CARBON DIOXIDE ENRICHMENT INDUCED DROUGHT TOLERANCE RESPONSES IN TOMATO (*Solanum lycopersicum* L.) AND AMARANTHUS (*Amaranthus tricolor* L.)

by

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DECLARATION

I, hereby declare that this thesis entitled **"Carbon dioxide enrichment induced drought tolerance responses in tomato (*Solanum lycopersicum* L.) and amaranthus (*Amaranthus tricolor* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani **DHEERAJ CHATTI**

Date : (2014-11-245)

CERTIFICATE

Certified that this thesis entitled **"Carbon dioxide enrichment induced drought tolerance responses in tomato (*Solanum lycopersicum* L.) and amaranthus (*Amaranthus tricolor* L.)."** is a record of research work done independently by Mr. Dheeraj Chatti (2014-11-245) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associate ship to him.

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Dheeraj

CONTENTS

List of Tables

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
@	At the rate of
µg	Microgram
µm	Micrometer
^{o}C	Degree Celsius
m^{-2}	Per metre square
CD	Critical difference
Cm	Centimeter
Ml	Millilitre
M	Molar
EC	Enzyme commission
Ppm	Parts per million
o	Degree Celsius
M	Meter
µ	Micro
CRD	Completely Randomized Design
DNA	Deoxyribo nucleic acid
Rpm	Rotations per minute
et al.	and other Co workers
OD	Optical density
Fig.	Figure
G	Gram
i.e.	That is
KAU	Kerala Agricultural University
Mm	Millimeter
viz.	Namely

IPCC	Inter-governmental panel on climate change
NOAA	National Oceanographic and Atmospheric Administration
Mm	Milli meter
Ha	Hectare
FACE	Free Air CO_2 enrichment
µmol	Micromoles
Mmol	Millimoles
pCO2	Partial pressure of CO_2
µL	Microliter
kDa	Kilo Dalton
µ Enst.	Micro Einstein
Mg	Milligram
Nm	Nanometer
S	Seconds
A_{663}	Absorbance at 663nm
A_{645}	Absorbance at 645nm
A_{480}	Absorbance at 480nm
A_{510}	Absorbance at 510nm
A_{520}	Absorbance at 520nm
A_{460}	Absorbance at 460nm

1. INTRODUCTION

From the past 150 years, atmospheric CO_2 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 lead 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) 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^{o}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. Environmental Protection Agency considers many molecules like water vapor (H_2O), carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) as greenhouse gases. Of the major greenhouse gases, carbon dioxide is the most important anthropogenic component.

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. 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. 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.

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. The results of many studies indicate that lower evaporative flux density associated with high CO_2 induced stomatal closure results in increased net photosynthesis and better water use efficiency. Under elevated CO_2 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_2 conditions.

CO_2 is the 'food' that sustains essentially all plants on the face of the earth as well as those in the sea. Carbon dioxide being a primary substrate for photosynthesis, a rising concentration will have a direct effect on plant growth by enhancing the production of assimilates although not proportional. The indirect effects of rising carbon dioxide concentration include changes induced by other environmental variables which occur as a result of the effect of increased CO_2 on global climate. But there exists a spatial and species (C_3, C_4 and CAM) variation in CO_2 induced responses due to the variation in the availability of other growth resources. This necessitates site specific CO_2 enrichment studies with respect to specific crops. So designing improved production technologies with suitable varieties for a changing climatic scenario is highly significant.

Earlier researches on plant response to elevated CO_2 had been conducted under laboratory greenhouse or controlled field condition. Now a days, number of programmes are being carried out all over the world to study the impact of rising CO_2 on agricultural systems. Technologies such as FACE (Free Air CO2 enrichment), OTC (Open Top Chamber) and SPAR (Soil Plant Atmosphere Research) have been developed and are being currently used for crop response studies. In India studies

have been reported from IARI New Delhi, CRIDA Hyderabad, IGFRI Jhansi, NPL New Delhi, CRRI Cuttack, BHU, etc. CO_2 enrichment studies in Kerala are being carried out in CPCRI Kasargode and in College of Agriculture Vellayani.

Tomato (*Solanum lycopersicum*) is the widely cultivated vegetable in India and 2nd most important vegetable crop next to potato. Current world production is about 100 million ton fresh fruits from 3.7 million ha. It is a day neutral plant with optimum mean daily temperature of 18-25ºC. This crop is very sensitive to environmental factors like soil moisture status, temperature, salinity etc. The most sensitive periods of this crop is germination and early plant development phase and flowering stage. Under Hi-tech agricultural practices tomato is a highly chosen crop.

Amaranthus is the traditional leafy vegetable which has, over the centuries, provided rural communities with food and nutritional security. It is a hardy, drought tolerant plant and is with a great potential for adaptation to impending climate change. Frequent application of water is required, related to the stage of the growth of the crop and the moisture retaining capacity of the soil. But it can grow on a wide range of soil types and soil moisture levels.

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

2. REVIEW OF LITERATURE

Agricultural productivity is decreasing worldwide due to detrimental effects of various biotic and abiotic stresses. Drought, which is the most important environmental stress, severely impairs plant growth and development, limits plant production and the performance of crop plants more than any other environmental factor. Plant experiences drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. Available water resources have been found decreasing in recent years for successful crop production. Furthermore, in view of various climatic change models scientists suggested that crop losses due to increasing water shortage will further aggravate its impacts in many regions of world it has become imperative to take actions for improving the drought resistance ability of crop plants and to ensure higher crop yields against unfavorable environmental stresses.

According to the Intergovernmental Panel on Climate Change (IPCC), by the year 2050, the current atmospheric CO_2 level of 384 μmol l^{-1} (800 Gt) is predicted to rise to 1000 Gt. This time only humans are the drivers of these changes and not glacial-interglacial cycles. Human-caused increases in atmospheric CO_2 concentration are thought to be largely responsible for recent increases in global mean surface temperatures and are expected to increase by 1.4 to over 5ºC by 2100 (Intergovernmental Panel on Climate Change, 2007, 2012). 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 (Schiermeier, 2008) and can increase potential evapotranspiration, leading to a more severe water deficit in arid and semiarid areas.

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. 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. 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.

Under elevated CO_2 conditions, plants adapt many mechanisms to cope up with the stress factors. Plant growth is nearly always stimulated by elevation of CO_2. Photosynthesis increases, more plant biomass accumulates per unit of water consumed, and economic yield is enhanced. The profitable use of supplemental CO_2 over years of greenhouse practice points to the value of CO_2 for plant production. In the agricultural context, the growing season has been shortened for some crops with the application of more CO_2; less water use was generally observed but not always and it is under further study.

Important stresses including drought, temperature, salinity, and air pollution have been shown to be ameliorated when CO_2 levels are elevated. Plant responses to CO_2 are known to interact with other environmental factors, e.g. light, temperature, soil water, and humidity. Elevated CO_2 decreases stomatal conductance and transpiration in C_3 and C_4 species and greatly improves water-use efficiency in all plants. Experimental studies have shown that economic yield for most crops increases by about 33% for a doubling of ambient CO_2 concentration.

Evidence shows that plant growth and productivity responses to elevated CO_2 are constrained by drought, depending on its severity and duration as well as on the plant species (Morgan and others 2001; Luo and others, 2006; Xu and others, 2007; Leakey and others, 2012). Elevated CO_2 levels may enhance plant diversity and productivity in an entire ecosystem by decreasing stomatal conductance (g_s) and consequently increasing water use efficiency (WUE) and soil water availability (Owensby and others 1996; Nelson and others 2004; Morgan and others 2011). Thus,

plant growth and leaf area increase due to the improvement in water status by CO_2 enrichment under moderate drought conditions.

CO_2 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). CO_2 enrichment causes stimulation of photosynthesis, inhibition of photorespiration and increase in nitrogen use efficiency (NUE) and water use efficiency (WUE) (Bowes, 1991 and Drake *et al*., 1997), resulting in higher biomass production and changes in plant elemental composition.

In theory, increases in atmospheric levels of CO_2 above current levels can increase photosynthesis by decreasing photorespiration (fixation of O_2 rather than CO_2 by Rubisco), which increases with temperature and is higher in C_3 than C_4 and crassulacean acid metabolism (CAM) plants (Sage & Monson, 1999). In addition, rising CO_2 generally stimulates C_3 photosynthesis more than C_4. Doubling of the current ambient CO_2 concentration stimulated the growth of C_4 plants to the tune of 10–20% whereas that in C_3 plants was about 40–45% (Ghannoum *et al*., 2000). Elevated CO_2 increases photosynthesis, dry matter production and yield, substantially in C_3 species, but less in C_4.

C_3 photosynthesis is known to operate at less than optimal CO_2 levels and can show dramatic increase in carbon assimilation, growth and yields under elevated CO_2 conditions. As RuBISCO is substrate-limited by the current atmospheric CO_2 levels, this enzyme has the potential to respond to increases in CO_2 concentration; and have a metabolic control to alter the CO_2 flux during carbon assimilation (Bernacchi *et al*., 2003; Long *et al*., 2004).

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 CO_2 concentration will not only affect ecosystem

productivity in the future, but also the magnitude of C sequestration by plants and, consequently, the rate of CO_2 increase in the atmosphere.

Interactive studies on water availability and elevated CO_2 show that there will be a partial closure of stomata due to increased CO_2 concentration in the substomatal cavity decreasing partial pressure of CO_2 in the leaf and this CO_2 - dependent amplification of stomatal response could improve water use efficiency at the leaf and whole plant level. In a wide range of experiments, plants grown under elevated CO_2 had substantial decrease in stomatal conductance (g_s) showing acclimation of g_s to elevated CO_2. Decreased g_s might increase leaf temperature, which could increase the rates of transpiration. However, different experimental techniques used by Wullschleger *et al*. (1992) 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. 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.

GROWTH PARAMETERS

Since CO_2 is one of the substrates for the process of photosynthesis, this influences the growth rates and development of plant species. In most terrestrial plants increase in the rate of photosynthesis under elevated CO_2 was observed (Geissler *et al*., 2009) but growth responses may vary from 0 to 50% gain per season depending on the plant age, duration of observations and growth conditions (Beismann *et al*., 2002).

Elevated CO_2 increases plant biomass, root mass and total leaf area (Rogers *et al*., 1994; Curtis and Wang, 1998) and alters leaf net photosynthetic rate, stomatal

conductance and water use efficiency (WUE) (Gunderson and Wullschleger, 1994; *Saxe et al*., 1998).

In tomato Significant differences were observed in plant height, number of branches, leaf number, and leaf area in response to elevated CO_2 at the peak of the flowering stage (Mamatha *et al.,* 2014).

Dry weight of leaves, stems and rhizomes of ginger varieties were enhanced with rising CO_2. With an elevation in CO_2 concentration from 400 to 800 μmol mol^{-1}, total plant biomass was found increased in two ginger varieties i.e., 47.6% in Halia Bentong and 76.3% in Halia Bara. The order of increase of biomass in both varieties under elevated CO_2 concentration was rhizomes > leaves > stems.

Twenty two days old soybean plants grown under 10,000 mmol mol^{-1} CO_2 were found significantly taller than plants grown under 1200 and 400 mmol mol^{-1} CO_2. (Levine *et al*., 2008).

In sunflower, plant growth was markedly increased by elevated CO_2 but area per plant decreased by 6%, and leaf weight ratio specific leaf area and leaf area ratio were also found declined with elevated CO_2 (Tezara *et al*., 2002).

Height of Scots pine seedlings increased in response to elevated CO_2, whereas the final height in Norway spruce seedlings was found decreased under elevated CO_2 (Sallas, L., *et al*., 2003).

Number of Leaves

In most plants, leaves are the major site of food production for the plant. Structures within a leaf convert the energy in sunlight into chemical energy that the plant can use as food. Number of leaves in a plant indicates its physiological age. An increase in biomass due to increase in the number of branches or leaves has been reported in sweet potato and Japanese honey-suckle under CO_2 enrichment. (Bhattacharya, 1985; Sasek and Strain, 1991)

No significant effect of CO_2 enrichment was detected on the leaf growth rate of *Zostera noltii*. (Alexandre, A., *et al* 2012).

Carbon dioxide concentration had no effect on leaf fresh weight and number of Boston Fern micro cuttings. (Nowak, J. *et al* ,2006).

An increase in the number of leaves was reported in sweet potato (Bhattacharya, 1985) and in berseem (Pal, 2004) under elevated CO_2. Elevated CO_2 (800 μmol mol^{-1}) decreases the number of leaves by 23% and 14% in soybean compared with ambient CO_2 (380 μmol mol^{-1}) at 29 and 44 days after planting (Madhu and Hatfield, 2015).

Specific Leaf Area

Specific leaf area is the leaf area per unit leaf dry weight and it is inverse of specific leaf weight. It is the reduction in the leaf thickness of species achieved with height in net CO_2 exchange rate (CER) per unit leaf area from minimum leaf material (Rawson, 1992).

Leaf area expansion depends on leaf turgor, temperature, and assimilating supply for growth. Drought-induced reduction in leaf area is ascribed to suppression of leaf expansion through reduction in turgor and photosynthesis (Rucker *et al*., 1995).

Enriched CO_2 resulted in significant increase in leaf area at vegetative and 50% flowering stages in chickpea, but at pod maturity reverse trend was observed. (Saha, *et al*., 2014).

Drought stress decreased specific leaf area under elevated CO_2 in *Jatropha curca* and elevated CO_2 had little effect on leaf morphological variables. (Meng, 2013). CO_2 enrichment increased mustard plant leaf area by 52 and 23 % under well-watered and drought conditions, respectively (Mishra and others 1999). The elevated

CO_2 treatment decreased specific leaf area in Norway spruce, but had no effect on SLA of Scots pine. (Sallas *et al*., 2003).

Root Weight

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.

Increasing the atmospheric CO_2 stimulates root biomass more than above ground biomass or leaf area production in many annual plant species (Bernacchi *et al*., 2000). High carbon gain under CO_2 enrichment increased root length, diameter and number (Lee-Ho *et al*., 2007) and also stimulates lateral root production in winter wheat (Pritchard and Rogers, 2000). A shift in biomass allocation from leaves to roots can occur under CO_2 enrichment (Stulen and Den hertog, 1993).

For winter barley, higher root dry weight was observed under elevated CO_2 compared to ambient at early growth stages, but it was significantly lower at the last harvest.

Shoot Weight

Epron *et al*., 1995 reported that in *Fagus sylvatica*, shoot dry mass was significantly higher (90%) in the elevated CO_2 treatment than in the ambient CO_2 treatment. Leaf and root dry masses also showed significant increase (67% and 124% respectively) in the elevated CO_2 treatment compared to ambient CO_2 treatment.

Increasing atmospheric CO_2 significantly increased the final plant biomass, above ground biomass, leaf area and below ground biomass in *Larrea tridentate* (Obrist and Arnone, 2003).

Root Shoot Ratio

Root/shoot ratio is the simple calculation of the ratio of root dry mass to shoot (or stem) dry mass and serve as a measure of the preferential allocation of C to roots

or shoots. It is one measure to assess the overall health of plants. The partitioning pattern of photosynthates depends on plant development stage, plant species, and plant growth conditions along with physiological factors (Van veen *et al*., 1991).

Generally, when water availability is limited, the root: shoot ratio of plants increases because roots are less sensitive than shoots to growth inhibition by low water potentials (Wu and Cosgrove, 2000) .

Root shoot ratio was not significantly affected by higher CO_2 concentration in *Larrea tridentate*, a desert herb (Obrist and Arnone, 2003), tall grasses like Indian grass and Switch grass. (Mo *et al*., 1992).

Ellis, 1995 reported that, in tomato, the doubled ambient CO_2 treatment showed significantly lower root–shoot ratio (0.138), than the ambient CO_2 treatment (0.156).

Dry Matter Production

An increase in total dry matter production was reported in soybean (Pan, 1996), dry bean (Prasad, 2002), peanut and cowpea (Ellis, 1995) under elevated CO_2. Dry matter production of plants was found increased significantly under elevated CO_2 in soybean plants (Madhu and Hatfield, 2015).

In sunflower, the total biomass per plant was increased from 27.5 g in ambient CO_2 to 37.5 g in elevated CO_2, largest effect was on roots (53% increase) and then on stem (40% increase) with little effect on leaves (11%) (Tezara *et al*., 2002).

As reported by Reddy *et al.* (2010), elevated CO_2 on the respiratory rates were reduced in C_3 species, contributing to increase in biomass yield.

Under drought, the stimulation of plant growth by elevated CO_2 may be weakened, and even prohibited under severe drought. Compared to the well- watered condition, plant biomass obviously decreases with drought even under elevated CO_2 (Poorter and Perez, 2001; Xu and others, 2007).

In maize under elevated CO_2 conditions, there was no significant effect found on total dry matter production in wet conditions, but in dry conditions, there was a significant increase in total dry matter produced under elevated CO_2 (Manderscheid, R., 2011)

Pest Incidence

The increases in dry weight and stem diameter in *Phytophthora parasitica* infected plants grown in 700 ppm CO_2 relative to 350 ppm CO_2 suggested an enhanced tolerance to *Phytophthora parasitica* under elevated CO_2 conditions (Jwa and Walling, 2000).

Jasmonic acid (JA) signaling defense (JA is considered as the most important defense hormone involved in resistance against chewing insects) has been reported to be suppressed by elevated CO_2 (De Lucia, 2009) and CO_2-induced decreases in the expression of downstream genes of JA pathway (i.e., proteinase inhibitors) increased the consumption of soybean leaves by herbivorous insects (Zavala, J. A., 2008).

Proteinase inhibitors (PIs) of plants are able to reduce the feeding fitness of chewing insects by suppressing insect gut proteases. Wild type tomato plants grown under elevated CO_2 had reduced PI activity, the reduced resistance resulted in increased gut protease activities for *H. armigera* (Govind, G. M., *et al*.,2010).

The compensatory continuum hypothesis (CCH) predicts that plants growing in resource- rich or low-competition environments will be more tolerant to herbivores than those growing in resource-poor, stressful environments (Hawkes, 2001).

Plant tolerance to herbivorous insects can depend on the availability of carbon resources (Schwachtje, J., 2006). Elevated CO_2 increases C assimilation and causes re-allocation of C (especially sucrose) in plant tissue (Ainsworth, 2004). In the transport of sucrose from leaves to sink tissues via phloem, Sucrose phosphate synthase and Sucrose synthase are key regulatory enzymes. Because elevated CO_2 significantly increases plant growth and C metabolism, the CCH hypothesis would

predict that plant tolerance to herbivores would be increased in the resource-rich, elevated- CO_2 environment.

PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS:

Relative Water Content

Relative water content is the most appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. Leaf water status is intimately related to several leaf physiological variables, such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration (Kramer and Boyer, 1995).

Tognetti, *et al*. (2000) interpreted that, plants under elevated CO_2 conserve soil water either due to direct effects of elevated CO_2 on leaf conductance or by improved access to soil water due to deeper root system.

Yusuke, *et al*. (2007) reported that low stomatal conductance and high WUE were observed in Ginger (Zingiber officinale Roscoe) plants grown in elevated CO_2 conditions. Elevated CO_2 treatments significantly increased WUE in both varieties of ginger (Halia Bentong and Halia Bara) proving that ginger needs little water to maintain turgidity of the plant cells when enriched with carbon dioxide.

Manderscheid *et al.,* (2011) reported that under elevated carbon dioxide conditions (FACE), increased relative water content was observed in maize under water stress compared to ambient CO_2 concentration.

In a study conducted by Schwanz and Polle, 2001, on pendunculate oak (*Quercus rober*) and maritime pine (*Pinus pinaster*) grown under elevated CO_2 and drought conditions, under elevated CO_2 the loss of foliar water was observed 2 fold and 1.6 fold slower in oak and pine respectively than ambient CO_2.

Pigment Composition

The quantity of chlorophyll per unit area is an indication of photosynthetic capacity and productivity of a plant. Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate.

In a study conducted by Lin and Wang in 2002, doubled CO_2 decreased total chlorophyll content significantly in two spring wheat cultivars (*Triticum aestivum* L. Longchun 292 and Longchun 8139). Total chlorophyll content declined gradually initially after stress but under prolonged stress, total chlorophyll content lost rapidly.

Helyes *et al* , in year 2005 reported that, in tomato, lycopene content of fruits decreased with CO_2 enrichment. Lycopene content ranged from 81.7 to 124.4 mg / kg fresh weight with ambient level of CO_2 (360 μmol mol^{-1})., whereas in elevated CO_2 treatment (700 μmol mol^{-1}) it was between 70.7 and 108.4 mg / kg fresh weight. Elevated CO_2 level decreased lycopene content by 13% at the first harvest, 25% at the second harvest and 13% at the third harvest.

In a study conducted by Mamata, H. *et al*, 2014 in tomato, during flowering the plants grown at EC700 showed a lower chlorophyll content compared with EC550 and the control plants. Total chlorophyll content at EC700 was 15 and 14.5% lower in comparison with the control and EC550 treatments, respectively. Chlorophyll a and chlorophyll b content also showed the same trend.

Li *et al*., (2008) reported that, for cucumber seedlings grown in both ambient (380 ppm) and elevated CO_2 (760 ppm), leaf chlorophyll content decreased progressively and significantly under drought stresses. Chlorophyll content of seedling leaves not subjected to drought stress was found 15% and 16% higher than that of severe drought stressed seedlings in ambient and elevated CO_2, respectively. Elevated CO_2 was found to reduce chlorophyll content slightly but not significantly.

In a study conducted by Schwanz and Polle, (2001), on pendunculate oak (*Quercus rober*) and maritime pine (*Pinus pinaster*) grown under high elevated CO_2 and drought conditions, elevated CO_2 decreased chlorophyll and carotenoid content by 30% and 38% respectively compared to ambient CO_2 conditions under drought stress in pine tree species. In oak there is only a trend towards decreasing carotenoid content with increasing drought stress but no clear effect of CO_2 was found.

Stomatal Frequency

Stomata are the portals for gas exchange between the leaf mesophyll cells and the environment. They occupy between 0.5% and 5% of the leaf epidermis and are most abundant on the bottom or abaxial surface. They are the integrators of all environmental factors that affect the plant growth (Morison, 1998). Stomatal density is determined by stomatal initiation during ontogenesis and by epidermal cell expansion (Radoglou and Jarvis, 1990). CO_2 enrichment of 700 μmol mol^{-1} decreased the stomatal densities in the leaves of *Arabidopsis thaliana* (Woodward *et al*., 2002). Stomatal density decreased under elevated CO_2 as a consequence of an increase in leaf expansion, as stomatal index was not altered (Rey and Jarvis, 1997; Bettarini *et al*., 1998).

A wide range of responses are observed in crop plants with increasing CO_2 concentration. Induction of stomatal density is varied from the large reductions to large increases among species and even within the species (Woodward *et al*., 2002).

In a study conducted by Levine *et al*., (2008) on soybean, elevating CO_2 from 400 to 1200 ppm resulted in an overall decrease in day time stomatal conductance (g_s/day) Further increasing CO_2 to 10,000 ppm did not lead to a further decline in g_s/day, but rather increased stomatal conductance was recorded above those of 1200 ppm CO_2 grown plants. The number of stomata per square millimeter was 258, 259 and 285 for 400, 1200 and 10,000 ppm CO_2 - grown plants ,respectively. Although there was no difference in stomatal density between 400 and 1200 ppm plants, the stomatal density of plants grown at 10,000 ppm CO_2 observed 10% greater than those

of the control or 1200 ppm CO_2 plants. The SEM (Scanning Electron Microscope) images of the plant leaves also revealed that stomatal aperture in plants grown under 1200 ppm CO_2 appeared much smaller than those of plants grown at 400 and 10,000 ppm which is consistent with the result of gas exchange

As reported by Driscoll, *et al.* (2006), in maize, the number of stomata was found unaffected by CO_2 concentration. The size of the stomata was increased at 700 µL/L CO_2 compared with 350 µL/L CO_2. The stomatal index increased with doubling the CO_2 concentration on both leaf surfaces. The area occupied by stomata was found greater on the abaxial surface than the adaxial surface of the leaves under both CO_2 conditions.

In a study conducted by Sarker and Hara. (2011), on effects of elevated CO_2 and water stress on the adaptation of stomata and gas exchange in leaves of eggplant, eggplants grown under elevated CO_2 environment had reduced stomatal density in both adaxial and abaxial surfaces.

Transpiration Rate

Transpiration is the loss of water in the form of water vapour from the live aerial parts of the plant. It helps the plant to pull water up from the roots to supply photosynthates, to bring minerals from the roots for biosynthesis within the leaves, to cool the leaves and also to keep the plant cells turgid. The rate of transpiration is affected by a number of internal (plant factor) and external factors (light, temperature, humidity, wind, atmospheric pressure and water supply).

In a study conducted by Centritto (1999) on cherry, instantaneous transpiration efficiency (assimilation rate to transpiration rate ratio) was recorded significantly higher under elevated CO_2 than in ambient CO_2.

Sarker and Hara. (2011) said that, leaf transpiration rate was found decreased for eggplant grown under elevated CO_2 concentration. Water stress also markedly reduced the transpiration rate per unit leaf surface area. Under elevated CO_2

environment, eggplants had lower stomatal conductance than ambient CO_2 environment.

In an experiment conducted by Liang, 1994, CO_2 enriched *Alnus firma* trees grown under well watered conditions showed significantly lower stomatal conductance compared to well watered plants in ambient CO_2. In association with low stomatal conductance, transpiration rate was also found reduced by 21% at 900 ppm CO_2 level as compared to 350 ppm CO_2 level treatments.

Tezara, *et al* (2002) reported that, stomatal conductance of sunflower was influenced by water deficit and CO_2 during growth. For plants not subjected to water stress, stomatal conductance (g_s) under elevated CO_2 was 42% lower than those grown in ambient CO_2. With mild and severe water deficit, g_s was much lower than the well watered plants.

Elevated levels of CO_2 in *Podophyllum hexandrum* showed decreased levels of stomatal conductivity and specific leaf area (Chaturvedi *et al*., 2009).

Photosynthesis Rate

Photosynthetic rate is the rate at which CO_2 is fixed per unit leaf area per unit time and it is expressed as mmol CO_2 m^{-2} s^{-1}

Samarakoon and Gifford, (1995) reported that, sunflower plant reduced the impacts of water deficits on photosynthetic mechanism by stimulating the rate of photosynthesis by allowing the adjustment of cellular water balance, under elevated CO_2 conditions.

Ghasemzadeh and Jaafar, (2011) reported that, Photosynthesis rate was increased in two varieties of ginger *Halia Bentong* and *Halia Bara* by 65% and 46% under elevated CO_2 concentration. The increase in carboxylation activity of ribulose 1,5-bisphosphate carboxylase oxygenase enzyme (rubisco) in leaves under elevated carbon dioxide level increased net photosynthesis.

Li, *et al*. (2013) reported that, in soyabean, under drought conditions, photosynthetic rate was increased by elevated CO_2 at all the three stages i.e flowering, pod and seed filling stages.

In a study conducted by Sarker and Hara (2011) on effects of elevated CO_2 and water stress on the adaptation of stomata and gas exchange in leaves of eggplant, net photosynthetic rate of leaves exposed to elevated CO_2 was observed greater than ambient CO_2, irrespective of their soil moisture status. Withholding water reduced photosynthetic rate of leaves at both CO_2 concentrations but fall at ambient CO_2 concentration was proportionally greater than elevated CO_2.

CO_2 enrichment increased net photosynthetic rate in *Alnus firma* under well watered conditions. Leaves of 900 and 600 μ mol mol^{-1} plants had an average of 98% and 67% photosynthetic rate respectively. (Liang, 1994)

Elevated CO_2 may alleviate the high temperature damage to photosynthesis because with higher CO_2 concentrations, there is a interaction between improved plant water status and protection of photosynthesis against high-temperature damage (Poorter and Perez-Soba 2001).

For mustard plants, 20 % increase in photosynthetic rate was observed due to elevated CO_2 in the well-watered condition whereas 69 % increase was recorded in drought conditions. (Mishra and others 1999).

Total Soluble Protein

Proteins and amino acids make up to 10% of the total dry mass of plant roots and shoots (Rejsek *et al*., 2010). Growth at elevated CO_2 can result in a large decline in Rubisco protein up to 60% (Sage *et al*., 1989; Besford *et al*., 1990).

Lin and Wang in 2002 reported that, elevated CO_2 decreased soluble protein content in spring wheat cultivars. Decrease in soluble protein contents could be largely due to a decline in ribulose-1,5-bisphosphate carboxylase/oxygenase

(Rubisco) protein. Though doubled CO_2 decreased total protein content under well watering conditions, the decreases in protein contents in plants grown under doubled CO_2 were delayed after stress. These 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.

In sun flower, Tezara *et al.,* 2002 reported that total soluble protein content of leaves of well watered plants were significantly reduced by (17%) in elevated CO_2 (700 ppm) compared to ambient CO_2 (350 ppm).

Reduction in soluble protein content was observed under elevated CO_2 in conifer seedlings, but the soluble protein concentration did not decrease significantly. (Sallas, 2003)

Schwanz and Polle, in 2001 said that when pine tree (*Pinus pinaster*) and pendunculate oak (*Quercus robur*) are subjected to water stress and elevated CO_2, protein content was decreased by 25% in elevated CO_2 conditions compared to ambient CO_2 conditions in pine. In oak there was only a trend towards decreasing protein with increasing drought stress, but no significant CO_2 effect was observed.

In a study conducted by Driscoll *et al*. (2005) in maize, protein content was observed low in plants grown under 700 µL L^{-1} CO_2 treatment compared to plants grown at 350 µL L^{-1} CO_2 treatment.

The soluble protein recorded was found to be higher in leaves of *Stylosanthes hamata* grown under 600ppm CO_2 (Baig *et al*., 2012). Under elevated CO_2 concentration of 700 µmol mol^{-1} a decrease in total soluble protein of barley pnultimate leaves and wheat flag leaves were reported. (Richard And James, 1997).

Starch and Reducing Sugars

Significant increase in foliar carbohydrate content is usually observed at elevated CO_2, even when plants are free from artificial restriction of sink development (Long *et al*., 2004).

Li, *et al.* (2013) reported that, elevated CO_2 increased carbohydrates accumulation in tomato plants. The leaf carbohydrates determinations showed that the starch, total soluble sugar, and sucrose concentrations increased significantly in plants exposed to 800 µmol mol^{-1} CO_2. The concentrations of the three carbohydrates were increased by 90%, 60% and 44%, respectively compared to control.

In a study conducted by Centritto, M., *et al.,* (1999) on cherry seedlings, leaf starch concentration was strongly enhanced by elevated CO_2 and influenced by water stress treatments. The increase in starch in the well watered seedlings ranged from 33% (day 80) to 198% (day 69), whereas in the droughty seedlings the increase was significant only on day 115 (61%).

Ghasemzadeh and Jaafar, 2011 reported that, elevated carbon dioxide concentration had significant effect on total soluble carbohydrate (TSC) and starch content in two ginger varieties i.e . *Halia Bara* and *Halia Bentong.* Maximum TSC content was observed in *Halia Bara* (38.43 mg/g dry weight) and *Halia dentong* (38.22 mg/g dry weight) leaves grown under 800 µmol mol^{-1} CO_2 and maximum starch content was observed in *Halia bentong* rhizomes (583.5 mg/g dry weight) and *Halia bara* rhizomes (553.3 mg/g dry weight) grown under 800 µmol mol−1 CO_2. Elevated CO_2 concentration enhanced TSC and starch content in all parts of both varieties. Due to elevated CO_2, carbohydrates accumulate in plant tissues, as their usage intensity is lower than their production under these conditions

Levine, *et al*. (2008) said that, in soybean, under native lighting (550 photosynthetic photo flux (PPF), increasing atmospheric CO_2 from 400 to 1200 and 10,000 µmol mol^{-1} increased starch accumulation by 65% and 165%, respectively. A

24h acclimation to reduced light intensity (150PPF) dramatically reduced the starch levels for all 3 CO_2 treatments

Saha, S., *et al*., 2015 reported that, CO_2 enrichment resulted in increase in the water soluble carbohydrate concentration in leaves especially during vegetative (18%,) and 50% flowering stages (46%). At pod maturity, the water soluble carbohydrate concentration in leaves decreased.

In a study conducted by Yelle (1989) on acclimation of two tomato species *Lycopersicon esculentum* Mill. cv Vedettos and *Lycopersicon chmielewskii* to high atmospheric CO_2, tomato plants of both the species grown at 900 , uL L^{-1} CO_2 contained more starch, sucrose and glucose + fructose than the control.

CO_2 enrichment enhances the concentration of total carbohydrates in plants (Ibrahim and Jaafer., 2012). When alfalfa plants were grown under CO_2 enrichment (700 μmolmol^{-1}) under different levels of temperature total soluble sugar content was enhanced and total starch content remained unchanged (Aranjuelo *et al*., 2005).

It is widely agreed that plant growth in CO_2 enriched atmospheres enhances the accumulation of both leaf starch and soluble carbohydrates (De Souza *et al*., 2008; Norby *et al*., 1986).

Phenol Content

Phenolics are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against stress. These secondary metabolites play important roles in plant development, particularly in lignin and pigment biosynthesis. They also provide structural integrity and scaffolding support to plants.

Accumulation of total phenolics in *L. pumila* was influenced by the interaction effect between CO_2 and plant parts. Total phenolics was observed to be higher in the leaf at 1,200 μmol/mol CO_2 (1.259 mg/g) followed by leaf-800

µmol/mol CO_2 (1.167 mg), leaf-400 µmol/mol CO_2 (0.835 mg/g), stem-1,200 µmol/mol CO_2 (0.862 mg/g). (Ibrahim, 2012).

Mamata *et al* (2014) reported that, in tomato (*Lycopersicon esculentum* Mill) cv. Arka Ashish decreased phenols and antioxidants activity was observed in elevated CO_2 conditions, which might be due to lower stress experienced by the plants at EC as observed by the higher water potentials of these plants.

Koricheva *et al*. (1998) reported that the total phenolic concentration increased in temperate species when grown under elevated CO_2 although responses varied among species and environmental conditions (Kinney and Lindroth, 1997). In a two year study with open-top chambers using Japonica rice variety, a reduction in phenolic concentration was reported during seedling stage whereas an increase was reported during maturity stage under elevated CO_2 concentration of 550 µmol mol^{-1} (Goufo, 2014).

Free Amino Acid

Increase in soluble amino acids under CO_2 enrichment was noticed as ample carbon was available to support amino acid synthesis (Sicher, 2008). Soluble amino acids were increased in young soybean and tobacco leaves exposed to atmospheric CO_2 enrichment (Geiger *et al*., 1998; Ainsworth *et al*., 2007) in tobacco and soybean and barley (Manderscheid *et al*., 1995).

Increasing amino acid content can be related to degradation of proteins under elevated CO_2 conditions and hydrolysis to free amino acids (Wrigley *et al*., 1988).

Membrane Integrity

Cellular membrane modification is a major impact of plant environmental stress, which results in cellular membrane perturbed function or total dysfunction. Cellular membrane dysfunction due to stress is well expressed in increased permeability and leakage of ions out. High temperature due to elevated CO_2 can alter

the physical state of the membrane, and lead to fluidization and disintegration (Los and Murata, 2004).

No major differences were observed in membrane integrity in the cases of two spring wheat cultivars (*Triticum aestivum* L. Longchun 292 and Longchun 8139) grown under ambient or doubled CO_2. (Lin and Wang, 2002).

Stable Isotope Discrimination

Carbon isotope discrimination can be defined as the molar ratio of $^{13}C/^{12}C$ (Ra) in atmospheric CO_2- the carbon source for plants divided by the same ratio in the plant product (Rp) (Farquhar and Richards, 1984). Atmospheric pCO_2 has been shown to influence multiple aspects of plant biology like growth, water use efficiency, chemical profiles in plant cells etc. The basis of the biochemical discrimination against ^{13}C in C_3 plants lies with the primary carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) which discriminates against ^{13}C because of the intrinsically lower reactivity of ^{13}C compared with $_{12}C$ (Farquhar *et al*., 1982)

The isotopic composition of carbon in whole plant and plant organs can provide an integrated long term view of carbon assimilation by the plant. The isotopes are unevenly distributed among and within different compounds and this isotopic distribution can reveal information about the physical, chemical, and metabolic processes involved in carbon transformations. Several physical factors like stomatal conductance and carboxylation have been shown to influence the integrated balance of isotopic discrimination in plants (Henderson *et al*., 1998).

Records of Δ ʒ Cp^{13} in oak trees have been reported to show a positive correlation with increasing CO_2 over the last 160 years (Gagen *et al*., 2007; Loader *et al*., 2008; Mc Carroll *et al*., 2009).

Studies showed a positive correlation between Δ ʒ ^{13}Cp and p CO_2 (Saurer *et al*., 2003; Hietz *et al*., 2005; Sharma and Williams, 2009), negative correlation

(Beerling and Woodward, 1993) and no correlation (Jahren *et al*., 2008) was reported in various fossil studies.

SOD and Ascorbic Acid

Plant cells involve complex antioxidant defence mechanisms against oxidative stress generated under stress conditions (Matsuura and Fett-Neto, 2013). Antioxidative activity can be non-enzymatic and enzymatic (Bartels and Sunkar, 2005). Non-enzymatic antioxidants include vitamin C, vitamin E, glutathione, flavonoids, alkaloids, carotenoids etc and Enzymatic antioxidants include catalase, superoxide dismutase, peroxidase and metallothionein (Seki *et al*., 2001).

Three months exposure to elevated CO_2 concentration of 720µL L^{-1} in open top field chambers reduced the activities of superoxide dismutase and catalase by an average of 23% and 39% respectively in soybean (Pritchard *et al*., 2000).

Polle *et al*. (1997) showed that two years of atmospheric CO_2 enrichment reduced the activities of several key antioxidative enzymes including catalase and superoxide dismutase in beech seedlings.

Lin and Wang, 2002 reported that, activities of three SOD forms (Cu/ZnSOD, FeSOD, MnSOD) declined significantly after stress for 10 days, in two spring wheat cultivars (*Triticum aestivum* L. Longchun 292 and Longchun 8139), regardless of ambient or doubled CO_2. No significant changes were observed in the ratios of GSH/GSSG and AS/DHA in MnSOD. Doubled CO_2 significantly decreased the ratios of GSH/GSSG and AS/DHA in *Triticum aestivum* L. Longchun 8139.

In bean sprouts, a mere one hour per day doubling of atmospheric CO_2 concentration actually doubled vitamin C over a 7 day period (Tajiri, 1985).

MOLECULAR STUDIES:

As reported by Nie *et al*., 1995, in spring wheat leaves grown under elevated CO_2 concentration of 550μmol mol^{-1} the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBISCO) content declined by 60%. Reduction in total ribulose1,5-bisphosphate carboxylase/oxygenase (RuBISCO) activity along with plant age was observed lower in the elevated CO_2 (100 Pa) compared to the ambient CO2 treatment (Hanhong and Richard, 2004). RuBISCO activity and RuBISCO protein in barley penultimate leaves and wheat flag leaves were decreased under elevated CO_2 concentration of 700 μmol mol-1 (Richard and James, 1997). In black gram, enhanced CO_2 concentration was found to decrease the intensity of 52 kDa and 51.4 kDa polypeptide at vegetative and flowering stages (Sathish *et al*., 2014). Several investigations suggest that most prominent change in leaf photosynthetic apparatus under elevated CO_2 is a decrease in the amount of RuBISCO protein (Drake *et al*., 1997).

RuBISCO content of sunflower leaves of well watered plants reduced by 25% by growth in elevated CO_2 compared to ambient CO_2. But in severe water deficit conditions, RuBISCO content decreased more in plants grown in ambient CO_2 than elevated CO_2 (Tezara *et al.,* 2002).

3. MATERIALS AND METHODS

The experiment entitled Carbon dioxide enrichment induced drought tolerance responses in tomato (*Solanum lycopersicum* L.) and amaranthus (*Amaranthus tricolor* L.) was undertaken with the main objective to study the physiological basis of varietal responses of tomato and amaranthus to water stress conditions and to study their modifications under elevated CO_2 environments.

For this, two pot culture experiments were conducted with three varieties of tomato i.e, Manulakshmi, Vellayani Vijay, Anagha and three varieties of amaranthus i.e, Arun, CO -1 and Renusree at the Department of Plant Physiology, College of Agriculture, Vellayani. The technology used for subjecting the plants to elevated CO_2 environments is the Open Top Chambers (OTC) system. One month old potted plants of tomato and 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.

3.1 EXPERIMENT DETAILS

3.1.1 Location

The field experiment was conducted in Open Top Chambers located at College of Agriculture Vellayani, situated at 8°5'N latitude and $76^0$9'E longitude and an altitude of 29 m above mean sea level.

3.1.2 Season

The experiments were conducted from August, 2015 to September, 2015 on tomato and from February, 2016 to March, 2016 on amaranthus in Open Top Chambers.

3.1.3 Planting material

One month old tomato plants of variety Manulakshmi, Vellayani vijay, Anagha and amaranthus plants of variety Arun, CO-1 and Renusree were used for the study. The planting materials were procured from Department of Olericulture, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala.

3.1.4 Layout of the Experiment

The experiment was laid out in CRD with three treatments three replications and two stress levels.

3.1.5 Technique for CO2 enrichment

Technology used for creating CO_2 enriched environment is Top Chambers (OTC). Open Top Chambers (OTC) are square type chambers constructed to maintain near natural conditions and elevated CO_2 conditions for experimental purposes. 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, 45^0slope and $1m^2$ opening at the top. 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. Elevated CO_2 was released into the chamber from a CO_2 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^OC relative humidity of 65.96% and solar radiation of 384.65µ Enst. 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.

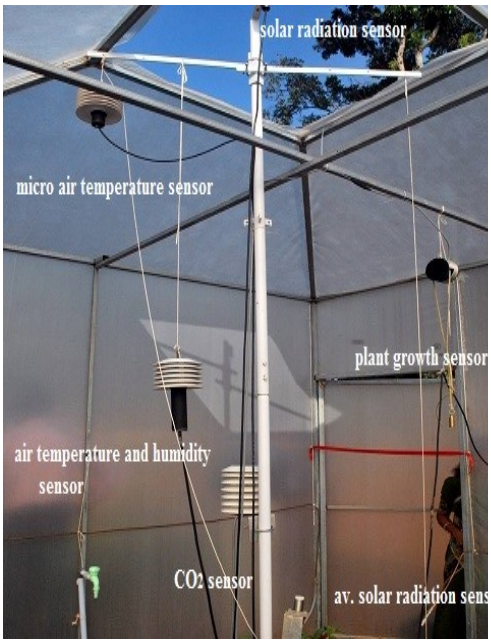


Plate 1. Open Top Chamber for CO_2 enrichment

Plate 2. Tomato plants kept in open top chamber

Plate 3. Amaranthus plants kept in open top chamber

The elevated CO_2 concentration of 600 ppm was selected by referring IPCC (2007) which suggested that atmospheric concentrations of carbon dioxide has been steadily rising with an average annual increase rate of about 2 ppm and continued to rise to 500 - 1000 ppm by the year 2100.

3.1.6 Treatments

T1 - OTC with elevated CO_2 concentration (OTC EC) – 600 ppm

T2 - OTC with ambient CO_2 concentration (OTC AC)

T3 - Open control (C)

3.1.7 Preparation and Planting

The experiment was conducted in pots filled with potting mixture containing farm yard manure, sand and soil in the ratio of 1:1:1. The experiment was laid out in CRD. The potted plants were kept in OTCs during the experimental period.

3.2 OBSERVATIONS

3.2.1 Growth Parameters

3.2.1.1 *Number of Leaves*

Total numbers of leaves in the treatment plants were counted after stress and re-watering.

3.2.1.2 *Specific Leaf Area*

From each plant, fully expanded third leaf (from main stem apex) was collected. Leaflets were separated, petioles were discarded and area was measured. Leaflets were oven dried at 80^0C for 2 days and the dry weight was taken. SLA was calculated using the formula;

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

3.2.1.3 *Root 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.

3.2.1.4 *Shoot Weight*

Shoot weight was measured by weighing the above ground part of the plants in a weighing balance.

3.2.1.5 *Root Shoot Ratio*

Ratio of weights of dried roots and shoots of sample plants were calculated and mean values were calculated.

3.2.1.9 *Dry matter Production*

The sum of root and shoot dry weights were taken as the total dry matter yield.

3.2.1.10 *pest incidence*

Pest incidence was recorded at weekly intervals.

3.2.2. Physiological and Biochemical parameters

3.2.2.1. *Relative Water Content*

Relative water content was estimated as per the method given by Barr and Weatherly (1962) by measuring the fresh weight, turgid weight and dry weight of known number of leaf discs from the experimental plants. After measuring the fresh weight of the sample, leaf discs were 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 80ºC for 3 consecutive days.

The RWC was calculated using the following formula;

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

3.2.2.2 *Pigment Composition*

Estimation of Chlorophyll and Carotenoids

Chlorophyll content of leaf samples were estimated as per the procedure described by Arnon (1949). A weighed quantity of leaf sample (0.5g) was taken from third fully expanded leaf and it was cut into small bits. These bits were put into test tubes and incubated overnight at room temperature with 10 ml DMSO: 80% acetone mixture (1:1 v/v). The coloured solution was transferred 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.

$$Chla = \left(12.7 \times A_{663} - 2.69 \times A_{645}\right) \times \frac{V}{1000} \times \frac{1}{fresh\ weight}$$

$$Chlb = \left(22.9 \times A_{645} - 4.68 \times A_{663}\right) \times \frac{V}{1000} \times \frac{1}{fresh\ weight}$$

$$TotalChl\,(a+b) = \left(8.02 \times A_{663} - 20.2 \times A_{645}\right) \times \frac{V}{1000} \times \frac{1}{fresh\ weight}$$

$$Carotenoid = \left(\frac{7.6 \times A_{480} - 1.49 \times A_{510} \times V}{w \times 1000}\right)$$

3.2.2.3 *Stomatal Frequency*

Stomatal count 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.

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

3.2.2.4 ***Transpiration rate***

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

3.2.2.5 ***Photosynthetic rate***

Photosynthetic Rate was measured using portable photosynthetic system (CIRAS-3 SW) available in Department of Plant Physiology, College of Agriculture, Vellayani.

3.2.2.6 ***Estimation of Total Soluble Protein***

The total soluble protein content of leaf samples was estimated using simple protein dye binding assay of Bradford (1976) using bovine serum albumin (BSA) as the standard. One hundred milligram of CBB 250 was dissolved in 50 ml of 95% ethanol. To this 100 ml of 85% (w/v) ortho phosphoric acid was added. The resulting solution was diluted to a final volume of 200 ml with distilled water. 0.1g of leaf samples were taken from third fully opened leaves and was ground to a thin paste and soluble protein was extracted with 10 ml of phosphate buffer (pH 7.8).

The extract was centrifuged at 5000 rpm for 10 minutes. To the 20µl of the supernatant a known volume (5 ml) of diluted dye binding solution was added. The solution was mixed well and allowed to develop a blue colour for at least 5 min but no longer than 30 min and the absorbance was measured at 596 nm. The protein

content was calculated using the BSA standard in the range of (10-100µg). The protein content was expressed as mg/g FW.

3.2.2.7 ***Estimation of Starch***

The estimation of starch in plants was done following the Anthrone method (Mc Cready et al., 1950). 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 does 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 0oC for 20 min. This solution was centrifuged and the supernatant was saved. The extraction was repeated using fresh perchloric acid. The supernatants 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 change 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.

3.2.2.8 ***Estimation of Reducing Sugars***

The estimation of reducing sugars in plants was done following Dinirto Salicylic acid (DNS) method (Somogyi, 1952). 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 80^0C. 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 glucose standard (0 to 500µg) was run and a standard curve was plotted. The amount of reducing sugars in the sample was calculated from the standard graph.

3.2.2.9 ***Estimation of Phenols***

Estimation of phenols was done by Folin-Ciocalteau method (Mayr *et al*., 1995). 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 centrifuged 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 minutes 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.

3.2.2.10 ***Estimation of Total Free Amino Acid***

The total free amino acids were estimated by the Ninhydrin method (Moore and Stein, 1948). The plant sample was weighed (500 mg) and ground in a

mortar and pestle. To this homogenate 5-10 ml of 80% ethanol was added. The solution was filtered and centrifuged. The filtrate or the supernatant was saved for further use. This extraction was repeated twice with the residue and the supernatants were pooled. The volume was reduced by evaporation and the extract was used for the quantitative estimation of total free amino acids. Ninhydrin solution (1 ml) was added to 1 ml of extract and the volume was made up to 2 ml using distilled water. The test tube was heated in a boiling water bath for 20 minutes. The contents were mixed after adding 5 ml of the diluents (equal volumes of water and n-propanol). The intensity of the purple colour was read at 570 nm, against a reagent blank, after incubation of 15 minutes. The reagent blank was prepared as above by taking 0.1 ml of 80% ethanol, instead of extract. The standard Leucine (50mg) was dissolved in 50 ml of distilled water in a volumetric flask.

The stock standard of 10 ml was diluted to 100 ml in another volumetric flask to make the working standard solution. A series of volume from 0.1 to 1 ml of this standard solution was prepared to give a concentration range of 10µg- 100µg. The procedure was followed as that of sample and the absorbance of purple colour was read at 570 nm. A standard curve was drawn using absorbance versus concentration. The concentration of total free amino acid in the sample was determined from the standard graph and was expressed as % equivalent of leucine.

3.2.2.12 ***Membrane Integrity***

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 3hours. 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^{o} c) for 15 minutes. Absorbance of bathing medium is again read out at 273 nm to indicate final absorbance.

% leakage = Initial absorbance of bathing medium / Final absorbance of bathing medium x 100

3.2.2.13 ***Stable Isotope Discrimination***

The third fully opened leaves of experimental plants were collected, oven dried at 800C and were ground to a very fine powder. The samples were sent to the National Facility for stable isotope studies at the Department of Crop Physiology UAS GKVK Bangalore where they were analysed using the isotope ratio mass spectrophotometer (IRMS) coupled with the elemental analyzer for the continuous flow measurement of carbon isotope ratios in plant samples.

3.2.3.3 ***Superoxide dismutase***

Superoxide dismutase (SOD) activity was quantified following the method described by Kakkar *et al*. (1984). 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 assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2 ml of the enzyme preparation and water in a total volume of 2.8 ml. The reaction was initiated by the addition of 0.2ml of NADH.

The mixture was incubated at 30°C for 90 second and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of n-butanol, allowed to stand for 10 minute and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

3.2.3.4 *Estimation of Ascorbic Acid*

The ascorbic acid content in plants was estimated volumetrically by the method explained by Sadasivam and Manickam (2008). 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 (V1 ml). End point was noted on 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 homogenate was filtered through a double layered cheese cloth. 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 (V2 ml). The amount of ascorbic acid is calculated as follows:

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

3.2.3. Molecular studies

SDS - PAGE Electrophoresis separation of soluble protein and Rubisco in black pepper leaves were carried out as per the procedure described by Laemelli (1970).

One gram of leaf samples were homogenized in1.5 ml of phosphate buffer at 4^0C. The extract was centrifuged at 5000 rpm for 15 minutes. The supernatant was mixed with chilled acetone in the ratio 1:1 and the protein was allowed to precipitate by keeping the mixture at 4^0C for 30 minutes. The sample was centrifuged at 3600 rpm for 10 minutes. The supernatant was removed and the pellet was re suspended in 50

µL of denaturing buffer and vortexed. The homogenate was centrifuged at 5000 rpm for 15 minute .The supernatant was mixed with 10 µL of sample buffer and kept in a boiling water bath for 3 minutes. These samples were subjected to electrophoresis using SDS- PAGE.

Reagents

a) Acrylamide stock (30%)

Acrylamide - 29.2 g

Bis-acrylamide - 0.8 g

Double distilled water - 100 ml

b) Separating (resolving) gel buffer stock (1.5M Tris - HCl, pH 8.8)

Tris base (18.15g) was dissolved in approximately 50 ml of double distilled water. The pH was adjusted to 8.8 with 6 N HCl and made up the volume to 100 ml with double distilled water and stored at 40C.

c) Stacking gel buffer stock (0.5M Tris - HCl pH 6.8)

Tris base (6.0 g) was dissolved in approximately 60 ml of double distilled water and adjusted the pH to 6.8 with 6 N HCl and the volume was made up to 100 ml with double distilled water and stored at 40C.

d) Polymerising agents

Ammonium per sulphate (APS) 10 per cent prepared freshly before use.

TEMED –Fresh from refrigeration.

e) Electrode buffer pH 8.3

Tris base - 6.0 g

Glycine - 28.8 g

SDS - 2.0 g

Double distilled water - 2 L

f) Sample buffer

Double distilled water - 2.6 ml

0.5 M Tris HCl pH 6.8 - 1.0 ml

2-mercapto ethanol - 0.8 ml

Glycerol - 1.6 ml

SDS 20% (w/v) - 1.6 ml

0.5% Bromophenol blue - 0.4 ml

g) Staining solution

Coomassie brilliant blue R 250 - 0.1 g

Methanol - 40.0 ml

Glacial acetic acid - 10.0ml

Double distilled water - 50.0 ml

h) Destaining solution

As above without Coomassie brilliant blue

Procedure

Separating gel was first casted followed by stacking gel by mixing the various solutions as indicated below

a) Preparation of separating gel (12%)

Double distilled water - 6.7 ml

Tris HCl, pH 8.8 - 5.0 ml

SDS 10% - 0.2 ml

Acrylamide stock - 8.0 ml

The above solution was mixed well and de gassed for 3 minutes and then the following were added immediately.

Freshly prepared 10% ammonium per sulphate (APS) - 0.10 ml

Tetra methyl ethylenediamine (TEMED) - 0.01 ml

The separating gel was mixed well and poured immediately between glass plates and a layer of water was added above the polymerising solution to quicken the polymerising process

b) Preparation of stacking gel

Double distilled water - 6.1ml

Tris HCl, pH 6.8 - 2.5 ml

SDS 10% - 0.2 ml

Acrylamide stock - 1.3 ml

The solution was mixed well, degased and the following were added

APS 10% - 0.05 ml

TEMED - 0.1 ml

The water layered over the separating gel was removed and washed with a little electrode buffer and then the stacking gel was poured over the polymerized separating gel, after keeping the comb in position.

After polymerization, the comb was removed and the samples were loaded into the wells. Standards with known molecular weights was also loaded to one well. The electrophoresis was performed at 100 V till the dye reached the separating gel. Then the voltage was increased in 200 V and continued till the dye reached the bottom of the gel. Immediately after electrophoresis the gel was removed from the glass plates and incubated in the staining solution overnight with uniform shaking. Then the gel was transferred to the destaining solution. The protein appeared as bands and the gel was photographed after placing it on a transilluminator (Appligene Model White/ UV TMW- 20).

4. RESULTS

The current programme was undertaken with the main objective of assessing the "Carbon dioxide enrichment induced drought tolerance responses in tomato (*Solanum lycopersicum* L.) and amaranthus (*Amaranthus tricolor* L.)".The technology used for CO_2 enrichment was Open Top Chamber (OTC) system. Two Open Top Chambers were used, one with CO_2 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). Two pot culture experiments were conducted with three varieties of tomato i.e, Manulakshmi, Vellayani Vijay, Anagha and three varieties of amaranthus i.e, Arun, CO -1 and Renusree. One month old potted plants of tomato and 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 results based on statistically analysed data pertaining to the experiment conducted during the course of investigation are presented below.

4.1. EFFWCT OF ELEVATED CO_2 IN TOMATO (*solanum lycopersicum L.)*

4.1.1. Effect of Elevated CO_2 on Growth Parameters in Tomato

4.1.1.1 *Number of Leaves*

Effect of elevated CO_2 on number of leaves in tomato is presented in Table 1. Significantly higher mean value for number of leaves was observed under treatment T2 (15.38) compared to treatment T3 (10.00) after stress. Under elevated CO_2, leaf number was recorded as 9.77. Among the varieties, highest mean value for number of leaves was observed for Anagha.

After re-watering, higher mean value for number of leaves was observed under treatment T2 (17.88) compared to treatment T3 and treatment T1 (Table 2).

4.1.1.2 *Specific Leaf Area*

As presented in Table 3**,** Stress induced reduction in specific leaf area was observed less in treatment T1 compared to treatment T2 and treatment T3. Reduction in specific leaf area was found under elevated CO_2 (294.10 $cm^2 g^{-1}$) compared to open control (319.73 $cm^2 g^{-1}$) and control chamber (346.09 $cm^2 g^{-1}$). Among the varieties, highest specific leaf area was observed for variety Manulakshmi (347.77 $cm^2 g^{-1}$) and lowest was observed for variety Vellayani Vijay (280.75 $cm^2 g^{-1}$).

After re-watering also specific leaf area was observed highest for control chamber (368.33 $cm^2 g^{-1}$) followed by open control (365 $cm^2 g^{-1}$) and elevated CO_2 (334.16 $cm^2 g^{-1}$). Among the varieties, highest specific leaf area was observed for variety Manulakshmi (422.22 $cm^2 g^{-1}$) and lowest was observed for variety Vellayani Vijay (308.27 $cm^2 g^{-1}$) (Table 4).

4.1.1.3 *Root Weight*

Effect of elevated CO_2 on root weight was presented in table number 5. Reduction in root weight due to water stress was observed in varieties under all the treatments after stress. After stress, higher root weight was maintained under treatment T1 (1.32 g) followed by treatment T2 (1.28 g) and treatment T3 (0.87 g). Among the varieties, Vellayani Vijay recorded higher root weight (1.55 g) compared to Anagha (1.06 g) and it was significantly higher compared to Manulakshmi (0.85 g).

As shown in Table 6, after re-watering, higher root weight was observed under elevated CO_2 (1.30 g) compared to open control (1.11 g) and among the varieties, Vellayani Vijay was found to maintain higher root weight (1.64 g) followed by Anagha (1.17 g) and Manulakshmi (1.13 g).

4.1.1.4 *Shoot Weight*

As shown in Table 7, higher shoot weight was observed under treatment T1 (4.42 g) followed by treatment T2 (3.98 g) and treatment T3 (3.54 g) after stress and among the varieties, higher shoot weight was observed for the variety Vellayani Vijay (4.56 g) followed by Manulakshmi (3.85 g) and Anagha (3.53 g).

After re-watering (Table 8), higher shoot weight was observed under elevated CO_2 (4.09 g) followed by control chamber (3.02 g) and open control (2.04 g). Among the varieties, higher shoot weight was observed for Vellayani Vijay (3.54 g). Extent of re-gain in shoot weight from water stress was observed more for variety Vellayani Vijay under treatment T1 compared to treatment T3.

4.1.1.5 *Root Shoot Ratio*

Root shoot ratio under elevated CO_2 (0.40) was observed higher compared to treatment open control (0.35) after stress. Among the varieties, higher root shoot ratio was recorded for Vellayani Vijay (0.47) compared to Anagha (0.37) and Manulakshmi (0.40) as presented in Table 9.

After re-watering, lower root shoot ratio was observed under treatment T1 (0.34) followed by treatment T3 (0.56) and treatment T2 (0.62). Among the varieties, higher root shoot ratio was observed for the variety Manulakshmi (0.70) compared to Vellayani Vijay (0.64) and it was found significantly higher compared to Anagha (0.47) (Table 10).

4.1.1.6 *Dry Matter Production*

Effect of elevated CO_2 on dry matter production was shown in Table 11. After stress, water stress induced reduction in dry matter production under elevated CO_2 was found to be less compared to open control. Dry matter production was recorded significantly higher under treatment T1 (5.74 g) compared to treatment T3 (4.41 g)

and lower compared to treatment T2 (5.94 g). Among the varieties, dry matter production was recorded significantly higher for Vellayani Vijay compared to both Manulakshmi and Anagha.

After re-watering (Table 12), highest recovery in dry matter production from stress was observed under elevated CO_2 for the variety vellayani vijay. Dry matter production was observed significantly higher under elevated CO_2 (5.40 g) compared to treatment open control (3.16 g). Among the varieties, highest dry matter production was recorded for the variety Vellayani Vijay (5.19 g) followed by Anagha (4.21 g) and Manulakshmi (3.72 g).

4.1.1.7 *Pest Incidence*

Incidence of pests like mealy bugs (*Ferrisia virgate*) and serpentine leaf miner (*Liriomyza trifoli*) were observed in potted plants of all the varieties of tomato under open control. Measures were taken to control the pest incidence at initial stages of identification.

4.1.2 Effect of Elevated CO_2 on Physiological and Biochemical Parameters in Tomato:

4.1.2.1 *Relative Water Content*

After the stress, highest relative water content was registered for varieties under treatment T1 (80.69 %) followed by treatment T3 (79.78 %) and treatment T2 (79.45 %). Among the varieties, higher relative water content was observed for the variety Vellayani Vijay (80.79 %) compared to Manulakshmi (79.97 %) and it was significantly higher compared to Anagha (79.16 %) (Table 13).

After re-watering, significantly higher relative water content was recorded under elevated CO_2 (86.20 %) compared to control chamber (83.28 %) and open control (82.38 %). Among the varieties, relative water content was observed higher

for the variety Vellayani Vijay (85.35 %) compared to Manulakshmi (85.08 %) and it was significantly higher compared to Anagha (82.38 %) (Table 14).

4.1.2.2 ***Pigment Composition***

4.2.2.2.1 Chlorophyll a

After stress (Table 15), chlorophyll a content was found to be significantly superior under treatment T1 (0.69 mg/g) than treatment T3 (0.50 mg/g). Highest mean value of chlorophyll a content among the varieties was recorded for Vellayani Vijay (0.66 mg/g).

After re-watering (Table 16), highest mean value of Chlorophyll a content was recorded for treatment T1 (0.15 mg/g) followed by treatment T2 (0.13 mg/g) and treatment T3 (0.06 mg/g).

4.2.2.2.2. Chlorophyll b

As presented in Table 17, highest chlorophyll b content was recorded under elevated CO_2 (0.30 mg/g) followed by control chamber (0.26 mg/g) and open control (0.20 mg/g). Variety Vellayani Vijay (0.29 mg/g) recorded highest chlorophyll b content among the varieties.

Significantly higher chlorophyll b content was observed under elevated CO_2 (0.09 mg/g) than open control (0.05 mg/g) after re-watering. No significant difference in chlorophyll b content was observed among the varieties (Table 18).

4.2.2.2.3 Total chlorophyll

Elevated CO_2 was found to have significant and positive influence on total chlorophyll content after stress (Table 19). Under elevated CO_2, significantly higher total chlorophyll content (1.00 mg/g) was recorded compared to open control (0.70 mg/g).

Significant and positive influence of elevated CO_2 on total chlorophyll content was found to be continued after re-watering (Table 20). Significantly superior total chlorophyll content was recorded under treatment T1 (0.24 mg/g) than treatment T3 (0.11 mg/g) .

4.2.2.2.4 Carotenoid Content

Elevated CO_2 was found to have no influence on carotenoid content after stress (Table 21) but after re-watering (Table 22), highest carotenoid content was recorded under treatment T1 (elevated CO_2) (0.21 mg/g) which was significantly superior to treatment T2 (control chamber) (0.15 mg/g) and treatment T3 (0.14 mg/g) (open control).

4.1.2.3 *Stomatal Frequency*

As shown in Table 23, Significantly lower stomatal frequency was observed for varieties under treatment T1 (555.85 no cm^{-2}) compared to treatment T2 (610.94 no cm^{-2}) and treatment T3 (658.18 no cm^{-2}) after stress. Stomatal frequency among the varieties was observed significantly lower for the variety Vellayani Vijay (512.91 no cm^{-2}) compared to Manulakshmi (634 no cm^{-2}) and Anagha (679 no cm^{-2}).

After re-watering, significantly lower stomatal frequency was recorded under elevated CO_2 (624.11 no cm^{-2}) compared to open control (692.02 no cm^{-2}) and among varieties, significantly lower stomatal frequency was observed for the variety Vellayani Vijay (586.66 no cm^{-2}) compared to Manulakshmi (666.4 no cm^{-2}) and Anagha (707.85 no cm^{-2}) (Table 24).

4.1.2.4 *Transpiration Rate*

Effect of elevated CO_2 on transpiration rate was presented in Table 25. Significant reduction in transpiration rate was observed for varieties under treatment T1 (8.13 mmol water m^{-2} s^{-1}) compared to treatment T2 (13.26 mmol water m^{-2} s^{-1})

and treatment T3 (23.27 mmol water m^{-2} s^{-1}) after stress. Among the varieties lowest transpiration rate was observed for the variety Vellayani Vijay and highest was observed for the variety Manulakshmi.

After re-watering also (Table 26), significantly lower transpiration rate was observed for varieties under elevated CO_2 (8.66 mmol water m^{-2} s^{-1}) followed by control chamber (12.07 mmol water m^{-2} s^{-1}) and open control (15.52 mmol water m^{-2} s^{-1}) and among the varieties, lowest transpiration rate was observed for the variety Vellayani Vijay (10.88 mmol water m^{-2} s^{-1}) followed by Anagha (12.94 mmol water m^{-2} s^{-1}) and Manulakshmi (12.43 mmol water m^{-2} s^{-1}).

4.1.2.5 *Photosynthetic Rate*

Elevated CO_2 was found to have highly significant effect on Photosynthetic rate on all the varieties after stress. Significant increase in Photosynthetic rate was noticed under elevated CO_2 (18.69 mmol CO_2 m^{-2} s^{-1}) compared to control chamber (14.87 mmol CO_2 m^{-2} s^{-1}) and open control (13.56 mmol CO_2 m^{-2} s^{-1}). Among all varieties, highest mean value for photosynthetic rate was recorded for the variety Vellayani Vijay (17.45 mmol CO_2 m^{-2} s^{-1}) (Table 27).

Same trend of significant increase in photosynthetic rate under treatment elevated CO_2 (23.43 mmol CO_2 m^{-2} s^{-1}) compared to control chamber (19.06 mmol CO_2 m^{-2} s^{-1}) and open control (17.77 mmol CO_2 m^{-2} s^{-1}) was observed after re-watering (Table 28). Among all varieties, highest photosynthetic rate was recorded for the variety Vellayani Vijay (21.91 mmol CO_2 m^{-2} s^{-1}), which was significantly higher compared to variety Manulakshmi (19.73 mmol CO_2 m^{-2} s^{-1}).

4.1.2.6 *Total Soluble Protein*

Effect of elevated CO_2 on total soluble protein was represented in Table 29. Stress induced reduction in protein content was found lower in treatment T1 (elevated CO_2) compared to treatment T3 (open control). After stress, reduction in protein

content was found under treatment T1(elevated CO_2) compared to treatment T3 (open control) and treatment T2 (control chamber). Lowest total soluble protein content was recorded under treatment T1 (elevated CO_2) (14.41 mg/g) followed by treatment T3 (open control) (18.77 mg/g) and treatment T2 (19.35 mg/g) (control chamber). Among the varieties, highest mean value for total soluble protein was recorded for variety Vellayani Vijay (21.37 mg/g) which was significantly higher compared to variety Manulakshmi (14.15 mg/g).

After re-watering (Table 30), lowest mean value for total soluble protein content was recorded under treatment T1 (elevated CO_2) (14.76 mg/g) followed by treatment T3 (open control) (18.56 mg/g) and treatment T2 (control chamber) (19.30 mg/g). Extent of recovery in total soluble protein content from stress was observed more under treatment T1(elevated CO_2) compared to treatment T3 (open control). Among the varieties, highest protein content was observed for the variety Vellayani Vijay (19.33 mg/g) followed by Manulakshmi (16.88 mg/g) and Anagha (16.42 mg/g).

4.1.2.7 *Starch*

After stress (Table 31), highest mean value for starch content was observed under treatment T1 (4.63 mg/g) followed by treatment T2 (3.92 mg/g) and treatment T3 (3.63 mg/g). Among the varieties, significantly higher starch content was recorded for the variety Manulakshmi (5.52 mg/g) than Vellayani Vijay (3.68 mg/g) and Anagha (2.99 mg/g).

After re-watering (Table 32), Treatment T1 (6.93 mg/g) was observed holding significantly higher mean value for starch content followed by treatment T2 (3.40 mg/g) and treatment T3 (3.23 mg/g) and among the varieties, highest starch content was recorded for varieties Manulakshmi (4.78 mg/g) and Vellayani Vijay (4.77 mg/g) than Anagha (4.02 mg/g).

4.1.2.8 *Reducing Sugars*

Starch content was found increased significantly under elevated CO_2 treatment after stress (Table 33). Significantly highest mean value for reducing sugars was observed under elevated CO_2 (15.13 mg/g) followed by control chamber (13.55 mg/g) and open control (13.95 mg/g). There was no significant difference observed in reducing sugars content among the varieties.

Significantly higher reducing sugars was observed under elevated CO_2 (15.62 mg/g) compared to control chamber (14.30 mg/g) and open control (14.38 mg/g) and among the varieties, Anagha (15.13 mg/g) registered highest mean value for reducing sugars followed by Manulakshmi (14.51 mg/g) and Vellayani Vijay (14.66 mg/g) as presented in Table 34.

4.1.2.9 *Phenol Content*

Elevated CO_2 was found to have positive influence on phenol content after stress (Table 35). Highest phenol content was recorded under treatment T1 (2.86 mg/g) followed by treatment T2 (2.43 mg/g) and treatment T3 (1.89 mg/g). There was no significant difference observed among the varieties.

Increasing trend in phenol content was continued under elevated CO_2 after re-watering also. As shown in Table 36, highest phenol content was observed under treatment T1 (28.77 mg/g) followed by treatment T2 (27.77 mg/g) and treatment T3 (26.72 mg/g). Mean values of phenol content for all the varieties were found on par.

4.1.2.10 *Free Amino Acid Content*

After stress, an increasing trend of free amino acid content was observed under elevated CO_2 treatment. Free amino acid content under elevated CO_2 (1.57 mg/g) was found significantly higher compared to control chamber (1.14 mg/g)

and open control (0.89 mg/g). Higher free amino acid content was observed for the variety Vellayani Vijay (1.36 mg/g) compared to Manulakshmi (1.13 mg/g) and Anagha (1.11 mg/g) though not significant (Table 37).

After recovery (Table 38), a decreasing trend of free amino acid content was noticed under elevated CO_2 (5.61 mg/g) compared to open control (5.93 mg/g) and control chamber (6.32 mg/g). Among the varieties, significantly higher free amino acid content was recorded for the variety Anagha (7.33 mg/g) compared to Vellayani Vijay (6.03 mg/g) and Manulakshmi (4.51 mg/g).

4.1.2.11 ***Membrane Integrity***

Membrane integrity was expressed in terms of % leakage in Table 39. Decreasing trend of % leakage was observed under elevated CO_2 compared to control chamber and open control after stress. Per cent leakage was recorded lower under treatment T1 (4.76 %) compared to treatment T2 (5.48 %) and it was observed significantly lower compared to treatment T3 (6.24 %). Among the varieties, lowest % leakage was observed for the variety Vellayani Vijay (5.24 %).

After recovery, % leakage was observed lower under treatment T1 (4.03 %) compared to treatment T2 (4.30 %) and it was significantly lower compared to treatment T3 (4.74 %). Among the varieties, lowest % leakage was recorded for the variety Vellayani Vijay (3.84 %) and it was significantly lower compared to Anagha (5.28 %) (Table 40).

4.1.2.12 ***Stable Isotopic Discrimination***

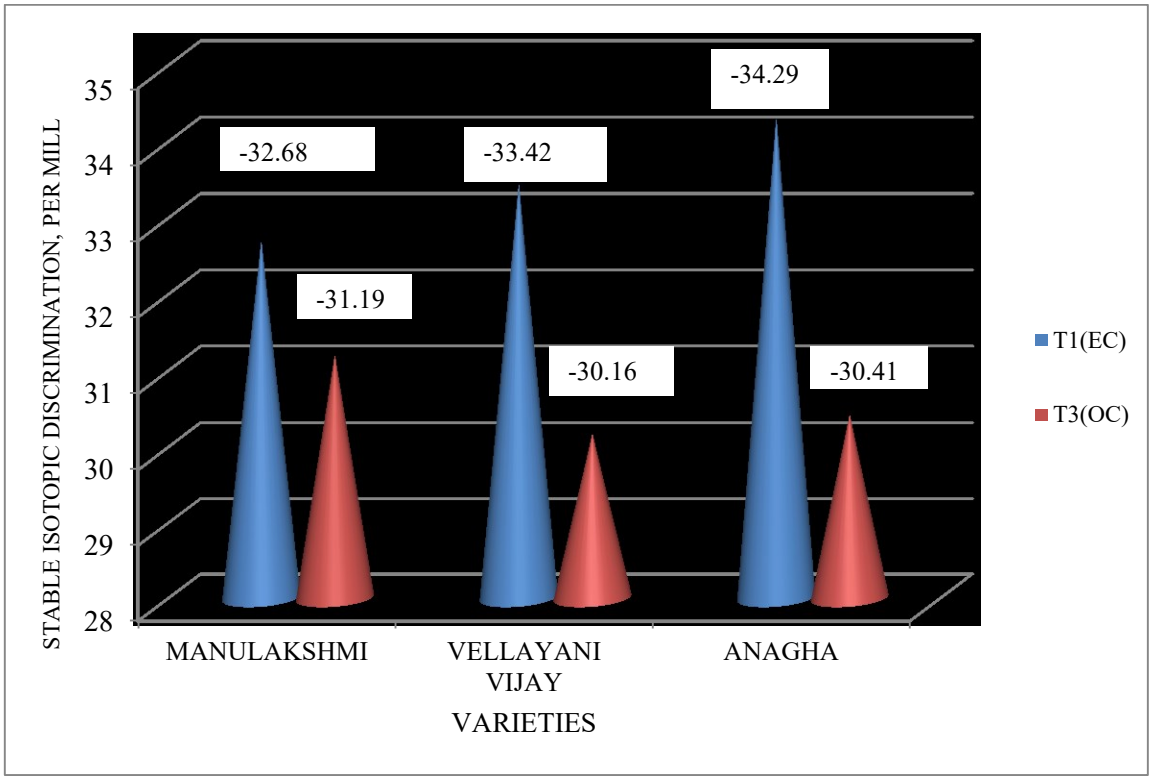


Fig 1. Effect of elevated CO_2 on stable isotopic discrimination (per mill) in tomato

Effect of elevated CO_2 on stable isotopic discrimination in tomato is presented in Fig 1. More negative stable isotopic discriminations were recorded for varieties Manulakshmi (-32.68) ,Vellayani Vijay (-33.42) and Anagha (-34.29) under treatment T1 (elevated CO_2) compared to treatment T3 (open control).

4.1.2.13 *SOD*

As shown in Table 41, significant increase in SOD activity was observed under elevated CO_2 after stress. Significantly higher SOD content was observed under elevated CO_2 (0.66 $g^{-1}minute^{-1}$) compared to control chamber (0.45 $g^{-1}minute^{-1}$) and open control (0.41 $g^{-1}minute^{-1}$). Among the varieties, highest SOD activity was recorded for the variety Vellayani Vijay (0.59 $g^{-1}minute^{-1}$) and it was significantly higher compared to Anagha (0.36 $g^{-1}minute^{-1}$).

Increasing trend of SOD activity under elevated CO_2 was found continued after re-watering (Table 42). Higher SOD activity was recorded under elevated CO_2 (0.43 $g^{-1}minute^{-1}$) compared to control chamber (0.37 $g^{-1}minute^{-1}$) and open control (0.37 $g^{-1}minute^{-1}$). Among the varieties, SOD activity was found significantly higher for Vellayani Vijay (0.46 $g^{-1}minute^{-1}$) compared to Manulakshmi (0.39 $g^{-1}minute^{-1}$) and Anagha (0.32 $g^{-1}minute^{-1}$).

4.1.2.14 *Ascorbic Acid*

Elevated CO_2 was shown to have positive influence on ascorbic acid content after stress (Table 43). Ascorbic acid content was recorded significantly higher under treatment T1 (10.39 mg/100g) compared to treatment T3 (9.90 mg/100g). Among the varieties, highest ascorbic acid content was recorded for the variety Vellayani Vijay (10.80 mg/100g) which was significantly higher compared to variety Manulakshmi (9.64 mg/100g).

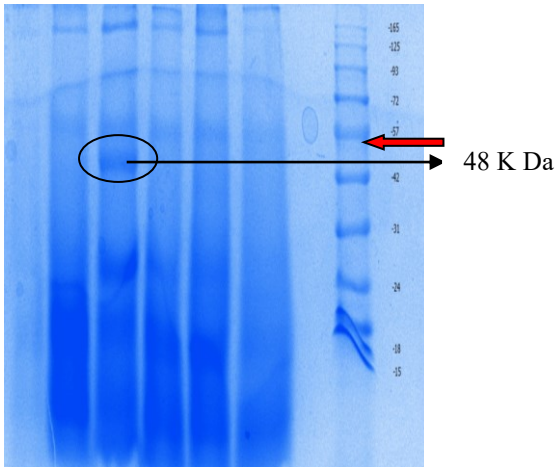
After re-watering also, increasing trend in ascorbic acid content under elevated CO_2 was found continued. Higher ascorbic acid content was observed under treatment T1 (13.65 mg/100g) followed by treatment T3 (12.03 mg/100g) and treatment T2 (11.34 mg/100g).

Among the varieties, highest ascorbic acid content was recorded for the variety Manulakshmi (13.88 mg/100g) and it was significantly higher than variety Anagha (10.88 mg/100g) (Table 44).

4.1.2 Effect of Elevated CO_2 on Protein Profiling and RuBISCO in Tomato

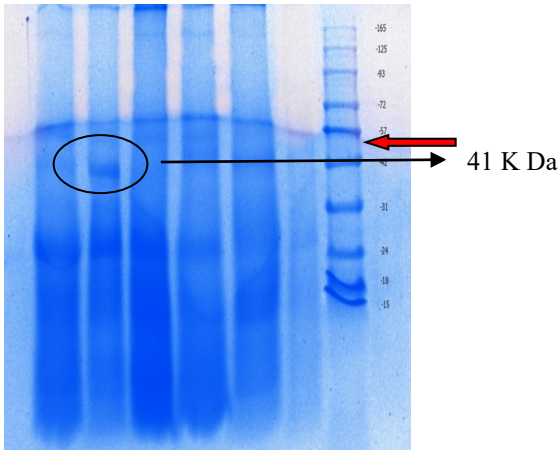
In the present study, the electrophoresis analysis of proteins using SDS PAGE revealed that elevated CO_2 induced the production of a few new proteins under water stress. The protein content and profile varied with different varieties in response to elevated CO_2 level. In elevated CO_2, formation of a few new bands of molecular weight nearly 48 K Da, 41 K Da and 45 K Da were observed under water stress for tomato varieties Anagha, Vellayani Vijay and Manulakshmi, whereas no changes in RuBISCO activity was observed under elevated CO_2 (Plate. 4).

Anagha



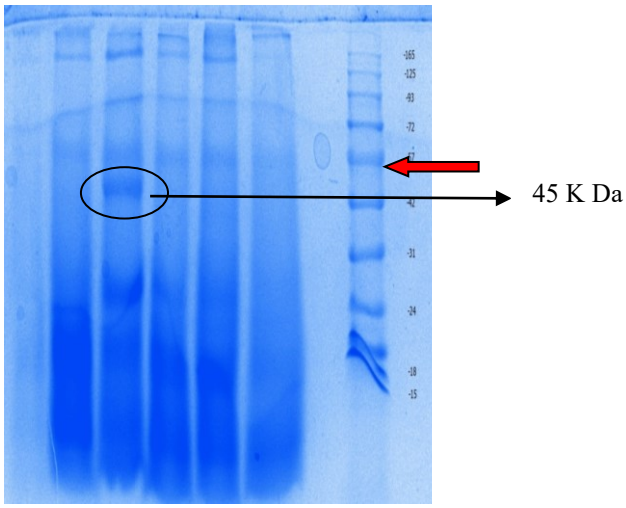
1 2 3 4 5

Vellayani Vijay



1 2 3 4 5

CO-1



1 2 3 4 5

Plate 4. Protein profiling in tomato

1 .EC, Unstressed

2. EC, Stressed

3. EC, Unstressed

4. EC, Recovered

5. Open control

RuBISCO (56 KDa)

Table 1: Effect of elevated CO_2 on number of leaves after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	8.00	9.00	8.33	10.00	7.66	9.00	8.66
V2	10.00	9.00	10.00	16.33	10.66	9.66	10.94
V3	10.66	12.00	21.66	26.00	11.66	11.33	15.55
MEAN(S)	9.55	10.00	13.33	17.44	10.00	10.00	GM
MEAN(T)	9.77		15.38		10.00		11.72
CD(0.05): CD(T) =2.98, CD(V) = 2.98, CD(T*V) = 3.32, CD(S*T) = 4.21, CD(V*S)=4.21							

T1 - OTC with Elevated CO_2 Concentration (OTC Ec)

T2 - OTC with Ambient CO_2 Concentration (OTC Ac)

T3 - Open Control

S1 - Without Stress

S2 - With Stress

V1 - Manulakshmi

V2 - Vellayani Vijay

V3 - Anagha

GM - Grand Mean

Table 2: Effect of elevated CO_2 on number of leaves after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	10.00	11.00	10.66	12.66	10.33	11.33	11.00
V2	12.00	11.66	12.33	18.66	12.00	12.00	13.11
V3	13.66	14.00	24.33	28.66	13.66	14.00	18.05
MEAN(S)	11.88	12.22	15.77	20.00	12.00	12.44	GM
MEAN(T)	12.05		17.88		12.22		14.05
CD(0.05): T = 3.14 ,V = 3.14, T*V = 3.49, S*T = 3.43, V*S= 3.43							

Table 3. Effect of elevated CO_2 on specific leaf area ($cm^2\ g^{-1}$) after stress in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	296.00	371.46	544.58	285.80	447.41	303.40	347.77
V2	233.55	260.00	376.58	240.80	217.80	355.80	280.75
V3	215.33	388.25	363.21	255.60	315.83	278.16	304.40
MEAN(S)	248.29	339.90	428.12	264.06	327.01	312.45	GM
MEAN(T)	294.10		346.09		319.73		319.97
CD (0.05): T =59.72, V = 59.72, T*V = 66.56, S*T = 84.42, V*S = 84.42							

Table 4. Effect of elevated CO_2 on specific leaf area ($cm^2\ g^{-1}$) after re-watering in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	339.33	391.66	581.66	344.00	521.00	355.66	422.22
V2	265.00	298.33	381.66	256.33	265.33	383.00	308.27
V3	317.33	393.33	372.66	273.66	343.33	321.66	337.00
MEAN(S)	307.22	361.11	445.33	291.33	376.55	353.44	GM
MEAN(T)	334.16		368.33		365.00		355.83
CD(0.05): T = 63.89, V = 63.89, T*V = 71.27, S*T = 90.36, V*S = 90.36							

Table 5. Effect of elevated CO_2 on root weight (g) after stress in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.68	0.63	1.25	1.00	0.89	0.68	0.85
V2	1.70	1.64	2.90	0.93	1.14	1.00	1.55
V3	2.85	0.42	0.99	0.64	0.96	0.54	1.06
MEAN(S)	1.74	0.89	1.71	0.86	0.99	0.74	GM
MEAN(T)	1.32		1.28		0.87		1.16
CD(0.05): T = 0.52 , V = 0.52, T*V = 0.58, S*T = 0.74, V*S= 0.74							

Table 6. Effect of elevated CO_2 on root weight (g) after re-watering in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.69	0.62	1.71	1.16	0.95	1.66	1.13
V2	1.64	1.82	2.63	1.40	1.23	1.16	1.64
V3	2.63	0.45	1.45	0.84	0.82	0.83	1.17
MEAN(S)	1.65	0.96	1.93	1.13	1.00	1.22	GM
MEAN(T)	1.30		1.53		1.11		1.31
CD(0.05): T = 0.47 , V = 0.47, T*V = 0.52, S*T = 0.64, V*S = 0.64							

Table 7. Effect of elevated CO_2 on shoot weight (g) after stress in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	2.61	4.98	6.60	3.39	2.66	2.87	3.85
V2	5.43	3.31	4.72	4.60	5.63	3.68	4.56
V3	6.71	3.48	3.55	1.06	4.20	2.21	3.53
MEAN(S)	4.91	3.92	4.96	3.01	4.16	2.92	GM
MEAN(T)	4.42		3.98		3.54		3.98
CD(0.05) : T =1.41, V = 1.41, T*V = 1.55, S*T = 2.02, V*S = 2.02							

Table 8. Effect of elevated CO_2 on shoot weight (g) after re-watering in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	5.65	2.97	2.05	1.24	1.50	2.11	2.58
V2	2.48	5.45	3.79	5.18	1.86	2.46	3.54
V3	6.16	1.84	4.03	1.86	2.35	1.99	3.04
MEAN(S)	4.76	3.42	3.29	2.76	1.90	2.18	GM
MEAN(T)	4.09		3.02		2.04		3.05
CD(0.05): T = 1.50 , V = 1.50, T*V = 1.68, S*T = 2.15, V*S = 2.15							

Table 9. Effect of elevated CO_2 on root shoot ratio after stress in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.26	0.25	0.19	0.41	0.33	0.23	0.37
V2	0.30	0.52	0.66	0.24	0.20	0.35	0.47
V3	0.41	0.12	0.29	0.58	0.23	0.23	0.40
MEAN(S)	0.32	0.29	0.38	0.41	0.25	0.27	GM
MEAN(T)	0.40		0.49		0.35		
CD(0.05): T = 0.19, V = 0.19, T*V = 0.21, S*T = 0.25, V*S = 0.25							

Table 10. Effect of elevated CO_2 on root shoot ratio after re-watering in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.12	0.21	0.85	0.93	0.62	0.88	0.70
V2	0.68	0.37	0.75	0.33	0.65	0.47	0.64
V3	0.41	0.24	0.36	0.50	0.34	0.41	0.47
MEAN(S)	0.41	0.27	0.65	0.59	0.54	0.58	GM
MEAN(T)	0.34		0.62		0.56		0.51
CD(0.05) T = 0.20, V = 0.20, T*V = 0.22, S*T = 0.28, V*S = 0.28							

Table 11. Effect of elevated CO_2 on dry matter production (g) after stress in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	3.29	5.62	7.85	4.39	3.55	3.55	4.71
V2	7.14	4.95	7.62	9.52	6.77	4.69	6.78
V3	9.56	3.90	4.54	1.70	5.16	2.76	4.60
MEAN(S)	6.66	4.82	6.67	5.20	5.16	3.66	GM
MEAN(T)	5.74		5.94		4.41		5.36
CD(0.05): T =1.85, V = 1.85, T*V = 2.07, S*T = 1.68, V*S = 1.68							

Table 12. Effect of elevated CO_2 on dry matter production (g) after re-watering in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	6.34	3.59	3.77	2.40	2.46	3.77	3.72
V2	4.12	7.27	6.43	6.59	3.09	3.62	5.19
V3	8.79	2.29	5.48	2.70	3.18	2.82	4.21
MEAN(S)	6.42	4.38	5.22	3.90	2.91	3.40	GM
MEAN(T)	5.40		4.56		3.16		4.37
CD(0.05): T = 1.63, V = 1.63, T*V = 1.82, S*T = 2.32, V*S = 2.32							

Table 13. Effect of elevated CO_2 on relative water content (%) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	85.97	78.13	84.32	74.20	85.41	76.73	79.97
V2	87.18	74.17	85.64	74.26	84.79	73.82	80.79
V3	85.15	73.53	84.93	73.37	84.51	73.45	79.16
MEAN(S)	86.10	75.28	84.96	73.94	84.90	74.67	GM
MEAN(T)	80.69		79.45		79.78		79.97
CD(0.05): T = 1.20, V = 1.20, T*V = 1.35, S*T = 1.72, CD V*S = 1.72							

Table 14. Effect of elevated CO_2 on relative water content (%) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	89.28	86.06	85.26	8.48	83.47	82.79	85.08
V2	89.07	85.88	86.71	82.65	85.51	82.27	85.35
V3	86.00	80.94	84.26	80.33	82.60	80.18	82.38
MEAN(S)	88.11	84.29	85.41	82.15	83.86	81.74	GM
MEAN(T)	86.20		83.78		82.80		84.26
CD(0.05): T =1.37, V = 1.37, T*V = 1.55, S*T = 1.97, V*S = 1.97							

Table 15. Effect of elevated CO_2 on chlorophyll a (mg/g) content after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.76	0.59	0.71	0.59	0.39	0.53	0.59
V2	0.76	0.79	0.74	0.63	0.44	0.58	0.66
V3	0.66	0.58	0.56	0.66	0.51	0.55	0.59
MEAN(S)	0.73	0.66	0.67	0.63	0.44	0.55	GM
MEAN(T)	0.69		0.65		0.50		0.61
CD (0.05): T = 0.12 , V = 0.12, T*V = 0.13, S*T = 0.17, V*S = 0.17							

Table 16. Effect of elevated CO_2 on chlorophyll a (mg/g) content after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.32	0.10	0.07	0.10	0.05	0.05	0.12
V2	0.13	0.14	0.07	0.20	0.06	0.04	0.11
V3	0.11	0.11	0.17	0.15	0.09	0.05	0.11
MEAN(S)	0.19	0.12	0.10	0.15	0.07	0.05	GM
MEAN(T)	0.15		0.13		0.06		0.11
CD (0.05): T = 0.09, V = 0.09, T*V = 0.10, S*T = 0.12, V*S = 0.12							

Table 17. Effect of elevated CO_2 on chlorophyll b content (mg/g) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.32	0.23	0.30	0.25	0.28	0.13	0.25
V2	0.30	0.46	0.30	0.27	0.15	0.26	0.29
V3	0.31	0.18	0.21	0.24	0.20	0.18	0.22
MEAN(S)	0.31	0.29	0.27	0.26	0.21	0.19	GM
MEAN(T)	0.30		0.26		0.20		0.25
CD (0.05) = T = 0.12, V = 0.12, T*V = 0.13, S*T = 0.17, V*S = 0.17							

Table 18. Effect of elevated CO_2 on chlorophyll b (mg/g) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.10	0.07	0.04	0.09	0.07	0.04	0.07
V2	0.11	0.09	0.08	0.14	0.04	0.02	0.08
V3	0.08	0.09	0.12	0.09	0.08	0.05	0.08
MEAN(S)	0.10	0.08	0.08	0.11	0.06	0.04	GM
MEAN(T)	0.09		0.09		0.05		0.08
CD (0.05): T = 0.02, V = 0.02, T*V = 0.02, S*T = 0.03, V*S = 0.03							

Table 19. Effect of elevated CO_2 on total chlorophyll content (mg/g) after stress in

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.09	0.83	1.01	0.85	0.67	0.63	0.85
V2	1.06	1.25	1.04	0.91	0.59	0.85	0.95
V3	0.97	0.77	0.78	0.91	0.71	0.74	0.81
MEAN(S)	1.04	0.95	0.94	0.89	0.66	0.74	GM
MEAN(T)	1.00		0.92		0.70		0.87
CD (0.05): T = 0.22, V = 0.22, T*V = 0.25, S*T = 0.32, V*S = 0.32							

Table 20. Effect of elevated CO_2 on total chlorophyll content (mg/g) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.43	0.17	0.12	0.20	0.12	0.09	0.19
V2	0.24	0.23	0.15	0.35	0.11	0.06	0.19
V3	0.19	0.21	0.29	0.24	0.17	0.11	0.20
MEAN(S)	0.29	0.20	0.19	0.26	0.13	0.09	GM
MEAN(T)	0.24		0.22		0.11		0.19
CD (0.05): T = 0.10, V = 0.10, T*V = 0.11, S*T = 0.15, V*S = 0.15							

Table 21. Effect of elevated CO_2 on carotenoid content (mg/g) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.28	0.24	0.37	0.28	0.33	0.26	0.29
V2	0.34	0.48	0.40	0.38	0.31	0.47	0.39
V3	0.29	0.35	0.31	0.28	0.37	0.25	0.31
MEAN(S)	0.30	0.35	0.36	0.31	0.33	0.33	GM
MEAN(T)	0.33		0.33		0.33		0.33
CD (0.05): T = 0.10, V = 0.10, T*V = 0.11, S*T = 0.15, V*S = 0.15							

Table 22. Effect of elevated CO_2 on carotenoid content (mg/g) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.20	0.17	0.10	0.14	0.13	0.15	0.15
V2	0.21	0.20	0.13	0.18	0.15	0.13	0.17
V3	0.19	0.26	0.14	0.22	0.13	0.17	0.18
MEAN(S)	0.20	0.21	0.12	0.18	0.14	0.15	GM
MEAN(T)	0.21		0.15		0.14		0.17
CD (0.05): T = 0.04, V = 0.04, T*V = 0.04, S*T = 0.06, V*S = 0.06							

Table 23. Effect of elevated CO_2 on stomatal frequency (no cm^{-2}) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	611.52	561.36	670.39	610.80	709.50	640.74	634.00
V2	475.10	420.77	543.76	469.27	624.62	543.97	512.91
V3	613.26	659.11	656.36	715.08	686.98	740.27	679.01
MEAN(S)	566.62	547.08	623.50	598.38	674.70	641.66	GM
MEAN(T)	555.85		610.94		658.18		608.66
CD (0.05): T = 36.44, V = 36.44, T*V = 40.63, S*T = 51.50, V*S= 51.50							

Table 24. Effect of elevated CO_2 on stomatal frequency (no cm^{-2}) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	631.92	625.89	691.39	639.25	741.86	668.10	666.40
V2	578.10	538.72	639.22	486.60	662.76	614.56	586.66
V3	613.65	738.39	688.81	741.43	721.89	742.97	707.85
MEAN(S)	607.89	634.33	673.14	622.42	708.84	675.21	GM
MEAN(T)	624.11		647.78		692.02		653.64
CD (0.05): T = 43.20, V = 43.20, T*V = 48.17, S*T = 61.05, V*S = 61.05							

Table 25. Effect of elevated CO_2 on transpiration rate (mmol water m^{-2} s^{-1}) after stress in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	11.33	5.89	13.51	14.12	26.39	27.25	16.41
V2	9.15	6.21	14.32	14.06	22.65	22.33	14.78
V3	11.12	5.14	13.33	10.22	19.66	21.36	14.88
MEAN(S)	10.53	5.74	13.72	12.8	22.9	23.64	GM
MEAN(T)	8.13		13.26		23.27		15.35
CD(0.05): T = 4.77, V = 4.77, T*V = 3.24, S*T = 0.51, V*S = 0.51							

Table 26. Effect of elevated CO_2 on transpiration rate (mmol water m^{-2} s^{-1}) after re-watering in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	9.21	9.13	13.36	14.23	12.33	16.35	12.43
V2	8.36	8.44	9.66	9.54	15.64	13.66	10.88
V3	7.55	9.33	12.41	13.22	17.11	18.06	12.94
MEAN(S)	8.37	8.96	11.81	12.33	15.02	16.02	GM
MEAN(T)	8.66		12.07		15.52		12.08
CD(0.05): T = 2.55, V = 2.55, T*V = 2.32, S*T = 1.12, V*S = 1.12							

Table 27. Effect of elevated CO_2 on photosynthesis rate (mmol CO_2 m^{-2} s^{-1}) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	18.32	16.12	13.11	12.69	14.56	12.22	14.52
V2	27.45	18.63	15.32	15.11	17.14	11.09	17.45
V3	16.27	15.12	18.36	14.65	13.25	13.14	15.13
MEAN(S)	20.68	16.70	15.60	14.15	14.98	12.15	GM
MEAN(T)	18.69		14.87		13.56		15.69
CD(0.05): T = 2.72, V = 2.72, T*V = 3.32, S*T = 2.41, V*S = 2.41							

Table 28. Effect of elevated CO_2 on photosynthesis rate (mmol CO_2 m^{-2} s^{-1}) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	20.54	21.31	18.65	20.55	17.12	20.21	19.73
V2	29.32	27.56	24.26	16.35	16.28	17.74	21.91
V3	19.23	22.66	16.35	18.22	20.11	15.23	18.63
MEAN(S)	23.03	23.84	19.75	18.37	17.83	17.72	GM
MEAN(T)	23.43		19.06		17.77		19.88
CD(0.05) T = 2.98, V = 2.98, T*V = 3.47, S*T = 1.21, V*S = 1.21							

Table 29. Effect of elevated CO_2 on total soluble protein content (mg/g) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	12.00	5.51	19.34	17.57	26.71	3.74	14.15
V2	12.69	23.72	22.31	17.66	29.80	22.06	21.37
V3	23.55	9.00	21.62	17.63	17.72	12.60	17.02
MEAN(S)	16.08	12.74	21.09	17.62	24.74	12.80	GM
MEAN(T)	14.41		19.35		18.77		17.51
CD(0.05): T= 5.80 , V = 5.80, T*V = 6.50, S*T = 8.21, V*S = 8.21							

Table 30. Effect of elevated CO_2 on total soluble protein content (mg/g) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	14.57	11.25	21.95	19.07	18.09	16.36	16.88
V2	13.73	22.87	24.39	16.53	20.40	18.04	19.33
V3	14.31	11.85	11.89	22.00	20.04	18.45	16.42
MEAN(S)	14.20	15.32	19.41	19.20	19.51	17.62	GM
MEAN(T)	14.76		19.30		18.56		17.54
CD(0.05): T = 3.98 , V = 3.98, T*V = 4.44, S*T = 5.63, V*S = 5.63							

Table 31. Effect of elevated CO_2 on starch content (mg/g) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	6.18	8.64	6.86	4.52	1.65	5.24	5.52
V2	1.80	4.33	1.54	4.71	1.04	8.63	3.68
V3	2.59	4.27	1.92	3.93	2.24	3.00	2.99
MEAN(S)	3.52	5.75	3.44	4.39	1.65	5.62	GM
MEAN(T)	4.63		3.92		3.63		4.06
CD(0.05): T = 1.47, V = 1.47, T*V = 1.64, S*T = 2.08, V*S = 2.08							

Table 32. Effect of elevated CO_2 on starch content (mg/g) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	7.31	8.81	2.43	4.14	3.01	2.98	4.78
V2	4.80	9.91	2.70	5.48	3.15	2.54	4.77
V3	7.13	3.65	4.40	1.25	3.85	3.87	4.02
MEAN(S)	6.41	7.46	3.18	3.63	3.34	3.13	GM
MEAN(T)	6.93		3.40		3.23		4.06
CD (0.05): T = 0.88 , V = 0.88, T*V = 0.98, S*T = 1.24, V*S = 1.24							

Table 33. Effect of elevated CO_2 on reducing sugars content (mg/g) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	14.86	14.97	14.06	14.46	13.80	13.10	14.21
V2	15.00	15.73	14.56	12.50	13.70	13.46	14.16
V3	15.26	15.00	13.73	14.36	14.53	12.70	14.26
MEAN(S)	15.04	15.23	14.12	13.77	14.01	13.08	GM
MEAN(T)	15.13		13.95		13.55		14.21
CD(0.05): T = 0.91 ,V = 0.91, T*V = 1.01, S*T = 1.29, V*S = 1.29							

Table 34. Effect of elevated CO_2 on reducing sugars content (mg/g) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	15.26	15.46	14.13	13.73	14.50	13.96	14.51
V2	15.03	16.33	14.03	14.50	13.53	14.26	14.66
V3	15.56	15.80	14.96	14.43	14.76	15.26	15.13
MEAN(S)	15.28	15.96	14.37	14.22	14.26	14.50	GM
MEAN(T)	15.62		14.30		14.38		14.77
CD(0.05): T = 0.80 , V = 0.80, T*V = 0.89, S*T = 1.13, V*S = 1.13							

Table 35. Effect of elevated CO_2 on phenol content (mg/g) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	2.44	2.49	2.60	2.63	1.93	2.01	2.35
V2	2.98	3.39	1.86	1.95	1.90	1.98	2.34
V3	2.88	2.97	2.67	2.85	1.76	1.79	2.48
MEAN(S)	2.77	2.95	2.37	2.48	1.86	1.92	GM
MEAN(T)	2.86		2.43		1.89		2.39
CD(0.05) T =1.04, V = 1.04, T*V = 1.17, S*T = 0.345, V*S = 0.345							

Table 36. Effect of elevated CO_2 on phenol content (mg/g) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	28.66	34.00	22.00	35.33	27.00	26.66	28.94
V2	21.66	28.00	30.00	28.33	25.66	28.66	27.05
V3	31.33	29.00	23.00	28.00	28.66	23.66	27.27
MEAN(S)	27.22	30.33	25.00	30.55	27.11	26.33	GM
MEAN(T)	28.77		27.77		26.72		27.75
CD(0.05) T = 6.92 ,V = 6.92, T*V = 7.72, S*T = 9.81, V*S = 9.81							

Table 37. Effect of elevated CO_2 on free amino acid content (mg/g) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.65	1.60	0.97	0.91	0.80	0.87	1.13
V2	1.25	2.09	1.02	1.80	0.90	1.09	1.36
V3	1.74	1.11	1.28	0.86	1.04	0.66	1.11
MEAN(S)	1.54	1.60	1.09	1.19	0.91	0.87	GM
MEAN(T)	1.57		1.14		0.89		1.20
CD(0.05) T = 0.36 , V = 0.36, T*V = 0.40, S*T = 0.51,V*S = 0.51							

Table 38. Effect of elevated CO_2 on free amino acid content (mg/g) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	3.76	4.76	4.26	5.16	5.06	4.03	4.51
V2	5.26	6.43	6.16	6.76	6.00	5.56	6.03
V3	6.13	7.33	7.00	8.60	8.10	6.86	7.33
MEAN(S)	5.05	6.17	5.81	6.84	6.38	5.48	GM
MEAN(T)	5.61		6.32		5.93		5.96
CD(0.05) T = 0.55, V = 0.55, T*V = 0.60, S*T = 0.77, V*S = 0.77							

Table 39. Effect of elevated CO_2 on membrane integrity (% leakage) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	3.38	6.20	3.94	7.45	3.67	8.45	5.51
V2	3.05	6.07	3.27	7.33	3.79	7.95	5.24
V3	3.11	6.73	3.56	7.33	4.42	9.19	5.72
MEAN(S)	3.18	6.33	3.59	7.37	3.96	8.53	GM
MEAN(T)	4.76		5.48		6.24		5.49
CD(0.05): T = 0.73, V = 0.73, T*V = 0.83, S*T = 1.07, V*S = 1.07							

Table 40. Effect of elevated CO_2 on membrane integrity (% leakage) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	3.37	3.99	3.97	3.82	4.00	4.61	3.96
V2	3.47	3.70	3.82	4.17	3.51	4.37	3.84
V3	3.82	5.83	4.18	5.87	5.25	6.73	5.28
MEAN(S)	3.55	4.51	3.99	4.62	4.25	5.24	GM
MEAN(T)	4.03		4.30		4.74		4.36
CD(0.05): T = 0.58, V = 1.58, T*V = 6.42, S*T = 0.81, V*S = 0.81							

Table 41. Effect of elevated CO_2 on SOD activity ($g^{-1}minute^{-1}$) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.49	0.74	0.48	0.62	0.44	0.54	0.55
V2	0.44	1.35	0.27	0.47	0.44	0.57	0.59
V3	0.38	0.54	0.30	0.52	0.20	0.23	0.36
MEAN(S)	0.44	0.88	0.35	0.54	0.36	0.45	GM
MEAN(T)	0.66		0.45		0.41		0.54
CD(0.05): T = 0.12, V = 0.12, T*V = 0.13, S*T = 0.17, V*S = 0.17							

Table 42. Effect of elevated CO_2 on SOD activity ($g^{-1}minute^{-1}$) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.22	0.47	0.38	0.44	0.46	0.37	0.39
V2	0.64	0.67	0.51	0.18	0.39	0.36	0.46
V3	0.30	0.28	0.27	0.44	0.34	0.30	0.32
MEAN(S)	0.39	0.47	0.39	0.35	0.40	0.35	GM
MEAN(T)	0.43		0.37		0.37		0.39
CD (0.05): T = 0.06, V = 0.06, T*V = 0.07, ST = 0.09, VS = 0.09							

Table 43. Effect of elevated CO_2 on ascorbic acid content (mg/100g) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	6.94	6.94	11.11	8.33	8.33	9.72	9.64
V2	9.72	11.11	6.94	12.50	9.72	8.33	10.80
V3	9.72	10.04	8.33	7.27	9.72	9.88	10.24
MEAN(S)	8.79	9.83	10.18	8.44	8.33	9.31	GM
MEAN(T)	10.39		10.39		9.90		
CD(0.05) T = 1.04, V = 1.04, T*V = 1.17, S*T = 0.345, V*S = 0.345							

Table 44**.** Effect of elevated CO_2 on ascorbic acid content (mg/100g) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	15.27	19.44	13.88	11.11	11.11	12.50	13.88
V2	13.88	12.50	9.72	11.11	13.88	12.50	12.26
V3	11.11	9.72	12.50	9.72	11.11	11.11	10.88
MEAN(S)	13.42	13.88	12.03	10.64	12.03	12.03	GM
MEAN(T)	13.65		11.34		12.03		12.34
CD (0.05) : T = 2.36 ,V = 2.36, T*V = 2.63, S*T = 3.31, V*S = 3.31							

4.2. EFFECT OF ELEVATED CO_2 ON AMARANTHUS (*Amaranthus tricolor* L.)

4.2.1. GROWTH PARAMETERS

4.2.1.1. *Number of Leaves*

Effect of elevated CO_2 on number of leaves after stress in amaranthus is presented in Table 45. Significantly higher mean value for number of leaves was observed in control chamber (12.05) than elevated CO_2 (11.88) and open control) (11.05). Among the varieties, highest mean value for number of leaves was observed for variety Renusree (13.83).

After re-watering (Table 46), higher mean value for number of leaves was observed in control chamber (14.72) than elevated CO_2 (14.11) and it was significantly higher compared to open control (13.22). Among the varieties, highest mean value for number of leaves was recorded for variety Renusree (16.00).

4.2.1.2. *Specific Leaf Area*

Higher specific leaf area was recorded under treatment T1 (193.36 cm^2 g^{-1}) compared to treatment T2 (180.82 cm^2 g^{-1}) and treatment T3 (171.81 cm^2 g^{-1}) after stress. Among the varieties, highest specific leaf area was recorded for the variety CO-1 (234.23 cm^2 g^{-1}) (Table 47)

After re-watering (Table 48), same increasing trend of specific leaf area was observed under treatment T1 (231.02) compared to treatment T2 (228.17cm^2 g^{-1}) and treatment T3 (227.35 cm^2 g^{-1}). Among the varieties, variety CO-1 recorded highest specific leaf area (297.3 cm^2 g^{-1}).

4.2.1.3. *Root Weight*

As presented in Table 49, significantly higher root weight was observed under elevated CO_2 (0.92 g) compared to control chamber (0.69g) and open control

(0.53 g) after stress. 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).

After re-watering (Table 50), significantly higher root weight was observed under elevated CO_2 (0.22 g) compared to open control (0.16g) 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).

4.2.1.4. *Shoot Weight*

After stress (Table 51), shoot weight was found significantly higher under treatment T1 (6.88 g) than treatment T2 (5.31 g) and treatment T3 (4.45 g). Significantly higher shoot weight was recorded for the variety CO-1 (7.69 g) after re-watering compared to Arun and Renusree.

After re-watering (Table 52), significantly higher shoot weight was observed under treatment T1 (0.75 g) compared to treatment T3 (0.63 g). 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).

4.2.1.4. *Root Shoot Ratio*

Significant reduction of root shoot ratio was observed under elevated CO_2 (0.25) than open control (0.76) after stress. Highest root shoot ratio was observed for the variety Arun (0.49) (Table 53).

Highest root shoot ratio was observed under elevated CO_2 (0.31) for the variety Arun (0.36) after re-watering (Table 54)

4.2.1.5. *Dry Matter Production*

As presented in table number 55, 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. Among the varieties, highest mean value for dry matter production was recorded for varieties CO-1 (0.76 g) and Renusree (0.75 g).

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). 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 56).

4.2.1.6. *Pest Incidence*

Potted plants of all the varieties of amaranthus were found to be slightly infested with pests like amaranthus leaf webber *(Hymenia recurvalis)* and serpentine leaf miner (*Liriomyza huidobrensis)* in the initial stages of experiment. Control measures were taken to make the experimental area pest free. Pest incidence was found comparatively higher in open control where as no to very less infestation was observed in open Top chambers with elevated and ambient CO_2 concentrations.

4.2.2 Physiological and Biochemical Parameters

4.2.2.1 *Relative Water Content*

After stress (Table 57), 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 %) (Table 58).

4.2.2.2. *Pigment Composition*

4.2.2.2.1 *Chlorophyll a*

As depicted in Table 59, 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 (Table 60), significant enhancement in chlorophyll a content was recorded under elevated CO_2 (0.65 mg/g) compared to open control (0.41 mg/g).

4.2.2.2.2 *Chlorophyll b*

After stress (Table 61), 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) (Table 62).

4.2.2.2.3 *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) (Table 63).

After re-watering (Table 64), 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 (0.63 mg/g).

4.2.2.2.4 *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 (Table 65).

After re-watering (Table 66), 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.

4.2.2.3. *Stomatal Frequency*

Stomatal frequency was found reducing under elevated CO_2 after stress (Table 67). 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 (Table 68), 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}).

4.2.2.4. *Transpiration Rate*

After stress (Table 69), 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}) and Renusree (16.23 mmol water m^{-2} s^{-1}) (Table 70)

4.2.2.5. *Photosynthesis Rate*

After two days of water stress (Table 71), 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 CO_2 m^{-2} s^{-1}) which was significantly higher than Renusree (7.35 mmol CO_2 m^{-2} s^{-1}).

After re-watering also (Table 72), photosynthesis rate was significantly higher under elevated CO_2 (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}).

4.2.2.6. *Total Soluble Protein*

As presented in Table 73, reduction in total soluble protein content was observed under elevated CO_2 after stress. Protein content was observed lower under treatment T1 (13.90 mg g^{-1}) followed by treatment T2 (14.45 mg g^{-1}) and treatment T3 (15.73 mg g^{-1}). Among the varieties, highest protein content was recorded for the variety Renusree (15.79 mg g^{-1}) which was significantly higher than Arun (12.55 mg g^{-1}).

After re-watering (Table 74), significant reduction in protein content under treatment T1 (16.40 mg g^{-1}) was observed followed by treatment T2 (21.60 mg g^{-1}) and treatment T3 (25.56 mg g^{-1}). Among the varieties, significantly higher total soluble protein content was recorded for CO-1 (27.90 mg g^{-1}) compared to Anagha and Renusree.

4.2.2.7 *Starch*

Starch content under elevated CO_2 (3.22 mg g^{-1}) was observed lower compared to open control (3.54 mg g^{-1}) and significantly higher compared to control chamber (2.28 mg g^{-1}) after stress. Highest starch content among varieties was recorded for Arun (3.39 mg g^{-1}) followed by CO-1(2.98 mg g^{-1}) and Renusree (2.67 mg g^{-1}) (Table 75).

After re-watering (Table 76), highest starch content was recorded under elevated CO_2 (2.78 mg g^{-1}) which was significantly higher than control chamber (1.97 mg g^{-1}). Among the varieties, significantly higher starch content was recorded for the variety Arun (2.80 mg g^{-1}) compared to CO-1 and Renusree.

4.2.2.8 *Reducing Sugars*

As presented in Table 77, 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}). 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 (Table 78), 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}).

4.2.2.9. *Phenol Content*

Elevated CO_2 was found to have highly significant effect on phenol content after stress (Table 79). Significant increase in phenol content was observed under elevated CO_2 (25.46 mg g^{-1}) followed by control chamber (7.10 mg g^{-1}) and open control (1.49 mg g^{-1}). Among the varieties, highest phenol content was recorded for the variety CO-1(14.05 mg g^{-1}) followed by Arun (10.94 mg g^{-1}) and Renusree (9.07 mg g^{-1}).

After re-watering (Table 80), highest phenol content was observed under elevated CO_2 (7.36 mg g^{-1}), which was significantly higher compared to control chamber (2.75 mg g^{-1}). Among the varieties highest phenol content was recorded for Renusree (5.33 mg g^{-1}).

4.2.2.10. *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}) (Table 81).

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}) (Table 82).

4.2.2.11. *Membrane Integrity*

Membrane integrity after stress was expressed in terms of % leakage in Table 83. Per cent leakage was observed significantly lower under elevated CO_2 (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 CO_2 (3.54 %) compared to control chamber (4.51 %) and open control (6.19 %) after re-watering (Table 84). Among the varieties, significantly lower % leakage was recorded for Arun (2.90 %) and CO-1 (2.99 %) compared to Renusree (8.35 %).

4.2.2.12. *Stable Isotope Discrimination*

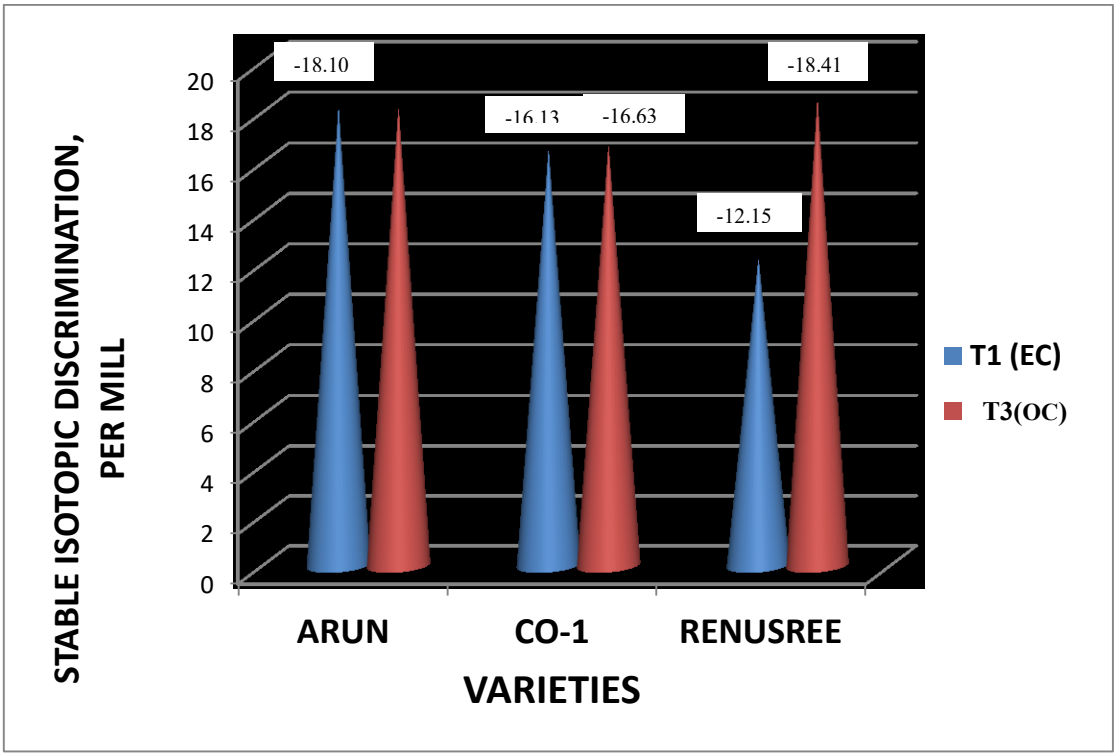


Fig 2: Effect of elevated CO_2 on stable isotopic discrimination, per mill in amaranthus

Effect of elevated CO_2 on stable isotopic discrimination in amaranthus was presented in figure 2. No significant variation in stable isotopic discrimination was observed under elevated CO_2 compared to open control for all the varieties of amaranthus.

4.2.2.13. *SOD*

Elevated CO_2 was found to have positive and significant influence on SOD activity after stress (Table 85). Significantly higher SOD activity was recorded under treatment T1 (1.65 $g^{-1}minute^{-1}$) than treatment T2 (0.93 $g^{-1}minute^{-1}$) and treatment T3

(0.84 $g^{-1}minute^{-1}$). CO-1 recorded highest SOD activity (1.33 $g^{-1}minute^{-1}$) among the varieties and it was significantly higher than Renusree (0.82 $g^{-1}minute^{-1}$).

As presented in Table 86, SOD activity under T1 (elevated co2) (2.05 $g^{-1}minute^{-1}$) was observed higher compared to T3 (open control) (1.94 $g^{-1}minute^{-1}$) and lower compared to treatment T2 (control chamber) (2.59 $g^{-1}minute^{-1}$) after re-watering. Among the varieties, highest SOD was recorded for the variety CO-1(2.59 $g^{-1}minute^{-1}$) which was significantly higher than Renusree (1.94 $g^{-1}minute^{-1}$).

4.2.2.14. *Ascorbic Acid*

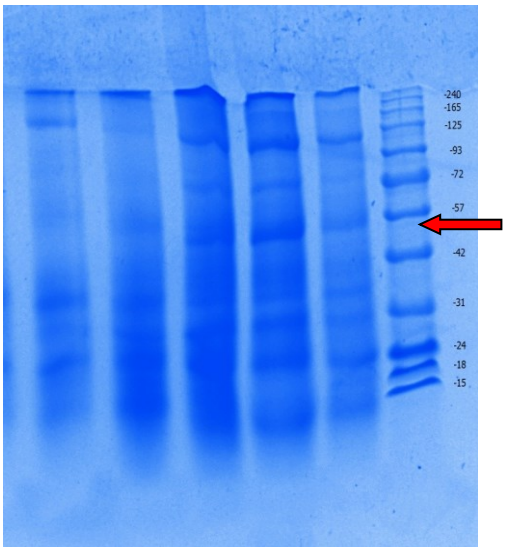
After stress (Table 87), Higher ascorbic acid content was observed under elevated CO_2 (116.31 mg g^{-1}) compared to open control (106.94 mg g^{-1}) which was significantly higher than control chamber (98.61 mg g^{-1}). Among the varieties, highest ascorbic acid content was recorded for the variety 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 82).

4.2.3 Effect of Elevated CO_2 on Protein Profiling and RuBISCO in Amaranthus

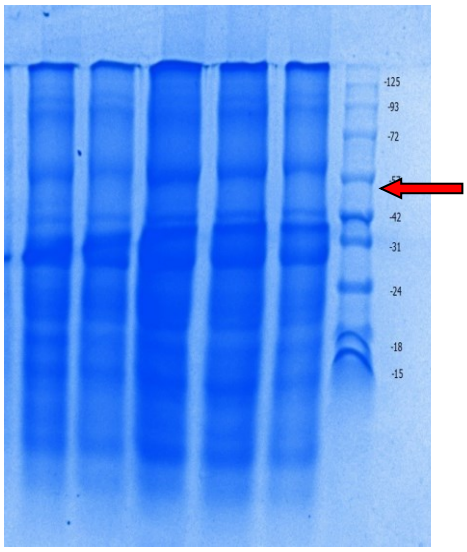
In the present study, the electrophoresis analysis of proteins using SDS PAGE revealed that elevated CO_2 induced no changes in protein profiling and RuBISCO expression levels in amaranthus (Plate. 5).

ARUN



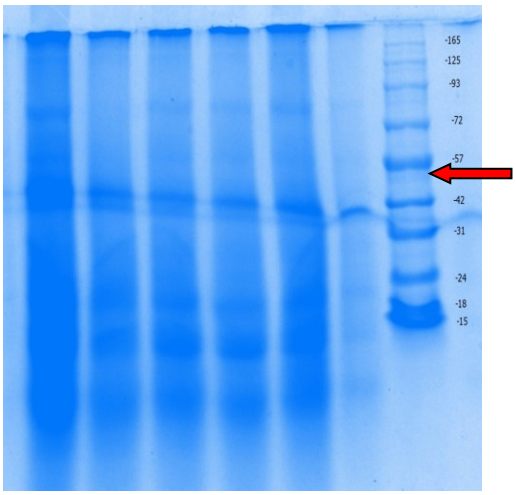
1 2 3 4 5

CO - 1



1 2 3 4 5

Renusree



1 2 3 4 5

Plate 4. Protein profiling in amaranthus

1 . EC Unstressed

2. EC Stressed

3. EC Unstressed

4. EC Recovered

5. Open control

RuBISCO (56 KDa)

Table 45. Effect of elevated CO_2 on number of leaves after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	10.33	13.00	12.00	12.33	12.00	11.00	11.77
V2	9.66	9.00	10.00	10.66	8.00	8.66	9.33
V3	14.33	14.66	12.00	15.33	12.33	14.33	13.83
MEAN(S)	11.44	12.22	11.33	12.77	10.77	11.33	GM
MEAN(T)	11.88		12.05		11.05		11.64
CD(0.05): T = 1.04, V = 1.04, T*V = 1.17, S*T = 0.345, V*S = 0.345							

Table 46. Effect of elevated CO_2 on number of leaves after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	12.00	14.66	14.66	15.00	14.66	13.00	14.00
V2	11.66	12.00	13.33	13.66	10.33	11.33	12.05
V3	17.00	17.33	14.33	17.33	14.33	15.66	16.00
MEAN(S)	13.56	14.66	14.11	15.33	13.11	13.33	GM
MEAN(T)	14.11		14.72		13.22		14.01
CD (0.05): T = 1.31 , V = 1.31, T*V = 1.46, S*T = 1.86, V*S = 1.86							

T1 - OTC with elevated CO_2 concentration (OTC Ec)

T2 - OTC with ambient CO_2 concentration (OTC Ac)

T3 – Open control

S1 – With stress

S2 – Without stress

V1 - Arun

V2 – CO-1

V3 - Renusree

GM – Grand Mean

Table 47. Effect of elevated CO_2 on specific leaf area ($cm^2 g^{-1}$) after stress in amaranthus:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	181.60	132.80	157.91	139.08	145.41	194.12	158.49
V2	340.36	178.62	279.63	189.62	185.00	232.16	234.23
V3	193.00	133.77	121.00	197.66	129.75	144.44	153.27
MEAN(S)	238.37	148.40	186.18	175.45	153.38	190.24	GM
MEAN(T)	193.36		180.82		171.81		182.00
CD (0.05): T = 32.05, V = 32.05, T*V = 35.73, S*T = 45.35, V*S = 45.35							

Table 48**.** Effect of elevated CO_2 on specific leaf area ($cm^2 g^{-1}$) after re-watering in amaranthus:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	203.66	154.10	216.66	154.86	169.20	224.50	187.16
V2	375.86	238.96	326.30	258.20	268.33	316.16	297.30
V3	234.50	179.06	177.36	235.66	162.20	223.73	202.08
MEAN(S)	271.34	190.71	240.11	216.24	199.91	254.80	GM
MEAN(T)	231.02		228.17		227.35		228.85
CD (0.05): T = 32.5, V = 32.5, T*V = 36.34, S*T = 46.08, V*S = 46.08							

Table 49. Effect of elevated CO_2 on root weight (g) after stress in amaranthus:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.11	0.97	0.69	0.59	0.60	0.48	0.74
V2	1.04	1.11	0.86	0.87	0.67	0.47	0.83
V3	0.48	0.85	0.37	0.75	0.33	0.64	0.57
MEAN(S)	0.88	0.97	0.64	0.74	0.53	0.53	GM
MEAN(T)	0.92		0.69		0.53		0.71
CD (0.05): T = 0.21 , V = 0.21, T*V = 0.11, S*T = 0.30, V*S = 0.30							

Table 50. Effect of elevated CO_2 on root weight (g) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.26	0.18	0.23	0.19	0.18	0.17	0.20
V2	0.22	0.28	0.22	0.25	0.19	0.17	0.22
V3	0.13	0.26	0.10	0.19	0.11	0.16	0.16
MEAN(S)	0.20	0.24	0.18	0.21	0.16	0.17	GM
MEAN(T)	0.22		0.20		0.16		0.19
CD (0.05): T = 0.06 , V = 0.06, T*V = 0.07, S*T = 0.09, V*S)= 0.09							

Table 51. Effect of elevated CO_2 on shoot weight (g) after stress in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	6.92	4.21	6.47	3.42	4.79	2.13	4.65
V2	12.70	7.33	6.81	6.53	6.87	5.94	7.69
V3	5.82	4.30	5.12	3.52	4.10	2.86	4.29
MEAN(S)	8.43	5.28	6.13	4.49	5.25	3.64	GM
MEAN(T)	6.88		5.31		4.45		5.54
CD (0.05): T = 0.98 , V = 0.98, T*V = 1.10, S*T = 1.39, V*S = 1.39							

Table 52. Effect of elevated CO_2 on shoot weight (g) after re-watering in tomato:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.73	0.51	0.68	0.49	0.60	0.47	0.58
V2	0.97	0.99	0.87	0.80	0.77	0.88	0.88
V3	0.63	0.67	0.69	0.52	0.61	0.45	0.59
MEAN(S)	0.77	0.72	0.74	0.60	0.66	0.60	GM
MEAN(T)	0.75		0.67		0.63		0.68
CD (0.05): T = 0.11 , V = 0.11, T*V = 0.13, S*T = 0.16, CD V*S = 0.16							

Table 53. Effect of elevated CO_2 on root shoot ratio after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.23	0.36	0.22	0.30	0.67	1.16	0.49
V2	0.17	0.20	0.16	0.20	1.00	0.64	0.39
V3	0.22	0.29	0.13	0.29	0.33	0.74	0.33
MEAN(S)	0.21	0.28	0.17	0.26	0.67	0.85	GM
MEAN(T)	0.25		0.22		0.76		0.41
CD (0.05): T = 0.29, V = 0.29, T*V = 0.32, S*T = 0.40, V*S = 0.40							

Table 54. Effect of elevated CO_2 on root shoot ratio after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.36	0.36	0.34	0.41	0.30	0.36	0.36
V2	0.25	0.28	0.27	0.32	0.26	0.19	0.26
V3	0.21	0.40	0.15	0.37	0.18	0.36	0.28
MEAN(S)	0.27	0.35	0.26	0.36	0.25	0.31	GM
MEAN(T)	0.31		0.31		0.28		0.303
CD (0.05): T = 0.10, V = 0.10, T*V = 0.12, S*T = 0.15, V*S = 0.15							

Table 55. Effect of elevated CO_2 on dry matter production (g) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.04	0.78	0.84	0.52	0.31	0.22	0.62
V2	1.38	1.08	0.81	0.77	0.27	0.23	0.76
V3	0.79	0.85	1.37	0.78	0.38	0.32	0.75
MEAN(S)	1.07	0.90	1.01	0.69	0.32	0.25	GM
MEAN(T)	0.99		0.85		0.29		0.71
CD (0.05): T = 0.13 , V = 0.13, T*V = 0.15, S*T = 0.19, V*S = 0.19							

Table 56. Effect of elevated CO_2 on dry matter production (g) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.00	063	0.91	0.68	0.79	0.64	0.78
V2	1.19	1.27	1.09	1.05	0.96	1.05	1.10
V3	0.76	0.94	0.79	0.71	0.72	0.61	0.76
MEAN(S)	0.98	0.96	0.93	0.81	0.82	0.77	GM
MEAN(T)	0.97		0.87		0.80		0.88
CD (0.05): T = 0.12, V = 0.12, T*V = 0.14, S*T = 0.18, V*S = 0.18							

Table 57**.** Effect of elevated CO_2 on relative water content (%) after stress in amaranthus:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	85.49	75.35	80.60	77.31	85.49	75.35	79.93
V2	93.97	92.97	91.42	82.92	93.90	93.04	91.37
V3	91.15	84.52	90.09	88.83	91.15	84.52	88.38
MEAN(S)	9.21	84.28	87.37	83.02	90.18	84.30	GM
MEAN(T)	87.24		85.20		87.24		86.56
CD (0.05): T = 3.43 , V = 3.43, T*V = 3.82, S*T = 4.85, V*S = 4.85							

Table 58. Effect of elevated CO_2 on relative water content (%) after re-watering in amaranthus:

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	95.08	95.08	93.85	93.82	94.65	86.86	93.22
V2	96.11	95.91	94.23	95.36	93.52	90.11	94.21
V3	95.05	85.81	95.27	82.37	94.65	82.38	89.26
MEAN(S)	95.41	92.27	94.45	90.52	94.27	86.45	GM
MEAN(T)	93.84		92.48		90.36		92.23
CD (0.05): T = 2.83, V = 2.83, T*V = 3.19, S*T = 4.04, V*S = 4.04							

Table 59. Effect of elevated CO_2 on chlorophyll a (mg/g) content after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.70	0.43	0.54	0.61	0.22	0.34	0.54
V2	0.69	0.52	0.69	0.82	0.70	0.65	0.74
V3	0.45	0.28	0.42	0.34	0.36	0.30	0.43
MEAN(S)	0.61	0.41	0.55	0.59	0.43	0.42	GM
MEAN(T)	0.51		0.57		0.42		0.50
CD (0.05): T = 0.51, V = 1.46, T*V = 0.163, S*T = 0.20, V*S = 0.20							

Table 60. Effect of elevated CO_2 on Chlorophyll a (mg/g) content after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.03	0.65	0.84	0.35	0.49	0.34	0.62
V2	0.74	0.47	0.83	0.66	0.89	0.50	0.68
V3	0.15	0.86	0.18	0.22	0.11	0.13	0.28
MEAN(S)	0.64	0.66	0.62	0.43	0.50	0.32	GM
MEAN(T)	0.65		0.52		0.41		0.53
CD (0.05): T = 0.19, V = 0.19, T*V = 0.21, S*T = 0.27, V*S = 0.27							

Table 61. Effect of elevated CO_2 on chlorophyll b (mg/g) content after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.28	0.16	0.36	0.31	0.11	0.18	0.23
V2	0.28	0.20	0.34	0.42	0.46	0.32	0.34
V3	0.14	0.05	0.20	0.15	0.18	0.15	0.14
MEAN(S)	0.23	0.14	0.30	0.29	0.25	0.22	GM
MEAN(T)	0.18		0.30		0.23		0.24
CD (0.05): T = 0.06 , V = 0.06, T*V = 0.07, S*T = 0.09, V*S = 0.09							

Table 62. Effect of elevated CO_2 on Chlorophyll b (mg/g) content after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.60	0.33	0.42	0.08	0.24	0.18	0.31
V2	0.44	0.25	0.42	0.33	0.48	0.27	0.36
V3	0.09	1.24	0.09	0.15	0.07	0.07	0.28
MEAN(S)	0.38	0.61	0.31	0.18	0.26	0.17	GM
MEAN(T)	0.49		0.25		0.22		
CD (0.05): T = 0.07 , V = 0.07, T*V = 0.08, S*T = 0.10, V*S = 0.10							

Table 63. Effect of elevated CO_2 on carotenoid content (mg/g) after stress in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.53	0.47	0.57	0.52	0.62	0.55	0.54
V2	0.48	0.48	0.57	0.53	0.65	0.56	0.54
V3	0.45	0.40	0.46	0.46	0.52	0.48	0.46
MEAN(S)	0.48	0.45	0.54	0.50	0.59	0.53	GM
MEAN(T)	0.47		0.52		0.56		0.52
CD (0.05): T = 0.08 , V = 0.08, T*V = 0.09, S*T = 0.12, V*S = 0.12							

Table 64. Effect of elevated CO_2 on carotenoid content (mg/g) after re-watering in tomato

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.75	0.71	0.75	0.59	0.72	0.65	0.69
V2	0.76	0.71	0.75	0.73	0.73	0.78	0.74
V3	0.31	0.44	0.40	0.48	0.27	0.30	0.37
MEAN(S)	0.61	0.62	0.63	0.60	0.57	0.56	GM
MEAN(T)	0.61		0.62		0.57		
CD (0.05): T = 1.03 , V = 1.03, T*V = 0.04, S*T = 0.05, V*S = 0.05							

Table 65. Effect of elevated CO_2 on total chlorophyll content (mg/g) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	0.98	0.60	0.90	0.93	0.34	0.53	0.71
V2	0.98	0.73	1.03	1.24	1.17	0.94	1.02
V3	0.59	0.34	0.62	0.49	0.55	0.46	0.51
MEAN(S)	0.85	0.55	0.85	0.89	0.68	0.64	GM
MEAN(T)	0.70		0.87		0.66		0.74
CD (0.05): T = 0.21, V = 0.21, T*V = 0.23, S*T = 0.29, V*S = 0.29							

Table 66. Effect of elevated CO_2 on total chlorophyll content (mg/g) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.64	0.99	1.26	0.57	0.74	0.52	0.95
V2	1.18	0.72	1.25	0.99	1.37	0.78	1.05
V3	0.24	1.34	0.28	0.37	0.18	0.21	0.44
MEAN(S)	1.02	1.01	0.93	0.65	0.76	0.50	GM
MEAN(T)	1.02		0.79		0.63		0.81
CD (0.05): T = 0.15, V = 0.15, T*V = 0.18, S*T = 0.22, V*S = 0.22							

Table 67. Effect of elevated CO_2 on stomatal frequency (no cm^{-2}) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	658.93	612.05	733.39	674.37	701.74	638.57	669.84
V2	515.77	462.67	670.54	602.76	569.45	489.92	551.85
V3	669.26	721.11	648.42	712.42	685.39	745.47	697.01
MEAN(S)	614.65	598.61	684.11	663.18	652.19	624.65	GM
MEAN(T)	606.63		673.65		638.42		639.57
CD (0.05): T = 42.21, V = 42.21, T*V = 47.09, S*T = 59.52, V*S = 59.52							

Table 68. Effect of elevated CO_2 on stomatal frequency (no cm^{-2}) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	664.28	666.54	734.64	666.54	727.86	708.55	634.0
V2	615.51	568.69	688.31	523.45	689.64	531.68	512.91
V3	649.70	754.34	727.93	697.81	740.58	749.06	679.01
MEAN(S)	643.16	663.17	716.96	629.27	719.96	663.10	GM
MEAN(T)	555.85		610.94		658.18		608.66
CD (0.05): T = 45.82, V = 45.82, T*V = 51.10, S*T = 64.71, V*S = 64.71							

Table 69. Effect of elevated CO_2 on transpiration rate (mmol water m^{-2} s^{-1}) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	2.36	1.23	13.31	10.24	12.33	11.14	8.43
V2	1.66	1.33	6.23	3.55	18.21	18.65	8.27
V3	2.15	0.95	8.45	7.33	19.36	14.24	8.74
MEAN(S)	2.05	1.17	9.33	7.04	16.63	14.67	GM
MEAN(T)	1.61		8.18		15.65		8.48
CD (0.05): T = 6.52, V = 6.52, T*V = 3.42, S*T = 1.03, V*S = 1.03							

Table 70. Effect of elevated CO_2 on transpiration rate (mmol water m^{-2} s^{-1}) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	3.77	4.37	16.33	15.56	12.36	13.51	10.98
V2	3.36	3.45	12.37	9.23	21.33	14.65	10.73
V3	4.12	5.12	18.36	12.26	17.36	18.12	75.34
MEAN(S)	3.75	4.13	15.68	12.35	17.01	15.42	GM
MEAN(T)	3.94		14.01		16.23		32.35
CD (0.05): T = 5.47, V = 5.47, T*V = 2.32, S*T = 2.11, V*S = 2.11							

Table 71. Effect of elevated CO_2 on photosynthesis rate (mmol CO_2 m^{-2} $s^{-1)}$after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	16.21	14.35	15.36	17.33	20.12	12.33	15.95
V2	22.56	16.26	18.69	16.35	16.25	9.65	16.62
V3	15.36	16.66	13.22	13.45	16.22	13.36	44.13
MEAN(S)	18.04	15.75	15.75	15.71	17.53	11.78	GM
MEAN(T)	16.89		15.73		14.65		25.56
CD (0.05): T = 1.22, V = 1.22, T*V = 0.73, S*T = 1.21, V*S = 1.21							

Table 72**.** Effect of elevated CO_2 on photosynthesis rate (mmol CO_2 m^{-2} s^{-1}) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	9.56	13.36	12.36	9.66	9.35	10.32	10.76
V2	19.25	15.66	14.22	8.69	11.36	9.57	13.12
V3	14.35	16.33	13.55	12.65	13.23	12.12	13.70
MEAN(S)	14.38	15.11	13.37	10.33	11.31	10.67	GM
MEAN(T)	14.74		11.85		10.99		12.52
CD (0.05): T = 3.38, V = 3.38, T*V = 1.32, S*T = 0.78, V*S = 0.78							

Table 73. Effect of elevated CO_2 on total soluble protein content (mg/g) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	14.54	10.14	11.49	8.10	17.93	13.08	12.55
V2	16.35	13.64	16.80	15.79	16.35	15.56	15.75
V3	19.29	9.46	17.60	16.92	18.27	13.19	15.79
MEAN(S)	16.73	11.08	15.30	13.60	17.52	13.94	GM
MEAN(T)	13.90		14.45		15.73		14.69
CD (0.05): T = 2.69, V = 2.69, T*V = 3.00, S*T = 3.81, V*S = 3.81							

Table 74. Effect of elevated CO_2 on total soluble protein content (mg/g) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	10.59	15.49	18.27	18.95	20.53	23.36	17.87
V2	18.88	20.99	26.52	32.30	35.45	33.30	27.90
V3	12.71	19.75	13.40	20.16	15.03	25.66	17.78
MEAN(S)	14.06	18.74	19.40	23.80	23.67	27.44	GM
MEAN(T)	16.40		21.60		25.56		21.19
CD (0.05): T = 1.68, V = 1.68, T*V = 1.87, S*T = 2.37, V*S = 2.37							

Table 75. Effect of elevated CO_2 on starch content (mg/g) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	2.78	2.33	2.88	2.72	6.87	2.78	3.39
V2	3.75	3.39	1.66	2.19	4.57	2.28	2.98
V3	4.24	2.80	2.19	2.00	3.12	1.64	2.67
MEAN(S)	3.59	2.84	2.24	2.31	4.85	2.23	GM
MEAN(T)	3.22		2.28		3.54		3.01
CD (0.05): T = 0.61 , V = 0.61, T*V = 0.68, S*T = 0.86, V*S = 0.86							

Table 76. Effect of elevated CO_2 on starch content (mg/g) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	3.99	3.00	1.82	2.43	2.36	3.16	2.80
V2	2.95	1.75	1.61	1.43	2.39	3.13	2.21
V3	1.97	3.00	2.04	1.90	2.52	2.54	2.27
MEAN(S)	2.97	2.58	2.04	1.90	2.52	2.54	GM
MEAN(T)	2.78		1.97		2.53		2.43
CD (0.05): T = 0.48 , V = 0.48, T*V = 0.54, S*T = 0.68, V*S) = 0.86							

Table 77. Effect of elevated cCO_2 on reducing sugars content (mg/g) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	19.43	15.80	15.53	14.10	12.36	12.53	14.96
V2	16.43	14.80	14.12	12.23	12.70	10.63	13.48
V3	14.86	14.56	12.76	12.86	9.70	10.46	12.53
MEAN(S)	16.91	15.05	14.13	13.06	11.58	11.21	GM
MEAN(T)	15.98		13.60		11.40		13.66
CD (0.05): T = 0.09 , V = 0.09, T*V = 0.16, S*T = 0.13, V*S = 0.13							

Table 78. Effect of elevated CO_2 on reducing sugars content (mg/g) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	21.5	19.76	18.26	18.20	11.16	12.46	16.89
V2	22.66	18.33	19.36	16.16	14.26	11.93	17.20
V3	19.53	17.76	14.46	13.43	12.66	10.76	14.77
MEAN(S)	21.33	18.78	17.36	15.93	12.70	11.72	GM
MEAN(T)	20.01		16.65		12.21		16.29
CD (0.05): T = 0.29, V = 0.29, T*V = 0.32, S*T = 0.41, V*S = 0.41							

Table 79. Effect of elevated CO_2 on phenol content (mg/g) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	11.98	20.59	17.13	12.33	1.49	2.12	10.94
V2	25.49	46.30	8.12	1.49	1.35	1.56	14.05
V3	31.81	16.61	2.77	0.80	0.77	1.66	9.07
MEAN(S)	23.09	27.83	9.34	4.87	1.20	1.78	GM
MEAN(T)	25.46		7.10		1.49		11.35
CD (0.05): T = 5.85, V = 5.85, T*V = 6.52, S*T = 8.27, V*S = 8.27							

Table 80. Effect of elevated CO_2 on phenol content (mg/g) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	2.82	3.32	2.40	7.38	0.99	11.95	4.81
V2	7.95	9.80	0.62	1.59	7.60	3.89	5.24
V3	9.88	10.37	1.29	3.22	3.49	3.74	5.33
MEAN(S)	6.88	7.83	1.44	4.06	4.03	6.53	GM
MEAN(T)	7.36		2.75		5.28		5.13
CD (0.05): T = 2.54, V = 2.54, T*V = 4.40, S*T = 3.59, V*S = 3.59							

Table 81. Effect of elevated CO_2 on free amino acid content (mg/g) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.32	1.08	0.92	0.92	0.98	1.21	1.07
V2	1.56	1.24	1.30	1.00	0.80	0.89	1.13
V3	1.02	0.89	0.89	0.76	0.74	0.70	0.83
MEAN(S)	1.30	1.07	1.03	0.89	0.84	0.93	GM
MEAN(T)	1.19		0.96		0.89		
CD (0.05): T = 0.09 ,V = 0.09, T*V = 0.09, S*T = 0.12, V*S = 0.12							

Table 82. Effect of elevated CO_2 on free amino acid content (mg/g) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.39	1.24	1.03	0.98	1.15	1.10	1.15
V2	1.65	1.33	0.98	1.12	1.26	1.22	1.26
V3	1.05	1.01	0.86	0.79	0.95	0.86	0.92
MEAN(S)	1.36	1.19	0.96	0.96	1.12	1.09	GM
MEAN(T)	1.28		0.96		1.09		1.11
CD (T) = 0.09 , CD(V) = 0.09, CD(T*V) = 0.10, CD(ST) = 0.13, CD (VS)= 0.13							

Table 83. Effect of elevated CO_2 on membrane integrity (% leakage) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	2.31	3.91	2.68	4.38	3.45	9.43	4.36
V2	3.77	5.15	3.83	6.23	4.74	6.81	5.09
V3	6.96	14.64	5.15	10.64	9.35	16.66	10.58
MEAN(S)	4.35	7.90	3.89	7.10	5.85	10.97	GM
MEAN(T)	6.12		5.49		8.41		6.68
CD (0.05): T = 1.89 , V = 1.89, T*V = 2.10, S*T = 2.66, V*S = 2.66							

Table 84. Effect of elevated CO_2 on membrane integrity (% leakage) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.97	1.24	2.98	2.70	3.60	4.90	2.903
V2	2.86	2.23	2.77	2.29	4.32	3.49	2.99
V3	4.32	8.64	5.08	11.23	8.41	12.41	8.35
MEAN(S)	3.05	4.04	3.61	5.41	5.44	6.93	GM
MEAN(T)	3.54		4.51		6.19		4.75
CD (0.05): T= 0.80 , V = 0.80, T*V = 0.90, S*T = 1.14, V*S = 1.14							

Table 85. Effect of elevated CO_2 on SOD activity ($g^{-1}minute^{-1}$) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	1.10	2.67	0.66	1.56	0.45	1.23	1.28
V2	0.86	2.98	0.59	1.48	0.44	1.60	1.33
V3	0.51	1.80	0.41	0.88	0.49	0.82	0.82
MEAN(S)	0.82	2.48	0.55	1.30	0.46	1.22	GM
MEAN(T)	1.65		0.93		0.84		1.14
CD (0.05): T = 0.38, V = 0.38, T*V = 0.43, S*T = 0.54, V*S = 0.54							

Table 86. Effect of elevated CO_2 on SOD activity ($g^{-1}minute^{-1}$) after re-watering in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	2.11	2.27	1.91	2.19	2.12	1.69	2.05
V2	2.49	1.78	3.27	2.83	3.07	2.13	2.59
V3	2.66	2.19	1.87	1.37	2.00	1.54	1.94
MEAN(S)	2.42	2.08	2.35	2.13	2.39	1.79	GM
MEAN(T)	2.05		2.59		1.94		2.19
CD (0.05) T = 0.61, V = 0.61, T*V = 0.68, S*T = 0.86, V*S = 0.86							

Table 87. Effect of elevated CO_2 on ascorbic acid content (mg/100g) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	104.16	156.25	93.75	156.25	72.91	145.83	121.52
V2	145.83	177.08	62.50	145.83	131.25	145.83	134.72
V3	52.08	62.50	87.50	45.83	72.91	72.91	65.62
MEAN(S)	100.69	131.94	81.25	115.97	92.36	121.52	GM
MEAN(T)	116.31		98.61		106.94		107.29
CD (0.05) T = 13.70, V = 13.70, T*V = 15.27, S*T = 19.37, V*S = 19.37							

Table 88. Effect of elevated CO_2 on ascorbic acid content (mg/100g) after stress in amaranthus

	T1		T2		T3		MEAN(V)
VARIETIES	S1	S2	S1	S2	S1	S2	
V1	13.19	19.44	12.22	27.77	11.80	12.50	16.15
V2	43.75	20.83	25.00	20.83	45.83	16.66	28.81
V3	41.66	30.55	45.83	30.55	31.94	19.44	33.33
MEAN(S)	32.87	23.611	27.68	26.38	29.86	16.20	GM
MEAN(T)	28.24		27.03		23.03		26.10
CD (0.05): T = 7.45, V = 7.45, T*V = 8.31, S*T = 10.55, V*S = 10.55							

5. DISCUSSION

Based on reports by the IPCC (Intergovernmental Panel on Climate Change) atmospheric CO_2 concentration is rising. Increasing levels of atmospheric CO_2 can affect air temperature and precipitation patterns, thereby causing global change in many ways. Because of potential alteration in future climatic conditions, soil–water content may be affected in many regions of the globe which shows adverse effects on agriculture and food productivity altering the ecosystem balance

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. The steady increase in greenhouse gases might lead in future to higher temperatures and greater evaporative demands. In coming future with changing climate, drought occurrences will be more frequent, intense, and erratic, and will possibly affect regions not currently subjected to drought. Tomato (*Solanum lycopersicum*) is the widely cultivated vegetable in India and 2nd most important vegetable crop next to potato. This crop is very sensitive to environmental factors like soil moisture status, temperature, salinity etc. Amaranthus is the traditional leafy vegetable which has, over the centuries, provided rural communities with food and nutritional security. It is a hardy, drought tolerant plant and is with a great potential for adaptation to impending climate change.

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. 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. 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. There is no research report available about the interactive studies on water availability and elevated CO_2 in the case of tomato and amaranthus.

In the present programme, potted plants of tomato and amaranthuswere exposed to elevated CO_2 conditions. During their critical stages of development, plants were subjected to water stress and then were allowed to recover. Observations on growth, physiological and biochemical parameters were taken and also molecular studies were carried out after stress and recovery periods.

EFFECT OF ELEVATED CO_2 ON GROWTH PARAMETERS:

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_2. With CO_2 enrichment, Photosynthesis increases, plant biomass accumulated per unit of water consumed increases, and economic yield also gets enhanced. Increases in atmospheric levels of CO_2 above current levels can increase photosynthesis by decreasing photorespiration. Elevated CO_2 generally stimulates C_3 photosynthesis more than C_4. For C_3 plants the positive responses by CO_2 enrichment are mainly attributed by the competitive inhibition of photorespiration (Amthor and Loomis, 1996). The various growth parameters considered under this study include number of leaves, specific leaf area, root weight, shoot weight, root shoot ratio and dry matter production

Number of leaves, leaf size and anatomy are often altered under elevated CO_2 but the magnitude of these changes often decreases as leaves mature and hinges upon plant genetic plasticity, nutrient availability, temperature and phenology (Pritchard *et al.,* 1999).

In this experiment, elevated CO_2 had no influence on number of leaves for both tomato and amaranthus. This result was in agreement with the findings of Alexandre *et al* .(2012) in *Zostera noltii* and Nowak, *et al*. (2006) in Boston Fern micro cuttings.

Specific leaf area (SLA) is an indicator of leaf thickness. Exposure to elevated CO_2 can cause an increase in leaf thickness due to increased number of palisade cells, which contributed to leaf thickness (Thomas, 1983). The reduction in specific leaf area under elevated CO_2 can also be due to the high accumulation of starch and lower rate of leaf expansion.

In tomato, 8% and 8.44% Reduction in specific leaf area was found under elevated CO_2 compared to open control after stress and re-watering. The result was in accordance with a study conducted by Mishra and others (1999) in *Jatropha curca* where drought stress decreased specific leaf area under elevated CO_2.

In amaranthus, a rise by 11.14% and 1.58% in specific leaf area was recorded under elevated CO_2 compared to ambient CO_2 and open control after stress and recovery which was in accordance with the study conducted by (Sallas *et al*., 2003) in norway spruce.

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_2 enrichment can affect root physiology and morphology . Previous studies have shown that elevated CO_2 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 (Curtis *et al*., 1990).

In the present study highest root weight was maintained under elevated CO_2 than open control for tomato and amaranthus after stress and re-watering. In tomato a per cent increase in root weight by 34.09 and 14.61 was recorded under elevated CO_2

after stress and re-watering respectively. Whereas it was recorded as 42.39% and 27.27% increase in root weight under elevated CO_2 compared to open control for amaranthus after stress and re-watering respectively. This is in agreement with many studies conducted in winter wheat (Pritchard and Rogers, 2000) and many annual plant species (Bernacchi *et al*., 2000).

Shoot growth can be stimulated by exposure of plant canopies to high CO_2 concentration. The general consensus is that photosynthesis and C allocation to plant shoots increases as atmospheric CO_2 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 tomato and amaranthus under carbon dioxide enriched treatment compared to open control both after stress and re-watering. In tomato 19% and 50% increase in shoot weight after stress and recovery was observed respectively under elevated CO_2 in amaranthus it was recorded as 35% and 16%. This result was in agreement with former reports by Epron, D (1995) in *Fagus sylvatica* and Obrist and Arnone (2003) in *Larrea tridentate*.

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 (Madhu and Hatfield, 2013).

In this experiment, for tomato under elevated CO_2, no significant difference in root shoot ratio was observed after stress where as 39% reduction in root shoot ratio was observed after re-watering compared to open control. In amaranthus 67% reduction was observed after stress and a little increment by 5% was observed after re-watering.

These results were in accordance with the works done by Obrist and Arnone (2003) in *Larrea tridentate*, in tomato.

Elevated CO_2 stimulates photosynthesis in various intensities during different phenological phases (Mitchell *et al*., 1999) and its direct consequence is increased dry matter production (Lawlor and Mitchell, 1991; Ziska *et al*., 2004).

In present study, water stress induced reduction in dry matter production under elevated CO_2 was found to be less compared to open control. Dry matter production for tomato under elevated CO_2 was found 23.17% and 41.48% superior after stress and re-watering respectively. For amaranthus 70.7% and 17.52% increase in dry matter production was observed after stress and recovery respectively under elevated CO_2 compared to open control. This was in agreement with findings of Pan, (1996) in soybean, Prasad, (2002) in dry bean, Clifford *et al*., (2000) in peanut and Ellis (1995) in cowpea.

EFFECT OF ELEVATED CO_2 ON PHYSIOLOGICAL PARAMETERS:

The effect of CO_2 enrichment level on various physiological parameters like RWC, stomatal frequency, transpiration rate, photosynthetic rate, pigment composition, membrane integrity and stable isotope discrimination were analysed. The results of these parameters are discussed below.

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 of leaves is higher in the initial stages of leaf development and diminishes with dry matter accumulation and leaf maturaty. RWC related to water uptake by the roots as well as water loss by evapotranspiration

Plant water use efficiency is strongly influenced by stomatal density (Woodward and Kelly, 1995). Decreased stomatal opening can lead to improved water use efficiency (Guy and Reid, 1986; Clifford *et al*., 2000) and results in lower water stress of plants (Kimball, 1983).

In this experiment, RWC increased by 1.12% and 3.94% after stress and recovery respectively under elevated CO_2 for tomato. Among the tomato varieties, Vellayani Vijay recorded highest RWC.

In amaranthus, after stress no difference in RWC was observed between elevated CO_2 and open control. But 3.7 % of significant rise in RWC was recorded under elevated CO_2 after re-watering.

These results were found in agreement with research done by Yusuke *et al.* (2007) in ginger (Zingiber officinale Roscoe), Manderscheid R. *et al* .(2011) in maize under water stress and a study conducted by Schwanz and Polle, 2001, on Pendunculate Oak (*Quercus rober*) and Maritime Pine (*Pinus pinaster*).

Stomata are the integrators of all environmental factors that affect the plant growth (Morison, 1998). A wide range of responses are observed in crop plants with increasing CO_2 concentration. Induction of stomatal density is varied from the large reductions to large increases among species and even within the species (Woodward *et al*., 2002).

In the present study, significant reduction in stomatal frequency was observed after stress (15.54%) and re-watering (9.81%) in tomato compared to open control. a reduction of 4.97% after stress and 4.97% after recovery was recorded in amaranthus. Vellayani Vijay variety of tomato and CO-1 variety of amaranthus recorded lowest stomatal frequency under elevated CO_2.

Reduction in stomatal frequency under elevated CO_2 was reported by (Woodward *et al*., 2002) in the leaves of *Arabidopsis thaliana,* Levine *et al*. (2008) in soybean, Driscoll, *et al.* (2006) In Maize and Rey and Jarvis., (1997) in *Betula pendula* and *Fraxinus ornus*

Transpiration is the loss of water in the form of water vapour from the aerial parts of the plant and the rate of transpiration is affected by a number of internal

(plant factor) and external factors (light, temperature, humidity, wind, atmospheric pressure and water supply).

Plants respond to enriched CO_2 content by showing declined stomatal conductance, which typically leads to reduced rates of transpirational loss (Apple *et al.*, 2000). Elevated CO2 reduces transpiration by partially closing the stomata and decreasing stomatal conductance (Morison and Gifford, 1983 and Bunce, 2000). Douglas fir seedlings grown for three years in environmental chambers under CO2 concentration of 530ppm + 3.5°C resulted in 12% reduction of transpiration (Apple *et al*., 2000).

In this experiment there observed a significant reduction in transpiration rate after stress (65.06%) and re-watering (44.20%) in tomato under elevated carbon dioxide treatment compared to open control. In amaranthus 89.72% reduction in transpiration rate after stress and 75.72% reduction after recovery was observed under enriched CO_2 treatment compared to absolute control.

These results were found in accordance with studies conducted on cherry by Centritto (1999), egg plant by Sarker and Hara (2011), *Alnus firma* by Liang (1994), sunflower and *Podophyllum hexandrum* by Tezara *et al.* (2002) and Chaturvedi *et al*. (2009) respectively.

Elevated CO_2 may alleviate the high temperature damage to photosynthesis because with higher CO_2 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 CO_2, which can be alleviated under elevated CO_2 (Poorter and Perez-Soba, 2001). With elevated CO_2 concentration accompanied by high temperature, there was no increase in the risk observed of photo damage and down regulation of electrons in

rose plants (Urban *et al*., 2001), whereas enhanced photosynthesis and WUE in carrot plants were found with CO_2 enrichment. (Thiagarajan *et al*., 2007).

In the present study conducted on tomato under elevated CO_2, 27.44% and 24.15% enhancement in photosynthetic rate was observed after stress and re-watering respectively where as in amaranthus it was recorded as 13.26% and 25.44% increase after stress and re-watering respectively than control.

Similar results were observed by Samarakoon and Gifford (1995) in sunflower, Li, D. *et al*. (2013) in soybean, Sarker and Hara (2011) in egg plant and Mishra and others (1999) in mustard.

Plant productivity depends greatly on the amount of chlorophyll present in the chloroplast. Chlorophyll is the pigment that gives plant their characteristic green colour, it plays a unique role in the physiology, productivity and economy of green plants. The amount of chlorophyll in leaf tissues is influenced by nutrient availability and environmental stresses (Palta, 1990; Karacan, 2006 and Onwurah *et al*., 2007). Leaf chlorophyll content is a good indicator of photosynthesis activity, nutritional status, mutations and stress condition (Ghasemi *et al.,* 2011). Chlorophyll content of seedling leaves not subjected to drought stress was found 15% and 16% higher than that of severe drought stressed seedlings in ambient and elevated CO_2, respectively (Li *et al*., 2008).

In the present study conducted on tomato, increase in chlorophyll a (30%),chlorophyll b (60%), total chlorophyll (5.71%) and no change in carotenoid content was observed after stress under enriched CO_2 conditions. After re-watering, a per cent increase of 54.1, 33.33, 161 and 48.78 in chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content was recorded respectively under elevated CO_2 than open control.

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 CO_2 in comparison with control conditions.

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 (Robertson and Leech, 1995). Moreover, water use efficiency was observed better at high CO_2 which could have limited chlorophyll degradation. (Bazzaz, 1990).

Several contradictory results were also reported in the case of chlorophyll content under elevated CO_2. Decreased total Chlorophyll content was observed in two spring wheat cultivars by Lin, J. S and Wang, G. X in 2002 under elevated CO_2. Similar results were observed in tomato by Helyes *et al*.(2004) and Mamata *et al*, (2014) and in Pendunculate Oak by Peter Schwanz and Andrea Polle in 2001.

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 (Eckardt, 2008).

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. High temperature due to elevated CO_2 can alter the physical state of the membrane, and lead to fluidization and disintegration of membrane (Los and Murata, 2004).

In the present work, per cent reduction of 23.71 and 14.97 in leakage after stress and recovery was recorded in tomato under elevated CO_2. Whereas it was recorded as 27.22% and 42.81% reduction in leakage in the case of amaranthus after stress and re-watering respectively.

Several physical factors have been shown to influence the integrated balance of stomatal conductance and carboxylation and thus affect isotopic discrimination in plants (Henderson *et al*., 1998). In this study, carbon isotope discrimination values were found to be varying across the treatments and varieties. More negative stable isotopic discrimination was observed under elevated CO_2 compared to open control for all the 3 varieties of tomato,

EFFECT OF ELEVATED CO_2 ON BIOCHEMICAL PARAMETERS:

The major biochemical compounds studied in the current experiment are total soluble proteins, starch, reducing sugar, phenols, free amino acids, superoxide dismutase (SOD) and ascorbic acid.

Two types of metabolites are produced by plants i.e. primary and secondary metabolites. Exposure of plants to elevated CO_2 conditions influences both primary and secondary metabolites.(Ibrahim and Jaafar, 2012). As reported by Lin and Wang in (2002), elevated CO_2 decreased soluble protein content in spring wheat cultivars. decline in soluble protein contents could be largely due to a reduction in ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBISCO) protein. The reduction in protein contents in plants grown under doubled CO_2 were delayed after stress compared to control which suggested that drought-induced oxidative damage to protein had been significantly reduced by doubled CO_2, possibly by protecting the Rubisco protein from oxidative damage. Protein accumulation was found to be lowest in barley leaves (Robredo *et al*., 2011) enriched with high CO_2 concentration.

In the present experiment soluble protein content was found decreasing under elevated CO_2 after stress (23.22%) and re-watering (20.47%) in tomato compared to

control. In amaranthus, per cent reduction in total soluble protein content under enriched CO_2 chamber was recorded as 11.63 and 35.80 after stress and re-watering respectively in comparison with open control.

These results are in complete agreement with research done by Tezara *et al.,* (2002) in sun flower, Driscoll *et al*., (2005) in maize and Schwanz, P. and Polle, A., (2001) in pine tree (*Pinus pinaster*) under water stress and elevated CO_2, where they found reduction in protein content with CO_2 enrichment.

Under elevated CO_2 condition, carbohydrates accumulation in plant tissues is most pronounced since their intensity of usage is lower than their production under these conditions (Moore *et al.* 1998; Wolfie *et al.* 1998). Accumulation of carbohydrates in leaves is one of the most important responses observed in C_3 plants to elevated atmospheric CO_2 (Long *et al*., 2004). Elevated CO_2 conditions enhances the soluble sugar content of *Labisia pumila* (Ibrahim, 2011),

In the current study on tomato, under elevated CO_2 starch content was found to have an enhancement of 21.59% after stress and 53.39 % after re-watering than absolute control. In amaranthus, under enriched CO_2 treatment, there observed a decline by 9.03% in starch content after stress but after re-watering, 7.19% increment was observed compared to open control.

In the case of reducing sugars, significant rise by 10.44% and 7.93% was recorded after stress and recovery respectively in tomato under CO_2 enriched treatment in comparison with open control. For amaranthus also, a significant per cent rise of 28.66 and 38.9 was recorded under elevated CO_2 in comparison with open control.

Several reports on effect of elevated CO_2 on carbohydrate accumulation were made by several workers. Li *et al.,* 2013 reported that, elevated CO_2 (800 µmol mol^{-1} CO_2 increased carbohydrates accumulation in tomato plants. Centritto *et al.,*(1999) found that leaf starch concentration was strongly enhanced by elevated CO_2 and

influenced by water stress treatments in the cherry seedlings. Increased carbohydrate content with carbon dioxide enrichment was reported by Ghasemzadeh and Jaafar (2011) in ginger, Yelle (1989) in tomato and Ibrahim (2011) in alfalfa. Levine *et al*. (2008) reported increased starch content with CO_2 treatment in soybean. High carbohydrate accumulation was reported in strawberry under elevated CO_2 condition (Wang *et al*., 2003). Elevated CO_2 increases the accumulation of starch, total soluble sugars and reducing sugars in black gram during the flowering stage (Sathish *et al*., 2014). Lilley *et al*. (2001) reported that elevated CO_2 conditions increased non-structural carbohydrate contents by 28% for clover and 16% for phalaris.

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 (Poorter *et al*., 2001). Goncalves *et al*. (2009) reported elevated CO_2 induced increase in the total phenol content in wheat leaves. Similar reports were obtained by Saravanan and Karthi (2014).

Elevated CO_2 was shown to have significant impact on phenol content in the current study. In tomato a significant increase in phenol content by 33.91% after stress and 7.12% after re-watering was observed. Similarly 94.14% and 28.2% rise in phenol content was observed after stress and recovery respectively in amaranthus.

Koricheva *et al*. (1998) reported an increased total phenolic concentration in temperate species when grown under elevated CO_2. Contradictory to this, in tomato (*Lycopersicon esculentum* Mill) cv. Arka Ashish, Mamata *et al.* (2014) reported decreased phenols and antioxidants activity in elevated CO_2 conditions, which might be due to lower stress experienced by the plants at EC as observed by the higher water potentials of these plants.

Carbon dioxide enrichment enhances the accumulation of both leaf starch and soluble carbohydrates (De Souza *et al*., 2008; Norby *et al.,* 1986). 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 (Sicher, 2008). Ample carbon was available to support amino acid synthesis and to increase in soluble amino acids under CO_2 enrichment.

In the present study, free amino acid content under elevated CO_2 was found increasing significantly by 43.31% after stress in tomato. After re-watering, 5.39% decline in free amino acid content was recorded in elevated CO_2 compared to open control. In the case of amaranthus, significant increment of 25.21% and 14.84% free amino acid content was recorded under elevated CO_2 in comparison with open control after stress and re-watering respectively.

Increase in soluble amino acid content under CO_2 enrichment has been reported in soybean (Ainsworth *et al*., 2007), tobacco (Geiger *et al*., 1998).

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 mechanisms against reactive oxygen species (ROS) induced oxidative stress generated under stress conditions (Matsuura and Fett-Neto, 2013). Antioxidants are the substances that protect cell from the oxidative damage. Antioxidative activity can be non-enzymatic and enzymatic (Bartels and Sunkar, 2005). Non-enzymatic antioxidants include vitamin C, vitamin E, glutathione, flavonoids, alkaloids, carotenoids etc and enzymatic antioxidants include catalase, superoxide dismutase and peroxidase (Seki *et al*., 2001).

The antioxidants studied in this experiment are superoxide dismutase (SOD) and ascorbic acid. Elevated CO_2 was shown to have positive and significant influence on antioxidant production and activity. Under elevated CO_2 conditions, a significant

rise by 37.87% and 13.95% in SOD activity was recorded after stress and re-watering respectively in tomato. In amaranthus, 49.39% (after stress) and 5.36% (after re-watering) rise in SOD activity was recorded under elevated CO_2 compared to open control.

With CO_2 enrichment, ascorbic acid content was found enhancing by 47.16% and 11.86% in tomato after stress and recovery respectively. Similarly a per cent increase of 8.05 (after stress) and 18.44 (after re-watering) was observed in amaranthus under elevated CO_2 compared to control.

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 of Niewiadomska *et al*. (1999), Schwanz and Polle (2001) ; Lin and Wang (2002) etc. In bean sprouts, a mere one hour per day doubling of atmospheric CO_2 concentration over a 7 day period, doubled vitamin C content (Tajiri, 1985).

Several contradicting results were also reported. SOD activity declined significantly after water stress for 10 days in two spring wheat cultivars (*Triticum aestivum* L. Longchun 292 and Longchun 8139) regardless of ambient or doubled CO_2 (Lin and Wang, 2002). Polle *et al*. (1997) showed that two years of atmospheric CO_2 enrichment reduced the activities of several key antioxidative enzymes including catalase and superoxide dismutase in beech seedlings. Activities of superoxide dismutase, catalase and ascorbate peroxidase were declined under elevated CO_2 in *Catharanthus roseus* (Singh and Agrawal, 2015).

MOLECULAR STUDIES:

Under elevated CO_2, there can be imbalance in the supply and demand of carbohydrates resulting in their increased accumulation in the leaves (Stitt, 1991). Carbohydrate accumulation in the leaves has been shown to down regulate the expression of photosynthetic genes in higher plants under elevated CO_2 (Prentice *et al*., 2001). In the present study, the electrophoresis analysis of proteins using SDS PAGE revealed that elevated CO_2 induced the production of a few new proteins under water stress. The protein content and profile varied with different varieties in response to elevated CO_2 level. In elevated CO_2, formation of a few new proteins of molecular weight nearly 42 K Da to 50 K Da were observed under water stress for tomato varieties Anagha, Vellayani Vijay and Manulakshmi which can be stress proteins imparting tolerance. CO_2 enrichment did not modify the expression levels of large or small sub units of RuBISCO in tomato. In the case of amaranthus, protein profile and RuBISCO sub unit expressions were not modified by the experimental treatments.

Many contradictory results were reported by several workers regarding the regulation of gene expressions as modified by CO_2 levels. In sunflower, RuBISCO content of well watered plants reduced by 25% in elevated CO_2 compared to ambient CO_2. But in severe water deficit conditions, RuBISCO content decreased more in plants grown in ambient CO_2 than elevated CO_2 (Tezara *et al.,* 2002). Pandurangam *et al*. (2006) said that photosynthetic acclimation to elevated CO_2 concentration due to down regulation of RuBISCO is by the limitation imposed on RuBISCO small subunit gene expression as a consequence of high sugar content. RuBISCO activity and Rubisco protein in barley penultimate leaves and wheat flag leaves were decreased under elevated CO_2 concentration of 700 μmol mol^{-1} (Richard and James, 1997).

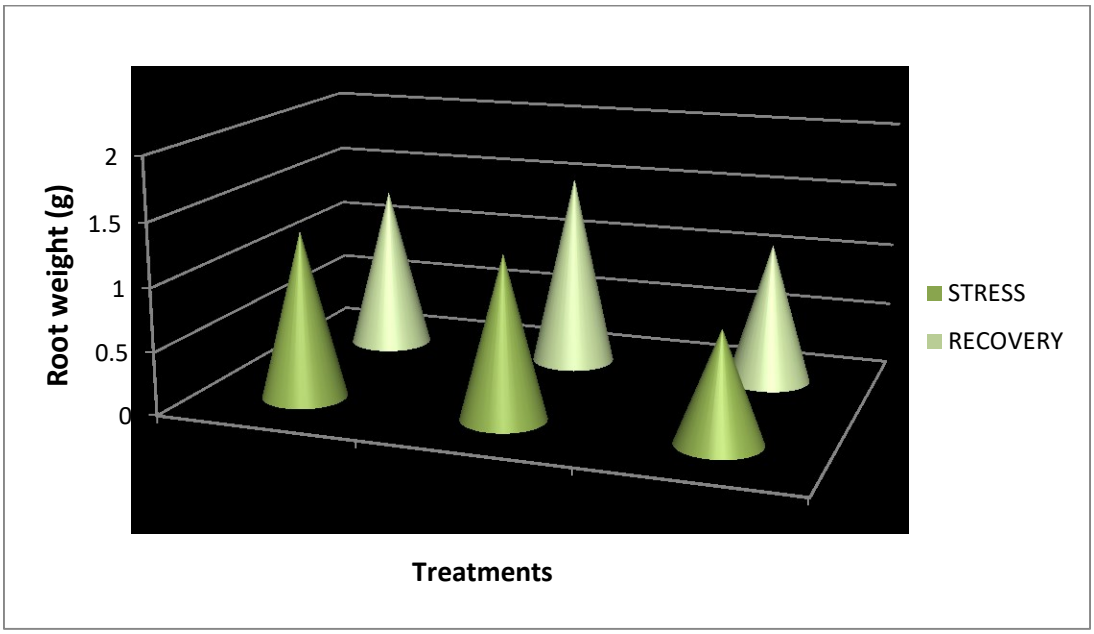


Fig 3. Effect of elevated CO_2 on root weight (g) in tomato

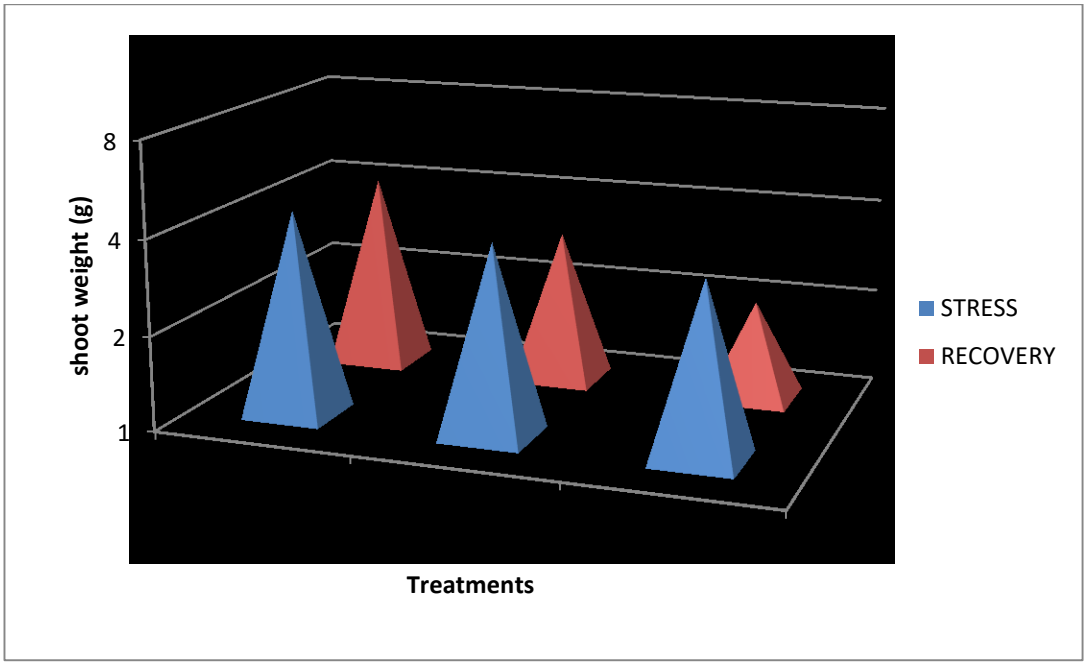


Fig 4. Effect of elevated CO_2 on shoot weight (g) in tomato

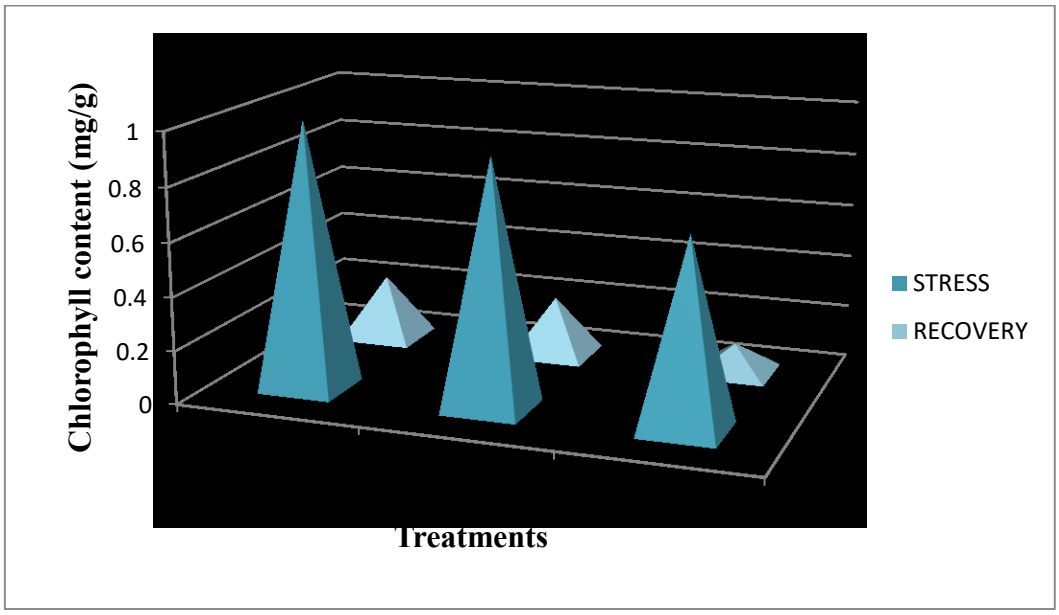


Fig 5. Effect of elevated CO_2 on total chlorophyll content (mg/g) in tomato

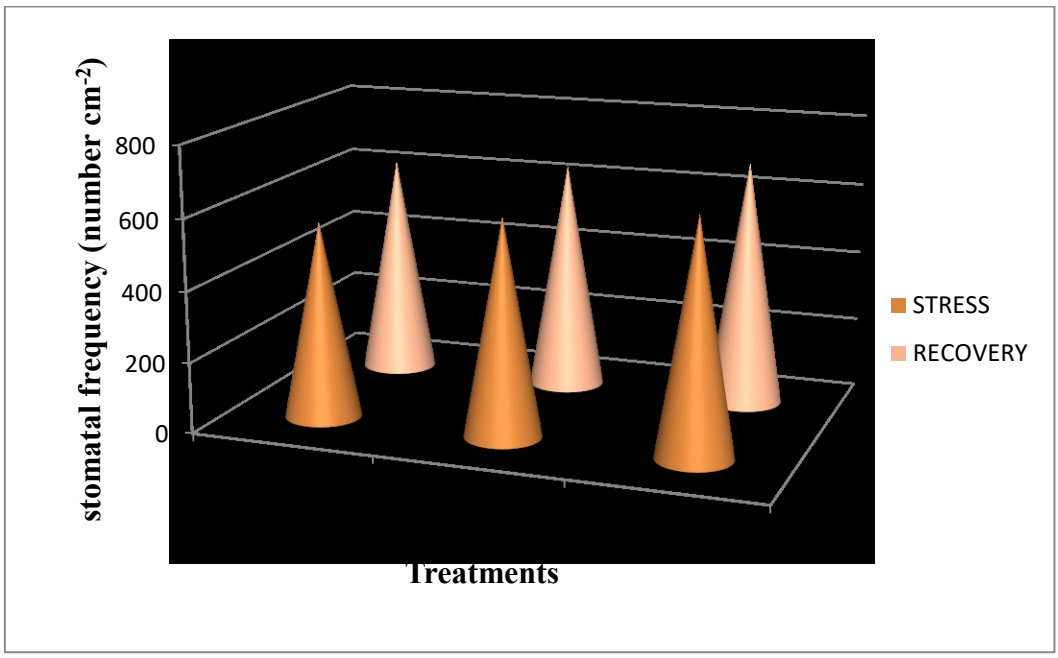


Fig 6. Effect of elevated CO_2 on stomatal frequency (number cm^{-2}) in tomato

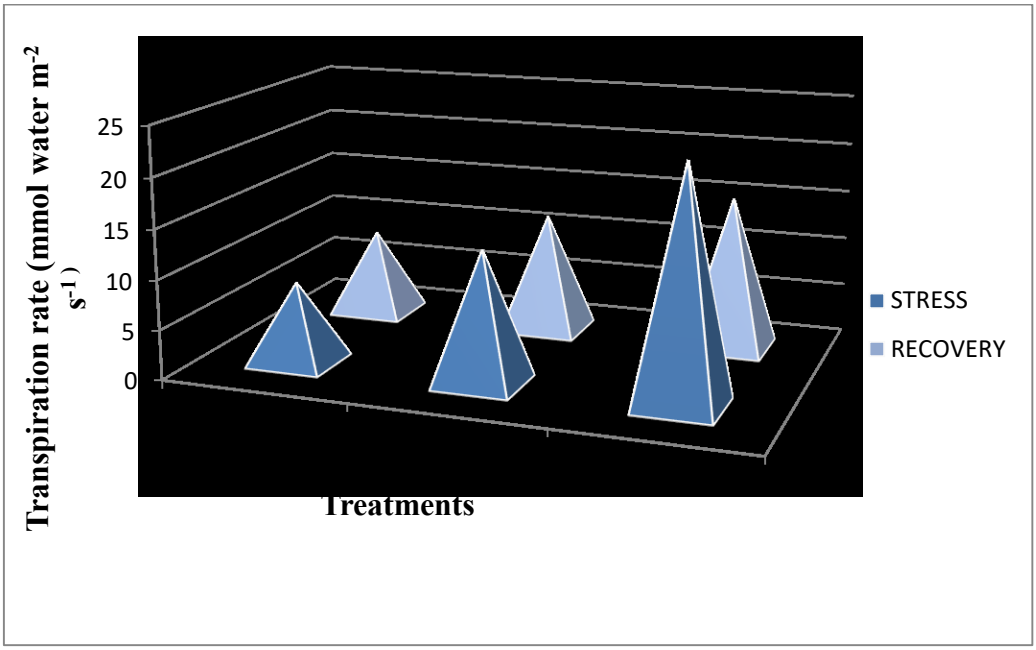


Fig 7. Effect of elevated CO_2 on transpiration rate (mmol water m^{-2} s^{-1}) in tomato

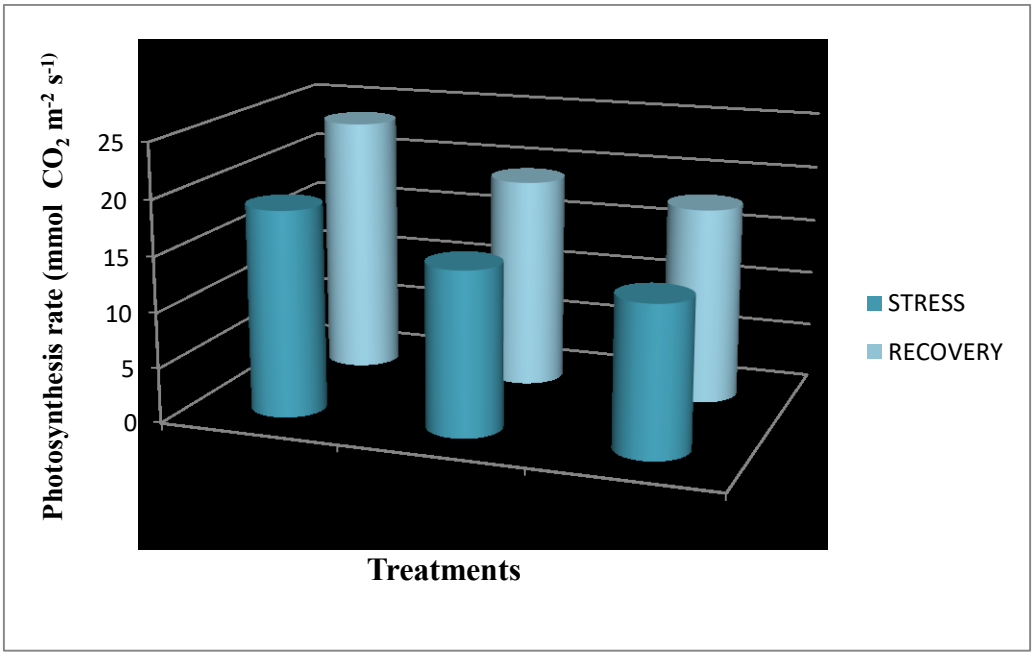


Fig 8. Effect of elevated CO_2 on photosynthesis rate (mmol CO_2 m^{-2} s^{-1}) in tomato

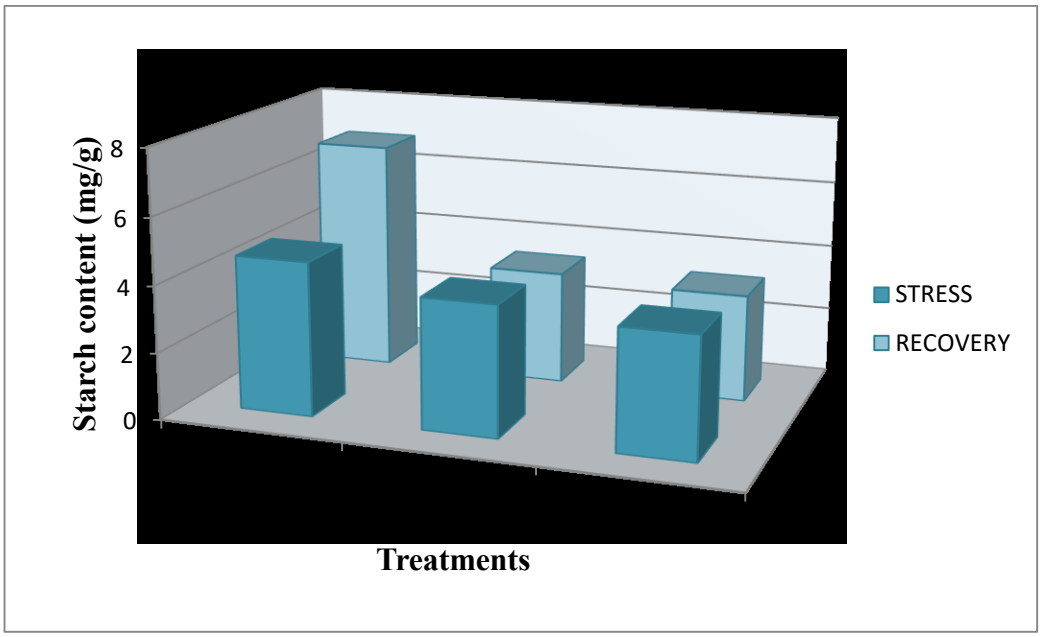


Fig 9. Effect of elevated CO_2 on starch content (mg/g) in tomato

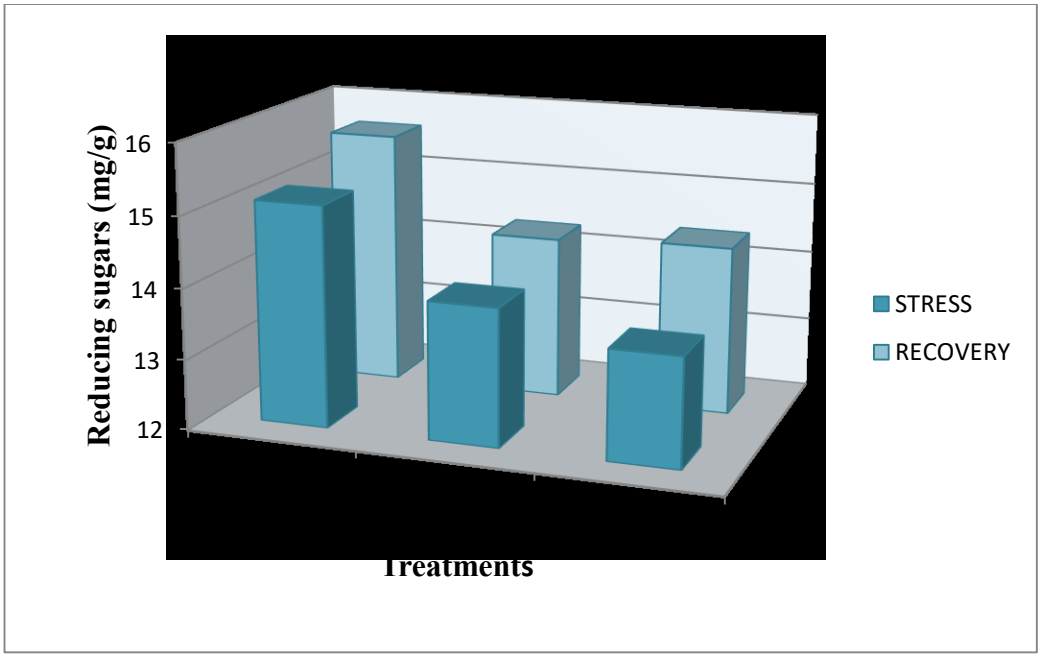


Fig 10. Effect of elevated CO_2 on reducing sugar content (mg/g) in tomato

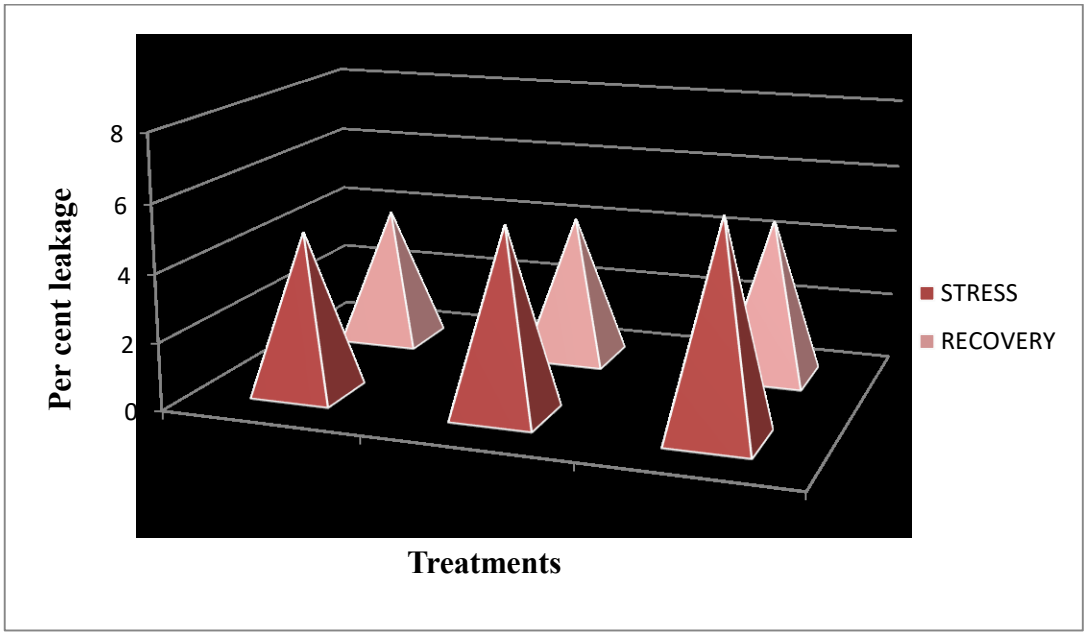


Fig 11. Effect of elevated CO_2 on per cent leakage in tomato

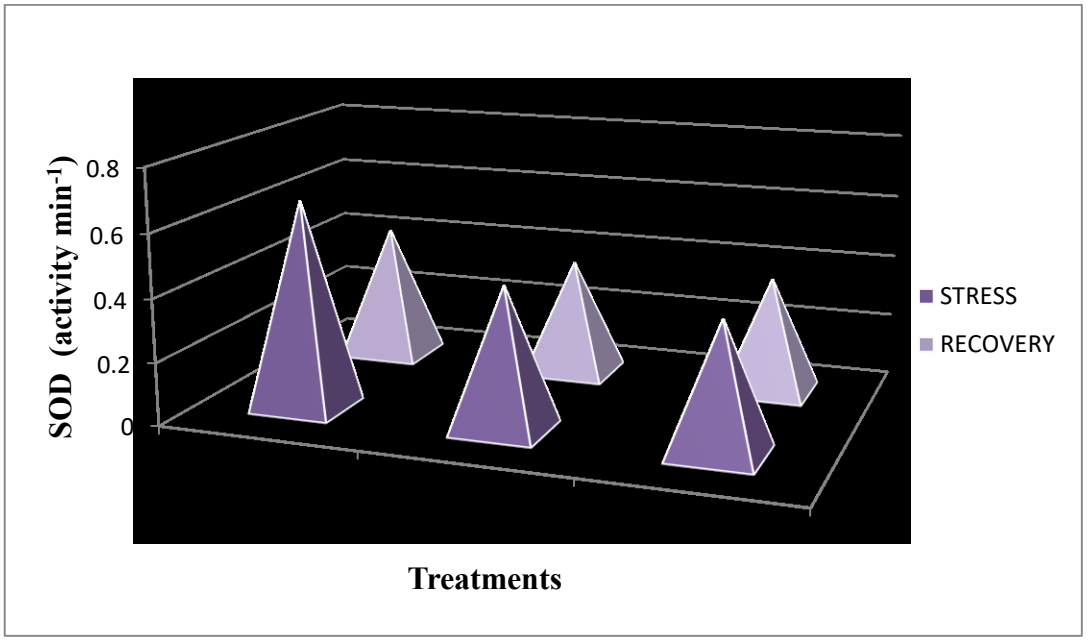


Fig 12. Effect of elevated CO_2 on SOD (activity min^{-1})

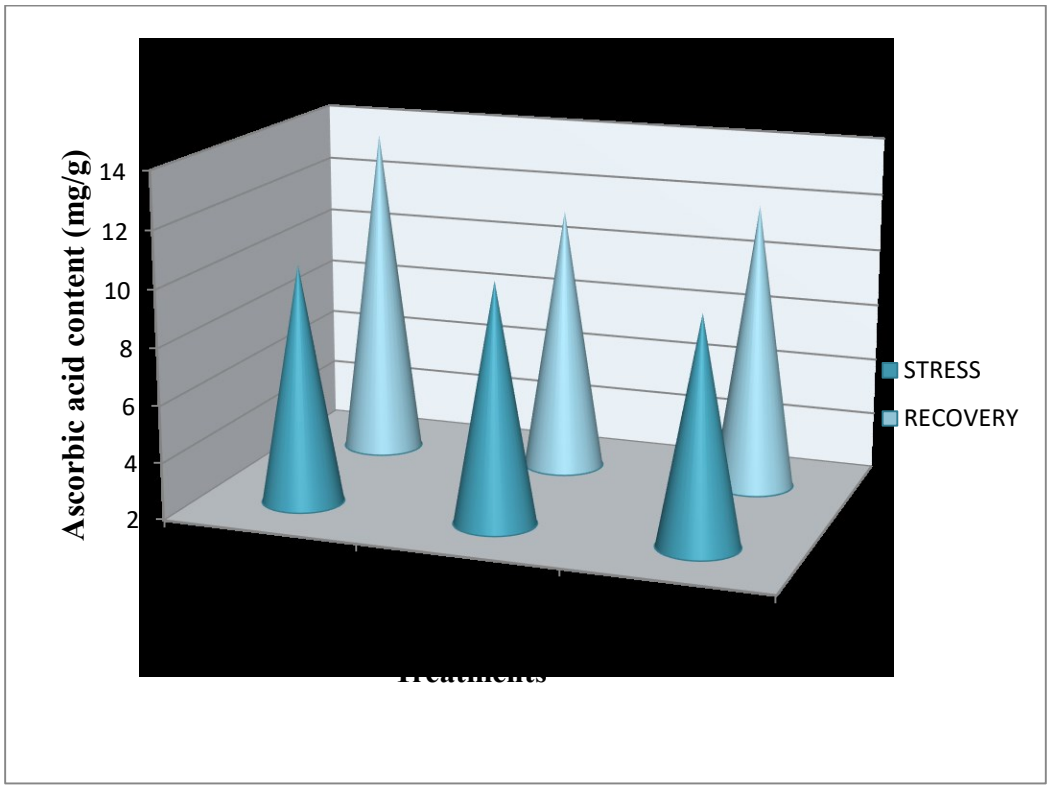


Fig 13. Effect of elevated CO_2 on ascorbic acid content (mg/g) in tomato

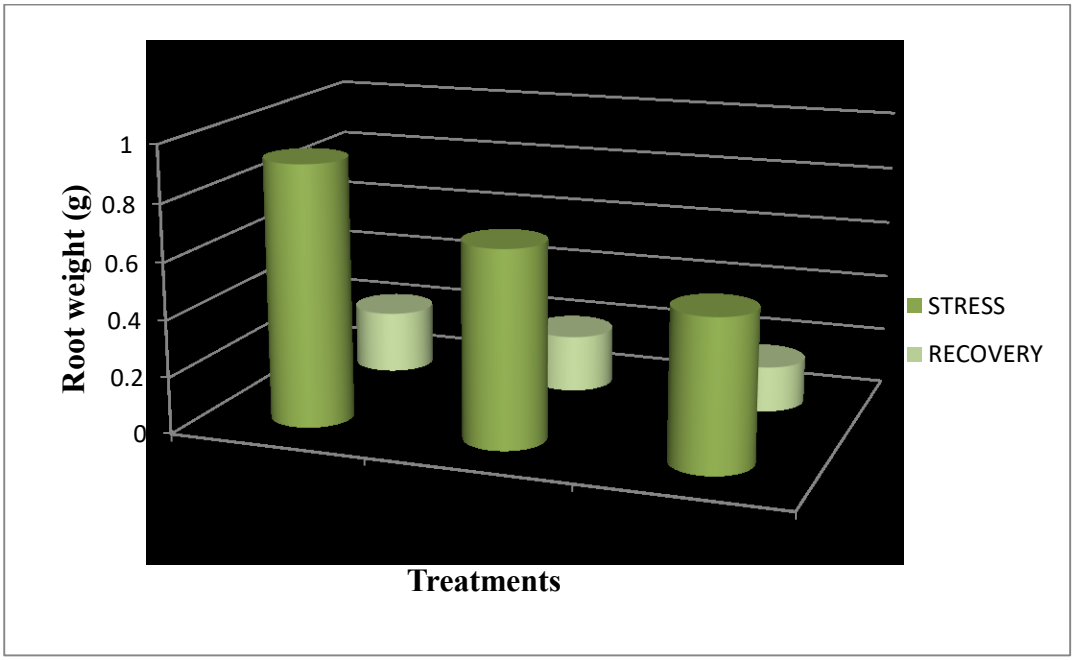


Fig 14. Effect of elevated CO_2 on root weight (g) in amaranthus

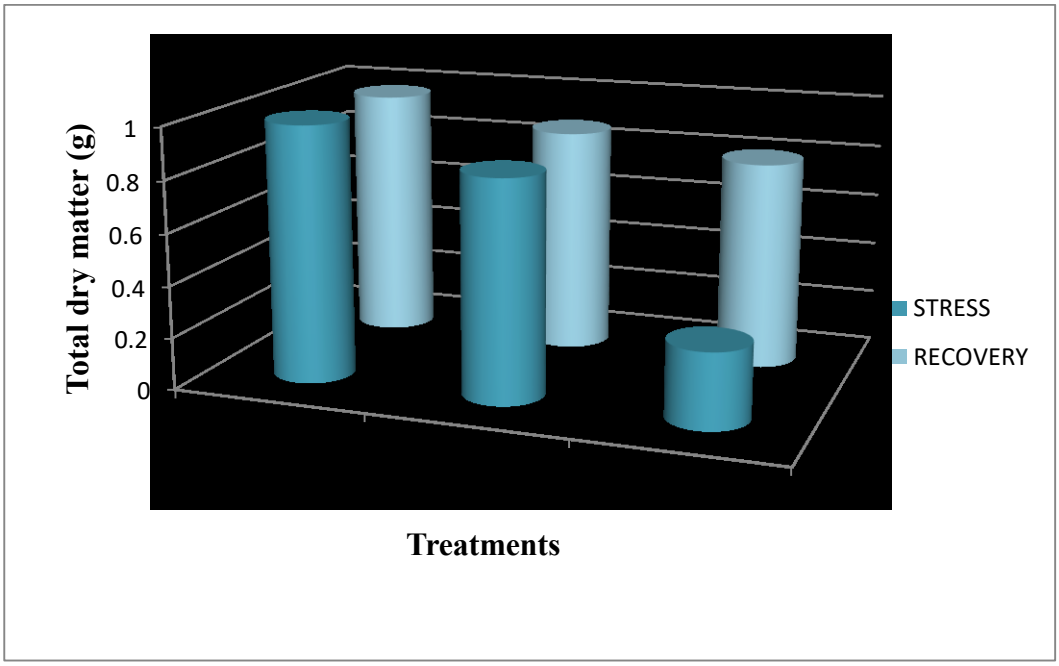


Fig 15. Effect of elevated CO_2 on total dry matter (g) in amaranthus

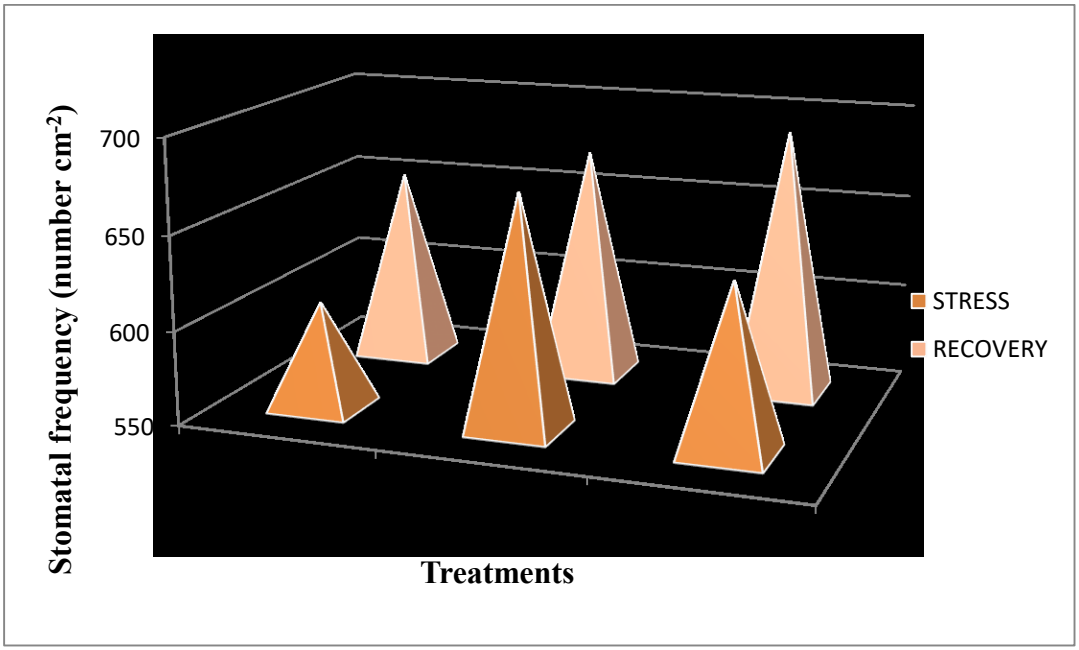


Fig 16. Effect of elevated CO_2 on stomatal frequency (number cm^{-2}) in amaranthus

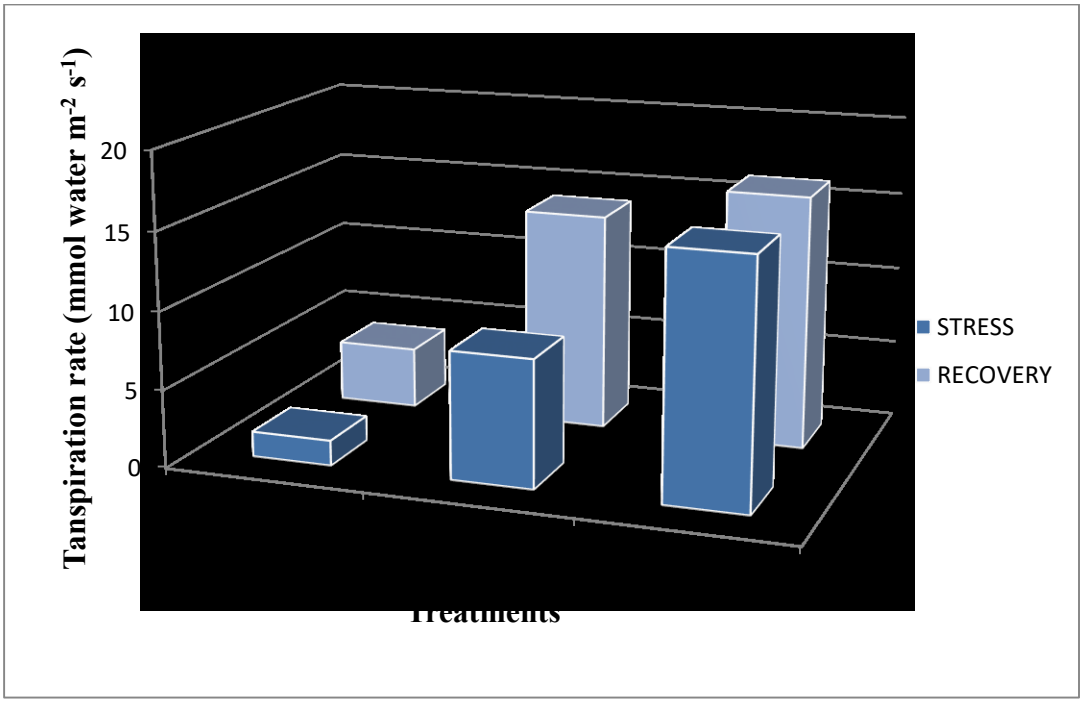


Fig 17. Effect of elevated CO_2 on transpiration rate (mmol water m^{-2} s^{-1}) in amaranthus

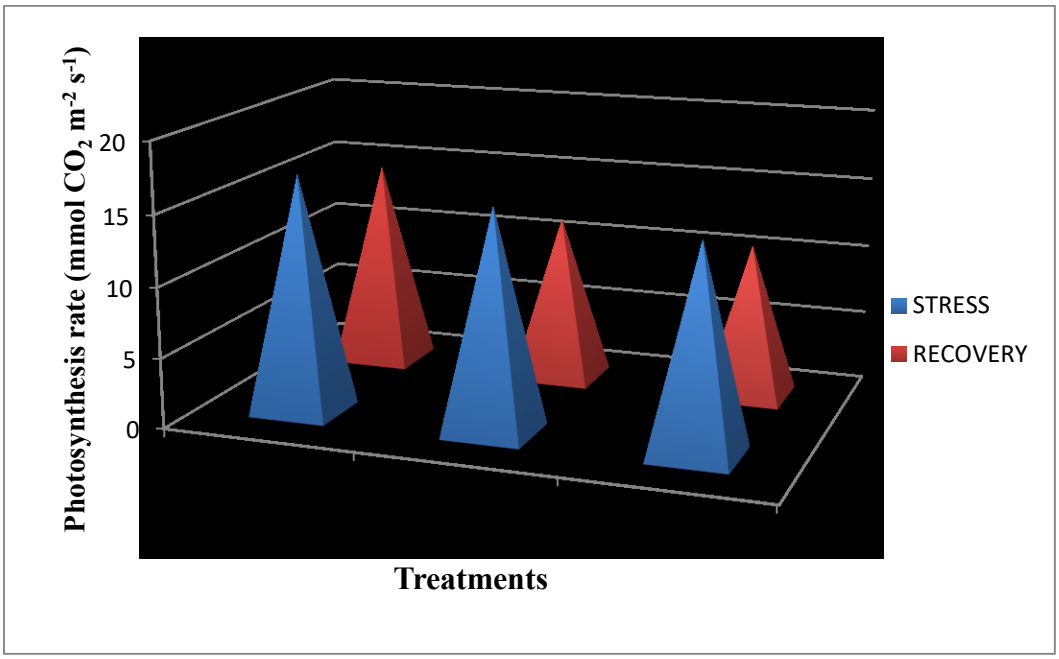


Fig 18. Effect of elevated CO_2 on photosynthesis rate (mmol CO_2 m^{-2} s^{-1}) in amaranthus

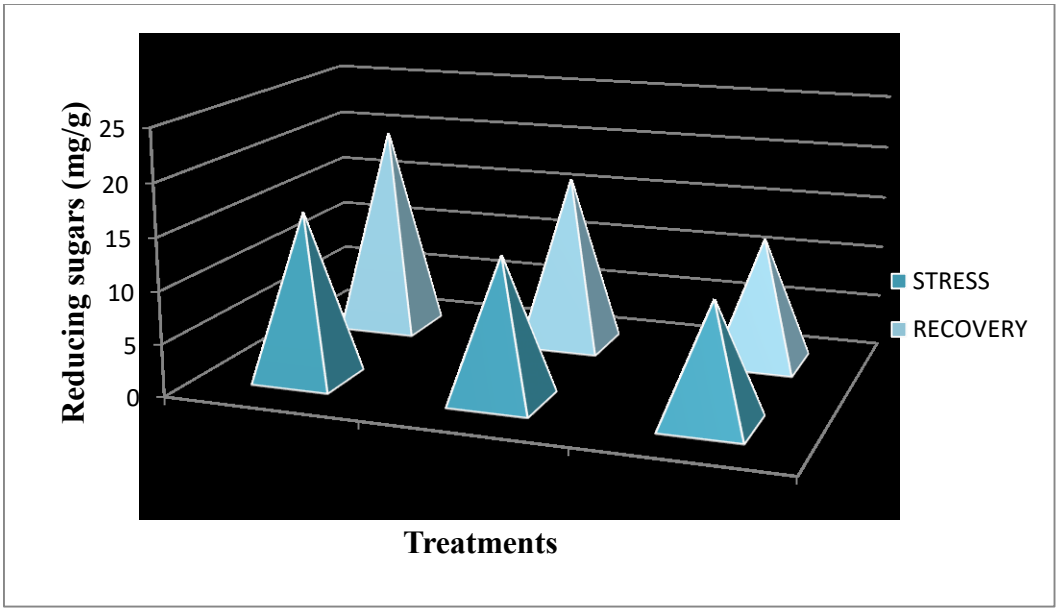


Fig 19. Effect of elevated CO_2 on reducing sugars (mg/g) in amaranthus

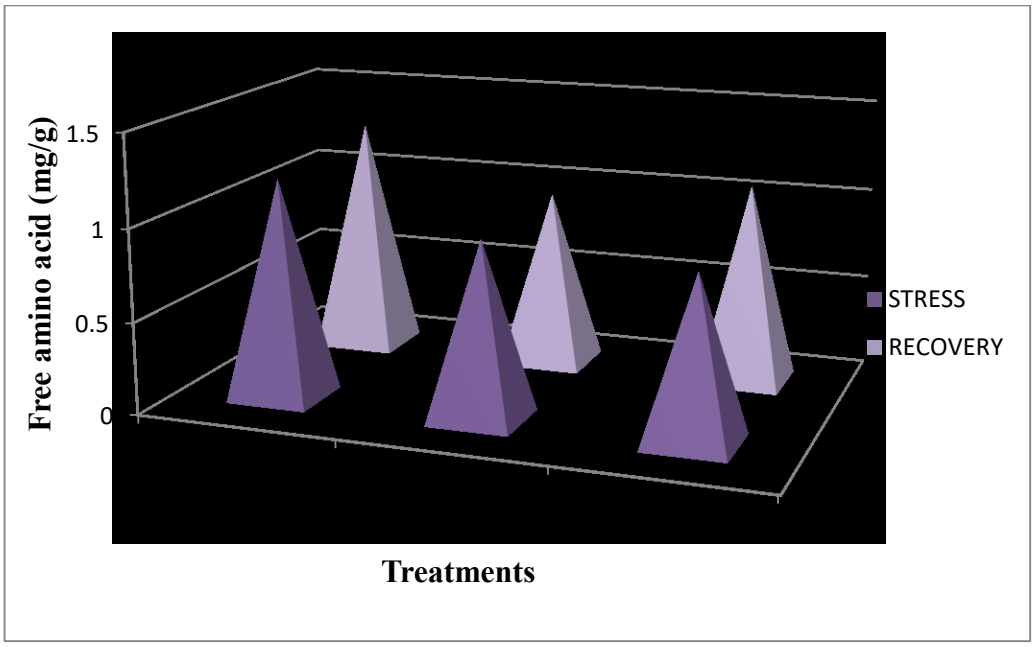


Fig 20. Effect of elevated CO_2 on free amino acid content (mg/g) in amaranthus

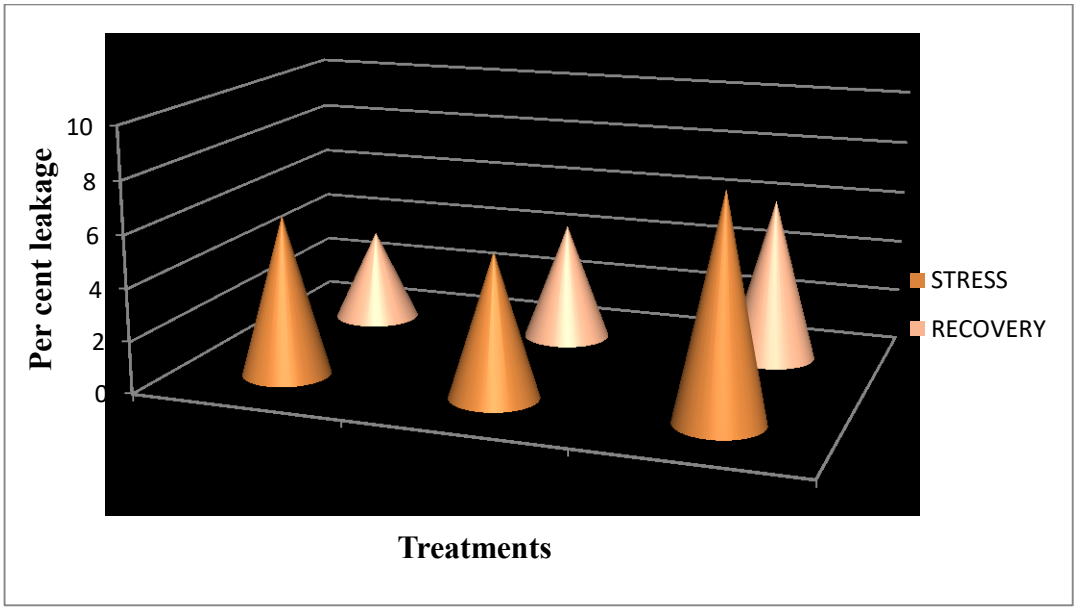


Fig 21. Effect of elevated CO_2 on per cent leakage in amaranthus

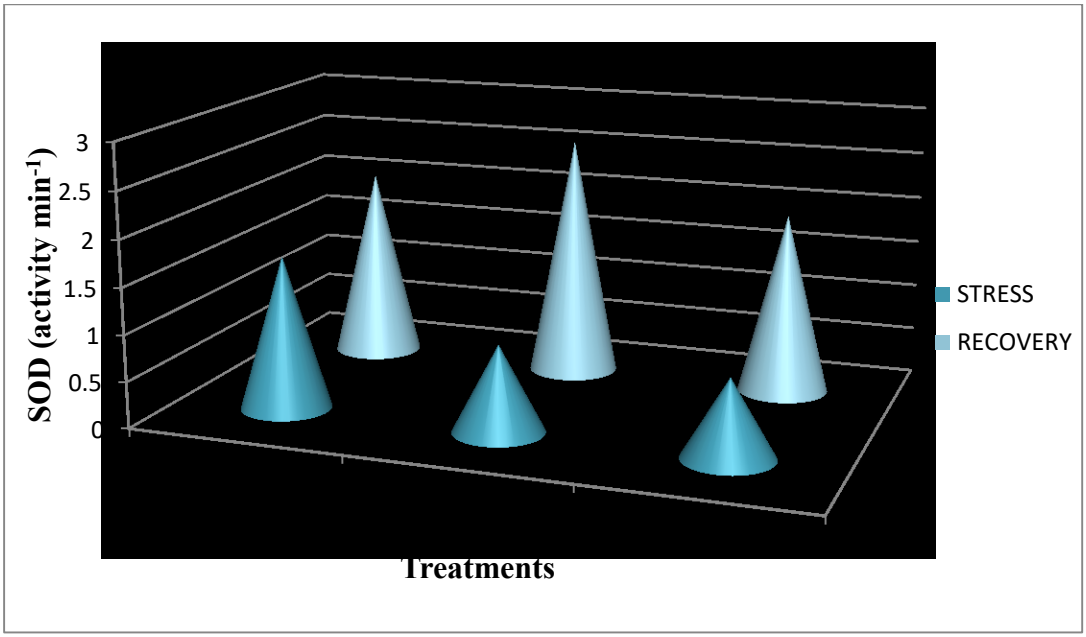


Fig 22. Effect of elevated CO_2 on SOD (activity min $^{-}$1) in amaranthus

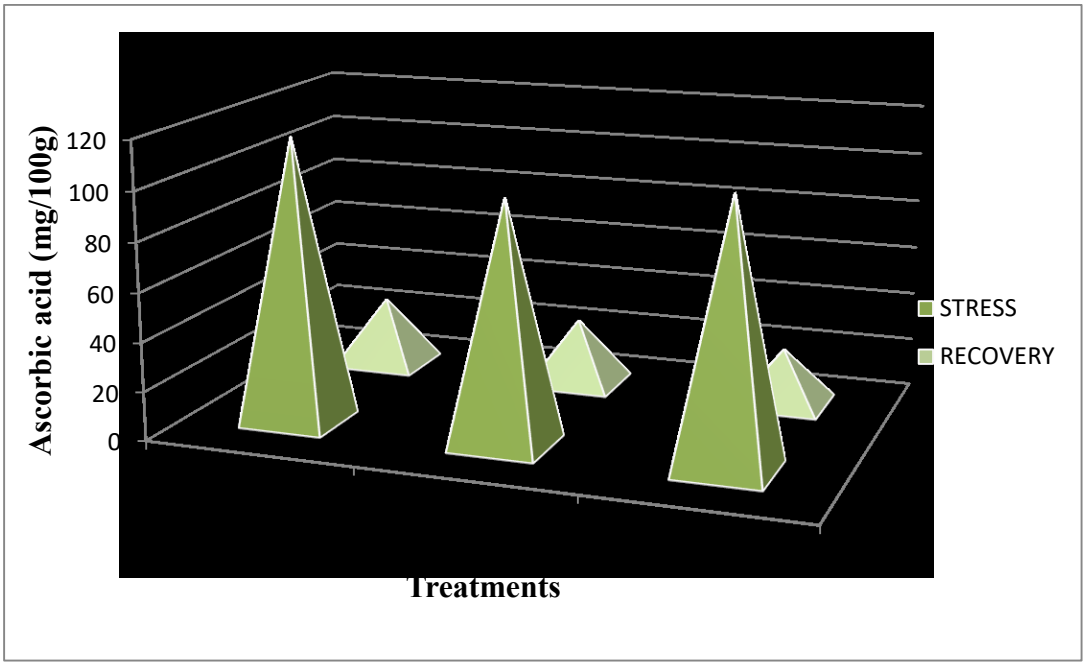


Fig 23. Effect of elevated CO_2 on ascorbic acid content (mg/100g) in amaranthus

6. SUMMARY

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. 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 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.

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. The steady increase in greenhouse gases might lead in future to higher temperatures and greater evaporative demands.

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. The results of many studies indicate that lower evaporative flux density associated with high CO_2 induced stomatal closure and results in increased net photosynthesis and better water use efficiency. Under elevated CO_2 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 and phytochemical profiles were also observed under elevated CO_2 conditions.

Tomato (*Solanum lycopersicum*) is the widely cultivated vegetable in India and 2nd most important vegetable crop next to potato. Current world production is about 100 million ton fresh fruits from 3.7 million ha. Amaranthus is the traditional leafy vegetable which has provided rural communities with food and nutritional security

over the centuries. It is a hardy, drought tolerant plant and is with a great potential for adaptation to impending climate change. The challenges extended by the changing climate situations along with the progressively reducing water availability, make studies on drought tolerance responses as modified by elevated CO_2 environments highly significant.

In this context, the current programme "Carbon dioxide enrichment induced drought tolerance responses in tomato (*Solanum lycopersicum* L.) and amaranthus (*Amaranthus tricolor* L.)" attempts to study the physiological basis of varietal responses of tomato and amaranthus to water stress conditions and to study their modifications under elevated CO_2 environments. This investigation will help to design improved production technologies with suitable varieties for a changing climatic scenario.

Two pot culture experiments were conducted with three varieties of tomato i.e, Manulakshmi, Vellayani Vijay, Anagha and three varieties of amaranthus i.e, Arun, CO -1 and Renusree. The technology used for CO_2 enrichment was Open Top Chamber (OTC) system. Two open top chambers were used, one with CO2 concentration of 600 ppm (T1) and a second control chamber with ambient CO2 level to assess the chamber effect. A set of experimental plants was maintained in the open field as control (T3). The experiments were laid out in CRD with 18 treatments and three replications. One month old potted plants of tomato and 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 observations on growth parameters after stress in tomato revealed a reduction in specific leaf area by 8% after under elevated CO_2 condition compared to absolute control. Root and shoot dry weights were also found to be higher by 34.1 %

and 19 % under elevated CO_2 resulting an increase in root shoot ratio by 5 %. Dry matter production was recorded 23.17% higher under elevated CO_2. Among the physiological and biochemical parameters studied, Highest relative water content was recorded under elevated CO_2 (80.69%). Carbon dioxide enrichment significantly lowered the stomatal frequency by 15.54 % and transpiration rates by 65.06 % . Significant increase in photosynthetic rate and total chlorophyll contents by 13.26 % and 30 % was registered under elevated CO_2 conditions, whereas no change in carotenoid content was observed. Per cent leakage was found significantly lower (23.71%) under CO_2 enriched treatment compared to control. Among physiological parameters, a marked rise in starch and phenol content was noticed by 21.59% and 33.91% respectively under elevated CO_2. Significant increase in reducing sugars, free amino acid, SOD and ascorbic acid contents by 10.44%, 43.31%, 37.87% and 47.16% was recorded in elevated CO_2. Protein content was found decreasing under elevated CO_2 by 23.22%.

Elevated CO_2 was found to have a positive impact on recovery responses also. Root shoot ratio and free amino acid content was found lower by 39 % and 5.39 % after re-watering. Among the three different varieties of tomato, Vellayani Vijay was found to be the best performing variety under elevated carbon dioxide treatment with highest root weight (1.55 g), shoot weight (4.56 g), total dry matter production (6.78 g), total chlorophyll (30 mg/g), photosynthesis rate (18.69 mmol CO_2 m^{-2} s^{-1}) RWC (80.79%). Transpiration rate (8.13 mmol water m^{-2} s^{-1}), stomatal frequency (512.91 number cm^{-2}) and per cent leakage (5.24%) were recorded lowest for the variety Vellayani Vijay among all the varieties. Among the biochemical parameters, highest protein (21.37 mg/g), free amino acid (1.36 mg/g), SOD (0.59 activity $minute^{-1}$) and ascorbic acid content (10.80 mg/g) was registered for the variety Vellayani Vijay compared to Manulakshmi and Anagha.

In the case of amaranthus, after imposing water stress, SLA (193.36 cm^2 g^{-1}) was found to be highest under elevated CO_2. Root weight, shoot weight and total dry

matter production were found enhanced by 42.39%, 27.27% and 35.31% under elevated CO_2 in comparison with control. Lower stomatal frequency (606.63 number cm^{-2}) , transpiration rate (1.61 mmol water m^{-2} s^{-1}) and per cent leakage (6.12%) were observed prominently under elevated CO_2 compared to open control. Photosynthetic rate (16.89 mmol CO_2 m^{-2} s^{-1}) was recorded significantly higher under elevated CO_2. Significant increment in reducing sugars by 28.6 %, phenol by 94.14 %, free amino acid content by 25.21% was recorded under elevated CO_2. SOD and ascorbic acid content was found increased by 49.39 % and 8.05 % under elevated CO_2 treatment compared with control. In the case of recovery responses also, elevated CO_2 was found to have positive influence on growth, physiological and biochemical parameters.

Variety CO-1 of amaranthus was identified as best performing variety under elevated CO_2 treatment with highest root weight (0.83 g), shoot weight (7.69 g), total dry matter (0.62 g), RWC (91.37 %), total chlorophyll content (1.02 mg/g) and photosynthetic rate (16.62 mmol CO_2 m^{-2} s^{-1}). Lowest stomatal frequency (551.85 number cm^{-2}) and transpiration rate (1.61 mmol water m^{-2} s^{-1}) were also recorded for the variety CO-1. Among the biochemical parameters, highest free amino acid (1.13 mg/g), phenol (25.46 mg/g), SOD (1.33 activity $minute^{-1}$) and ascorbic acid content (134.72 mg/g) was recorded for the variety CO-1.

CONCLUSION

The present investigation was carried out with the objective to study the physiological basis of varietal responses of tomato and amaranthus to water stress conditions and to study their modifications under elevated CO_2 environments. Considering all the physiological, biochemical and molecular 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 tomato and amaranthus. It was achieved mainly due to better photosynthetic rate and activation of defense

mechanisms. High total dry matter content in tomato for the variety Vellayani Vijay and 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 maintaining high root weight which helps in efficient water absorption, maintaining lower stomatal frequency and transpiration rate which helps in efficient water saving and accumulation of more antioxidants like SOD , phenol and 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.

FUTURE LINE OF WORK

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 envisaged 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.

The present investigation has shown the existence of varietal variations in water stress tolerance levels of both tomato and amaranths and the defense mechanisms are proved mainly to be the greater accumulation and activation of anti-oxidants. There should be further investigations into the genetic variations in species and varietal sensitivity towards elevated CO_2-stress interactions. These studies can be extended to field level using suitable technologies like FACE.

For developing tolerant varieties, discerning the underlying mechanisms is highly essential. Analyzing oxidative stress markers like membrane leakage, protein oxidation, lipid peroxidation etc: and focusing on individual molecules from various anti-oxidant defense pathways can be undertaken towards this direction.

Efforts should also be taken towards developing technologies for exploiting the positive impacts of elevated CO_2 environment on crop growth and development.

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8. ABSTRACT

The study entitled "Carbon dioxide enrichment induced drought tolerance responses in tomato (*Solanum lycopersicum* L.) and amaranthus (*Amaranthus tricolor* L.)" was undertaken with the objective to study the physiological basis of varietal responses of tomato and amaranthus to water stress conditions and to study their modifications under elevated CO_2 environment. The experiments were conducted from August, 2015 to September, 2015 on tomato and from February, 2016 to March, 2016 on amaranthus. Two pot culture experiments were conducted with three varieties of tomato i.e, Manulakshmi, Vellayani Vijay, Anagha and three varieties of amaranthus i.e, Arun, CO -1 and Renusree. The technology used for CO_2 enrichment was Open Top Chamber (OTC) system established under Department of Plant Physiology, college of Agriculture, Vellayani.

Carbon dioxide was released from CO_2 cylinders to one of the two OTC s bringing the CO_2 level to 600 ppm and the second OTC worked as control at ambient CO_2 for chamber effect. The experiments were laid out in CRD with 18 treatments and three replications. One month old potted plants of tomato and 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.

In tomato, higher values were recorded for total dry matter production (5.74 g), shoot weight (4.42 g), root weight (1.32 g), root shoot ratio (0.40), relative water content (RWC) (80.69 %), membrane integrity (4.76% loss), superoxide dismutase (SOD) (0.66 activity g^{-1} min^{-1}), phenol (2.86 mg/g), total chlorophyll content (1.00 mg/g), reducing sugar (15.13 mg/g), starch (4.63 mg/g), and photosynthetic rate

(18.69 µmol $CO_2/m^2/s$) under elevated CO_2 compared to control after stress. Parameters like stomatal frequency, transpiration rate and protein content recorded lower values (555.85 number/cm^2 , 8.13 mmol water/m^2/s, 14.41 mg/g respectively) under CO_2 enriched treatments. Lower stable isotopic discrimination was observed under elevated CO_2 compared to open control. Elevated CO_2 was found to have a positive impact on recovery responses also. Mealy bugs, scales and serpentine leaf miner infestations were found to be more in open control compared to elevated CO_2 treatment. Protein profiling revealed that elevated CO_2 induced the production of formation of a few new proteins of molecular weight nearly 42 K Da to 50 K Da were observed under water stress for tomato varieties Anagha, Vellayani Vijay and Manulakshmi which can be stress proteins imparting tolerance and no changes were observed in expression levels of RuBISCO small or large sub units expression Among the three varieties of tomato, Vellayani Vijay was found to be the best responding variety to elevated CO_2.

In the case of amaranthus, significantly higher values were recorded for root weight (0.92 g), shoot weight (6.88 g), total dry matter production (5.74 g), SOD (1.65 activity g^{-1} min^{-1}) and reducing sugars (15.13 mg/g). Parameters like leaf number, free amino acid, chlorophyll, ascorbic acid and membrane integrity showed an increasing trend though not significant. Stomatal frequency and transpiration rate were lower under elevated CO_2. Even in the case of amaranthus, elevated CO_2 was found to have positive impact on recovery responses. Leaf webber and mite incidences were more in elevated CO_2 treatment compared to control. Among the three varieties of amaranthus, CO-1 maintained highest root weight, shoot weight and dry matter production compared to Arun and Renusree. Protein profile and RuBISCO sub unit expressions were not modified by the experimental treatments.

In the present study, CO_2 enrichment was revealed to have a role in improving the stress tolerance and recovery responses in the case of tomato and amaranthus. Considering all the physiological and biochemical studies carried out in the case of

tomato and amaranthus, the better stress tolerance under elevated CO_2 was found to be achieved mainly through better photosynthetic rate and activation of defense mechanisms, especially activation of antioxidants. The study also demonstrated the varietal variation existing in CO_2 enrichment induced drought tolerance responses in tomato and amaranthus which will help in the selection of suitable varieties for a changing climatic scenario.