



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Effect of drought stress on chlorophyll content, protein and total free amino acid in [*Cajanuscajan*(L.)Millspaugh]

S.Punyasheeladevi^{1*}, B.Sujatha²

1. Research scholar, Department of Botany Andhra University, Visakhapatnam, Andhrapradesh.

2. Professor, P.G. Department of Botany Andhra University, Visakhapatnam, Andhrapradesh.

ABSTRACT

Physiological and biochemical analysis responses of 6-day-old seedlings of two pigeon pea (*Cajanuscajan*(L.) millspaugh) cultivars namely ICPL-85063 (short duration, lakshmi) and ICPL - 87119 (medium duration, Asha) were studied. Stress was applied with polyethylene glycol (PEG-6000) and water potentials were: zero (control), -0.3Mpa (50mM), -1.1Mpa (100mM) and -2.3Mpa (150mM). In this study, the water stress effects on different parameters like chlorophyll content, protein and total amino acid content was measured following the standard methods. Total free amino acid content registered higher values in ICPL-85063. The protein and total chlorophyll content was observed as lower values when compared to their controls in lakshmi cultivar. It was observed that amino acid content was highest in stress level was -2.3Mpa and the least exist in blank. The results of chlorophyll content demonstrate a concentration dependant decline with increasing concentration of polyethylene glycol (PEG-6000). ICPL-85063 cultivar showed better performance in total amino acid content than Asha cultivar. It was established that the applied doses of PEG-6000 caused stress on young pigeon pea plants, which found suppression of photosynthesis.

Keywords: *CajanusCajan*, water potential, water stress, amino acid, protein, chlorophyll content, polyethylene glycol, drought.

*Corresponding Author Email: svnspdevi@gmail.com

Received 23 July 2014, Accepted 1 August 2014

Please cite this article as: Punyasheeladevi S *et al.*, Effect of drought stress on chlorophyll content, protein and total free amino acid in [*Cajanuscajan*(L.)Millspaugh]. American Journal of PharmTech Research 2014.

INTRODUCTION

Plants are subjected to various abiotic stresses due to unfavorable environmental condition that affect their growth, metabolism and yield ¹ and drought is one of the major abiotic stresses which limit the crop production in arid and semi arid tropics like India. Pigeon pea (*Cajanuscajan* (L). Millspaugh) is one of the major grain legume crops of the tropics and sub tropics. It is the most important pulse crop which is cultivated in the gross cropped area (3.58 million ha) under pulses providing 20% of the national pulse production (2.51 m tones).this accounts for 90% of the world`s pigeon pea ² . Pigeon pea plays an important role in food security balanced diet and alleviation of poverty by providing source of food, feed, and fodder ³ fuel wood, rearing lac insects⁴ hedges, windbreaks, soil conservation, green manuring and roofing. It is a major source of protein of about 20% of the world population ⁵ and is an abundant source of minerals and vitamins⁶.

PEG is used successfully to decrease the water potentials of plants as it doesn`t enter in to the root⁷. This neutral polymer is being widely used to impose water stress in plants. Responses of plants to water deficit result in alteration of chlorophyll content. Simulation of drought stress by PEG (polyethylene glycol) includes drought stress on the plants ⁸, and significant deviation from the control continues to increase with the increasing solute potential (ψ_s) ⁹. PEG-6000 has long been utilized as a reliable maker under laboratory conditions for testing the drought tolerant genotypes. This is because polyethylene glycol acts as a non- penetrating osmotic agent resulting in to increasing solute potential (ψ_s) and blockage of absorption of water by the root system^{8,10,11}. Drought screening using some seed technological parameters has been found to be quite useful in a number of crops¹² under laboratory conditions. This technique can be further extended to test drought tolerance in other genotypes¹³. Several physiological processes are found to be effected by water stress, both at whole plant and cellular levels¹⁴.

Inhibition of leaf growth is a primary whole plant response to water stress, which has been reported in maize, barley and rice seedlings¹⁵. Drought is undoubtedly one of the most important environmental stresses limiting the productivity of the plants around the world¹⁶. Drought stress decreases the rate of photosynthesis¹⁷. Plants grown under drought condition have a lower stomatal conductance in order to conserve water Cosequently, CO_2 fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for growth and yield of plants. Diffusive resistance of the stomata to CO_2 entry probably is the main factor limiting photosynthesis under drought ¹⁸. Certainly under mild or moderate drought stress stomatal closure (causing reduced leaf

internal CO₂ concentration) is the major reason for reduced rates of leaf photosynthesis¹⁹. The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplast caused by active oxygen species²⁰.

MATERIALS AND METHODS

Two locally cultivated pigeon pea cultivars were selected and seeds were obtained from Regional Agricultural Research station Guntur, Andhra Pradesh, India. Two cv of pigeon pea ICPL87119 (tall) and ICPL85063 (short) were selected for present investigation. The seeds of healthy and uniform size were selected and surface sterilized with 0.05M mercuric chloride for 2min. washed thoroughly with glass distilled water and then soaked in distilled water for 2hrs. The soaked seeds were then spread over plastic trays (approximately 50 seeds per tray) lined with two layered what man no 1 filter paper containing different concentrations of polyethylene glycol 6000 representing 0mM, 50mM, 100mM and 150mM. The seeds raised in distilled water were referred to as controls. Ten ml of each test solution was added separately to each tray and the filter papers were replaced on every alternate day during the study period. The seeds of the two cultivars were allowed to germinate at 30±2°C for 6 days under a photoperiod of 18h. The analysis was made in different parts of the seedlings like root and shoot which are separated prior to the experiment where as for various photosynthetic parameters shoot and leaf is considered. All the experiments were replicated 3 times. The concentration of PEG 6000 (g/kg of water) for each water stress was determined using the equation of Michel and Kaufmann²¹.

Total chlorophyll content

Total chlorophyll content was estimated by the method of²². About 200mg of fresh shoot of seedlings grown in different water potentials were ground in a mortar using 80% acetone in the presence of a little quantity of acid washed sand and a pinch of calcium carbonate. The completely homogenized material was centrifuged and the supernatant was diluted suitably to a known volume with 80% acetone taking precautions without exposing it to light. The absorbance of the solution was read at two wavelength 645nm and 663nm using ECIL's junior spectrophotometer GSB66B. The amount of total chlorophyll per gram of shoot tissue as well per shoot according to the formula.

$$\text{Mg total chlorophyll/g tissue} = (20.2 \times \text{OD. At } 645 \div 8.02 \times \text{OD at } 663) \times V/1000 \times W$$

In the above equation O.D represents the optical density of the chlorophyll extract at the specific indicated wavelength V the final volume of the 80% acetone chlorophyll extract and W the fresh weight in grams of the tissue.

Total soluble proteins

Total protein content was estimated by adopting the method of ²³. Extraction Five hundred mg of sample was ground and macerated thoroughly by using a suitable volume of 10% (w/v) trichloro acetic acid. The homogenate was centrifuged at 5000 rpm for 20 min and the supernatant was discarded. This process was repeated twice. The pellet was suspended in 5 ml of 0.1N NaOH solution and was used for the estimation of proteins.

Estimation

One ml of protein extract was taken into a clean dry test tube. To this 5.0 ml of reagent 'C' was added, mixed thoroughly and allowed to stand at room temperature for 10 min. Then 0.5 ml of reagent 'D' was added rapidly with immediate mixing and allowed to incubate for 30 min at room temperature. The color developed was read at 660 nm using Systronics 112 spectrophotometer. A similarly treated blank was used for zero setting. The protein content was calculated from a standard curve prepared from bovine serum albumin.

Preparation of reagents

Reagent A: 2% Na₂CO₃ in 0.1 N NaOH.

Reagent B: 0.5% CuSO₄ in 1% sodium potassium tartrate solution.

Reagent C: It is a mixture of 50 ml of reagent A + 1 ml of reagent B.

Reagent D: 1N Folin –Ciocalteus phenol reagent.

Total free Amino acids:

Total free amino acids were determined following the method ²⁴.

Extraction:

100 mg of plant samples were homogenised in 80% ethanol. The supernatant after centrifugation was evaporated in a boiling water bath. The residue was dissolved in 15 ml 2.2 pH citrate buffer. The supernatant after centrifugation was neutralised to the end point of methyl red with 1N NaOH.

Estimation:

To 1 ml of Amino acid extract 2 ml of ninhydrin reagent was added. After keeping the aluminum caps, the test tubes were kept shaking for 2 or 3 min and were heated in vigorous boiling water bath for 20 min. The colour intensity was read at 570 nm using the mixture of neutralized citrate buffer and ninhydrin reagent as blank on Shimadzu (UV-240) spectrophotometer ²⁴. Standard curve was prepared by using leucine.

Preparation of ninhydrin reagent:

The ninhydrin reagent was prepared directly in the brown bottles. Forty seven ml of ethylene glycol was poured into the bottle. To it 1.25 g of carefully weighed ninhydrin ²⁴ was added. The

solution was shaken until the ninhydrin was completely dissolved. To this solution 1% stannous chloride in acetate buffer (pH 5.1 ± 0.03) was added. The ninhydrin reagent was ready for use 2 to 3h after preparation.

RESULTS AND DISCUSSION:

Effects of drought stress on total chlorophyll content:

Osmotic stress generated by polyethylene glycol (PEG-6000) generally reduce photosynthetic rate²⁵. Total chlorophyll content in both the cultivars of pigeon pea declined with the water stress increase. Total chlorophyll content of the shoots of the seedlings of two pigeon pea cultivars decrease with the increasing concentration of polyethylene glycol and registered lower values when compared to their respective controls (figure 1). Decreased or unchanged chlorophyll level during drought stress has been reported in other species depending on the duration and severity of drought²⁶. In the present investigation there was a significant decreased in the total chlorophyll content in both the cultivars. The results demonstrate a concentration dependent decline in chlorophyll content with increasing concentration of polyethylene glycol-6000(PEG-6000). Reduction in chlorophyll level by water stress has been shown in a few systems^{27,28}. Decrease in the total chlorophyll content by PEG -6000 has also been noticed by²⁹ in black gram³⁰ in wheat seedlings. Reduction in chlorophyll content due to water stress was previously reported in tea leaves³¹.

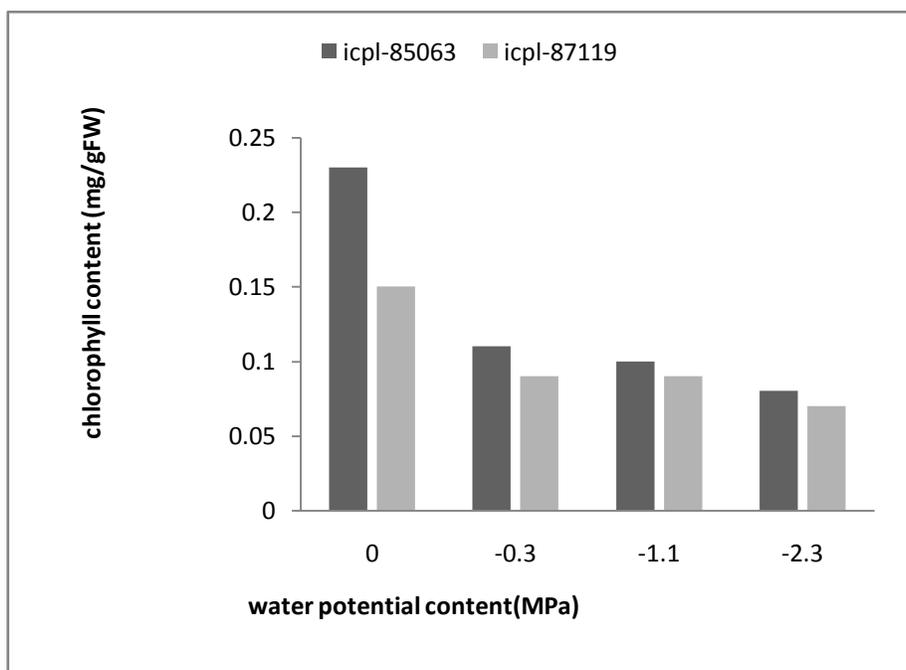


Figure 1: Change in the chlorophyll content in the shoots of two pigeon pea cultivars and are subjected to water stress

Effect of drought stress on protein content:

In the present study the results obtained with lower protein content in ICPL-85063 (fig2A) are in agreement with the findings of Baruahand *et al.* ³² who also reported a lower protein content in drought stressed groundnut. The capacity for protein synthesis also decreases considerably as observed in response to water stress³³. Lakshmi cultivar showed decreased root protein content when compared to the cvAsha.

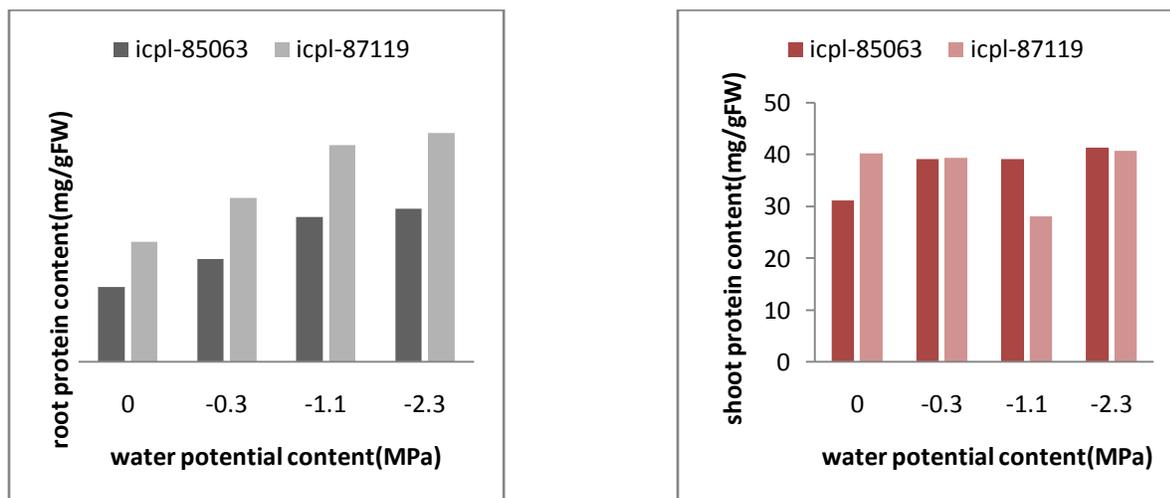


Figure: 2A. Effect of different water potentials on protein contents (mg/g FW) in the roots and shoots of two pigeon pea cultivars

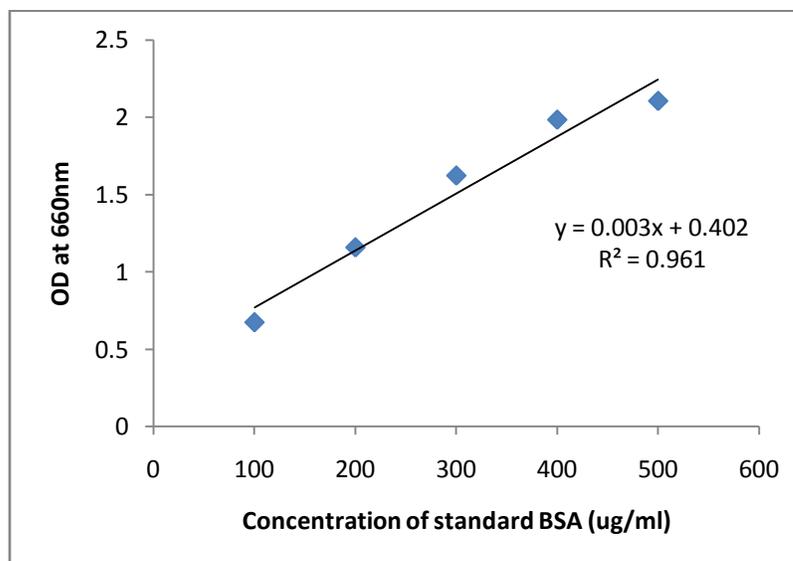


Figure: 2B Calibration plot for Protein content

Effect of water stress on total free amino acid:

The accumulation of total free amino acid content (figure: 3A) was higher in ICPL-85063 than cv. ICPL -87119. In water potential -2.3 Mpa (PEG-150 mM) shoot amino acid content increased in cv. ICPL - 85063. The results showed the increased values of total free amino acid content in

ICPL- 85063 than the Asha cultivar. Both the cultivars showed increased values at the highest water stress and at the same treatment ICPL-85063 was the highest in the accumulation of free amino acid.

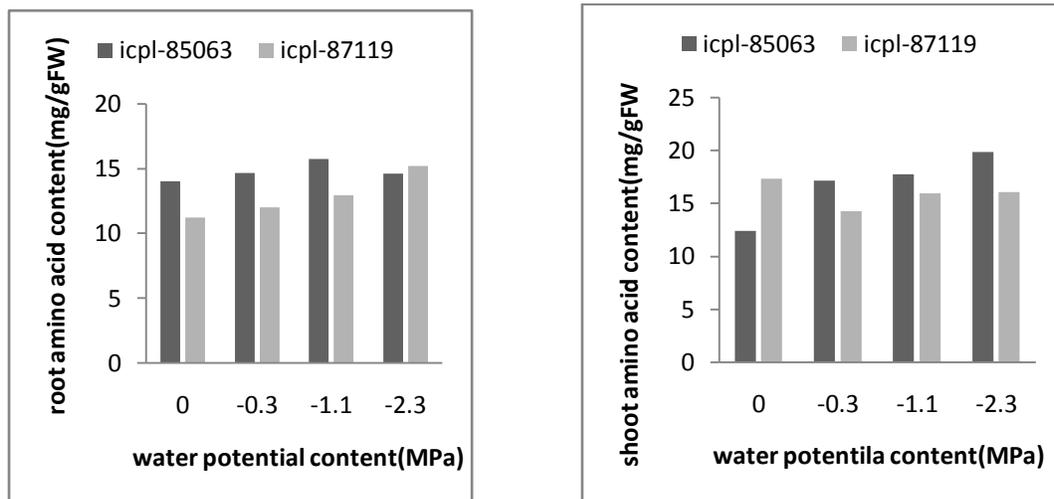


Figure: 3A.Effect of different water potential on total amino acid content (mg/g FW) in the roots and shoots of two pigeon pea cultivars.

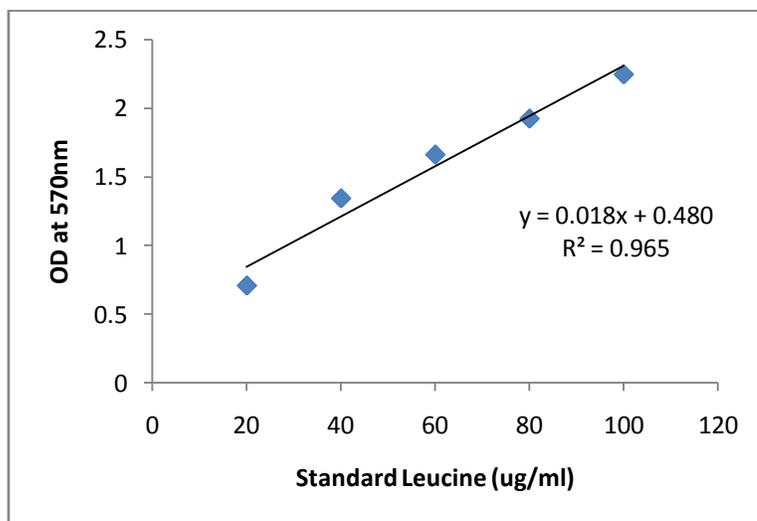


Figure: 3B. Calibration plot for total amino acid content

CONCLUSION:

Present results indicate that water stress induced by PEG-6000 cause significant physiological and biochemical changes in pigeon pea. The short cv ICPL- 85063 accumulated higher amounts of total free amino acids compared with the tall cv ICPL-87119. The physiological and biochemical parameters responses of drought adapted two cultivars pigeon pea to limited water supply showed similar patterns of decreased total chlorophyll content and protein content. Differences between varieties were mainly found in total free amino acid. From the results we can understand that cv

lakshmi is the best drought sustaining than the cv Asha .

REFERENCES:

- 1 Kaur, N and A.K. Gupta. Signal transduction pathways under abiotic stresses in plant. *Current Sci.* 2005;88 (11):1771-1778.
- 2 Johansen, C.1990. Pigeon pea: Mineral nutrition. In: Y.L. Nene, S.H. Hall and V.K. Sheila. *The Pigeon pea.* CAB International, Welling ford, UK. PP. 211.
- 3 Rao SC, Coleman SW and Mayeux HS. Forage production and nutritive value of selected pigeon pea ecotypes in the southern Great Plains. *Crop Sci.*2002;42: 1259-1263.
- 4 Zhenghong L, Saxena KB, ChaohongZ,Jianyun Z, Yong G, Xuxiao Z and Shiyong Y. 2001 Pigeon pea: excellent host for Lac production. *ICPN.* 8:58-60.
- 5 Thu TT ,Mai TTX, Dewaele E, Farsi S, Tadesse Y, Angenon G and Jacobs M. In vitro regeneration and transformation of pigeon pea [*Cajanuscajan (L) Mill. Sp.*].*Mol. Breeding.* 2003;11:159-168.
- 6 Saxena KB, Kumar RV and Rao PV. Pigeon pea nutrition and its improvement. *Quality improvement in field Crops.* Food products Press, 2002: 227-260.
- 7 Zgallai, H., K. Steppe and R. Lemeur, Photosynthetic, physiology and Biochemical Responses of Tomato Plants to Polyethylene glycol induced water deficit.*J. Integr. Plant Biol.* 2005;47(12):1470-1478.
- 8 Y.Jiang, S.E.Macdonald and J.J. Zwiazak, ``Effect of cold storage and Water Stress on water Relations and Gas Exchange of White Spruce (*piceaglauca*) Seedlings. *Tree physiology,* 1995;15(4):267-273.
- 9 A. Ranjbarfordoei, R. Samson, P. V. Damne and R. Lemeur, “Effects of Drought Stress Induced by polyethylene glycol on Pigment content and Photosynthetic Gas Exchange of *Pistaciakhinjuk* and *P mutica*,” *Photosynthetic,* 2000;38(3): 443-447. doi:10. 1023/A: 1010946209484
- 10 O.Chezen, W. hartwig and P.M. Newman, “The different effects of PEG-6000 and NaCl on leaf development Are Associated with Differential Inhibition of root Water Transport,.*Plant Cell,* 1995; 18(7):727-735.
- 11 M. Ashraf and J. W.O’Leary, “effect of drought stress on Growth, Water Relations and Gas Exchange of Two Lines of Sunflower Differing in Degree of Salt Tolerance,” *Int J Plant Sci* 1996;157(6): 729-732. doi:10.1086/297395.

- 12 K. Singh and B. S. Afria, "Seed Technological Approach for Evaluation of Drought Tolerance in Wheat Germplasm," In: T.P. Yadav and C. Ram, Eds., Proc. Nation Semin. Seed Sci Tech, HAU, Hissar, 1988, pp. 72-178.
- 13 K. Singh and B.L. Kakralya, "Seed physiological Approach for Evaluation of Drought Tolerance in Groundnut Stress and Environmental Plant Physiology," In: K. K. Bora, K. Singh and A. Kumar, EDS., Pointer Publishers, Jaipur, Rajasthan, 2001, pp. 45-152.
- 14 Morghan, J.M.(1984) Osmoregulation and Water stress in higher plants. Annu. Rev. Plant Physiol., 35:299-319.
- 15 Lu, Z. and Neumann, P.M. Water –Stressed maize, barley and rice seedlings show species diversity in mechanisms of leaf growth inhibition. J.Exp. Bot., 1998;49:1945-1952.
- 16 Bohnert HJ, Nelson DE, Jensen RG. Adaptations to environmental stress. Plant Cell 1995; 7:1099-1111.
- 17 Michel, B.E. and M.R. Kaufmann. The osmotic potential of polyethylene glycol6000, plant physiol. 1973; 51: 914-916.
- 18 Kawamitsu Y, Driscoll T, Boyer JS. Photosynthesis during desiccation in an Inter tidal algae and a Land Plant. Plant Cell Physiol. 2000;41(3):344-353.
- 19 Boyer JS. Leaf enlargement and metabolic rates corn, Soyabean, and Sunflower at various leaf Water potentials. Plant Physiol 1970; 46:233-235.
- 20 Chaves MM. Effects of water deficits on Carbon assimilation. J. Exp. Bot. 1991;42:1-16.
- 21 Smirnoff N. Antioxidant systems and plant response to the environment. In: Smirnoff V (Ed.), Environment and scientific publishers, Oxford, UK 1995.
- 22 Arnon DI. Copper enzymes in isolated chloroplast Polyphenol oxidase in Beta vulgaris,- Plant physiol. 1949;24; 1-15.
- 23 O. H. Lowery, N. J. Rosenbrough, A. L. Farr and R. J. Randall, "Protein Measurement with Folin Phenol Reagent," The Journal of Biological Chemistry, 1951;193(1): 277-283.
- 24 Stanford Moore and William H. Stein. (1948). Photometric ninyhydrin method for use in the chromatography of amino acids. J. Biol Chem. 176:367-388.
- 25 J. Zhang, and M. B. Khirkham, "Water relations of Water Stressed Split Root C4 and C3 Plants," American Journal of Botany, 1995; 82(1):1220-1229. doi:10.2307/2446244.
- 26 Kpyoarissis A, Petropoulou Y, Manetas Y (1995) Summer Survival of leaves in a soft – leaved shrub (phlomisfruticosa L. Labaitae) under mediterranean field condition : avoidance of photoinhibitory damage through decreased chlorophyll contents. Journal of Experimental Botany 46:1825-1831.

- 27 Albert, R.S. and Thornber, J.P. Water Stress effect on the content and organization of chlorophyll in mesophyll and bundle sheath chloroplast. *Plant physiology* 1977;59:351-353.
- 28 Tomati, U., Veri, G. and Gall, E. Effect of Water status on photosynthesis and nitrate reductase activity in maize plants. *Review in Agronomy*, 1978;12:119-122.
- 29 Pratap, V and Sharma, Y.K. (2010) Impact of osmotic stress on seed germination and seedling growth in black gram (*Phaseolus mungo*) *Journal of Environmental Biology*, 31(5):721-726.
- 30 Guo, R., Hao, W.P., Gong, C.Z., Zhong, X.L. and Gu, F.X. Effects of Water Stress on Germination and Growth of Wheat, photosynthetic efficiency and accumulation of Metabolites. Chapter 13 in book "Soil Processes and current trends in Quality Assessment" edited by Maria C. and Hernandez Soriano. 2013:367-380.
- 31 Jeyaramaraj, P.R. Meenakshi, S.N., Kumar, R.S., Joshi, S.D. and Ramasubramanian, B. Water deficit induced oxidative damage in tea (*Camellia sinensis*) plants, *J. plant physiol.*, 2005;162:413-419.
- 32 K. K. Baruahand, K. Singh and A. Kumar, "Evaluation of Drought Tolerance in groundnut stress and Environmental Plant Physiology." Pointer Publishers, Jaipur, Rajasthan, 1998, pp. 145-152.
- 33 T.C. Hsiao, "Rapid Changes in the levels of polyribosomes in Zea mays in Response to Water Stress," *Plant physiology*, 1970;46(2):281-285.

AJPTR is

- Peer-reviewed
- bimonthly
- Rapid publication

Submit your manuscript at: editor@ajptr.com

