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Research Article

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES IN *AMARANTHUS* (*AMARANTHUS TRICOLOR* L.) TO DIFFERENT SOIL MOISTURE REGIMES UNDER ELEVATED CARBON DIOXIDE CONCENTRATIONS

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Abstract: Raising CO_2 leads to higher yielding; more vigorous crops an unexpected boon. Agricultural productivity depends on key inputs prevailing CO_2, temperature and water that are the key inputs. Global warming increases temperature by 2-3°C, CO_2 and other gases concentration. The present programme was an attempt to study the modifications brought in the developmental pattern of *amaranthus* by elevated CO_2 concentration. Two weeks old potted plants were shifted to OTCs (CO_2 concentration of 600ppm maintained). All the three sets of plants were maintained at field capacity (FC) initially. Soil moisture levels were brought down to 80% and 70%, in the second and third sets 30 days after planting and were maintained for a period of 30 days at these soil moisture regimes in OTCs. Plant responses in terms of growth parameters, leaf characters and dry matter accumulation were analyzed. Increasing CO_2 concentration in the atmosphere can have a positive influence on the plant growth and development. The result indicated an improvement in growth performances of *amaranthus* under mild and severe moisture stress conditions (80% and 70% Field capacity).

Keywords: Elevated CO_2, Cowpea, Growth, Open Top Chambers, Field capacity

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Introduction

Raised CO_2 heats the atmosphere by absorbing reflected solar radiation by earth surface. Carbon dioxide is the most important resource for crop growth. According to the Intergovernmental Panel on Climate Change (IPCC), by the year 2050, the current atmospheric [1, 2]. CO_2 level of 400 µmol l^{-1} (800 Gt) is predicted to rise to 1000 Gt [3]. Considering the role of CO_2, in bringing about the projected changes in climate and importance of *amaranthus* a study was undertaken to analyse the growth performances under elevated CO_2 condition under different soil moisture regimes.

Materials and Methods

A pot culture experiment was conducted on *amaranthus* variety Arun at the Department of Plant Physiology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvanthapuram, 695522, Kerala, India. Technology used for creating CO_2 enriched environment is OTC [Plate-1].

OTCs used for the present study are square type chambers constructed to maintain near natural conditions with elevated CO_2 concentration for experimental purposes. The basic structure of OTC is built of metal frame and installed in the experimental field. OTCs are covered with a 200-micron UV poly sheet. Two such chambers were built in the experimental field; one serves to impose CO_2 enrichment (Ec) and the other serves as control chamber (Ac) to study the chamber effects and in contrast one set of plants grown in open field condition (C) to mimic the natural environment.

The chamber was constructed with 3 x 3 x 3-dimension, 460 slope and 1m^2 opening at the top. Potted plants were maintained in OTC as well as in filed condition. One-month old potted plants (2 plants/pot) were shifted to OTC and were maintained at FC initially. Soil moisture levels were brought down to 80% and 70%, 1 week after shifting and maintained at these soils' moisture levels for a period of 2 weeks.

Plate-1 Open Top Chamber facility for CO_2 enrichment studies

Plate-2 *Amaranthus* plants under different nutrient levels in OTC

Pots were filled with potting mixture consisting of 1:1:1 ratio of farm yard manure, sand and soil. The period of CO_2 enrichment was from 9.00 am to 5.00 pm. Elevated CO_2 was released into the chamber from a CO_2 cylinder in a controlled manner. Real time basis sensors were used to measurement of microclimatic parameters (temperature, humidity and light) were done within and outside the OTCs [Plate-2]. Observations were taken at the end of crop period. Total chlorophyll contents of leaf samples of *amaranthus* were estimated following the standard methodology and expressed as mg g^{-1} of fresh weight [4]. For calculating specific leaf area, third fully expanded leaf (from main stem apex) was collected. Leaflets were separated, petioles were discarded and area was measured. Leaflets were dried at 80°C for 2 days and the dry weight was taken.

Chlorophyll pigments (mg g^{-1})

A weighed quantity of leaf sample (0.5g) was taken from fully expanded third leaf and cut into small bits. These bits were put in test tubes and incubated overnight at room temperature, after pouring 10ml DMSO: 80% acetone mixture (1:1v/v). The coloured solution was decanted into a measuring cylinder and made up to 25 ml with the DMSO-acetone mixture. The absorbance was measured at 663, 645, 480 and 510nm. The chlorophyll content was measured by substituting the absorbance values in the given formulae.

$$Total\ Chl\ (a+b) = (8.02 \times A_{663} - 20.0 \times A_{645}) \times V/1000 \times 1/Fresh\ weight$$

Specific leaf area was calculated using the formula. The roots of plants were cut at the base level and washed free of adhering soil with low jet of 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. The sum of root and shoot dry weights were taken as the total dry matter accumulation. Pod yield was determined at the time of harvest and was expressed on fresh weight basis.

$$SLA\ (cm^2/g) = Leaf\ area\ /\ Dry\ weight$$

Relative Water Content (%)

Relative water content was estimate by measuring the fresh weight, turgid weight and dry weight of known number of leaf discs from the experimental plants [5]. After measuring the fresh weight of the sample, it was submerged in distilled water for 3 hours and then the turgid weight was taken. The dry weight of the sample was measured after keeping the samples in oven at 80°C for 3 consecutive days. The RWC was calculated using the following formula.

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

Estimation of Starch (mg g^{-1})

The estimation of starch in plants was done following the Anthrone method. A known quantity of plant sample (0.1g) was homogenized in hot 80% ethanol to remove sugars. The homogenate was centrifuged and residue was retained. The residue was washed repeatedly with hot 80% ethanol till the washing did not give any colour with anthrone reagent. Then the residue was dried well over a water bath. The dried residue was mixed with 5ml water and 6.5 ml 52% Perchloric acid and was extracted at 0°C for 20 min. This solution was centrifuged and the supernatant was saved. The extraction was repeated using fresh Perchloric acid. The supernatant after centrifugation was pooled and made up to 100 ml.

An aliquot of 0.1 ml of the supernatant was taken and again made up to 1 ml using distilled water. The standard was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard solution and made up the volume to 1 ml in each tube using distilled water. Anthrone reagent (4 ml) was added to both the sample and standard test tubes. These test tubes were heated for eight minutes in a boiling water bath and cooled rapidly. The intensity of colour changed from green to dark green was measured at 630 nm. The glucose content in the sample was calculated using the standard curve. This value was multiplied by a factor of 0.9 to arrive at the starch content.

Estimation of Reducing Sugars (mg g^{-1})

The estimation of reducing sugars in plants was done following Dinitrosalicylic acid (DNS) method. The sample was weighed (100 mg) and the sugars were extracted with hot 80% ethanol, twice. The supernatant was collected and evaporated by keeping it on a boiling water bath at 80°C. The sugars were dissolved by adding 10 ml water. Aliquots of 0.5 to 3 ml were pipetted out into test tubes and the volume was equalized to 3ml with distilled water in all the test tubes. To this 3 ml of DNS reagent was added. The test tubes were heated in a boiling water bath for 5 minutes. Rochelle salt solution (40%, w/v) (1 ml) was added to the test tubes when the contents were hot. Then the test tubes were cooled and the intensity of dark red colour was read at 510 nm. A series of the standard, glucose, (0 to 500µg) were run and a standard curve was plotted. The amount of reducing sugars in the sample was calculated from the standard graph.

Transpiration Rate (mmol water m^{-2} s^{-1})

Transpiration rate was measured using the SAI^{-1} Porometer of company (Delta T Devices) and expressed as mmoles $m^{-2}s^{-1}$.

Estimation of Phenols (mg g^{-1})

Quantification of phenols was done by Folin-Ciocalteau method [6]. Phenol was estimated from 0.5g of leaf samples and reflexed in 10 ml 80% methanol for 20 min. The tissue was ground thoroughly in a mortar with pestle and filtered through a double layered cheese cloth. The filtrate was subjected to centrifugation at 1000 rpm for 10 min. The supernatant was collected and made to a known volume using 80% methanol. 0.1 ml aliquot was drawn to a test tube and made up to 3 ml using 80% methanol. To this, 0.5 ml of Folin-Ciocalteau reagent and 2 ml 20% Na_2CO_3 were added. It was kept in a boiling water bath for 5 min till a white precipitate was formed and was then again centrifuged at 5000 rpm for 5 min. The absorbance of the clear supernatant was read at 650 nm against the blank. Standard curve was prepared using different concentrations of catechol and expressed in catechol equivalents as microgram per gram leaf tissue on fresh weight basis

Membrane Integrity (%)

Leaf discs (10 no) from the third fully opened leaves were taken in a 50ml beaker with 10ml distilled water. Initial EC was measured for detecting the small degree of leakage by the discs caused by the punching treatment using conductivity electrode (ECa). After 30 minutes incubation the leakage of solutes in this bathing medium was measured (ECb). Then the beakers were boiled at 100°C for 10 minute and the EC was again recorded (ECc). The membrane integrity of leaf tissues was calculated using the following formula.

$$Percentage\ of\ leakage = \frac{ECb - ECa}{ECc} \times 100$$

Superoxide dismutase (g^{-1}minute^{-1})

Superoxide dismutase (SOD) activity was quantified following the method. Leaf samples (0.5g) from third fully opened leaves were ground with 3.0 ml of potassium phosphate buffer, centrifuged at 2000rpm for 10 minutes and the supernatants were used for the assay. The mixture was incubated at 30°C for 90 second and was arrested by the addition of 1.0ml of Glacial acetic acid. The reaction mixture was then shaken with 4.0 ml of n-butanol, allowed to stand for 10 minute and centrifuged [7].

Estimation of Ascorbic Acid (mg 100 g^{-1})

The Ascorbic acid content in plants was estimated volumetric method. Working standard solution of 5ml containing 100µg/ml of Ascorbic acid was pipetted out into a 100 ml conical flask. 4% Oxalic acid was added to it and titrated against 2,6-dichlorophenol indophenol dye (V_1 ml) [8]. End point was noted as appearance of pink colour which persisted for a few minutes. The sample (0.5g) was weighed and ground in a mortar with pestle using 15ml 4% Oxalic acid.

The filtrate was made up to a known volume and centrifuged at 10,000 rpm for 10 min. The supernatant was collected and made up to 25ml using Oxalic acid. 5.0 ml aliquot was pipetted into a conical flask to which 10ml of 4% Oxalic acid was added. This was titrated against dichlorophenol indophenol (DCPIP) solution, until the appearance of pink colour (V_2 ml).

The amount of Ascorbic acid is calculated as follows:

$$Ascorbic\ acid = \frac{0.5mg}{V_1 ml} \times \frac{V_2}{5ml} \times \frac{100}{Weight\ of\ sample}$$

Statistical analysis

The experiment used a CRD with three treatments and each treatment was analysed with three replications. Statistical analysis was performed using ANOVA.P values d ≤ 0.05 were considered as significant.

Results and Discussion

Alteration in growth performance of *amaranthus* under elevated CO_2 studied by analyzing the parameters *viz*, leaf number, leaf area, shoot weight, root weight and dry matter accumulation. The various growth parameters considered under this study includes number of leaves, specific leaf area, root weight, shoot weight, root shoot ratio and dry matter production. Exposure to elevated CO_2 concentration resulted in better growth performances of *amaranthus* under mild (80% Field Capacity) and severe moisture stress conditions (70% Field Capacity).

The effect of elevated CO_2 on number of leaves under moisture stress is presented in [Table-1]. Highest mean value for number of leaves was observed under elevated CO_2 (41.88) followed by plants under open control (39.44) and ambient chamber (38.77). Under elevated CO_2 highest value was observed in 100% field capacity (100% FC) (47.00) followed by S2-80 % FC (41.00) and S3-75% FC (37.66). Under all the moisture regimes CO_2 had significant influence on improvement of number of leaves.

Table-1 *Effect of elevated CO_2 on number of leaves under moisture stress in amaranthus*

Varieties	Number of leaves			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	47.00	42.00	43.66	44.22
S2:80% FC	41.00	39.01	38.33	39.44
S3:70% FC	37.66	35.33	36.33	36.44
Mean	41.88	38.77	39.44	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.429	0.147	0.208	
S	0.429	0.147	0.208	
T x S	0.743	0.255	0.360	

Table-2 *Effect of elevated CO_2 on specific leaf area under moisture stress in amaranthus*

Varieties	Specific leaf area ($cm^2\ g^{-1}$)			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	236.39	205.34	201.84	217.85
S2:80% FC	171.25	155.62	148.95	158.60
S3:70% FC	149.02	134.26	157.59	146.95
Mean	185.42	165.21	162.79	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	2.385	1.067	2.234	
S	2.385	1.067	2.234	
T x S	4.132	2.268	2.969	

Effect of elevated CO_2 on specific leaf area under moisture stress is presented in [Table-2]. Highest mean value for specific leaf area was observed under elevated CO_2 (185.42) followed by plants under ambient chamber (165.21) and open control (162.79). Under elevated CO_2 highest value was observed in S1 (236.39) followed by S2 (171.25) and S3 (149.02). The mean values of all the treatments-T1, T2 and T3 were found to be on par.

Plants respond to enriched CO_2 content by showing declined stomatal conductance, which typically lead to reduced rates of transpirational loss [9].

Significantly higher mean value for plant height was observed under (87.22) followed by plants under ambient chamber (78.33) and open control (84.87) [Table-3]. Under elevated CO_2 highest value was observed in S1 (112.00) followed by S2 (79.66) and S3 (72.32). Under all the moisture regimes CO_2 had significant influence on improvement of plant height.

[Table-4] shows effect of elevated CO_2 on root weight (g) under moisture stress in *amaranthus*. Highest mean value of root weight (g) was observed under elevated CO_2 (1.096 g) followed by plants under open control (0.879) and ambient chamber (0.855). Under elevated CO_2 highest value was observed in S1 (2.133) followed by S2 (0.840) and S3 (0.416). The mean values of all the treatment-T1, T2 and T3 were found to be on par.

Table-3 *Effect of elevated CO_2 on plant height under moisture stress in amaranthus*

Varieties	Plant height (cm)			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	112.00	98.34	106.66	105.87
S2:80% FC	79.66	72.02	77.33	76.33
S3:70% FC	72.32	64.11	70.66	68.22
Mean	87.22	78.33	84.87	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.576	0.192	0.272	
S	0.576	0.192	0.272	
T x S	0.998	0.333	0.471	

Table-4 *Effect of elevated CO_2 on root weight under moisture stress in amaranthus*

Varieties	Root weight (g/plant)			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	2.133	1.630	1.450	1.738
S2:80% FC	0.840	0.650	0.790	0.760
S3:70% FC	0.416	0.283	0.398	0.338
Mean	1.096	0.855	0.879	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.081	0.027	0.038	
S	0.081	0.027	0.038	
T x S	0.140	0.047	0.066	

CO_2 enrichment was also found to influence the root hydraulic conductance. Eggplant (*Solanum melongena* L.) roots showed low hydraulic conductance at repetitive stress due to synthesis and accumulation of suberin in the root cells under elevated CO_2 and this change in root structure may occur from synthesizing suberin lipids in endo- and exo-dermis layers regardless of soil water availability or stress intensity. Lowering conductance is highly desirable for agricultural plants when water supply is limited in the growth and development stage [10]. These observations suggest that increasing CO_2 concentration in the global atmosphere might be beneficial for better use of soil water through enhanced root production and water uptake, lowered transpiration loss and reduced hydraulic conductance.

The effect of elevated CO_2 on shoot weight under moisture stress is presented in [Table-5]. Though not significant, there is improvement of shoot weight (g) under moisture stress in *amaranthus*. Highest mean value of shoot weight (g) was observed under elevated CO_2 (4.694) followed by plants under open control (4.595) and ambient chamber (3.890). Under elevated carbon dioxide, highest value was observed in S1-100% field capacity (6.313) followed by S2-80 % field capacity (4.740) and S3-75% field capacity (3.031).

Table-5 *Effect of elevated CO_2 on shoot weight under moisture stress in amaranthus*

Varieties	Shoot weight (g/plant)			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	6.313	5.383	6.113	5.936
S2:80% FC	4.740	3.590	4.646	4.125
S3:70% FC	3.031	2.696	3.026	2.921
Mean	4.694	3.890	4.595	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.278	0.095	0.135	
S	0.278	0.095	0.135	
T x S	0.482	0.165	0.234	

Table-6 *Effect of elevated CO_2 on root shoot ratio under moisture stress in amaranthus*

Varieties	Root shoot ratio			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	0.337	0.302	0.237	0.292
S2:80% FC	0.197	0.018	0.170	0.128
S3:70% FC	0.130	0.010	0.116	0.068
Mean	0.221	0.110	0.174	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.002	0.001	0.001	
S	0.002	0.001	0.001	
T x S	0.004	0.002	0.002	

Highest mean value of root shoot ratio was observed under elevated CO_2 (0.221) followed by plants under open control (0.174) and ambient chamber (0.110) [Table-6]. Under elevated CO_2 highest value was observed in 100% field capacity (0.337) followed by 80 % field capacity (0.197) and 75% field capacity (0.130). There were significant increases in Root Shoot Ratios under all the moisture regimes through CO_2 enrichment.

The effect of elevated CO_2 on dry matter production (g) under moisture stress is presented in [Table-7]. Highest mean value for dry matter production was observed under elevated CO_2 5.99) followed by plants under open control (5.53) and ambient chamber (4.54). There were significant differences among all the treatments with regard to dry matter production. Under elevated CO_2 significantly higher value was observed in S1 (8.44) followed by S2 (4.82) and S3 (4.71).

A meta-analysis of the effects of elevated CO_2 on woody plant species found that biomass responses were strongly affected by environmental stress factors and to a lesser degree by duration of CO_2 exposure [11]. They reported that belowground biomass responses to CO_2 were highly significant but were affected by stress and length of exposure to increased CO_2. In addition to the effects of CO_2 on photosynthesis and C allocation elevated CO_2 can impact growth through improved plant water relations [12]. From a physiological standpoint, increased WUE can be represent as one of the most significant plant responses to elevated CO_2 [13].

Table-7 *Effect of elevated CO_2 on dry matter production under moisture stress in amaranthus*

Varieties	Dry matter production (g)			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	8.44	7.01	7.56	7.67
S2:80% FC	4.82	3.65	4.42	4.19
S3:70% FC	4.71	2.97	4.63	3.58
Mean	5.99	4.54	5.53	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.032	0.011	0.015	
S	0.032	0.011	0.015	
T x S	0.055	0.019	0.027	

Table-8 *Effect of elevated CO_2 on relative water content under moisture stress in amaranthus*

Varieties	Relative water content			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	96.44	91.7	93.29	93.81
S2:80% FC	84.98	88.81	82.53	85.43
S3:70% FC	79.37	89.47	78.34	82.72
Mean	93.13	89.96	90.21	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.838	0.287	0.406	
S	0.838	0.287	0.406	
T x S	1.452	0.498	0.704	

Effect of elevated CO_2 on relative water content under moisture stress is presented in [Table-8]. Highest mean value for relative water content was observed under elevated CO_2 (93.13) followed by plants under open control (90.21) and ambient chamber (89.96). Under elevated CO_2 highest value was observed in S1 (96.44) followed by S2 (84.98) and S3 (79.37). Under all the moisture regimes elevated CO_2 had significant influence on Relative Water Content. But CO_2 enrichment did not influence plants in ambient chamber significantly with respect to Relative Water Content.

Relative water content is a useful indicator of the state of water balance of a plant [14]. 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.

[Table-9] shows significant enhancement in total chlorophyll content was recorded under moisture stress. Significantly higher value for total chlorophyll was observed under elevated CO_2 (1.321) followed by plants under open control (1.277) and ambient chamber (1.194). Under elevated CO_2 highest value was observed in S1 (1.513) followed by S2 (1.245) and S3 (1.206). Under all the moisture regimes elevated CO_2 had significant influence on improvement of total chlorophyll content. An increasing trend of chlorophyll a, chlorophyll b and total chlorophyll content by 36.92%, 55.10% and 6.55% were recorded in tomato under elevated CO_2 in comparison with control conditions [15].

The function of majority of chlorophyll is to absorb light and transfer the energy by resonance to a special pair of chlorophyll pair in the reaction centre of the photosystems [16]. Leaf chlorophyll content is a good indicator of photosynthesis activity, mutations, stress condition and nutritional status of plants [17].

The increase in chlorophyll content in elevated CO_2 grown plants could be explained by the larger size and number of chloroplasts present in the tissues exposed to high CO_2 levels [18]. Moreover, water use efficiency was observed better at high CO_2 which could have limited chlorophyll degradation [19].

Table-9 *Effect of elevated CO_2 on total chlorophyll content under moisture stress in amaranthus*

Varieties	Total chlorophyll content (mg g^{-1})			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	1.513	1.338	1.442	1.464
S2:80% FC	1.245	1.138	1.202	1.161
S3:70% FC	1.206	1.105	1.189	1.143
Mean	1.321	1.194	1.277	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.067	0.023	0.033	
S	0.067	0.023	0.033	
T x S	0.117	0.040	0.040	

Table-10 *Effect of elevated CO_2 on transpiration rate under moisture stress in amaranthus*

Varieties	Transpiration rate (mmol water m^{-2} s^{-1})			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	3.13	16.46	19.34	12.97
S2:80% FC	2.09	15.34	16.09	11.17
S3:70% FC	1.41	12.02	12.8	8.74
Mean	2.21	14.61	16.07	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.571	0.196	0.277	
S	0.571	0.196	0.277	
T x S	0.989	0.339	0.479	

[Table-10] shows effect of elevated CO_2 on transpiration rate (mmol water m^{-2} s^{-1}) of *amaranthus*. Highly significant reduction in transpiration rate was induced by elevated CO_2 condition under all the moisture levels of soil. The mean values recorded are treatment T1 (2.212) followed by treatment T2 (14.610) and T3 (16.078). Highest values for transpiration rate were recorded under S1, S2 and S3 under elevated CO_2 conditions. Plants respond to enriched CO_2 content by showing declined stomatal conductance, which typically lead to reduced rates of transpirational loss. Elevated CO_2 reduces transpiration by partially closing the stomata and decreasing stomatal conductance [20]. Douglas fir seedlings grown for three years in environmental chambers under CO_2 concentration of 530ppm resulted in 12% reduction of transpiration. Effect of elevated CO_2 on total soluble protein content under moisture stress is presented in [Table-11]. Elevated CO_2 exposure resulted in reduction of total soluble protein content in plants grown under all the three different soil levels - S1, S2 and S3 compared to open condition. Highest mean value for total soluble protein content (mg/g) was recorded under open control condition (19.56) followed by plants under ambient chamber (17.43) and elevated CO_2 condition (16.54). Under open control condition, highest value was observed in S1-100% field capacity (24.63) followed by S2-80 % field capacity (17.41) and S3-75% field capacity (16.65). T1, T2 and T3 didn't show any significant difference on total soluble protein content.

Table-11 *Effect of elevated CO_2 on total soluble protein under moisture stress in amaranthus*

Varieties	Total soluble protein (mg/g)			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	19.15	22.02	24.63	21.93
S2:80% FC	15.42	15.88	17.41	16.23
S3:70% FC	15.06	14.41	16.65	14.75
Mean	16.54	17.43	19.56	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	1.269	0.435	0.615	
S	1.269	0.435	0.615	
T x S	2.428	0.735	1.066	

Table-12 *Effect of elevated CO2 on starch content under moisture stress in amaranthus*

Varieties	Starch content (mg g^{-1})			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	4.14	3.29	3.52	3.65
S2:80% FC	2.76	2.41	2.54	2.60
S3:70% FC	2.56	2.37	2.46	2.43
Mean	3.25	2.69	2.74	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.340	0.115	0.160	
S	0.340	0.115	0.160	
T x S	0.680	0.199	0.280	

Exposure of plants to elevated CO_2 conditions influences both primary and secondary metabolites [21]. Elevated CO_2 decreased soluble protein content in spring wheat cultivars.

Reduction 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 lower in barley leaves enriched with high CO_2 concentration [22].

These results are in complete agreement with research done in sun flower, in maize and pine tree (Pinus pinaster) under water stress and elevated CO_2, where they found reduction in protein content with CO_2 enrichment [23, 24 and 25].

[Table-12] shows effect of elevated CO_2 on starch (mg g^{-1}) content under moisture stress in *amaranthus*. Highest mean value for starch content was observed under elevated CO_2 (3.25) followed by plants under open control (2.74) and ambient chamber (2.69) CO_2 enrichment had significant influence on starch content and didn't show significant difference. Under elevated CO_2 highest value was observed in S1 (4.14) followed by S2 (2.76) and S3 (2.56).

Effect of elevated CO_2 on reducing sugar (mg g^{-1}) under moisture stress is presented in [Table-13]. Highest mean value for reducing sugar was observed under elevated CO_2 (18.11) followed by plants under open control (16.33) and ambient chamber (15.97) CO_2 enrichment had significant influence on reducing sugar content. Under elevated CO_2 highest value was observed in S1 (23.14) followed by S2 (17.61) and S3 (13.56). S2 and S3 did not show any significant difference upon exposure to increased CO_2 concentration.

Highest mean value for reducing sugar under elevated CO_2 in the case of Cerrado species (*Chrysolaena obovata*) [26]. Highest mean value for reducing sugars was observed under elevated CO_2 in the case of tomato [27].

Table-13 *Effect of elevated CO_2 on reducing sugars under moisture stress in amaranthus*

Varieties	Reducing sugars (mg g^{-1})			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	23.14	18.76	20.07	20.66
S2:80% FC	17.61	16.9	15.77	16.76
S3:70% FC	13.56	12.23	13.46	13.19
Mean	18.11	15.97	16.33	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	1.782	0.611	0.864	
S	1.782	0.611	0.864	
T x S	2.448	1.058	1.496	

Table-14 *Effect of elevated CO_2 on phenol content under moisture stress in amaranthus*

Varieties	Phenol content (mg g^{-1})			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	8.74	3.29	4.95	5.66
S2:80% FC	6.92	2.74	3.93	4.53
S3:70% FC	3.53	1.76	3.19	2.82
Mean	6.4	2.59	4.024	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.525	0.180	0.254	
S	0.525	0.180	0.254	
T x S	0.909	0.312	0.441	

Elevated CO_2 expose resulted in significant increase in phenol (mg g^{-1}) content of *amaranthus* [Table-14]. Highest mean values in phenol content was observed under elevated CO_2 (6.40) followed by plants under open control (4.02) and ambient chamber (2.59). Under elevated CO_2 highest phenol content value was observed in S1 (8.74) followed by S2 (6.92) and S3 (3.53). Under all the moisture regimes, elevated CO_2 had significant influence on accumulation of phenol in *amaranthus*.

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 [28]. Elevated CO_2 increase in the total phenol content in wheat leaves [29]. Similar reports were recorded in the case of tomato [30].

Effect of elevated CO_2 on Membrane integrity (% leakage) under moisture stress is presented in [Table-15]. Highest mean value for membrane integrity (% leakage) was recorded under open control condition (6.558) followed by plants under ambient chamber (5.152) and elevated CO_2 condition (3.855). Under open control condition highest value was observed in S1-100% FC (7.886) followed by S2-80 % FC (6.011 %) and S3-75% FC (5.780 %). T1, T2 and T3 are show significant influence on improvement on membrane integrity (% leakage) of *amaranthus*.

Table-15 *Effect of elevated CO_2 on membrane integrity (% leakage) under moisture stress in amaranthus*

Varieties	Membrane integrity (% leakage)			
	T1 (OTC Ec)	T2 (OTC Ac)	T3 (Open control)	Mean
S1:100 % FC	5.07	6.47	7.88	6.47
S2:80% FC	3.48	5.19	6.01	4.89
S3:70% FC	3.01	3.78	5.78	4.19
Mean	3.85	5.15	6.55	
Factors	CD (0.05)	SE±(m)	SE±(d)	
T	0.447	0.153	0.217	
S	0.447	0.153	0.217	
T x S	0.794	0.265	0.375	

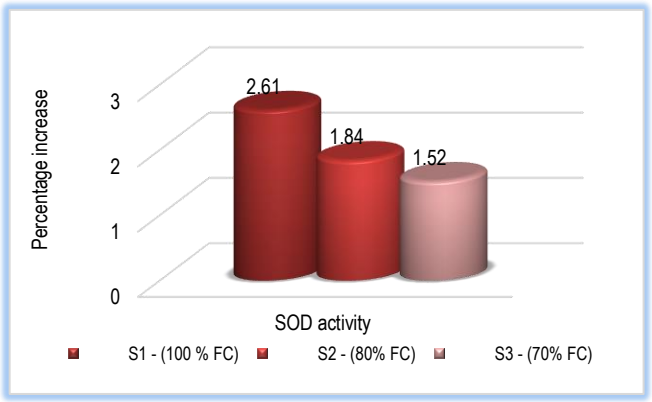

Fig-1 Effect of elevated CO_2 on SOD activity under moisture stress in *amaranthus*

[Fig-1] shows effect of elevated CO_2 on SOD (g^{-1}minute^{-1}) activity under moisture stress in *amaranthus*. Highest mean value for SOD activity was observed under elevated CO_2 (1.988) followed by plants under ambient chamber (0.978) and open control (1.065). Under elevated CO_2 highest value was observed in S1 (2.610) followed by S2 (1.842) and S3 (1.526). Elevated CO_2 had improved SOD activity significantly in *amaranthus* both under 80% FC and 70% FC.

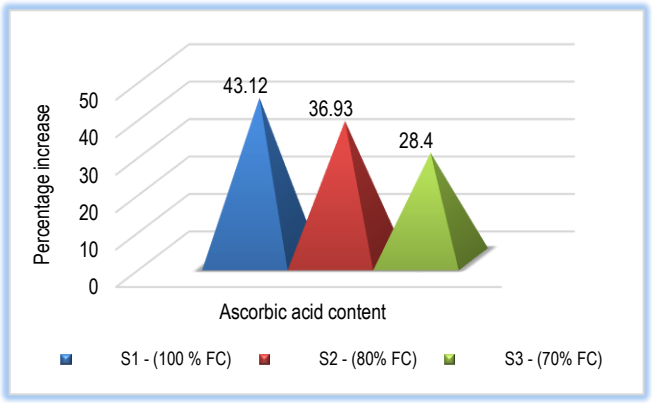

Fig-2 Effect of elevated CO_2 on Ascorbic acid content under moisture stress in *amaranthus*

Effect of elevated CO_2 on Ascorbic acid content under moisture stress is presented in [Fig-2]. Highest mean values for Ascorbic acid was observed under elevated CO_2 (36.15) followed by plants under open control (33.70) and ambient chamber (32.23). Under elevated CO_2 highest value was observed in 100% FC (43.12) followed by 80 % FC (36.93) and 75% FC (28.40). The data showed CO_2 induced increase in ascorbic content under moisture stress condition at significant level. This can be explained on the basis of developmental and physiological effect of the stress hormone Abscisic acid (ABA). ABA plays primary regulatory roles in the response of plants to stress factors, particularly, water stress. ABA induced stomatal closure plays important role in saving water in plant tissues under moisture stress.

ABA influences many other aspects of plant development by interacting with auxin, cytokinin, gibberllin and ethylene. Higher CO_2 concentration was found to improve tissue water status in terms of RWC. Though stomatal frequency was not modified significantly, there was reduction in transpiration rate. Membrane damage was found to be less under stress condition in both cowpea and *amaranthus* upon exposure to CO_2. Starch, Free amino acid, reducing sugar and phenol contents were significantly increasing even under stress condition in both the systems. The production of enzymatic and non-enzymatic antioxidants like SOD and Ascorbic acid was modified under elevated CO_2 concentration condition especially with a significant increase in the case of SOD under moisture stress condition.

Conclusion

Plant productivity is determined both by the amount of water available and the efficiency by which the water is used by the plant. With the continuous rise in atmospheric CO_2 and global climate change, it is essential to know how water use pattern of different species would change with the environment and CO_2 levels, to predict the associated changes in productivity. In this study elevated CO_2 concentration improved the growth performances of *amaranthus* under mild and severe moisture stress conditions in terms of increased number of leaves, specific leaf area, root weight, shoot weight, root shoot ratio and dry matter production both under 80% and 70% FCs.

Application of research: Understanding response of *Amaranthus* the most to the predicted environmental condition in terms of growth and development under water stress.

Research Category: Climate change (CO_2 enrichment- simulation study)

Table legend: Treatments found Significant at 1% and 5% level of significance
T-Treatment, S-Moisture levels, FC- Field capacity, T1-OTC with Elevated CO_2 Concentration (OTC Ec),
T2-OTC with Ambient CO_2 Concentration (OTC Ac), T3-Open Control

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Study area / Sample Collection: Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvanthapuram, 695522, Kerala, India

Cultivar/Variety name: *Amaranthus*- Arun Variety

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