRD with three treatments three replications and two stress levels.

The sum of root and shoot dry weights were taken as the total dry matter yield. Relative water content was estimated as per the method given by Barr and Weatherly (1962) [3] by measuring the fresh weight, turgid weight and dry weight of known number of leaf discs from the experimental plants [4].

The RWC was calculated using the following formula

\[ RWC = \frac{Fresh \ weight - Dry \ weight}{Turgid \ weight - Dry \ weight} \times 100 \]

Chlorophyll content of leaf samples were estimated by incubating a weighed quantity of leaf sample (0.5g) taken from third fully expanded leaves overnight at room temperature in 10 ml DMSO: 80% acetone mixture (1:1 v/v). The absorbance was measured at 663, 645, 480 and 510nm [5]. Stomatal frequency refers to the number of stomata per unit area of leaf. A thick mixture of thermocol and xylene was prepared and this was smeared on both the surfaces of leaves and allowed to dry. It was peeled gently after drying and the peel was observed under microscope and counted using a 40X objective and 10X eyepiece. The field of the microscope was measured using a stage micrometre and stomatal frequency per unit area was calculated using the formula.

\[ Stomatal \ frequency = \frac{No \ of \ stomata}{Area \ of \ the \ microscopic \ field} \]

Transpiration rate was measured using the SAI-1 Porometer of company Delta T Devices and expressed as mmols m⁻² s⁻¹. Photosynthetic Rate was measured using portable photosynthetic system (CIRAS-3 SW). The sum of root and shoot dry weights were taken as the total dry matter yield. Membrane integrity was calculated in terms of percentage leakage. Fully expanded leaves are excised with their petioles intact in water and allowed to regain turgidity by incubating in distilled water for 45 minutes. Turgid weight was taken and leaves were allowed to wilt for three hours. After 40 to 60% loss of the fresh weight, leaf punches of 1 cm diameter were taken and washed for 1-2 minutes to leach out their solutes from the cut ends and blotted on clean filter paper. 10 leaf punches were incubated in test tubes containing 20 ml distilled water for 3 hours. Leakage of the solutions in their bathing medium was estimated by recording its absorbance at 273 nm (initial leakage of solutes). Test tubes were incubated in hot water bath (100° c) for 15 minutes. Absorbance of bathing medium is again read out at 273 nm to indicate final absorbance.

\[ \% \ leakage = \frac{Initial \ absorbance \ of \ bathing \ medium}{Final \ absorbance \ of \ bathing \ medium} \times 100 \]

Results

Dry Matter Production

Significantly higher Dry matter production was found under elevated CO₂ (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 CO₂ (0.97 g), which was found significantly higher compared to treatment open control (0.80 g). 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).
Figure 1 Amaranthus plants kept in Open Top Chamber

Relative Water Content

After stress there was no change observed in relative water content between treatment T1 (87.24 %) and treatment T3 (87.24%). Relative water content in treatment T3 was recorded as 85.20 %. Among the varieties, highest relative water content was recorded for the variety CO-1 (91.37 %) which was significantly higher than variety Arun (79.93 %) and on par with variety Renusree (88.38 %). Significantly higher relative water content was recorded under treatment T1 (93.84 %) compared to treatment T3 (90.36 %) after re-watering. Among the varieties, highest relative water content was observed for variety CO-1 (94.21 %) and it was observed significantly higher than Renusree (89.26 %).

Membrane Integrity

Membrane integrity after stress was expressed in terms of % leakage. Per cent leakage was observed significantly lower under elevated CO₂ (6.12 %) compared to open control (8.41 %). Lowest % leakage was recorded for the variety Anagha, which was significantly lower than variety Renusree. Per cent leakage was found decreasing significantly under elevated CO₂ (3.54 %) compared to control chamber (4.51 %) and open control (6.19 %) after re-watering (Table 1). Among the varieties, significantly lower % leakage was recorded for Arun (2.90 %) and CO-1 (2.99 %) compared to Renusree (8.35 %).

Table 1 Effect of elevated CO₂ on various parameters after stress and re-watering in amaranthus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Elevated CO₂(T1)</th>
<th>Ambient CO₂(T2)</th>
<th>Open control (T3)</th>
<th>CD(0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stress Recovery</td>
<td>Stress Recovery</td>
<td>Stress Recovery</td>
<td>Stress Recovery</td>
</tr>
<tr>
<td>Dry matter production (g)</td>
<td>0.99 0.97</td>
<td>0.85 0.87</td>
<td>0.29 0.80</td>
<td>0.13 0.12</td>
</tr>
<tr>
<td>Relative water content (%)</td>
<td>87.24 93.84</td>
<td>85.20 92.48</td>
<td>87.24 90.36</td>
<td>3.43 2.83</td>
</tr>
<tr>
<td>Chlorophyll a (mg/g)</td>
<td>0.51 0.65</td>
<td>0.57 0.52</td>
<td>0.42 0.41</td>
<td>0.51 0.19</td>
</tr>
<tr>
<td>Chlorophyll b (mg/g)</td>
<td>0.18 0.49</td>
<td>0.30 0.25</td>
<td>0.23 0.22</td>
<td>0.06 0.07</td>
</tr>
<tr>
<td>Total chlorophyll (mg/g)</td>
<td>0.70 1.02</td>
<td>0.87 0.79</td>
<td>0.66 0.63</td>
<td>0.21 0.15</td>
</tr>
<tr>
<td>Carotenoid (mg/g)</td>
<td>0.47 0.61</td>
<td>0.52 0.62</td>
<td>0.56 0.57</td>
<td>0.08 1.03</td>
</tr>
<tr>
<td>Stomatal frequency(no cm⁻²)</td>
<td>606.63 555.85</td>
<td>673.65 610.94</td>
<td>638.42 658.18</td>
<td>42.21 45.82</td>
</tr>
<tr>
<td>Transpiration rate (mmol water m⁻² s⁻¹)</td>
<td>1.61 3.94</td>
<td>8.18 14.01</td>
<td>15.65 16.23</td>
<td>6.52 5.47</td>
</tr>
<tr>
<td>Photosynthesis rate (mmol CO₂ m⁻² s⁻¹)</td>
<td>16.89 14.74</td>
<td>15.73 11.85</td>
<td>14.65 10.99</td>
<td>1.22 3.38</td>
</tr>
<tr>
<td>Membrane integrity (% leakage)</td>
<td>6.12 3.54</td>
<td>5.49 4.51</td>
<td>8.41 6.19</td>
<td>1.89 0.80</td>
</tr>
</tbody>
</table>

Pigment Composition

Chlorophyll a
Elevated CO$_2$ (0.51 mg/g) was found to enhance chlorophyll a content compared to open control (0.42 mg/g) after stress. Among the varieties, CO-1 registered highest mean value for Chlorophyll a content (0.74 mg/g). After re-watering, significant enhancement in chlorophyll a content was recorded under elevated CO$_2$ (0.65 mg/g) compared to open control (0.41 mg/g).

**Chlorophyll b**

After stress, reduction in chlorophyll b content was observed under treatment T1 (0.18 mg/g) compared to treatment T3 and this reduction was found significant compared with treatment T2 (0.30 mg/g). Significantly high chlorophyll b content was recorded for the variety CO-1. But, after re-watering, significant enhancement in chlorophyll b content was observed under treatment T1 (0.49 mg/g) compared to treatment T2 (0.25 mg/g) and treatment T3 (0.22 mg/g). Superior chlorophyll b content was recorded for the variety CO-1 (0.36 mg/g) compared to Arun (0.31 mg/g) and Renusree (0.28 mg/g).

**Total Chlorophyll**

Total chlorophyll content under elevated CO$_2$ (0.70 mg/g) was found superior compared to open control (0.66 mg/g) and lower compared to (control chamber) (0.87 mg/g) after stress. Significantly superior total chlorophyll content was recorded for the variety CO-1 compared to Arun (0.71 mg/g). After re-watering, there found a significantly higher chlorophyll content under elevated CO$_2$ (1.02 mg/g) followed by control chamber (0.79 mg/g) and open control.

**Carotenoid Content**

Reducing trend of carotenoid content was observed under treatment T1 (0.70 mg/g). Carotenoid content was recorded significantly lower under treatment T1 (0.47 mg/g) compared to treatment T3 (0.56 mg/g) and T2 (0.52 mg/g treatment) after stress. After re-watering, no significant difference was observed between carotenoid content under treatment T1 (0.70 mg/g) and treatment T2 (0.52 mg/g treatment) but it was non significantly higher compared to treatment T3 (open control) (0.22 mg/g). Highest carotenoid content among the varieties was recorded for the variety CO-1, which was significantly superior compared to Renusree.

**Stomatal Frequency**

Stomatal frequency was found reducing under elevated CO$_2$ after stress. Lowest stomatal frequency was observed under treatment T1 (606.63 number cm$^{-2}$) followed by treatment T2 (673.65 number cm$^{-2}$) and treatment T3 (638.42 number cm$^{-2}$). Lowest stomatal frequency among the varieties was recorded for CO-1 (551.85 number cm$^{-2}$) followed by Arun (669.84 number cm$^{-2}$) and Renusree (697.01 number cm$^{-2}$). After re-watering also reducing trend of stomatal frequency under elevated CO$_2$ was found continued. Lowest stomatal frequency was recorded under elevated CO$_2$ (653.16 number cm$^{-2}$) followed by control chamber (673.11 number cm$^{-2}$) and open control (691.53 number cm$^{-2}$). Variety CO-1 recorded lowest stomatal frequency (602.88 number cm$^{-2}$) followed by Arun (694.73 number cm$^{-2}$) and Renusree (719.90 number cm$^{-2}$).

**Transpiration Rate**

After stress, significant reduction in transpiration rate was observed under treatment T1 (1.61 mmol water m$^{-2}$ s$^{-1}$) followed by treatment T2 (8.18 mmol water m$^{-2}$ s$^{-1}$) and treatment T3 (15.65 mmol water m$^{-2}$ s$^{-1}$). Among the varieties, lowest transpiration rate was recorded for CO-1 (8.27 mmol water m$^{-2}$ s$^{-1}$) followed by Arun (8.43 mmol water m$^{-2}$ s$^{-1}$) and Renusree (15.65 mmol water m$^{-2}$ s$^{-1}$). After re-watering, significantly lowest transpiration rate was recorded under treatment T1 (3.94 mmol water m$^{-2}$ s$^{-1}$) compared to treatment T2 (14.01 mmol water m$^{-2}$ s$^{-1}$) and treatment T3 (16.23 mmol water m$^{-2}$ s$^{-1}$). Lowest transpiration rate, among the varieties was recorded for CO-1 (10.73 mmol water m$^{-2}$ s$^{-1}$) followed by Arun (10.98 mmol water m$^{-2}$ s$^{-1}$).

**Photosynthesis Rate**
After two days of water stress, significant increase in photosynthesis rate was recorded under elevated CO2 (16.89 mmol CO2 m^{-2} s^{-1}) compared to open control (14.65 mmol CO2 m^{-2} s^{-1}). Highest photosynthesis rate was observed for the variety CO-1 (16.62 mmol CO2 m^{-2} s^{-1}) which was significantly higher than Renusree (7.35 mmol CO2 m^{-2} s^{-1}). After re-watering also (Table 2), photosynthesis rate was significantly higher under elevated CO2 (14.74 mmol CO2 m^{-2} s^{-1}) compared to open control (10.99 mmol CO2 m^{-2} s^{-1}). Highest photosynthesis rate was recorded for the variety Renusree (13.70 mmol CO2 m^{-2} s^{-1}).

Table 2 Varietal variation observed with elevated CO2 in various physiological parameters after stress and re-watering among three varieties of amaranthus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Arun</th>
<th>CO-1</th>
<th>Renusree</th>
<th>CD(0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stress</td>
<td>Recovery</td>
<td>Stress</td>
<td>Stress</td>
</tr>
<tr>
<td>Dry matter production (g)</td>
<td>0.99</td>
<td>0.97</td>
<td>0.85</td>
<td>0.87</td>
</tr>
<tr>
<td>Relative water content (%)</td>
<td>79.93</td>
<td>93.22</td>
<td>91.37</td>
<td>0.13</td>
</tr>
<tr>
<td>Chlorophyll a (mg/g)</td>
<td>0.54</td>
<td>0.62</td>
<td>0.74</td>
<td>3.43</td>
</tr>
<tr>
<td>Chlorophyll b (mg/g)</td>
<td>0.23</td>
<td>0.31</td>
<td>0.34</td>
<td>0.51</td>
</tr>
<tr>
<td>Total chlorophyll (mg/g)</td>
<td>0.71</td>
<td>0.95</td>
<td>1.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Carotenoid (mg/g)</td>
<td>0.54</td>
<td>0.69</td>
<td>0.54</td>
<td>0.21</td>
</tr>
<tr>
<td>Stomatal frequency (no cm^{-2})</td>
<td>669.84</td>
<td>634.0</td>
<td>551.85</td>
<td>0.08</td>
</tr>
<tr>
<td>Transpiration rate (mmol water m^{-2} s^{-1})</td>
<td>8.43</td>
<td>10.98</td>
<td>8.27</td>
<td>42.21</td>
</tr>
<tr>
<td>Photosynthesis rate (mmol CO2 m^{-2} s^{-1})</td>
<td>15.95</td>
<td>10.76</td>
<td>16.62</td>
<td>6.52</td>
</tr>
<tr>
<td>Membrane integrity (% leakage)</td>
<td>4.36</td>
<td>2.90</td>
<td>5.09</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Discussion
Relative water content (RWC), is an important character that influence plant water relations. Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful parameter for dehydration tolerance. RWC related to water uptake by the roots as well as water loss by evapotranspiration. In present experiment on amaranthus, after stress no difference in RWC was observed between elevated CO2 and open control. But 3.7 % of significant rise in RWC was recorded under elevated CO2 after re-watering. Plant water use efficiency is strongly influenced by stomatal density [6]. Decreased stomatal opening can lead to improved water use efficiency [7, 8] and results in lower water stress of plants [9]. In the present study, a reduction of 5.24% after stress and 14.85% after recovery in stomatal frequency was recorded in amaranthus (Figure 2). CO-1 variety of amaranthus recorded lowest stomatal frequency under elevated CO2. Reduction in stomatal frequency under elevated CO2 was reported in the leaves of Arabidopsis thaliana [10] and in soybean [11]. Plants respond to enriched CO2 content by showing declined stomatal conductance, which typically leads to reduced rates of transpirational loss [12]. Elevated CO2 reduces transpiration by partially closing the stomata and decreasing stomatal conductance. In present study on amaranthus 89.72% reduction in transpiration rate after stress and 75.72% reduction after recovery was observed under enriched CO2 treatment compared to absolute control (Figure 3). These results were found in accordance with studies conducted on egg plant [13, 14]. Elevated CO2 may alleviate the high temperature damage to photosynthesis because with higher CO2 concentrations, there is an interaction between improved plant water status and protection of photosynthesis against high-temperature damage. With the temperature rising above optimum, photosynthetic rate may be restrained by promoting oxygenation than carboxylation by decreasing the affinity of the Rubisco for CO2, which can be alleviated under elevated CO2 [15]. With elevated CO2 concentration accompanied by high temperature, there was no increase in the risk observed of photo damage and down regulation of electrons in rose plants, whereas enhanced photosynthesis and WUE in carrot plants were found with CO2 enrichment [16].

In the present study conducted on amaranthus under elevated CO2, 13.26% and 25.44% increase in photosynthetic rate after stress and re-watering respectively than control (Figure 4). Plant productivity depends greatly on the amount of chlorophyll present in the chloroplast. The amount of chlorophyll in leaf tissues is influenced by nutrient availability and environmental stresses [17, 18]. Leaf chlorophyll content is a good indicator of photosynthesis activity, nutritional status, mutations and stress condition. In the case of amaranthus after stress, 17.64% and 5.71% improvement in chlorophyll a & total chlorophyll content and a reduction in chlorophyll b & carotenoid content by
21.73% and 16.07% respectively was recorded under elevated carbon dioxide treatment. An increasing trend of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content by 36.92%, 55.10%, 161% and 6.55% was recorded respectively after re-watering under elevated CO₂ in comparison with control conditions. Several contradictory results were also reported in the case of chlorophyll content under elevated CO₂. Decreased total Chlorophyll content was observed in two spring wheat cultivars [19] under elevated CO₂. The plasma membrane is the selectively permeable lipid bilayer that surrounds the living cells. Being the first points of contact for environmental signals upon the cell, it plays an important role in stress responses. So the maintenance of membrane integrity is very important to thrive under stress conditions [20]. Modification in cellular membrane is a major impact of plant environmental stress, which results in perturbed function or total dysfunction of cellular membrane. Cellular membrane dysfunction due to stress can be well expressed as increased permeability and leakage of ions out from membrane. In the present work, per cent reduction of 27.22% and 42.81% in leakage was observed in the case of amaranthus after stress and re-watering respectively.

![Figure 2](image1.png)

**Figure 2** Effect of elevated CO₂ on stomatal frequency (no cm⁻²)

![Figure 3](image2.png)

**Figure 3** Effect of elevated CO₂ on transpiration rate (mmol water m⁻² s⁻¹)
Figure 4 Effect of elevated CO₂ on photosynthetic rate (mmol CO₂/m² s⁻¹) after stress and rewatering in amaranthus

Conclusion

The sensitivity of photosynthesis to each of the environmental variables including low water availability, high temperature, vapor pressure deficit and soil salinity is associated with the inevitable rise in atmospheric carbon dioxide. Plant growth responses to the increasing CO₂ concentration will not only affect ecosystem productivity in the future, but also the magnitude of C sequestration by plants and, consequently, the rate of CO₂ increase in the atmosphere. Considering all the physiological parameters and total dry matter content, it can be concluded that elevated carbon dioxide has a positive role in improving water stress tolerance and recovery responses in amaranthus. It was achieved mainly due to better photosynthetic rate and lower transpiration rate. High total dry matter content in amaranthus for the variety CO-1 was achieved in elevated CO₂ under water stress conditions because of activation of drought tolerance mechanisms like maintaining lower stomatal frequency, transpiration rate and higher membrane integrity which helps in efficient water saving. 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.

References


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