Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2015 Vol.4 (2) April -June, pp.67-70/Chhetri and Lepcha
Research Article

SIMULATED OSMOTIC STRESS INDUCES METABOLIC EFFECTS IN *RHODODENDRON ARBOREUM* SMITH

***Dhani Raj Chhetri and Dawa Lhendup Lepcha**
Department of Botany, Sikkim University, 6th Mile-Samdur, P.O. Tadong, Gangtok, Sikkim -737102, India
**Author for Correspondence*

ABSTRACT
Imposition of *in-vitro* abiotic stress on *Rhododendron arboreum* (*R. arboreum)* revealed rapid disruption of physiological and biochemical activities. Simulative environmental stress conditions i.e., drought induced by polyethylene glycol-6000 (PEG) increased the production of protein, soluble carbohydrates and proline with -0.5 MPa osmotic potential having a more pronounced effect on both protein and soluble carbohydrate production. PEG induced osmotic stress also led to membrane deterioration as evidenced by electrolyte leakage and membrane lipid peroxidation.

Keywords: *Abiotic Stress, Rhododendron Arboreum, Polyethylene Glycol, Water Stress, Osmotic Adjustment, Oxidative Damage*

INTRODUCTION
Rhododendron arboreum (*R. arboreum*) is an important ethnomedicinal plant of the Himalaya. The dried flowers of *R. arboreum* are used by Nepalese people for curing dysentery and diarrhea. A sip of the juice of *R. arboreum* flower is believed to dissolve fish bones stuck in the throat, *Rhododendrons* are one of the very few plants can grows up to an altitude of 5000 m in the Himalayas. *R. arboreum* was confirmed the most dominant species of the Himalaya (Tiwary and Chouhan, 2006). It is an excellent model for stress research because of its diversity of tolerance and susceptibility to environmental stress and possession of abilities to acclimate. Conditions of water stress can be created in the laboratory using polyethylene glycol, a biologically inert, non toxic chemical of high molecular weight. High molecular weight of PEG induces the condition of water stress similar to that caused by dry soil (Bajracharya, 1999). The cellular water deficit can result into changes in concentration of solutes, membrane structure, disruption of water potential and denaturation of proteins (Choudhuri and Choudhur, 1993). Cells subjected to water (osmotic) stress by exposure to hyperosmotic concentration of polyethylene glycol (PEG) responded with rapid accumulation of proline. Under stressed conditions, proline acts as a mediator of osmotic adjustment, a stabilizer of sub-cellular structures, a scavenger of free radicals and a contributor of cell wall structural proteins (Nanjo *et al.*, 1999). Abiotic stresses exert their effects directly or indirectly through the production of ROS and it was suggested that under prolonged oxidative conditions, active oxygen species would cause lipid peroxidation, DNA damage and protein denaturation (Scandalios, 1993). Cellular dehydration might be under the regulatory control of several proteins viz., membrane transport proteins, molecular chaperones and dehydrins that accumulates in response to dehydration.
Understanding the responses of plants to their external environment is an attractive target for improving stress tolerance. The objective of this study is to identify biochemical markers linked to stress tolerance traits so that the information may be used for future breeding and selection programme.

MATERIALS AND METHODS
Actively growing leafy twigs of *Rhododendron arboreum* Smith were collected from Darjeeling (87° 59′ – 88° 53′ E and 26° 31′ – 27° 13′ N). One leafy twig each was placed in Erlenmeyer flasks containing solution of PEG-6000 equivalent to -0.2 and -0.5 MPa osmotic stress (OS) levels. The flasks were covered with aluminium foil allowing only the end of the twigs to protrude. An appropriate control immersed in distilled water was also prepared. Young unopened leaves were harvested at the end of 7 and 14 days for analysis. Soluble protein was extracted from leaf tissue with 50 mM Tris-HCl buffer (pH 7.0)

and estimated following the method of Bradford (1976). Proline was extracted with 3% sulfosalicylic acid and estimated as per the method Bates *et al.*, (1973). Soluble carbohydrate was extracted from leaf tissue and estimated following the method of McCready *et al.*, (1950). Total phenol was extracted from the leaf sample and estimated as per the method of Malik and Singh (1980). The membrane injury index was measured as per the method of Sullivan (1972). Membrane lipid peroxidation was determined in terms of malondialdehyde (MDA) concentration in the leaf sample according to Heath and Packer (1968). Relative water content (RWC) from the sample leaves was determined as per Smart and Bingham (1974).

RESULTS AND DISCUSSION

On imposition of *in-vitro* osmotic stress on *R. arboreum*, the level of protein increased. The higher level of protein may be explained as a plant response to environmental stress. On extending the treatments to 14 days the rate of synthesis of protein declined than that in 7 day period (Table-1). The carbohydrate synthesis seemed directly proportional with the duration of treatment as well as extent of stress (Table-1). The role of carbohydrates in the development of stress tolerance has been demonstrated earlier (King *et al.*, 1988). A high level of proline accumulation in *R. arboreum* in osmotic stress conditions has been seen. The rate of increment was in direct proportion to the duration of treatment (Table-1).

Table 1: Effect of treatment with PEG-6000 (equivalent to -0.2 & -0.5 Mpa osmotic stress) on soluble proteins, soluble carbohydrates, proline and total phenol content (mg g $^{-1}$ fresh wt) of *R. arboreum*

Treatments	Control		$OS_{-0.2\ MPa}$		$OS_{-0.5\ MPa}$	
	Days after treatment		Days after treatment		Days after treatment	
Parameters	7	14	7	14	7	14
Soluble Protein (mg g $^{-1}$ fresh wt)	9.7 ± 0.79	10.2 ± 1.06	16.1 ± 1.03[a]	12.5 ± 0.44	17.6 ± 0.07[a]	12.3 ± 0.05
Soluble carbohydrate (mg g $^{-1}$ fresh wt)	5.5 ± 0.24	6.8 ± 0.17	7.7 ± 0.30[a]	11.0 ± 1.15[a]	10.5 ± 1.16[a]	16.5 ± 1.37[a]
Proline (mg g $^{-1}$ fresh wt)	2.6 ± 0.25	2.7 ± 0.20	5.7 ± 0.35[a]	6.5 ± 0.15[a]	3.1 ± 0.30	3.85 ± 0.18[a]
Total phenols (mg g $^{-1}$ fresh wt)	0.78 ± 0.03	0.40 ± 0.01	0.44 ± 0.007[a]	0.26 ± 0.06[a]	0.36 ± 0:01[a]	0.21 ± 0.01[a]

(The values are means± SE, n=3; [a]values significantly different at P ≤ 0.05)

Table 2: Effect of treatment with PEG-6000 (equivalent to -0.2 & -0.5 Mpa osmotic stress conditions) on relative water content (%), membrane injury index (%) and membrane lipid peroxidation expressed in terms of MDA accumulation (n mole g^{-1} fresh tissue) of *R. arboreum*

Treatments	Control		$OS_{-0.2\ MPa}$		$OS_{-0.5\ MPa}$	
	Days after treatment		Days after treatment		Days after treatment	
Parameters	7	14	7	14	7	14
Relative water content (%)	61.4 ± 1.40	60.6 ± 6.83	59.4 ± 0.80	49.9 ± 3.31	34.2 ± 1.42[a]	27.5 ± 1.80[a]
Membrane Injury Index (%)	61.0 ± 0.57	62.0 ± 1.00	72.0 ± 1.52[a]	79 ± 2.08[a]	62.0 ± 2.00	67 ± 2.08
MDA accumulation (n mole g $^{-1}$ fresh tissue)	10.96 ± 0.84	30.80 ± 2.70	18.54 ± 1.40[a]	32.41 ± 2.53	22.25 ± 1.65[a]	42.90 ± 1.45[a]

(The values are means± SE, n=3; [a]values significantly different at P ≤ 0.05)

It was found that the treatment at both -0.2 and -0.5 MPa OS uniformly raised the soluble protein level. However, over a prolonged period of treatment with the same stressors there is no rise in the protein levels. It may be due to breakdown of protein synthesis mechanism (Hsiao, 1970). In this study a positive linear correlation was seen between proline concentration and soluble sugar accumulation. These solutes can protect plants from stress through different mechanisms, including cellular osmotic adjustment, detoxification of reactive oxygen species, and protection of membrane integrity and stabilization of proteins (Ashraf and Foolad, 2007).

However, accumulation of proline may be a symptom of stress injury rather than an indication of stress tolerance (DeLacerda *et al.*, 2003).

A high level of proline in *R. arboreum* may be an indication of its accumulation in the cells as a stress response for offsetting the osmotic misbalance. Soluble carbohydrate also showed an increase in its content in the osmotic treatments. Secondary metabolites like polyphenols are the manifestation of stress response and its quantitative value may be used for quality determination of stress tolerant plants. During the present studies the level of total phenols almost halved at the end of the 14th day of stress treatment when compared with its content at 7th day. This was expected, because water is one of the raw materials of photosynthesis, and it directly impacts on organic synthesis of secondary metabolites (Cheruiyot *et al.*, 2007).

Determination of lipid peroxidation in terms of MDA accumulation revealed a gradual rise in MDA content from control to osmotic stressed conditions (Table-2). Abiotic stresses may cause hydrolysis of membrane components which cannot be repaired by other synthetic processes. The increased content of total phenols followed the same trend like that of MDA accumulation and the rate of increment also showed upward mobility with the increased duration of treatment (Table-1). Relative water content is an expression of water retaining capacity of plant organs. The RWC decreased with the stress treatments and in -0.5 MPa OS the rate of decrease was rather drastic (Table-2).

Lipid membranes are vulnerable targets for stress induced cellular damage and the extent of damage is commonly used as a measure of tolerance to the imposed stress. It was found that the membrane integrity was completely lost by stress treatment with -0.5 MPa OS. Significantly, ion leakage increased with increasing concentration of PEG which is in consonance with earlier results (Ackay *et al.*, 2010). MDA is a decomposition product of polyunsaturated fatty acids of biomembranes. In the present experiment, the accumulation of MDA was found to increase with the increased duration of stress treatment. This is in agreement with the results of Robert *et al.*, (1980). The results presented could contribute to our understanding of the physiology and biochemistry of osmotic stress and herald further studies on the abiotic stress tolerance mechanism in Rhododendrons.

ACKNOWLEDGEMENT

The Alpine Garden, 'Rhododendron Dale', Gandhi Road, Darjeeling is acknowledged for supplying the plant material

REFERENCES

Akcay UC, Ercan O, Kavas M, Yildiz L, Yilmaz C, Oktem HA and Yucel M (2010). Drought-induced oxidative damage and antioxidant responses in peanut (*Arachis hypogea* L.) seedlings. *Journal of Plant Growth Regulation* **61** 21-28.

Ashraf M and Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* **59** 206–216.

Bajracharya D (1999). *Experiments in Plant Physiology-a Laboratory Manual* (Narosa Publishing House, New Delhi).

Bates LS, Waldern RP and Teare ID (1973). Rapid estimation of free proline for water stress determination. *Plant Soil* **39** 205-207.

Bradford MM (1976). A rapid method for the quantitation of microgram quantities of proteins utilizing principle of protein-dye binding. *Analytical Biochemistry* **72** 248-254.

Chaudhuri K and Choudhuri MA (1993). Differential tolerance of seedlings of two jute species to NaCl salinity stress. *Indian Journal of Experimental Biology* **31** 90-92.

Cheruiyot EK, Mumera,LM, Ng'etich WK, Hassanali A and Wachira F (2007). Polyphenols as potential indicators for drought tolerance in tea (*Camellia sinensis* L.). *Biosciences Biotechnology and Biochemistry* **71** 2190-2197.

De-Lacerda CF, Cambraia J, Oliva MA, Ruiz HA and Prisco JT (2003). Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environmental and Experimental Botany* **49** 107–120.l

Heath RL and Packer L (1968). Photo oxidation in isolated chloroplasts: kinetics and stoichiometry of fatty oxidation. *Archieves of Biochemistry and Biophysics* **125** 189-198.

Hsiao TC (1970). Rapid changes in the levels of polyribosomes in *Zea mays* in response to water stress. *Plant Physiology* **46** 281-285.

King AI, Joice DC and Reid MS (1988). Role of carbohydrates in diurnal chilling sensitivity of tomato seedlings. *Plant Physiology* **86** 764-768.

Malik CP and Singh MB (1980). *Plant Enzymology and Histo Enzymology* (Kalyani Publishers, New Delhi).

McCready RN, Guggloz J, Silviera V and Owens AS (1950). Determination of starch and amylase in vegetables. *Analytical Chemistry* **22** 1156-1158.

Nanjo T, Kobayashi MY, Yoshiba Y, Kakubar K, Yamaguchi-Shinozaki Y and Shinozaki K (1999). Antisense suppression of the proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana. FEBS Letters* **461** 205–210.

Robert R, Stewart C and Bewly JD (1980). Lipid peroxidation associated with accelerated ageing of soybean cotyledons. *Plant Physiology* **65** 245-248.

Scandalios JG (1993). Oxygen stress and superoxide dismutase. *Plant Physiology* **101** 7-12.

Smart RE and Bingham GE (1974). Rapid estimates of relative water content. *Plant Physiology* **53** 258–260.

Sullivan CY (1972). Mechanism of heat and drought resistance in grain sorghum and methods of measurements. In: *Sorghum in Seventies,* edited by Rao NGP and Hase LR (Oxford and IBH publishing Company, New Delhi) 247-264.

Tiwari ON and Chauhan UN (2006). Rhododendron conservation in Sikkim Himalaya. *Current Science* **90** 532-541.