EFFECTS OF SOIL MOISTURE STRESS ON FREE PROLINE AND CHLOROPHYLL CONTENTS IN TOMATO (*Lycopersicon esculentum* Mill.)

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ABSTRACT

A study was conducted in the Agronomy farm of the Faculty of Agriculture, Eastern University, Sri Lanka to investigate the biochemical responses of moisture stress during the vegetative, flowering, early fruiting and fruit ripening stages of tomato (cv. KC-1). This experiment was layed out in the Randomized Complete Block Design with five treatments and four replications. Moisture stress was imposed for different treatments for a period of 4 days per treatment during the above growth stages. Moisture stress increased the proline content of tomato leaves. An increasing trend in proline content was observed with the age of plants when subjected to moisture stress. There was a decrease in the proline content 5-6 hours after supplying water and the values returned to normal after 48 hours of re-watering. Moisture stress reduced the chlorophylls a and b contents of tomato leaves. Plants which experienced moisture stress during the vegetative and flowering stages showed greater reduction in the chlorophyll content than those of the other growth stages. It was also observed that moisture stress caused higher reduction in chlorophyll a than chlorophyll b. The reason for this reduction needs to be investigated. There was a partial recovery in the chlorophylls a and b contents of plants after re-watering. The rate of recovery of chlorophyll content was faster during the vegetative stage than the flowering stage.

Key words: Chlorophyll, Growth stages, Moisture stress, Tomato

INTRODUCTION

Most of the agricultural crops are watered periodically during the growing season, either by natural rainfall or by irrigation. In the intervals of such watering, soil moisture stress sometimes becomes severe and limits plant growth and development (Techawongstien *et al.*, 1992). Water deficit affects every aspect of plant growth including anatomy, morphology, physiology and biochemistry. An induction of water stress in plants is known to trigger several biochemical changes. Free proline accumulation

and changes in the chlorophyll content of leaves are some of the important biochemical responses of tomato to moisture deficit stress.

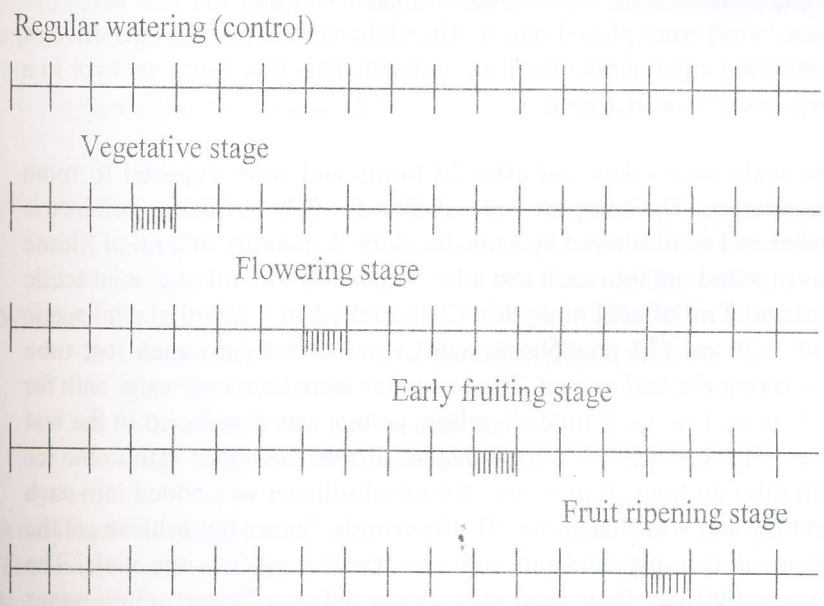
Free proline accumulation is found to be one of the most immediate responses of plants to moisture stress (Aspinall and Paleg, 1981). Cytoplasmic accumulation of this non-toxic amino acid is thought to be involved in osmotic adjustment of stressed tissues (Kavi Kishor *et al.*, 1995). The level of proline may vary with the length of time the tissue is under stress. Chlorophyll concentration is sensitive to leaf age, temperature and water balance of the whole plant. The synthesis of chlorophyll is highly sensitive to low leaf water potential. Small water deficits can have marked effects on chlorophyll accumulation in young tissues (Kozlowski, 1972).

There is very little information available on the biochemical changes on account of moisture stress in tropical crops. In view of this, tomato crop was chosen which is an important cash crop. Since the performance of tomato is very sensitive to irrigation practices, it was subjected to varying degrees of moisture stress during its growth cycle and the biochemical responses were studied. The present study therefore was conducted with the objectives of investigating the free proline and the chlorophyll contents of tomato under moisture stress and to estimate the extent of recovery of these parameters after re-watering.

MATERIALS AND METHODS

This experiment was conducted in the Agronomy farm of the Eastern University, Sri Lanka which is situated at 0 -75 m above mean sea level. The climate is warm (28° C - 32 °C) with an annual rainfall less than 1250 mm. A number of two nursery beds each having a dimension of 3m x 1m were prepared. A quantity of 10g tomato seeds (cv.KC-1) was treated with 'Captan' solution (2g l^{-1}). The seeds were then sown by row seeding to a depth of 0.5-1 cm. The seedlings were managed in the nursery beds according to the recommended practices of the Department of Agriculture. A number of twenty plots, each having a dimension of 5m x 5m were prepared. A distance of 1 m was maintained between plots to minimize the seepage of water from one plot to another during irrigation. Rain shelters were constructed to prevent the entry of rainwater into the experimental plots during rainy days. In addition, drainage channels were constructed around each plot to minimize the percolation of water. Polyethylene sheets were inserted around each plot to a depth of 30 cm to prevent the run off during irrigation. The 25 days old vigorous and uniform seedlings were transplanted on the main field at a spacing of 80 cm x 50 cm. There were five treatments and each one was replicated for four times. Treatment one was the control which received regular watering daily. In treatment - 2 tomato plants experienced moisture stress

for a period of four days during the vegetative stage. Treatment – 3 received moisture stress during the flowering stage. Treatment – 4 experienced moisture stress during the early fruiting stage and finally the plants were exposed to moisture stress in treatment – 5 during the fruit ripening stage. These treatments were arranged in a Randomized Complete Block Design (Fig.1). Moisture stress treatments were applied by withholding water completely at once. The experiment was managed in accordance with the recommended cultural practices (Technoguide, 2005).



T_1= Control- Regular watering at Field Capacity.

T_2= Moisture stress for 4 days during the vegetative stage, 35 days after sowing.

T_3= Moisture stress for 4 days during the flowering stage, 51 days after sowing.

T_4= Moisture stress for 4 days during the early fruiting stage, 67 days after sowing.

T_5= Moisture stress for 4 days during the fruit ripening stage, 83 days after sowing.

Figure 1: Diagrammatic representation of the manner in which moisture stress was applied at different growth stages of tomato

Proline determination

Proline determination was according to Bates (1973) with the following modifications. Three plants were randomly selected from each replicate of the treatments and five leaf discs / plant were obtained. As such, a number of 15 leaf discs each having a diameter of 2 cm were taken from each replicate of the treatments using a cork borer. The leaves were sampled on the 5th day from the commencement of each stress cycle to determine the effects of stress and on the same and 2nd day after re-watering to determine the extent of recovery. A quantity of 6 ml, 3% Sulphosalicilic acid was added into each vial and the leaf discs (15 discs / vial) were placed into it. The vials were kept in a regiform box containing equal amounts of ice and salt. This box was then kept in a deep freezer for 24 hours.

The vials were taken out after 24 hours and were exposed to room temperature. The samples were shaken for 2 hours using an electric shaker and were filtered by a muslin cloth. A quantity of 2 ml of filtrate was pipetted out into each test tube. A quantity of 2 ml of glacial acetic acid and 2 ml of acid ninhydrin (2.5g ninhydrin + 60 ml glacial acetic acid + 20 ml 6M phosphoric acid) were added into each test tube containing the leaf extract. The test tubes were kept in a water bath for an hour at 100^0 C. A reddish yellow colour was developed in the test tubes. These test tubes were transferred from the water bath to the ice bath after an hour. A quantity of 6 ml of toluene was added into each test tube and was shaken for 10–15 seconds. Soon after toluene got the colour, it was pipetted out and was transferred into the vials. The absorbance was then read at 520 nm using a Spectrophotometer (Camspec, M330BT). Standard proline concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm and 12 ppm were prepared to obtain a standard curve and similar procedure was followed. The absorbance of the standard solutions was read at 520 nm. The concentration of the proline was calculated per unit leaf area using the standard curve.

Chlorophyll determination

Fourty leaves representing 5 plants were randomly collected from each replicate of the treatments at different growth stages. The leaves were sampled on the 5th day from the onset of each stress cycle to determine the effects of stress and on the same and 2nd day after re-watering to estimate the extent of recovery. A quantity of 1g of fresh sub-sample leaves from the above sample was placed in a clean mortar. A quantity of 40ml of 80% (v/v) acetone was added and the tissues were ground to a fine pulp. The extract was filtered by a filter paper (Whatman No.1).

a fine pulp. The extract was filtered by a filter paper (Whatman No.1). The pulp was ground repeatedly with fresh 30ml aliquot of 80 % acetone. The second extract was filtered into the flask containing the first extract using a filter paper. The final volume of the filtrate was adjusted to 100ml by adding sufficient amount of 80% acetone. The optical density of the chlorophyll extract was recorded by a spectrophotometer (Camspec, M330BT) using 10 mm cuvettes. The wavelengths used were 645nm and 663nm. A quantity of 80% acetone was used as the solvent blank. The amount of chlorophyll present in the extract was calculated on the basis of milligrams of chlorophyll per gram of leaf tissue by using the following equations:

$$\text{mg chlorophyll a / g tissue} = [12.7\ (D_{663}) - 2.69\ (D_{645})] \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll b / g tissue} = [22.9\,(D_{645}) - 4.68\,(D_{663})] \times \frac{V}{1000 \times W}$$

Where,

D: Optical density reading of the chlorophyll extract at the specific wavelength

V: Final volume of the 80 % acetone – chlorophyll extract

W: Fresh weight of the tissue (g)

Analysis of data

The collected data were statistically analyzed using Analysis of Variance to determine the significance if any at the treatment level. The differences between treatments were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Free Proline content

It was found that there were significant differences in the free proline content of tomato plants between the stressed and the control treatments ($P<0.05$) during the vegetative, flowering, early fruiting and fruit ripening stages (Table 1). In the treatments where the stress cycles were experienced by plants during the above growth stages, the proline contents of the leaves on the 5th day from the commencement of the stress were significantly higher ($P<0.05$) than the control.

Table 1. The effects of soil moisture stress on the free proline content of tomato leaves at different growth stages

Treatments	Free proline content (mg cm^{-2})			
	Stages of growth			
	Vegetative	Flowering	Early fruiting	Fruit ripening
T_1	1.252^b	1.649^b	1.712^b	1.781^b
T_2	**2.340^a**	1.649^b	1.714^b	1.784^b
T_3	1.259^b	**2.804^a**	1.716^b	1.782^b
T_4	1.256^b	1.648^b	**2.891^a**	1.788^b
T_5	1.251^b	1.647^b	1.711^b	**2.938^a**

*Values in the same column followed by the same letter do not differ significantly ($P<0.05$).
*Values are the means of 12 plants in 4 replications.

Moisture stress increased the proline content of tomato leaves. An extensive body of literature shows the accumulation of proline under water stress conditions (Carceller *et al.*, 1999). Sean *et al*, (1998) observed 35 times increase in the concentration of proline in Ber (*Ziziphus Mauritiana*). This increase was shown during the drought period of 13 days. However, it was observed in the present experiment that the proline content increased within the range of 1.5 – 2 times irrespective of the stages of growth. This may be due to the short period of drought experienced by these plants. The reasons for the increase in proline content may be due to increase in the synthesis of proline or reduced rate of proline oxidation under moisture stress condition. Sundaresan and Sudhakaran (1995) stated that an increase in the activity of ornithine amino transferase, the key enzyme involved in the synthesis of proline occurred under moisture stress condition. They further stated that the reduced activity of proline oxidase under water stressed condition has been suggested to be one of the factors causing proline accumulation in the stressed tissues.

An increase in trend in the proline content was observed with the age of the plants when subjected to moisture stress. Mature plants when exposed to moisture stress would have accumulated high amounts of secondary metabolites and compatible solutes. As such, the proline contents also would have increased with maturity. The reduction in leaf water potential is highly correlated with the accumulation of proline. As stated by Lilley and Fukai (1994), decline in the leaf water potential was more rapid in the reproductive stage of rice than the vegetative stage. The tomato plants stressed during the vegetative stage showed the lowest amount

of proline than those of the other growth stages. Safeena *et al.* (2002) reported that higher amount of proline was accumulated during the flowering stage while it was lower during the seedling stage.

It was found that there was a fall in the proline content within 5 – 6 hours of supplying water and it returned to the normal within 48 hours of watering. A characteristic increase in the concentration of proline was observed during the induction of moisture stress followed by a rapid decline upon re-watering of these plants. Proline accumulated during an episode of water deficit is rapidly lost principally by oxidation to glutamate once the water deficit is eliminated (Stewart *et al.*, 1977).

Chlorophylls a and b contents

It was found that there were significant differences between the stressed and the control treatments ($P < 0.05$) in the chlorophylls a and b contents of tomato leaves during the vegetative, flowering, early fruiting and fruit ripening stages (Tables 2 and 3). In the treatments where the stress cycles were experienced by plants during the above growth stages, the chlorophyll a and b contents of tomato leaves on the 5th day from the commencement of the stress were significantly lower than the control values.

Table 2. The effects of soil moisture stress on the chlorophyll a content of tomato leaves at different growth stages

Treatments	Chlorophyll a content (mg g^{-1} fresh weight)			
	Stages of growth			
	Vegetative	Flowering	Early fruiting	Fruit ripening
T_1	14.186^a	14.030^a	13.836^a	12.558^a
T_2	**8.592**^{b}	13.272^b	13.805^a	12.550^a
T_3	14.196^a	**9.217**^{c}	13.550^b	12.300^b
T_4	14.199^a	14.093^a	**10.024**^{c}	12.177^c
T_5	14.196^a	14.090^a	13.812^a	**9.004**^{d}

* Values in the same column followed by the same letter do not differ significantly ($P<0.05$).

*Values are the means of 20 plants in 4 replications.

Table 3. The effects of soil moisture stress on the chlorophyll b content of tomato leaves at different growth stages

Treatments	Chlorophyll b content (mg g^{-1} fresh weight)			
	Stages of growth			
	Vegetative	Flowering	Early fruiting	Fruit ripening
T_1	12.387[a]	12.226[a]	11.810[a]	10.981[a]
T_2	**8.759**[b]	11.190[b]	11.719[a]	10.931[a]
T_3	12.392[a]	**9.394**[c]	11.048[b]	10.739[b]
T_4	12.398[a]	12.270[a]	**9.547**[c]	10.585[c]
T_5	12.392[a]	12.252[a]	11.891[a]	**8.417**[d]

* Values in the same column followed by the same letter do not differ significantly ($P<0.05$).

*Values are the means of 20 plants in 4 replications.

Moisture stress reduced the chlorophyll a and b contents of tomato leaves. As stated by Namanayake and Bandara (1998), chlorophyll a and b contents declined significantly under moisture stress condition. Majumdar *et al.* (1991) stated that chlorophyll loss is often assumed to be a symptom of stress injury. Chlorophyll contents of a crop are a determinant factor in the photosynthesis of plants. The high photosynthetic rates in seedlings can be correlated with the chlorophyll contents (Namanayake and Bandara, 1998). The reduction in chlorophyll content of the stressed plants would have been due to increased leaf temperature followed by destruction of the chloroplast membranes. Chlorophylls are present in the thylakoid membranes of the chloroplast. Kramer (1983) stated that the disorganization of thylakoid membranes in chloroplasts is correlated with the destruction of chlorophyll a and b under moisture stress condition. This photosynthetic apparatus of these plants would have severely damaged during the course of stress. Techawongestien *et al.* (1992) stated that the photosynthetic apparatus of plants may get damaged by water stress effects. The decline in chlorophyll content would be due to high leaf temperature which would probably be attributed to lowering of transpirational cooling with the onset of stress (Namanayake and Bandara, 1998).

The vegetative stage showed the highest percentage reduction in both chlorophyll a and b contents (Fig. 2). This shows that chlorophyll content is most sensitive to moisture stress during the vegetative stage. Chlorophyll degradation may be part of a process of stress – induced senescence. Extensive studies have demonstrated that water deficit results in an early senescence in annual plants during the vegetative and

flowering stages (Kyparissis *et al.*, 1995). A decrease in the chlorophylls a and b contents was also observed in the regularly watered plants towards maturity. This decrease would have been due to reduced metabolic activity of the tissues with the age of the plants.

It was observed that there was a partial recovery in the chlorophylls a and b contents of stressed leaves, 48 hours after re-watering. In the treatment where the stress cycle was experienced by plants during the vegetative stage (T_2), leaf chlorophyll content recovered by 15% in 48 hours following the relief of stress. The recovery was 12% in the T_3 treatment where the plants experienced moisture stress during the flowering stage. These partial recoveries have been due to repair of the chloroplast membranes as a result of regain in turgidity after re-watering.

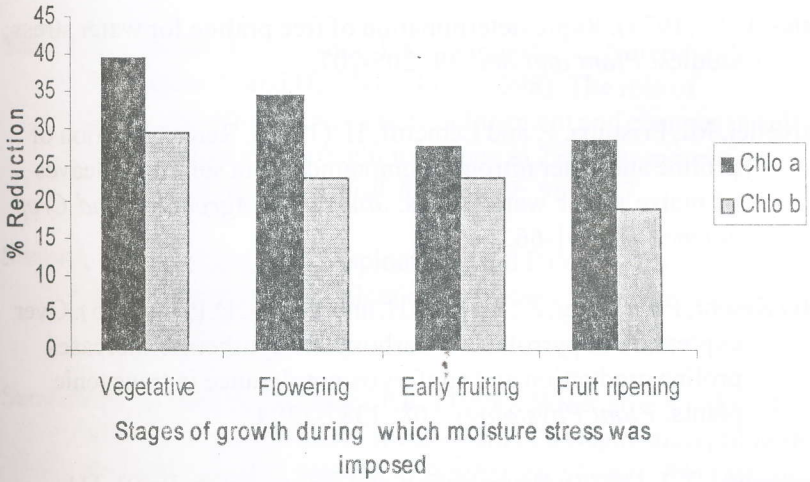


Figure 2: Percentage reduction in the chlorophylls a and b contents of tomato leaves at different growth stages

CONCLUSIONS

This experiment determined the extent to what moisture stress affected the free proline and chlorophyll contents of tomato leaves. The extents of recovery of proline and chlorophyll contents of leaves were also determined. Moisture stress increased the proline content and reduced the chlorophyll a and b contents of leaves. It was observed that there was a complete recovery in the proline content two days after withholding the stress. However, chlorophylls a and b contents did not recover completely on the 2nd day after the termination of the stress.

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REFERENCES

Aspinall, D. and Paleg, L.G. (1981). Physiology and Biochemistry of Drought Resistance in Plants. Pp. 205-240. London, UK: Academic Press.

Bates, L. S. (1973). Rapid determination of free praline for water stress studies. *Plant and Soil* 39: 205-207.

Carceller, M., Pristupa, P. and Lemcoff, H. (1999). Remobilization of proline and other nitrogen compounds from senescing leaves of maize under water stress. *Journal of Agronomy and Crop Science* 183: 61-66.

Kavi Kishor, P.B., Hong, Z., Mijo, G.H. and Verma, D.P.S. (1995). Over expression of pyrroline- 5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiology* 108: 1387-1394.

Kozlowski, T.T. (1972). Water deficit and plant growth. Pp 91-111. London, UK: Academic Press.

Kramer, P.J. (1983). Water Relations of Plants. Pp 342-373. London, U.K: Acedemic Press.

Kyparissis, A., Petropoulos, Y. and Manetas, Y. (1995). Avoidance of photoinhibitory damage through decreased chlorophyll contents. *Journal of Experimental Botany* 46: 1825-1831.

Lilley, J.M. and Fukai, S. (1994). Effect of timing and severity of water deficit on four diverse rice cultivars. II. Physiological responses to soil water deficit. *Field Crop Research*. 37: 215-223.

Majumdar, S., Ghosh, S., Glick, B.R. and Dumbroff, E.B. (1991). Activities of chlorophyllase,phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase in the primary leaves of soybean during senescence and drought. *Physiologia Plantarum* 81: 473-480.

Namanayake, N.P.A.D. and Bandara, D.C. (1998). Effect of water stress on coconut (*Cocos nucifera* L.) seedling under different soil types and compaction levels. *Tropical Agricultural Research* 10: 12-26.

Safeena, M.I.S., Bandara, D.C. and Sumanasinghe, V.A. (2002). Effect of salt stress on leaf area, rate of photosynthesis and proline accumulation of rice varieties at different growth stages. *Tropical Agricultural Research* 14: 87-96.

Sean, C.C., Stefan, K.A., Janet, E.C., Sangeeta, J., Narendra, S., Marianne, P. and Hamlyn, G.J. (1998). The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana*. *Journal of Experimental Botany* 19: 967-977.

Stewart, C.R., Bogges, S.F., Aspinall, D. and Paleg, L.G. (1977). Inhibition of proline oxidation by water stress. *Plant Physiology* 59: 930-932.

Sundaresan, S. and Sudhakaran, P.R. (1995). Water stress-induced alteration in the proline metabolism of drought-susceptible and drought–tolerant cassava (*Manihot esculenta*). *Physiologia Plantarum* 94: 635-642.4

Techawongestien, S., Nawata, E. and Shigenaga, S. (1992). Effects of water stress at various stage of plant development and growth and yield in chilli pepper. *Japanese Journal of Tropical Agriculture* 36 (1): 51-57.

Technoguide (2005). Department of Agriculture, Sri Lanka.