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Physiological and Molecular Basis of Drought Tolerance Responses in Tomato (*Solanum lycopersicum* L.) under Carbon Dioxide Enrichment

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A B S T R A C T

The sensitivity of photosynthesis to each of the environmental variables including low water availability, high temperature, 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. The level of CO_2 in the atmosphere is rising at an unprecedented rate. Under elevated CO_2 conditions, it has also been found that plants maintain higher total water potentials to increase biomass production and to be generally more drought tolerant. Physiological and molecular basis of varietal responses of tomato to water stress conditions and their modifications under elevated CO_2 environment was studied by conducting a pot culture experiment with three varieties of tomato i.e., Manulakshmi, Vellayani Vijay and Anagha in Open Top Chambers (OTC) system. Water stress conditions were imposed on one month old potted plants during their critical stages of development and then were allowed to recover. Various biochemical and growth parameters like free amino acids, reducing sugar content, total dry matter content and antioxidants like ascorbic acid content were analysed to understand the effect of CO_2 enrichment on drought tolerance. Electrophoresis analysis of proteins was performed using SDS PAGE. Elevated CO_2 was found to increase total dry matter production (5.74 g), reducing sugars (15.13 mg/g), free amino acids (1.57 mg/g) and ascorbic acid (10.31 mg/100g) content. Global warming and the demands of an increasing world population will increase water scarcity, which results in increased demand for water use efficient and drought tolerant crop plants. It has become imperative to elucidate the environmental stresses. 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 unfavourable conditions.

Introduction

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. 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. 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, 2012).

The sensitivity of photosynthesis to each of the environmental variables including low water availability, high temperature, 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. 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. 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.

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 18-25^{o}C. 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.

Considering the role of elevated CO_2 (EC) in the drought tolerance responses, the present investigation will help to understand the growth performance, productivity and water stress tolerance capacities of tomato under enriched CO_2 conditions. The results of this study will also help to design improved production technologies with suitable varieties for a changing climatic scenario.

Materials and Methods

To understand the Physiological and molecular basis of drought tolerance responses in tomato (*Solanum lycopersicum* L.) under elevated Carbon dioxide conditions three varieties of tomato i.e., Manulakshmi, Vellayani Vijay, Anagha at the Department of Plant Physiology, College of Agriculture, Vellayani, under Kerala Agricultural University. Technology used for enriching CO_2 was Open Top Chamber. Two Open Top Chambers, one with CO_2 level of 600 ppm (T1) for assessing elevated CO_2 effect and a second control chamber for assessing chamber effect (T2) were used. The basic structure of OTC was built of metal frame and covered with a 200 micron UV poly sheet. The chamber has 1m^2 opening at the top to reduce the temperature build up A set of experimental plants was maintained in the open field as control (T3). One month old potted plants of amaranthus were shifted to the CO_2 treatment conditions. Plants were maintained under well irrigated conditions for one week. Water stress conditions were imposed by withdrawing irrigation for two days after shifting and stress observations were taken. Thereafter plants were re-watered and on the 5th day of re-watering, recovery observations were taken. CO_2 was released into the chamber from a

CO_2 cylinder in a controlled manner. Microclimatic parameters (temperature, humidity and light) were measured within and outside the OTCs with the help of sensors on a real time basis. The experiment was laid out in CRD with three treatments three replications and two stress levels. The sum of root and shoot dry weights were taken as the total dry matter yield. The estimation of reducing sugars in plants was done following Dinirto Salicylic acid (DNS) method (Somogyi, 1952) and the total free amino acids were estimated by the Ninhydrin method (Moore and Stein, 1948). Both of these parameters were expressed in terms of mg/g. The ascorbic acid content in plants was estimated volumetrically by the method explained by Sadasivam and Manickam (2008) and expressed in terms of mg/100g. SDS - PAGE Electrophoresis separation of soluble protein and Rubisco in tomato leaves were carried out Laemelli (1970). The results based on statistically analysed data pertaining to the experiment conducted during the course of investigation are presented below (Table 1 and 2; Fig. 1–3).

Results and Discussion

Dry matter production

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, 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).

Reducing sugars

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).

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. After recovery, 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 variety Vellayani Vijay (6.03 mg/g) and Manulakshmi (4.51 mg/g).

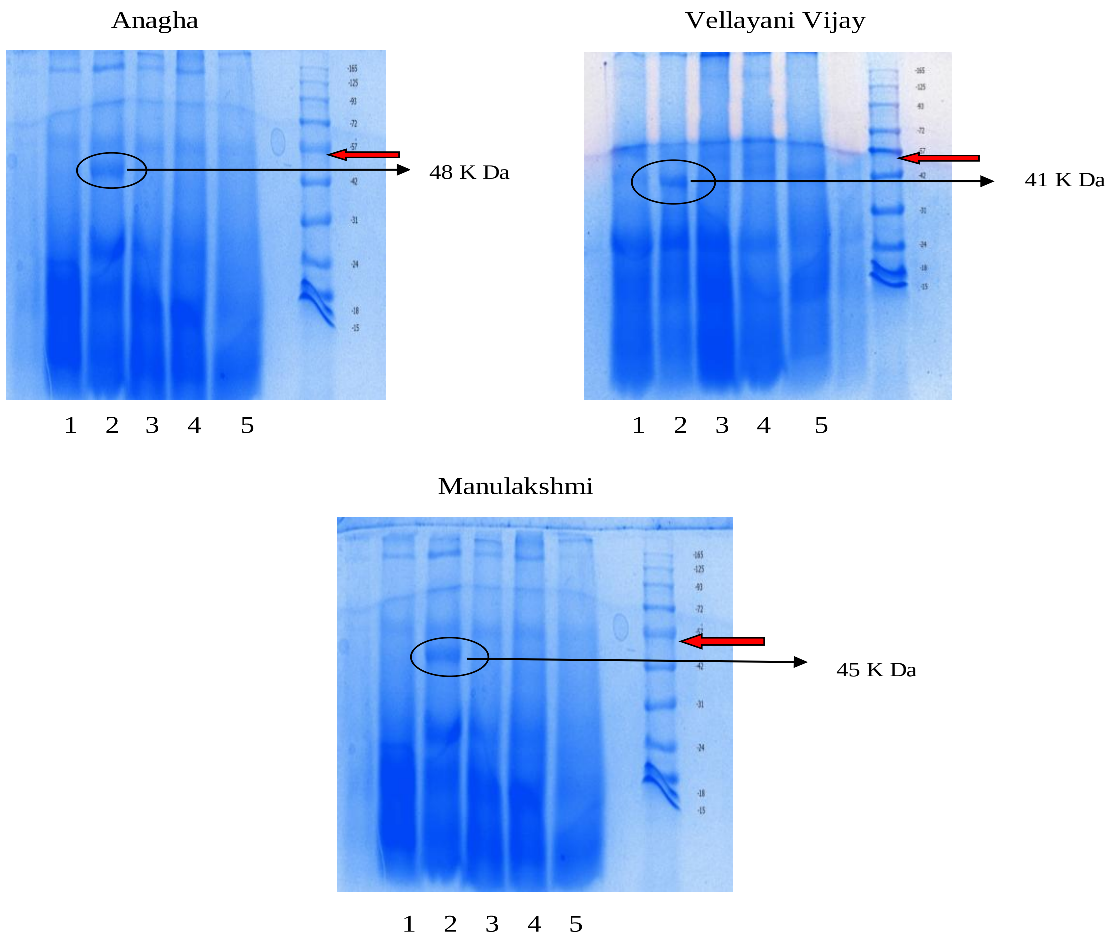


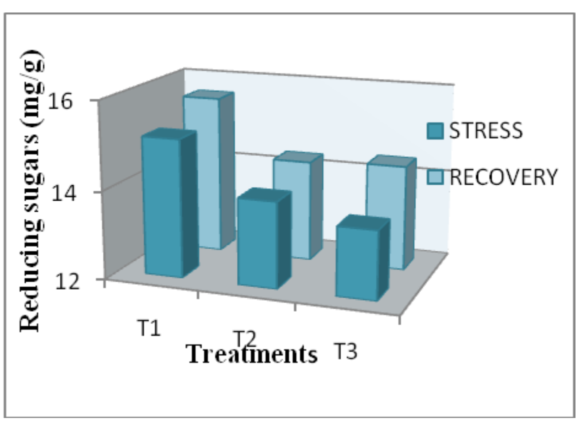
Figure 1. Protein profiling in 3 varieties of tomato

1. EC, Unstressed
2. EC, Stressed
3. EC, Unstressed
4. EC, Recovered
5. Open control

RuBISCO (56 KDa) ⟵

Fig.2 Effect of elevated CO_2 on reducing sugars (mg/g) after stress and re watering in tomato.

Fig.3 Effect of elevated CO_2 on ascorbic acid content (mg/100g) after stress and Re watering in tomato



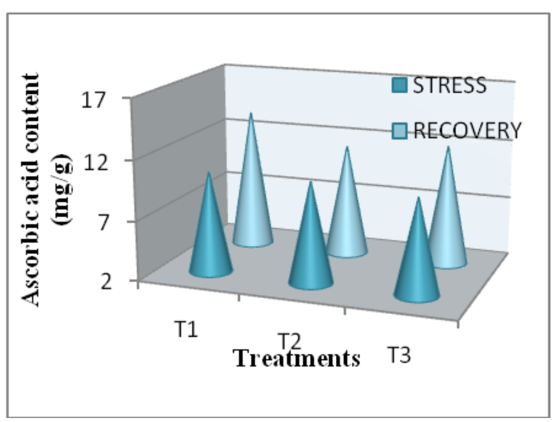


Table.1 Effect of elevated CO_2 on various biochemical and growth parameters after stress and re-watering in tomato

Parameter	Elevated CO_2(T1)		Ambient CO_2(T2)		Open control(T3)		CD(0.05)	
	Stress	Recovery	Stress	Recovery	Stress	Recovery	Stress	Recovery
Dry matter production (g)	5.74	5.40	5.94	4.56	4.41	3.16	1.85	1.63
Reducing sugars content (mg/g)	15.13	15.62	13.95	14.30	13.55	14.38	0.91	0.80
Free amino acid content (mg/g)	1.57	5.61	1.14	6.32	0.89	5.93	0.36	0.55
Ascorbic acid content (mg/100g)	10.39	13.65	10.39	11.34	9.90	12.03	1.04	2.36

Table.2 Varietal variation observed with elevated CO_2 biochemical and growth parameters after stress and re-watering in tomato

Parameter	Arun (V1)		CO-1(V2)		Renusree (V3)		CD(0.05)	
	Stress	Recovery	Stress	Recovery	Stress	Recovery	Stress	Recovery
Dry matter production (g)	4.71	3.72	6.78	5.19	4.60	4.21	1.85	1.63
Reducing sugars content (mg/g)	14.21	14.51	14.16	14.66	14.26	15.13	0.91	0.80
Free amino acid content (mg/g)	1.13	4.51	1.36	6.03	1.11	7.33	0.36	0.55
Ascorbic acid content (mg/100g)	9.64	13.88	10.80	12.26	10.24	10.88	1.04	2.36

Ascorbic acid

Elevated CO_2 was shown to have positive influence on ascorbic acid content after stress. 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).

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

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). In the present investigation, a significant rise in reducing sugars 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. 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), 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). Li *et al.*, 2013 reported that, elevated CO_2 (800 µmol mol^{-1} CO_2 increased carbohydrates accumulation in tomato plants.

CO_2 enrichment can increase the free amino acids. As the metabolism of carbohydrates is essential for the synthesis of amino acids, CO_2 enrichment effects on free amino acids can be similar to carbohydrates (Sicher, 2008). 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. Increase in soluble amino acid content under CO_2 enrichment has been reported in soybean (Ainsworth *et al.,* 2007), tobacco (Geiger *et al.,* 1998).

With CO_2 enrichment, ascorbic acid content was found enhancing by 47.16% and 11.86% in tomato after stress and recovery respectively. Oxidative stress does 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. 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).

Under elevated CO_2, there can be imbalance in the supply and demand of carbohydrates resulting in their increased accumulation in the leaves. Carbohydrate accumulation in the leaves has been shown to down regulate the expression of photosynthetic genes in higher plants under elevated CO_2 (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.

The present investigation was carried out with the objective to study the biochemical and molecular basis of varietal responses of tomato to water stress conditions and to study their modifications under elevated CO_2 environments. Considering the 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. Elevated CO_2 has improved total dry matter, reducing sugars, free amino acid and ascorbic acid content when compared to open control in all the three varieties. High total dry matter content in tomato for the variety Vellayani Vijay 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 and accumulation of more antioxidants like ascorbic acid which helps to fight against oxidative stress induced by drought. Varietal variation was found existing in Carbon dioxide enrichment induced drought tolerance responses which gives better scope for the selection of suitable varieties for a changing climatic scenario.

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