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METABOLIC CHANGES IN *RHODODENDRON ARBOREUM* SMITH IN RESPONSE TO ABIOTIC STRESS INDUCED BY SALINITY AND ABSCISIC ACID

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ABSTRACT

Studies on *in-vitro* abiotic stress on *Rhododendron arboreum* revealed rapid disruption in the physiological and biochemical activities when simulated stress conditions i.e., salinity and cold were induced by sodium chloride (NaCl) and abscisic acid (ABA). All the stress treatments increased the production of protein, soluble carbohydrates and proline. Salinity induced stress led to maximum membrane deterioration as evidenced by electrolyte leakage and membrane lipid peroxidation. Phenols, known for its antioxidant activity could maintain its level with 100 µM ABA treatment; in all the other cases, there was drastic downslide in the total phenol content. Carotenoids levels were generally enhanced and chlorophyll levels generally decreased in the given stress treatments. However, ABA caused stimulation of photo system-I activity.

KEYWORDS: Antioxidant Enzymes, Osmotic Adjustment, Oxidative Damage, *Rhododendron arboreum*

ABBREVIATIONS

ABA = Abscisic Acid, CAT = Catalase, EC = Electrical Conductivity, MDA = Malondialdehyde, OS = Osmotic Stress, PEG = Polyethylene Glycol, POX = Peroxidase, RWC = Relative Water Content

INTRODUCTION

Studies on the physiological and biochemical responses of *R. arboreum* under abiotic stress conditions are lacking. Understanding the responses of plants to their external environment is an attractive target for improving stress tolerance (Madhusudhan et al., 2002). An understanding of the physiological mechanisms and identification of specific characteristics conferring stress tolerance could play a major role in the development of new varieties suitable for such stress conditions (Chhetri and Mukherjee, 2003).

The effect of NaCl stress can be classified as osmotic, nutritional and ionic toxicity of which ionic toxicity is the primary stress and the other two are the secondary stresses. Under NaCl stress the plasma membrane permeability is disrupted which leads to reduced nutrient uptake. NaCl stress also mimics the effects of oxidative stress and results into enhanced peroxidative damage to the membrane systems mediated by oxygen radicals. Salt stress and dehydration stress show a high degree of similarity with respect to physiological, biochemical, molecular and genetic effects. Sub-lethal salt stress is ultimately an osmotic effect, which is apparently similar to that brought in by water deficit and to some extent by cold as well as heat stresses (Almoguera et al., 1995). Salinity affects various aspects of plant growth and metabolism such as osmotic adjustment, ion uptake, pigment content, protein and amino acid metabolism etc.

Exposure of plants to ABA induces the cold acclimation process and many cellular changes have been reported to

occur. These include alterations in soluble carbohydrate content, changes in cell protein profile and changes in membrane lipid composition. Many of the soluble compounds synthesized during cold acclimation are responsible for ameliorating the harmful effects of freeze-induced cellular dehydration. Plants develop freezing tolerance at non-acclimating temperatures when treated with exogenous ABA. Many of the genes or proteins expressed in low temperatures or with water deficit are also inducible by ABA (Taiz and Zeiger, 1998). This supports the role of ABA in cold and water deficit tolerance.

Many plants accumulate free proline in response to the imposition of environmental stresses such as drought, high salinity, and low temperature. 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. Thus proline is regarded as having multiple roles for stress tolerance in plants (Nanjo et al., 1999). It has been demonstrated that stress induced synthesis of proline is the result of *de novo* synthesis of amino acid and not from protein degradation. Proline is synthesized from L-glutamic acid via Δ^1-Pyrroline 5-carboxylate (P-5-C) by the mediation of enzymes, P-5-C synthetase and P-5-C reductase. The synthesis of P-5-C is induced by ABA during water stress and salinity. ABA also represses the enzyme proline dehydrogenase responsible for proline degradation, thus causing its accumulation.

Abiotic stresses exert their effects directly or indirectly through the production of ROS and ROS scavenging is a common response to most stresses (Srivalli et al., 2003). It was suggested that under prolonged oxidative conditions, active oxygen species would cause lipid peroxidation, DNA damage and protein denaturation (Scandalios, 1993).

The present work is an attempt towards understanding the metabolism in *R. arboreum* available in this part of the Himalaya. Effect of different abiotic stress conditions imposed in the laboratory viz., application of ABA that mimics the expressions of cold stress and NaCl that provides halophytic environment, have been tested. Improvement of plant stress resistance is highly important and should involve both conventional breeding and biotechnological approaches for improving biodiversity as well as crop production.

MATERIALS AND METHODS

Plant Material, and Stress Treatments

Fresh juvenile actively growing leafy twigs of *Rhododendron arboreum* Smith were collected from, Alpine Garden, situated between 87° 59′ – 88° 53′ East longitude and 26° 31′ – 27° 13′ North latitude in the Darjeeling hill (ca 2134 m amsl) of Eastern Himalayas in the morning and brought to the laboratory for pretreatment. One leafy twig each was placed in Erlenmeyer flasks containing abscisic acid (ABA) solution (50 and 100 µM l^{-1}) and NaCl (200 and 500 µM l^{-1}). The stems were cut under water periodically. The flasks were covered with brown paper and aluminium foil allowing only the end of the twigs to protrude. An appropriate control immersed in distilled water was also prepared. The experimental plant material were kept at 20 ˚C ± 2 ˚C with a RH of 78% ± 2% and a 16 h photoperiod. Young unopened leaves were harvested at the end of 7 and 14 days for analysis.

Determination of Proteins, Proline and Soluble Carbohydrates

For the estimation of protein 1 g of leaf tissue was homogenized in 19 ml of 50 mM Tris-HCl buffer (pH 7.0) with neutral sand. The sample was centrifuged at 15,000 rpm for 15 minutes. The pellet discarded and the protein was estimated from the supernatant following the method of Bradford (1976) with BSA as the standard. For the extraction of proline,

200 mg fresh leaf tissue was homogenized with 10 ml 3% sulfosalicylic acid and centrifuged at 4, 000 rpm for 10 min; the supernatant was used as the source for proline which was estimated with acid-Ninhydrin reagent using pure proline as standard as per the method Bates et al., (1973).

Soluble carbohydrate was extracted from 100mg of leaf tissue from each treatment which were homogenized separately with 5ml 80% ethanol and centrifuged at 3,000 rpm for 10 minutes. The supernatant was taken in a watch glass and evaporated to dryness and the chlorophyll was removed by washing with solvent ether. Residual material was taken in a test tube by washing the inner surface of the watch glass (three times) with 80% ethanol and making up the volume to 5 ml with the same. The sample served as the source of soluble carbohydrate. Estimation of soluble carbohydrates was done with anthrone reagent following the method of McCready et al., (1950).

Extraction and Estimation of Total Phenols

For the extraction of total phenols 1 g leaf tissue was homogenized in 10 ml 80% ethanol and centrifuged at 10,000 rpm for 20 minutes and the supernatant collected. The pellet was re-extracted with 5 ml of 80% ethanol and centrifuged at 10,000 rpm for 20 minutes. Both the supernatants were pooled together, taken in a watch glass and evaporated to dryness. The residue was dissolved in 5 ml of distilled water which served as crude phenol extract. Estimation of total phenol was done with the help of a standard curve prepared from 0-100 µg/ml catechol as per the method of Malik and Singh, (1980).

Determination of Antioxidant Enzyme Activities

For the extraction of catalase 500 mg plant material was homogenized with 10 ml 0.2 M chilled Na-phosphate buffer (pH-6.8) containing 1% PVP and centrifuged at 5,000 rpm for 10 minutes. The pellet was discarded and the supernatant was used as crude source of the enzyme extract. The enzyme assay was carried out according to the method of Snell and Snell (1971). For the estimation of peroxidase activity 500 mg tissue was homogenized with 5 ml 300 µm Na-phosphate buffer (pH-6.8) and centrifuged at 1,000 rpm for 10 minutes. The supernatant was used as the crude enzyme source and the enzyme activity was estimated following the method of Kar and Mishra (1976). The enzyme activities in both the cases were calculated by the formula of Fick and Qualset (1975) as given hereunder:

$$\text{Enzyme activity} = \Delta A \times Tv / t \times v \text{ units minute}^{-1} \text{ g}^{-1} \text{ fresh weight}$$

where, ΔA = O.D. difference of reaction set – control set,

Tv = total volume of enzyme extract,

t = time of incubation,

v = volume of enzyme extract taken for reaction

Determination of Membrane Injury Index and Membrane Lipid Peroxidation

For the measurement of membrane injury index 200 mg of leaf tissue was taken in glass vials and incubated with 15 ml deionised water at 25 °C. Electrical conductivity of the leachate was measured after 24 hrs with a direct reading conductivity meter. Subsequently, the tissue along with the leachate was autoclaved at 15 lb/cm^2 for 15 minutes and the conductivity was measured again. The injury index was calculated using the formula of Sullivan (1972) as follows:

$$\text{Percent injury} = 1 - \frac{1-(T_1/T_2)}{1-(C_1/C_2)} \times 100$$

where, C_1/C_2 is the EC of control sample before and after autoclaving,

T_1/T_2 is the EC of stressed sample before and after autoclaving

Extraction for the membrane lipid peroxidation was made from 200mg sample tissue which were homogenized in 5 ml of 0.1% TCA and centrifuged for 5 minutes at 10,000 rpm in Plastocrafts superspin-C centrifuge. The supernatant was collected as sample extract and lipid peroxidation was determined in terms of malondialdehyde (MDA) concentration according to Heath and Packer (1968).

Determination of Relative Water Content

For the determination of relative water content (RWC) ten discs from 2nd and 3rd leaves from the top of *Rhododendron arboreum* twigs were collected. After wet mass determination, the leaf discs were floated on distilled water for 24 h at 25°C. The hydrated discs were weighed to determine the turgid mass (TM). The leaf tissues were subsequently dried in an oven at 60 °C for 48 h and weighed to determine the dry masses. RWC was calculated as per Smart and Bingham (1974) using the following formula:

$$\text{RWC (\%)} = \frac{(\text{WM-DM})}{(\text{TM-DM})} \times 100$$

where, WM = wet mass, DM = dry mass and TM = turgid mass

Determination of Photosynthetic Pigments

Photosynthetic pigments (chlorophyll a, b and carotenoids) were extracted and estimated from fresh leaf samples of different cultivars. For the estimation of photosynthetic pigments 50 mg leaf tissue was homogenized with 5 ml 96% ethanol and centrifuged for 10 minutes at 5,000 rpm and the supernatant collected. Absorbance of the pigment extract was measured at 665, 649 and 470 nm. The different pigment contents were calculated following the method of Lichtenthaler and Welburn (1983) using the following formula:

Chlorophyll-a = $(13.95 \times A_{665} - 6.88 \times A_{649})$ µg ml^{-1}

Chlorophyll-b = $(24.96 \times A_{649} - 7.32 \times A_{665})$ µg ml^{-1}

Carotenoids = $(1000 \times A_{470} - 2.05 \times \text{chl.a} - 11.48 \times \text{chl.b})$ µg ml^{-1}

Chl-a, Chl-b and caroteinoids were finally expressed in terms of mg g^{-1} of tissue.

Duplicate assays involving three replications were performed for each determinations and standard error (±SE) of the means were calculated for n=3. A‘t’ test was used to compare the treatment value with the control value. The values which were significantly different as compared to the control value at 5% level of significance are superscripted with the letter ‘a’.

RESULTS

Soluble Proteins and Soluble Carbohydrates

During the study involving the in-vitro imposition of abiotic stress on *R. arboreum*, a relatively high level of protein content was found in all the treatments. The level of protein increased when the plants were subjected to any sort of dehydration stress. The highest level of protein was found in the leaves of *R. arboreum*. Only in treatment with 100 µM NaCl the increment in the protein level was not that appreciable. Besides the simple indication of overall vigor, the higher level of protein in *R. arboreum* may be explained as a plant response towards meeting the environmental stress conditions. When the treatments were extended over to 14 days the rate of synthesis of protein declined than that in 7 day period. However, it remained generally higher than the control. (Table 1). Several of the proteins induced in vegetative cells by water deficit and exposure to ABA (Taiz and Zeiger, 1998).

The role of carbohydrates in the development of stress tolerance has been demonstrated in tomato seedlings (King et al., 1988). The synthesis seemed directly proportional with the duration of treatment (Table 1). The highest levels of soluble carbohydrate content have been found in the treatment with 100 µM ABA during the present studies. The syntheses of low molecular weight solutes are accumulated due to stress response (Zuther et al., 2004).

Proline Content

The present finding indicates a high level of proline accumulation in *R. arboreum* in all the stress conditions. The rate of increment was in direct proportion to the duration of treatment. Only, in case of ABA treatment the level of proline remained almost identical over a two week period (Table 1). The role of proline in protecting plants from osmotic stress is well known. Proline level has been reported to increase in response to low temperature (Songstad et al. 1990).

Membrane Injury and Membrane Lipid Peroxidation

In the present study when membrane stability was tested in terms of membrane injury, it was found that all type of abiotic stress had considerable damaging effects on cell membrane. The water content increases and the dry matter content gradually declined with the increased duration of the treatment. This may be due to the fact that the cellular macromolecules cannot hold its own, for long under stressed conditions and gets degraded. Assessment of one of the products of lipid peroxidation in terms of MDA accumulation revealed a gradual rise in MDA content from control to salt stressed conditions (Table 2). Abiotic stresses may bring about hydrolysis of membrane components which cannot be repaired by other synthetic processes.

Total Phenol Content

Higher level of total phenol content was the hallmark of all the abiotic stress conditions applied during the present study. 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 3).

Activity of Antioxidant Enzymes, CAT and POX

The activity of the free radical scavenging enzymes catalase and peroxidase may be an indication of the vigor and the capacity for stress tolerance of plants. In the present study, the highest level of CAT and POX activity in *R. arboreum* has been recorded at- 100 µM ABA stress (Figure 1-2). In general the activity of catalase showed an increase in all the

stress conditions over the control value. The activity shows a steady increase with the increased duration of the stress treatments (Figure 1). The activity of peroxidase decreased due to the stress treatment, except in case of the ABA treatments. However, on increasing the duration of the treatment the activity was drastically reduced (Figure 2).

Relative Water Content (RWC) and Photosynthetic Pigments

Relative water content is an expression of water retaining capacity of plant organs. The RWC slightly decreased with all the stress treatments applied. The RWC showed increased decline with increasing duration of abiotic stress treatment (Table 3).

Photosynthetic pigments like chlorophyll-a either maintained its level or showed a decline during the stress treatments, with increasing duration of the treatment resulting in decline. Chlorophyll-b was invariably degraded on 14 days stress treatment and carotenoids either maintained its level or showed an increased content but as the treatment duration increased it showed a marked decline (Table 4). Impaired chlorophyll development may be due to interference with the synthesis of proteins, the structural components of chloroplasts (Nag et al., 1981)

DISCUSSIONS

In the present studies the soluble protein level was found to increase almost two fold in response to all osmotic stress like NaCl (200 µM and 500 µM) and ABA (50 µM and 100 µM) over a 7 day period. However, the salinity treatment to a level of 500µM may have become too toxic to raise the protein level to such extent. An abnormally high ratio of Na^+ to K^+ and high concentration of total salts inactivates enzymes and inhibits protein synthesis. ABA treatment at both 50 and 100µM concentrations uniformly raised the soluble protein level during this study. Application of ABA mimics cold stress conditions. Protein synthesis is necessary for the development of freezing tolerance and several distinct proteins accumulate during acclimation to cold as a result of changes in gene expression (Guy, 1990). Thus, ABA induced increase in protein synthesis is not that surprising. These proteins are thought to protect cells dehydrated by water shortage or freezing by stabilizing other proteins and membranes (Taiz and Zeiger, 1998). ABA induced synthesis of proteins responsible for salt stress tolerant properties in plants have already been reported. Significant level of cold tolerance can be induced by the application of ABA to intact plants, callus and suspension cultures. In Alfalfa, some induced proteins are common to both low temperature and ABA treatments (Mohapatra et al., 1988). However, over a prolonged period of treatment with the same stressors there is no rise in the protein levels which is evident even in the two week period of this study. It may be due to breakdown of protein synthesis mechanism or due to reduced incorporation of free amino acids into protein (Hsiao, 1970).

A common response of plants to environmental stresses is overproduction of different compatible organic solutes which are highly soluble and non-toxic at high cellular concentrations. Soluble sugars and proline are such organic solutes (Pei et al., 2010). There is strong correlation between increased cellular proline levels and the capacity to survive both water deficit and the effect of high environmental salinity (Sairam and Tyagi, 2004). A common observation is that proline protects the proteins against dehydration and maintains a favorable osmotic gradient in the cell. The synthesis of various low molecular weight sugars, such as glucose, fructose and sorbitol is a common feature of low temperature acclimation (Sakai and Larcher, 1987). Soluble carbohydrate showed an increase in its content in all the treatments performed here. It may be assumed that even the short exposure to abiotic stressors during the treatment was sufficient to synthesize new carbohydrates.

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 phenolics decreased gradually with the increasing duration of the treatment. Only in the treatment with 100 µM ABA the level of total phenols could be maintained. Interestingly enough in all the treatments the level of total phenols almost halved at the end of the 14^{th} day of stress treatment when compared with its content at 7^{th} day.

Injury to plants from salt stress may be due stress induced membrane damage, lipid peroxidation caused by free radicals (Cakmak and Horst, 1991). Lipid membranes are vulnerable targets for stress induced cellular damage and the extent of damage is commonly used to as a measure of tolerance to the imposed stress. Usually, there is a threshold level of salt concentration beyond which glycophytes begin to show growth inhibition, leaf discoloration and loss of dry weight. The plasma membrane can be the primary target of both osmotic and ionic stresses. NaCl stress also mimics the effects of oxidative stress as a secondary stress and results in enhanced peroxidative damage to the membrane system (Singh et al., 2003). MDA is a decomposition product of polyunsaturated fatty acids of biomembranes. It shows greater accumulation in response to abiotic stress conditions. In the present experiment, the accumulation of MDA was found to increase with the increased duration of stress treatments. This is in agreement with the results of Robert et al. (1980) and Cakmak and Horst (1991). Only ABA at 50 µM could prevent the increased accumulation of MDA.

The ability of plant tissues to mobilize enzymatic defense against uncontrolled production of reactive oxygen species (ROS) may be of great importance for plant survival under stress conditions. Catalase and peroxidase serve the indispensible role of preventing the accumulation of H_2O_2 (Singh et al., 2003). Similarly, an excessive synthesis of carotenoids in the photosynthetic tissue has been considered as part of cellular defense against the oxidative stress. In this study the activity of CAT increased with all the stress treatments and linearity was maintained between increased activities with increased duration of treatment. That may be because CAT does not require reducing equivalents for its function; its function might not be affected by prolonged stress unlike other mechanisms (Mittler 2002). Toxic H_2O_2 is produced in plants as a response to various stresses. POX acts as scavenging enzyme destroying H_2O_2. It is well known that peroxidases are often the first enzymes to alter their activities during stress, and in several cases enhanced activities have been observed under stress (Srivalli et al., 2003). High level of POX activity has been reported from *Picea asperata* (Yang et al. 2008) and *Capparis ovata* (Ozkur et al., 2009). However, in the present analysis POX activity showed a little or slightly more decline in all the stressors applied except ABA.

In general, both the abiotic stress treatments caused a decline in chlorophyll content, but the carotenoid level increased, giving the characteristics of senescent leaves. Among the photosynthetic pigments, chlorophyll-b showed a remarkable increase with 100 µM ABA and carotenoid content showed a trend of increasing accumulation with NaCl at 200 and 500 µM. With the increased duration of stress treatment there was gradual loss of carotenoids. Abrupt rise in chlorophyll-b levels by ABA suggests that ABA is probably involved in the stimulation of photo system-I activity. This is in consonance with earlier studies (Piotrowska et al., 2010). The results presented could contribute to our understanding of the mechanism of salinity and cold stress in Rhododendrons.

CONCLUSIONS

The essence in all these stress conditions to the plant is the deprivation of water, in one way or other. The studies on *R. arboreum*, investigating the biochemical basis of abiotic stress responses are rare. The present results may be

important to illustrate the role of different stress factors in the physiology of *R. arboreum*, a tree that dominates the Himalayan flora and shows a high level of adaptations to extreme cold, drought, desiccation, sunlight and windy conditions.

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APPENDICES

Table 1: Effect of Treatment with Different Stress Conditions (200 & 500 µm NaCl and 50 & 100 µm ABA) on Soluble Proteins, Soluble Carbohydrates and Proline Content (mg g $^{-1}$ Fresh Wt) of *R. arboretum* (The Values are Means± SE, N=3; ^{a}Values Significantly Different at $P \leq 0.05$)

Treatment	Soluble Protein (mg g $^{-1}$ Fresh Wt)		Soluble Carbohydrate (mg g $^{-1}$ Fresh Wt)		Proline (mg g $^{-1}$ Fresh Wt)	
	Days after Treatment		Days after Treatment		Days after Treatment	
	7	14	7	14	7	14
Control	9.7 ± 0.79	10.2 ± 1.06	5.5 ± 0.24	6.8 ± 0.17	2.6 ± 0.25	2.7 ± 0.20
$NaCl_{200}$	17.5 ± 0.40^{a}	12.4 ± 0.70	8.6 ± 0.94^{a}	9.8 ± 1.12	3.1 ± 0.25	3.9 ± 0.17^{a}
$NaCl_{500}$	11.4 ± 1.36	11.5 ± 0.45	7.3 ± 0.17^{a}	14.0 ± 0.40^{a}	6.4 ± 0.68^{a}	5.6 ± 0.68^{a}
ABA_{100}	16.2 ± 0.70^{a}	12.3 ±0.50	9.3 ± 0.75^{a}	14.9 ± 0.73^{a}	5.6 ± 0.32^{a}	5.2 ± 0.17^{a}

Table 2: Effect of Treatment with Different Stress Conditions (200 & 500 µm NaCl and 50 & 100 µm ABA) on Membrane Injury Index (%) and Membrane Lipid Peroxidation Expressed in Terms of MDA Accumulation (n Mole g^{-1} Fresh Tissue) of *R. arboreum*. (The Values are Means± SE, N=3; ^{a}Values Significantly Different at $P \leq 0.05$)

Treatment	Membrane Injury Index (%)		MDA Accumulation(n Mole g $^{-1}$ Fresh Tissue)	
	Days after Treatment		Days after Treatment	
	14	7	7	14
Control	61.0 ± 0.57	62.0 ± 1.00	10.96 ± 0.84	30.80 ± 2.70
$NaCl_{200}$	63.0 ± 1.15	70.0 ± 3.21	15.32 ± 1.49	33.28 ± 1.31
$NaCl_{500}$	69.00 ±.3.05	73.0 ± 1.15^{a}	16.77 ± 1.61^{a}	41.61 ± 3.78
ABA_{50}	61.0 ± 2.51	75.0 ± 2.51^{a}	10.80 ± 1.83	38.22 ± 3.61
ABA_{100}	64.0 ± 1.52	66.0 ± 2.08	15.32 ± 1.50	36.12 ± 2.93

Table 3: Effect of Different Stress Treatments (200 & 500 µm NaCl and 50 & 100 µm ABA) on Changes in Relative Water Content (%) and Total Phenol Content (Mg g $^{-1}$ Fresh Wt) of *R. arboreum*. (The Values are Means± SE, N=3; ^{a}Values Significantly Different at $P \leq 0.05$)

Treatment	Relative Water Content (%)		Total Phenols (mg g $^{-1}$ Fresh Wt)	
	Days after Treatment		Days after Treatment	
	7	7	7	14
Control	61.4 ± 1.40	60.6 ± 6.83	0.78 ± 0.03	0.40 ± 0.01
$NaCl_{200}$	56.3 ± 2.17	51.6 ± 1.22	0.40 ± 0.17	0.21 ± 0.02^{a}
$NaCl_{500}$	49.0 ± 2.51^{a}	38.4 ± 1.72^{a}	0.37 ± 0.02^{a}	0.25 ± 0.007^{a}
ABA_{50}	57.8 ± 1.63	57.0 ± 5.03	0.34 ± 0.02^{a}	0.20 ± 0.03^{a}
ABA_{100}	52.4 ± 1.10^{a}	48.3 ± 2.03	0.85 ± 0.03	0.41 ± 0.007

Table 4: Effect of Different Stress Treatments (200 & 500 µm NaCl and 50 & 100 µm ABA) on Changes in Photosynthetic Pigments: Chlorophyll-A, Chlorophyll-B and Carotenoids (mg g $^{-1}$ Fresh Wt) of *R. arboreum*. (The Values are Means± SE, n=3; ^{a}Values Significantly Different at $P \leq 0.05$)

Treatment	Chlorophyll-a (mg g $^{-1}$ Fresh Wt)		Chlorophyll-b (mg g $^{-1}$ Fresh Wt)		Carotenoids (mg g $^{-1}$ Fresh Wt)	
	Days after Treatment		Days after Treatment		Days after Treatment	
	7	14	7	14	7	14
Control	1.1 ± 0.20	1.2 ± 0.10	0.5 ± 0.11	0.5 ± 0.15	2.4 ± 0.30	2.5 ± 0.40
$NaCl_{200}$	1.5 ± 0.17	1.1 ± 0.10	1.1 ± 0.15^{a}	0.7 ± 0.15	2.6 ± 0.26	2.0 ± 0.20
$NaCl_{500}$	1.3± 0.26	1.4 ± 0.15	0.6 ± 0.10	0.3 ± 0.05	2.5 ± 0.15	1.8 ± 0.25
ABA_{50}	0.8± 0.05	1.2 ± 0.30	0.7 ± 0.17	0.5 ± 0.11	3.6 ± 0.26^{a}	2.1 ± 0.20
ABA_{100}	0.9±0.15	0.6 ± 0.10^{a}	1.7 ± 0.17^{a}	0.4 ± 0.11	2.8 ± 0.20	1.9 ± 0.17

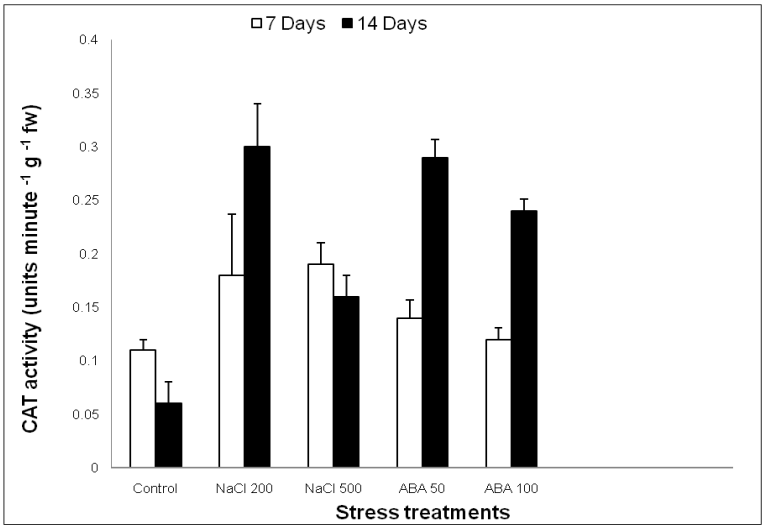

Figure 1: Changes in CAT Activity (Units Minute^{-1} g^{-1} FW) in Response to Different Stress Conditions (200 & 500 µm NaCl and 50 & 100 µm ABA) on *R. arboretum* (Values are Mean of Duplicate Assays, Each Consisting of 3 Replicates. Y-Error Bars Represent SE)

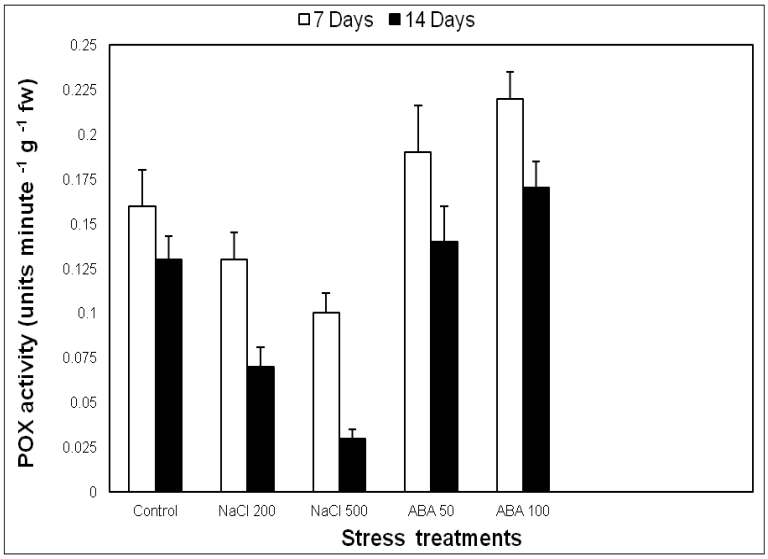

Figure 2: Changes in POX Activity (Units Minute^{-1} g^{-1} FW) in Response to Different Stress Conditions (200 & 500 µm NaCl and 50 & 100 µm ABA) on *R. arboretum* (Values are Mean of Duplicate Assays, Each Consisting of 3 Replicates. Y-Error Bars Represent SE)