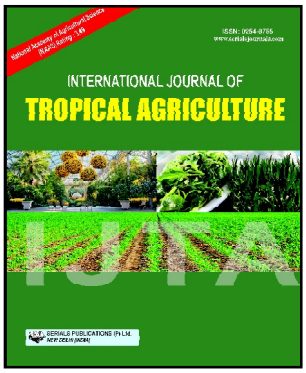

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Morphological, Physiological and Biochemical Responses of Two *Capsicum* Species Under Drought Stress

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Abstract: Drought stress is the most adverse crop environmental stress, accounting for 70% of potential agriculture yield losses worldwide. The present study attempted to characterize the drought stress responses in two species of *Capsicum*, viz, *C. annum and C. frutescens* at morphological, physiological and biochemical level. Plant height, number of nodes, length of internodes and length of leaves showed a significant reduction in drought stressed plants when compared to control plants. Estimation of photosynthetic pigments and carotenoids showed a significant reduction under drought stress. Total protein in plants significantly reduced under drought stress. However, total phenolics and proline were significantly increased under stress. Estimation of enzyme activity showed significant increase under drought stress.

Keywords: C. annum, C. frutescens, Drought Stress

INTRODUCTION

Modern agriculture is affected by a number of adverse environmental factors such as drought, extremes of temperature, light and salinity. In the natural environment, plants are well adapted to minimize damages which occur under these extreme conditions. The term 'stress' used by physiologists sometimes refers to the external environment factors capable of inducing a potentially injurious effect itself. Based on biological nature, stresses are of two types, biotic and abiotic. The biotic stress is due to pests, insects, pathogen, weeds, etc. The abiotic stress is mainly caused due to moisture, temperature, mineral toxicity, salinity, soil physiology, air pollution etc.

Different aspects of plant growth are affected by water stress. Low leaf water potential is known to affect photosynthesis, regulation of stomata,

respiration, cell expansion, cell wall synthesis and translocation. These changes result in reduction of growth and carbohydrate partitioning. When the growth of a major sink is sensitive to water stress, dry matter is preferentially to other parts which are strong sinks [1] or tolerate a greater level of stress. The most common change in the partitioning of assimilate is the increase in roots fraction of the total biomass. According to Forney and Breen [2] water stress limited the growth of stem and leaves more than roots while reducing the total dry matter production by the plant. On the other hand, water stress induces Ca deficiency in bulky storage organs such as fruits resulting in blossom end rot in tomatoes and capsicum as the transport of mineral is dependent on the rate of transpiration.

The changes in the water level alter the chemical composition of the plant. Water deficit causes surprisingly rapid changes in cell microstructure primarily in membrane ultra structures and causes disintegration of polysomes [3]. It is supposed that the loss of mRNA synthesis in one of the first results of water stress leading to a decrease of amino acid incorporation after disintegration of polysomes.

Capsicum is an important cash crop in India belonging to the family Solanaceae and is grown for its pungent fruits, which are used, both green and ripe to impact pungency to the food [4]. Chilies are indigenous to the American tropics and subtropics and the West Indies. *Capsicum* plants are herbaceous or semi- woody annuals or perennials. The crop is grown from almost the Sea level up to an attitude 1500 meters with an annual rainfall of 60-150cm. India is the world's largest exporter of chilies.

As a continent it has become an indispensable item in every Indian home. It is used in the treatment of diseases like dyspepsia, yellow fever and snake bite. It is also used in the preparation of chutneys and pickles. The ground ripe fruits constitute 'the red pepper of commerce' [5]. Pepper sauce is made by extracting the pulp by pressure and pickling in strong vinegar. The pungency or spicy taste of 'Capsaicin' ($C_{18}H_{27}NO_3$) contained in the skin and septa of the fruit. Chiles are a good source of Vitamin C.

Pepper (*Capsicum annum* L.) plants are sensitive to drought stress [6]. Water stress has been shown to adversely affect physiological and nutritional development and fruit yield of bell pepper [7]. Pepper performs well with adequate supplies of water during its growth cycle [8]. Very little research has been undertaken in the past on the growth and responses of *Capsicum* to water and osmotic stresses. Hence the present study has been designed with an objective to characterize the drought stress responses in two species of *Capsicum,* viz., C. *annum and C. frutescens* at morphological, physiological and biochemical level.

MATERIALS AND METHODS

The plant species selected for the present investigation were *Capsicum annum* and *Capsicum frutescence*. The seeds of *Capsicum annum* and *Capsicum frutescence* were collected from Kerala Agricultural University, Vellayani. The seeds of two varieties were germinated and grown in plastic bags.

After a growth period of one month the plants were kept in water stressed condition. One set of two plants was grown in well watered condition and was taken as control.

Morphological Characterization

Morphological characterization of treated and control were made immediately after the treatment period. Plant height, number of nodes, length of internodes, and length of leaves were measured as an indicator of plant growth and development.

Physiological characterization

Physiological traits were selected on the basis of plant water relations, which include leaf relative water content (RWC), Excised Leaf Water Retention

(ELWR), Initial Water Content (IWC), Relative Dry Weight (RDW) and Rate of Water Loss (RWL).

RWC, IWC and ELWR were measured using 3mm leaf disks from the stressed plants. For RWC, the leaf disks were weighed (Fresh Weight=FW) and then placed in distilled water for 4 hours and re-weighed to obtain Turgor Weight (TW). The turgid leaf pieces were oven dried, and obtained the Dry Weight (DW) after 24 hours of oven drying at 60^0C. RWC was calculated using the formula proposed by Ritchie et al. (1990):%RWC= (FW-DW)/ (TW-DW) x 100. IWC was calculated by measuring the fresh weight of leaf disks and dry weight after 24 hours of oven drying at 60^0C using the formula: IWC= (FW-DW) /FW x 100. For measuring ELWR, the leaf disks were weighed, and then kept at 30^0C for 4 hours and reweighed to obtain wilted weight. ELWR was calculated using the formula, ELWR= [1- (FW-WW4h/FW)] x 100, where WW4h is the wilted weight after 4 hours. Relative dry weight was measured using the formula,

RDW = DW / (TW-DW), where DW is the oven dried weight and TW is the turgid weight.

Rate of water loss (RWL) was calculated using the formula,

RWL=(FW-Ww) / DW, where FW is the fresh weight, DW is the dry weight and Ww is the wilted weight after a wilting of 5 hours.

Biochemical characterization

Selected biochemical parameters were used as a measure of salt stress responses in tomato. Amount of chlorophyll, carotenoids, total phenolics, proline and total protein were estimated from fresh leaf tissues harvested from the seedlings after the period of stress treatment.

Estimation of Chlorophyll

Chlorophyll pigments were estimated spectrophotometrically using the method of Arnon (1949).

Procedure

1g leaf sample was weighed out and washed. Leaf were sliced into small pieces and ground in a clean mortar and pestle. 80% of chilled acetone (10ml) was used as the grinding medium and filtered. The filtrate was centrifuged at 5000rpm for 5 minutes, the resultant supernatant was collected and made up to 10ml. 1ml volume of the supernatant was measured and taken which in turn was made up to 5ml using 80% acetone. The solution was read spectrophotometrically in the following wavelengths:490nm, 645nm, 663nm against 5ml of 80% acetone taken as blank.

Calculation

Amount of chlorophyll a , mg/g tissue = 12.7(A 663) – 2.69(A 645) x V/1000 x W

Amount of chlorophyll b , mg/g tissue = 22.9(A 645) – 4.68(A 663) x V/1000 x W

Amount of total chlorophyll, mg/g tissue

= 20.2(A 645) + 8.02(A 663) x V/1000 x W

Where, A – Absorbance at specific wave length

V – Final volume of chlorophyll extract in 80% acetone

W – Fresh weight of tissue extracted

Estimation of carotenoids

Total carotenoid content was also determined in the same chloroplast pigment extracted by 80% acetone, measuring the absorbance at 510nm and 480nm. The amount of total carotenoid was calculated according to the equation given by Lichtenthaler (1987) and expressed as milligram of carotenoid per gram of plant tissue according to the formula;

Amount of carotenoid, mg/g tissue = 7.6 (A 480) – 1.69 (A 510) x V/1000 x W

Where, A – Absorbance at specific wave length

V – Final volume of chlorophyll extract in 80% acetone

W – Fresh weight of tissue extracted

Estimation of total protein

The total protein in the leaf extract of three varieties was determined by the Bradford method using BSA as the standard. The following solutions were prepared to estimate the protein content.

- 0.1 M Phosphate buffer of pH 7.0
- Bradford reagent

Procedure

1g leaf tissue was homogenized in 0.1 M phosphate buffer (pH 7.0). It was filtered through three folded cheese cloth and filtrate was centrifuged at 5000rpm for 10 minutes. The supernatant was made up to 20 ml using phosphate buffer. 0.5 ml aliquots of the sample were pipette out in to the test tubes and made up to 1.5ml with phosphate buffer. To this 1,5ml Bradford reagent was added and incubated at room temperature for 5 minutes. The blank was prepared using 1.5ml phosphate buffer and 1.5ml Bradford reagent. OD was taken at 595nm.The standard graph of protein was prepared was prepared using known concentrations of Bovine Serum Albumin by the same method. The concentration of protein in the test sample was calculated by preparing the standard calibration curve (Fig 1).

Estimation of total phenol

The total phenolic contents of leaf extract was determined according to the method described by Malik and Singh (1980). Following solutions were prepared to estimate the concentration of phenol in the tomato leaves.

- 80% methanol: Mixed 80ml of methanol with 20ml of distilled water
- 20% Na_2CO_3: 20g Na_2CO_3 was dissolved in 100ml distilled water.
- Folin-Ciocalteau reagent

Procedure

1g fresh leaf tissue was homogenized with 10ml 80% methanol using mortar and pestle. The slurry was filtered using cheese cloth presoaked in 80% methanol. The filtrate was centrifuged at 5000rpm for 10 minutes. The resultant supernatant was collected and made up to 10ml using 80% methanol. From it 0.2 ml aliquot was taken and made up to 3 ml using 80% methanol. Blank was prepared using 80% methanol. 0.5ml Folin's reagent was added to each test tubes and incubated at room temperature. After 3 minutes, 2ml of 20% Na_2CO_3 was added to each test tube. The reaction mixture was incubated in a boiling water bath 5 minutes. The reaction mixture was centrifuged at 5000rpm. Supernatant was collected and was read at 650nm.A standard calibration plot was generated at 650nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot (Fig. 2) and expressed as mg catechol equivalent of phenol/g of sample.

Estimation of proline content

Total proline content of leaf extracts was determined according to the method described by Bates et al., (1973).

Procedure

Leaf samples (0.5g) were homogenized in 5ml of sulphosalycylic acid (3%) using mortar and pestle. About 2ml of Ninhydrin reagent was added to it. The reaction mixture was boiled in water bath at 100^0c for 30 minutes. After cooling the reaction mixture, 6 ml of toluene was added to it and the mixture was transferred to a separated and absorbance was read at 520nm in spectrophotometer against toluene blank. Concentration of proline was estimated with a standard curve of proline (Fig. 3).

Enzyme assays

After the stress treatment period, young leaves were collected for the assay of enzyme activities. Leaf samples were collected in plastic bags and brought to the laboratory. Leaves were then washed with distilled water and surface moisture was wiped out.

Assay of Catalase (CAT)

Catalase was measured according to the method suggested by Luck (1974). 0.5g leaf sample was weighed out in an electronic balance. It was ground in a pre-cooled mortar and pestle using 5ml of 0.1M phosphate buffer. The slurry was filtered through a two folded cheese cloth. The filtrate was centrifuged at 15000rpm for 20 minutes. This supernatant was used as the enzyme source. The assay mixture contained 2.6ml of 50mM potassium phosphate buffer (pH 7.0), 0.4ml of 1.5mM H_2O_2 and 0.04ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240nm. The enzyme activity was expressed in U mg^{-1} protein (U = 1mM of H_2O_2 reduction min^{-1} mg^{-1} protein).

The total enzyme activity = Difference in OD x total volume of enzyme extract / Enzyme extract pipettex weight of tissue taken

Assay of Polyphenol oxidase (PPO)

The enzyme activity was assayed using catechol as a substrate. The initial rate of formation of quinine is detected spectrophotometrically(Malik and Singh, 1994). 0.5g leaf sample was weighed out in an electronic balance. It was ground in a pre-cooled mortar and pestle using 5ml of 0.1ml phosphate buffer containing 10mM, ascorbic acid. The slurry was filtered through a two folded cheese cloth. The filtrate was centrifuged at 15,000rpm for 20 minutes. The supernatant was collected and made up to 10ml using extraction buffer. This supernatant was used as the enzyme source. To 0.1ml of enzyme extract, 2.9ml of 0.1M catechol (0.1M catechol in 0.1M phosphate buffer, pH 7) was added and the absorbance was taken at 420nm immediately and after 5 minutes against the blank containing 2.9ml catechol and 0.1ml extraction buffer.

Total enzyme activity = Difference in OD x Total volume of enzyme extract / Enzyme extract pipette x weight of tissue taken

Specific activity = Total enzyme activity / Total protein

Assay of Peroxidase (POD)

0.5g fresh plant tissue was homogenized in 3ml 0.1M Phosphate buffer (p H 7) using pre-cooled mortar and pestle. It was filtered through a double layered cheese cloth and centrifuged at 18,000rpm for 15 minutes at 5^0c. The supernatant was taken as enzyme source. The supernatant was made up to 20ml using extraction buffer. To 0.5 ml of the enzyme extract, 1ml phosphate buffer, 0.5ml guaiacol and 0.5ml H_2O_2 was added. The absorbance was recorded at 470nm and after 5 minutes against blank containing 1.5ml phosphate buffer, 0.5ml guaiacol and 0.5ml H_2O_2. Peroxidase activity was estimated according to Hemeda and Klein (1990).

The total enzyme activity = Difference in OD x Total volume of enzyme extract / Enzyme extracts pipette x Weight of tissue taken

Specific activity = Total enzyme activity / Total protein

RESULTS AND DISCUSSION

Effect of drought stress on plant growth and development

Effect of salt stress on plant growth and development were measured in terms of plant height, number of leaves, length of leaves and internodes. All the measurements were made from a random sample.

Plant height was 36.0 and 35.5 in the control plants of *C. annum* and *C. frutescence* respectively. After a period of two weeks of drought stress, the plants

showed an average of 34.0 and 35cm height for the two species *C. annum* and *C. frutescence* respectively. (Table 1) (Graph 1 and 2) Number of nodes was found to be reduced in treated plants in two species as in *C. annum* number of nodes were reduced from an average of 27.0 (in control) to 2.0 (in treated). Internodal length was also found to be reduced in the two species *C. annum* and *C. frutescence* as the former sowed a reduction from 3.5cm (in control) to 3.0cm (in treated) (Table.1)(Graph 1and 2). Significant reduction in leaf length was observed in *C. frutescence* under drought stress as leaf length was reduced from 3.9cm in control plants to 3.0cm in treated plants (Table 1) (Graph 1and 2).

In the present investigation, the colour intensity and the number of leaves in the plants subjected to water stress were reduced compared to the respective control plants. The leaf colour was dark green in control plants and in water deficit plants it changed to pale green, yellowish green and then to yellowish brown in both *Capsicum* species and later the leaves shed off. This shows chlorosis in the leaves of test plants under water stress (Table 1) (Graph 1 and 2).

Physiological Characterization

Physiological characterization of drought stress response was measured in terms of initial water content, relative water content, rate of water loss, relative dry weight and excised leaf water retention. These parameters revealed the water relations of the plant in response to salt stress. All the measured parameters showed significant reduction in treated plants when compared to control plants in both the species (Table 2) (Graph 3 and 4).

Biochemical characterization

Estimation of Photosynthetic pigments

Biochemical estimation of photosynthetic pigments showed significant reduction in Chlrophyll a, Chlorophyll b, total chlorophyll and carotenoid pigments under drought stress (Table.3). In both species, amount of chlorophyll and carotenoids reduced considerably under stress condition. In *C. annum*, total chlorophyll was reduced from 0.967 (in control) to 0.646 (in treated), while carotenoids showed a reduction from 0.32 to 0.251 (Table 3) (Graph 5 and 6).

Estimation of total protein, total phenolics and proline

Estimation of biochemical parameters such as total protein, phenolics and proline also showed significant variation between the treated and control plants in both species. Total protein in treated plants significantly reduced under drought stress, whereas amount of total phenolics and proline were significantly increased under stress when compared to control plants (Table 4). Both species showed significant increase in the amount of total phenolics and proline in treated plants when compared to control plants. In *C. annum*, total phenolics increased from 2.01mg/g leaf tissue (in control) to 2.41mg/g leaf tissue (in treated). Amount of proline showed an increase from 1.876 mg/g leaf tissue (in control) to 2.712mg/g leaf tissue (in treated). However, the total protein showed a reduction from 3.6 mg/g leaf tissue (in control) to 2.9mg/g leaf tissue (in treated) (Table 4 and Graph 5 and 6).

Biochemical estimation of Enzyme activity

Estimation of enzyme activity also showed significant increase in the activity of enzymes under drought stress. Two stress responsive antioxidant enzymes (Peroxidase and Catalase) along with polyphenol oxidase were assayed in the present study and found increased activity for all enzymes under drought stress in all both the species (Table.5). A significant increase in the enzyme activity was observed for all the enzymes under stress in both species. Polyphenol oxidase showed activity similar to that of two antioxidant enzymes under salt stress which shows its role in imparting salt tolerance to the cultivars studied (Graph 7 and 8).

Table 1
Morphological characterization of two Capsicum varieties under Drought stress

Treatment	*Plant height (cm)*		*Length of inter node(cm)*		*Number of leaves*		*Length of leaves (cm)*	
	Capsicum annum	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*
Control	36±	29±	3.5	3.8	27	25	3.6	3.9
Stress	34±	30±	3	3.6	22	21	3.2	3

Table 2
Physiological characterization of two Capsicum varieties under Drought stress

Treatment	*IWC (%)*		*RWC (%)*		*ELWR (%)*		*RDW (%)*		*RWL (%)*	
	Capsicum annum	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*
Control	87.31%	86.91%	89.33%	87.29%	97.66%	96.53%	56.21%	47.41%	64.61%	59.66%
Stress	85.26%	85.01%	77.32%	70.21%	95.35%	95.25%	29.61%	35.12%	47.22%	38.47%

Table 3
Estimation of chlorophyll and carotenoid pigments in two Capsicum varieties under drought stress

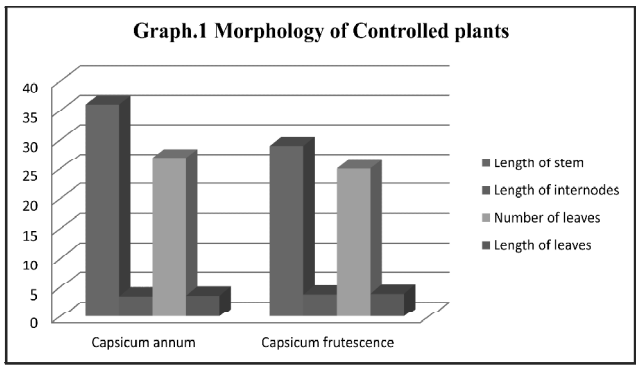
Treatment	*Amount of* Chl_a *(mg/g leaf tissue)*		*Amount of* Chl_b *(mg/g leaf tissue)*		*Amount of total chl (mg/g leaf tissue)*		*Amount of carotenoid (mg/g leaf tissue)*	
	Capsicum annum	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*
Control	0.612±	0.539±	0.373±	0.358±	0.967	0.889	0.32	0.2
Stress	0.432	0.481	0.262	0.220	0.646	0.508	0.251	0.2

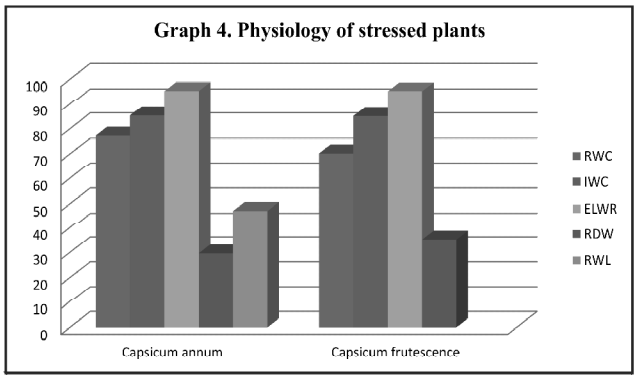
Table 4
Estimation of biochemical parameters in two varieties under Drought stress

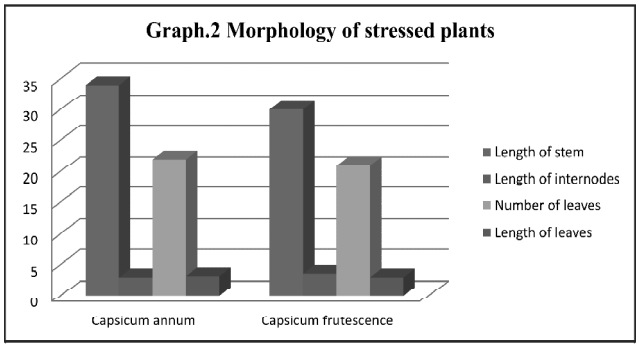
Treatment	*Amount of Protein (mg/g leaf tissue)*		*Amount of phenol (mg/g leaf tissue)*		*Amount of proline (mg/g leaf tissue*	
	Capsicum annum	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*
Control	3.6	2.9	2.01	2.17	1.876	2.045
Stress	2.6	2.01	2.41	2.21	2.712	2.835

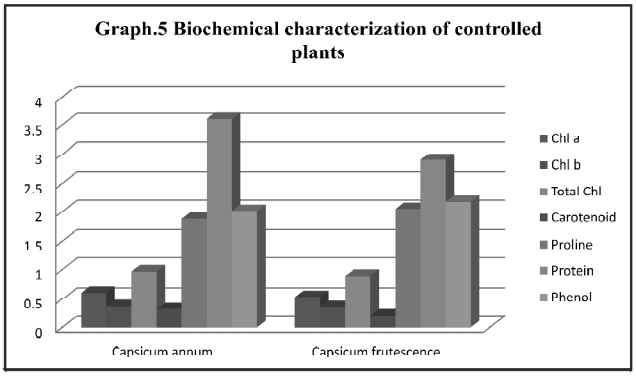
Table 5
Estimation of enzyme activity in two Capsicum varieties under drought stress

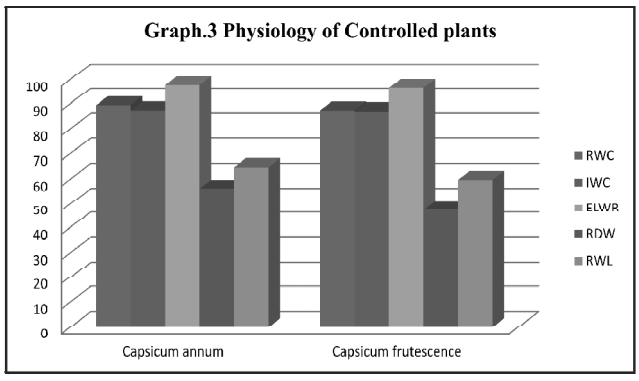
Treatment	*Peroxidase activity (unit min $^{-1}$ g $^{-1}$ FW)*		*Catalase activity (units mg $^{-1}$ protein min $^{-1}$)*		*Polyphenol activity (units mg $^{-1}$ protein min $^{-1}$)*	
	Capsicum annum	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*	*Capsicum annum*	*Capsicum frutescence*
Control	0.086	0.081	0.169	0.189	0.173	0.182
Stress	0.101	0.102	0.201	0.221	0.228	0.211

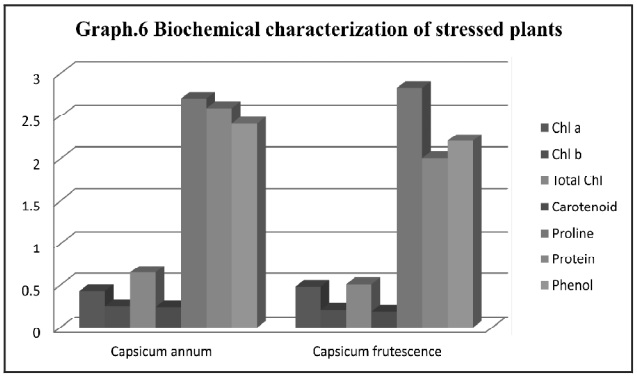


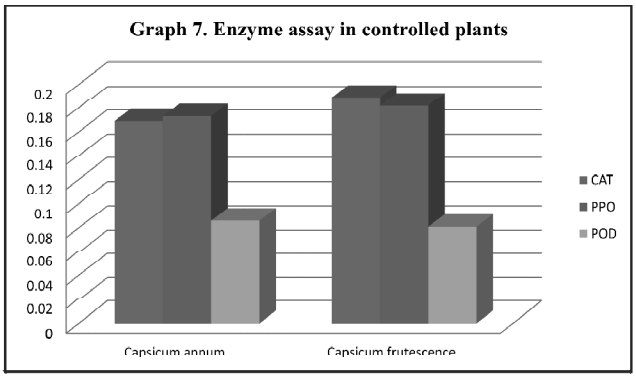


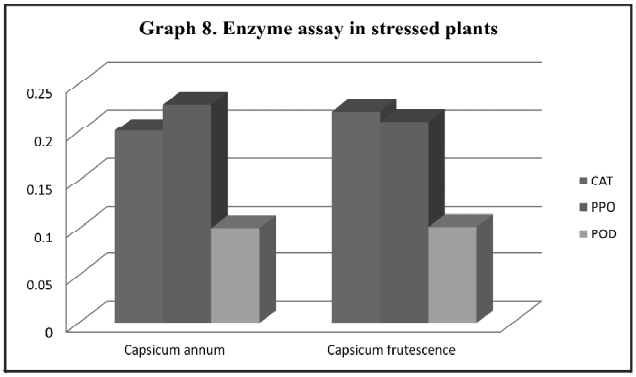












CONCLUSIONS

Drought stress affected the plant growth and development adversely in both species of *Capsicum*.Present study observed retardation of plant growth and development in *Capsicum* species under drought stress along with an increase in total phenolics, total proline and an increase in enzyme activity under drought stress. It can be concluded that the plant responds to drought stress by minimizing the plant size, leaf size, etc in order to survive under limited water availability. The plant tolerance is orchestrated by the increased production of secondary metabolite, especially, phenolics as well as by the accumulation of proline which act as a compatible solute for osmotic adjustment. Further works are needed for the better understanding of drought tolerance mechanism of the species.

REFERENCES

Wardlaw, I.F., 1969. The effect of water stress on translocation in relation to photosynthesis and growth. Aust. J. Biol. Sci., 22:1-16.

Forney, C.F., Breen, P.J., 1985. Dry matter partitioning and assimilation in fruiting and de blossomed strawberry. J. Amer. Soc. Hort. Sci., 110:181-185.

Hsicao, T.C (1970). Rapid changes in levels of polyribosomes in Zeemays in response to water stress. Plant Physiol, 46:281-285.

Heisar, C.B., 1976. Peppers. *Capsicum (S olanaceae)* In: Evolution of crop plants. Simmonds, N.W., ed. Longman, London.pp: 265-268.

Batal, K.M., Smittle, D.A., 1981. Response of bell pepper to irrigation, nitrogen and plant population. J. Amer.Soc.Hort. Sci, 106:259-262.

McGiffen, Jr. M.E., Masiunas, J.B., Huck, M.J., 1992. Tomato and night shade (*Solanumnigrum*L., and *S. ptycanthum*Dun). Effects on soil water content. J.AMER. Soc. Hort. Sci., 117:730-735.

Lurie, S., Ben-Yehoshua, S., 1986. Changes in membrane properties and ABA during senescence of harvested bell pepper fruit.J.Amer.Soc.Hort.Sci.,111:886-889.

Atkinson,C.J., Poliocarpo, M., Webster, A.D., 2000. Drought tolerance of clonal Malus determined from measurements of stomatal conductance and leaf water potential. Tree Physiol, 20:558-563.