

The Effects of Salicylic Acid and Ascorbic Acid on Some of Resistance Mechanisms to Drought Stress in *Echium amoenum*

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Abstract: Water is one of the most important environmental factors that regulate plant growth and development and water deficit is considered as the most important restricted factors for plant products, therefore many compounds were applied to minimize the harmful effects of this stress. Two of these compounds which have antioxidant characteristics are salicylic acid and ascorbic acid that the effects of them in this study were investigated. In order to investigate the relief effects of external salicylic acid and ascorbic acid on some of biochemical and physiological criteria of *Echium amoenum*, an experiment including different combination of three levels of water (1/3, 2/3 and 3/3 Field Capacity), three levels of salicylic acid (0, 1 and 3 mM) and three levels of ascorbic acid (0, 1 and 3 mM) with a completely randomized factorial design and three replications was done under green house conditions. The results showed that increasing the level of aridity the amount of protein reduced; however, lipid peroxidation, proline content, sugar content and ion leakage were increased. Results also concluded that drought stress affected the plants and activated protective mechanisms in them. The reduction of protein content in this investigation revealed that drought stress influenced metabolism and photosynthetic process as well. In general, adding salicylic acid and ascorbic acid significantly relieved the harsh effects of aridity on growth parameters of *Echium amoenum* plants and it seems two external acids were able to enhance the tolerant ability of the plant to aridity stress.

Key words: *Echium amoenum* · Drought stress · Salicylic acid · Ascorbic acid

INTRODUCTION

Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world [1,2]. To improve crop productivity, it is necessary to understand the mechanism of plant responses to drought conditions with the ultimate goal of improving crop performance in the vast areas of the world where rainfall is limiting or unreliable. In addition to the complexity of drought itself [1,2], plant's behavior responses to drought are complex and different mechanisms are adopted by plants when they encounter drought [3,4]. One mechanism utilized by the plants for overcome the water stress effects might be via accumulation of compatible osmolytes, such as proline [5,6] soluble sugars [7]. Production and accumulation of free amino acids, especially proline by plant tissue during drought, salt and water stress is an adaptive response. Proline has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm. Thus,

proline can be used as a metabolic marker in relation to stress [8]. Also protective mechanisms of proline still are not distinguished completely but his roles are more include: the mitigation of osmotic pressure fixation of macromolecules and broom of oxygen types [9]. Moreover, under drought stress the accumulation of total soluble sugars in different plant parts would be increased [10]. However the rate of additional production or accumulation of proline and soluble sugar is different in different plant parts.

Echium amoenum Fish. and C.A. Mey. belongs to the Boraginaceae family and is a biennial or perennial herb indigenous to the narrow zone of northern part of Iran and Caucasus, where it grows at an altitude ranging from 60-2200 m [11]. *Echium* genus has 4 species in Iran [12] and only *E.amoenum* has medicinal uses [13,14]. Dried violet-blue petals of *E. amoenum* has long been used as a tonic, tranquillizer, diaphoretic and as a remedy for cough, sore throat and pneumonia is known in traditional medicine of Iran as Gol-e-Gavzaban [13,15].

MATERIALS AND METHODS

In this study *Echium amoenum* plants were sown in plastic pots fully expanded leaves were appeared, Salicylic acid and ascorbic acid were applied to the plants at three levels of concentration (0, 1 and 3 mM) for a six weeks period. Three level of drought stress (1/3, 2/3 and 3/3 field capacity) were applied. Then the interaction effects of drought stress with Salicylic acid and ascorbic acid investigated on some of physiological and biochemical parameters.

Proline content in leaf tissue was determined according to method of Bates *et al.* [16]. 0.2 g of leaf fresh tissue was weighted and was abraded in china mortar of 10 ml sulfosalicylic acid 3%. The achieved juice was centrifuge device in 10000 G for 5 min. Then 2 ml of the above solution from centrifuge with 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent (1.25 g ninhydrin acid, 30 ml of glacial acetic acid, 20 ml NH_3PO_4 (6M)) and incubated for 1 h at 100°C. The reaction was stopped by placing the test tubes in cold water. The samples were rigorously mixed with 4 ml toluene. The light absorption of toluene phase was estimated at 520 nm using spectrophotometer. The proline concentration was determined using a standard curve. Results of measuring proline content was calculated and presented with $\mu\text{mol/l}$. Sugar content of leaf tissue and root was calculated with method Somogy [17]. The Results of measuring sugar content was calculated and presented with mg/l Protein percentage according to the method of Lowry *et al.*, [18]. For calculating the content of per oxidation in membrane fats, the concentration of Malon-Dialdehyde (MDA) from this reaction was measured. The measurement of MDA concentration was done according to Heat and Pacher [19] method. 0.2 g of leaf fresh tissue was weighted and was abraded in china mortar of 5 mL Trichloroacetic acid (TCA) 0.1%. The achieved juice was centrifuge device in 10000 G for 10 min. About 5.4 mL TCA solution Which is 20% and contains 0.5% Thiobarbituric acid (TBA) was added to 1 mL of the above solution from centrifuge. The achieved compound was heated in hot bath in 95°C for 30 min. Then become cool in ice immediately and again the compound was centrifuged in 10000 G for 10 min. The intensity absorption of this solution was read by spectrophotometer in wavelength of 532 nm. The specified compound for absorption this wavelength is red complex (MDA- TBA). The absorption of other nonspecific pigments was measured in 600 nm and reduced from this content for calculating MDA

concentration from silence coefficient was used and the results from measuring was calculated and presented according to milligram on liter. Ion leakage content for measuring leakage of cell membrane was evaluated by method of Marty *et al.* [20].

Statistical analysis: In this study, the total of experiments were done in different stages in Completely Randomized Design with 3 replications and test considers the reciprocal effect of Salicylic acid, ascorbic acid and drought on different parameters as factorial. The levels of 0(control), 1/3 FC and 2/3 FC of water stress were used and The levels of Salicylic acid and ascorbic acid factors were 0 mM(control), 1 mM and 3 mM. The comparison of means was done with Duncan test to SPSS 12.0 software in probability level of 1%. For drawing graph, we used Excel 2003 software.

RESULTS

In this study, drought stress cause increasing of proline content compared to check plants. Use of salicylic acid, ascorbic acid and the compounds of these 2 substances on the plant, which are in drought stress conditions caused an increasing of proline content of plant leaves (Fig a). Drought stress also increased sugar content in leaf and decreased in root. Salicylic acid, ascorbic acid and the compounds of these 2 treatment significantly caused mitigation and improved damage from drought stress on sugar concentration in root and leaf of *Echium* (Fig b). Moreover, Drought stress significantly decreased protein content considerably. But salicylic acid, ascorbic acid and the compounds of salicylic acid with ascorbic acid treatments on *Echium amoenum* leaves caused alleviating the effects of drought stress on protein content of leaf (Fig c).

The MDA and other aldehydes was significantly increased under drought stress. The treatment salicylic acid, ascorbic acid and drought stress significantly decreased Malon-Dialdehyde (MDA) and other aldehydes content (Fig d,e). Also drought stress significantly increased of ion leakage compared to check plants. Application of salicylic acid, ascorbic acid and the compounds of these 2 substances on the plant, which are in drought stress conditions cause decreasing of ion leakage of plant leaves (Fig f).

Fig. a-f) The effect of drought stress, salicylic acid and ascorbic acid on the proline content, sugar content, protein content, MDA content, other aldehyd and ion

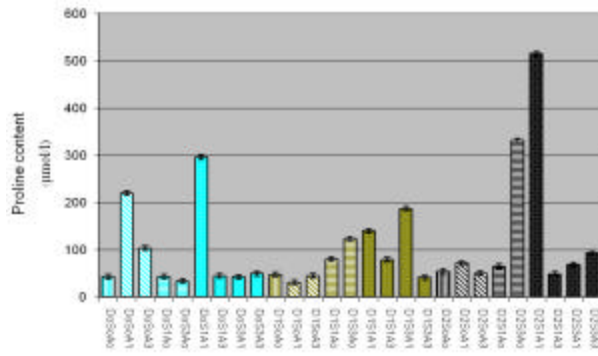


Fig a

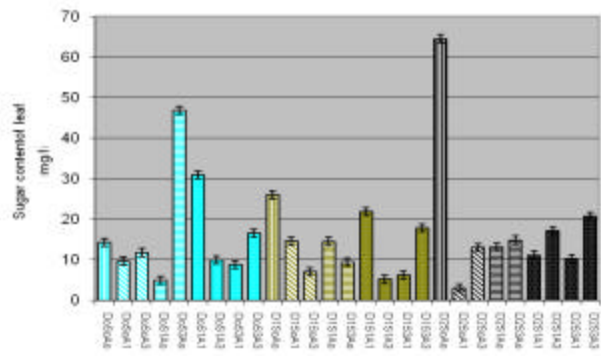


Fig b

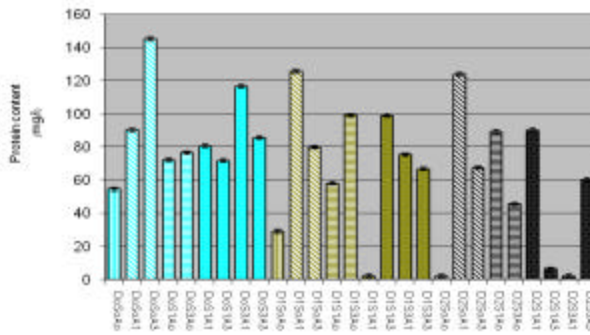


Fig c

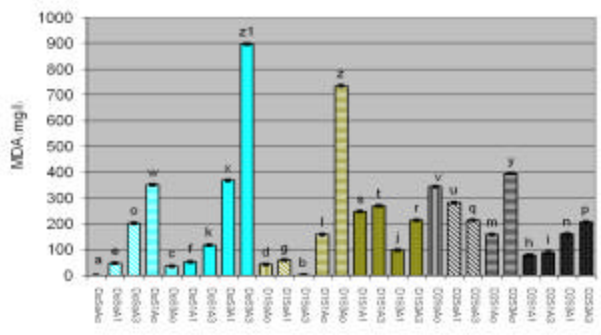


Fig d

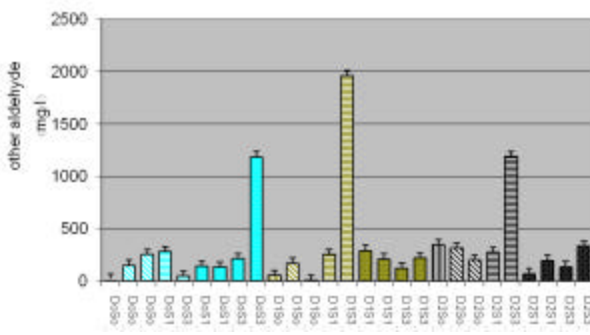


Fig e

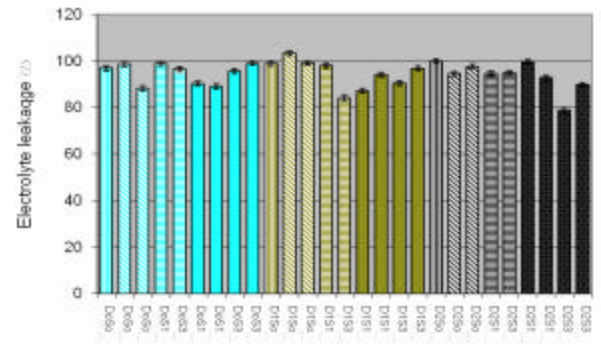


Fig f

Fig. a-f: The effect of drought stress, salicylic acid and ascorbic acid on the proline content, sugar content, protein content, MDA content, other aldehyd and ion leakage on leaf, respectively. (D₀,D₁, D₂ respectively drought about control, 1/3 , 2/3 field capacity) S₀,S₁,S₃ respectively salicylic acid 0,1,3 mM and A₀,A₁,A₃ respectively Ascorbic acid 0,1,3 mM)

leakage on leaf, respectively. (D₀, D₁, D₂ respectively drought about control, 1/3, 2/3 field capacity) S0, S1, S3 respectively salicylic acid 0, 1, 3 mM and A0, A1, A3 respectively Ascorbic acid 0, 1, 3 mM).

DISCUSSION

The increases in the concentration of proline and soluble carbohydrates in three rice cultivars leaves were found to be remarkable during drought stress. These results suggest that the production of these osmotic adjustments is a common response of plants under drought conditions. The role of proline in adaptation and survival of plants has been observed by Watanabe *et al.* [21] and Saruhan *et al.* [22]. Osmotic adjustment through the accumulation of cellular solutes, such as proline, has been suggested as one of the possible means for overcoming osmotic stress caused by the loss of water [8]. Proline is a non-protein amino acid that forms in most tissues subjected to water stress and together with sugar, it is readily metabolized upon recovery from drought [23]. In addition to acting as an osmo-protectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger [24], as a solute that protects macromolecules against denaturation and as a means of reducing acidity in the cell [25], who reported that proline content was increased in drought stress condition [26].

Sairam *et al.* [27] reported that in increasing proline leads to increasing drought and salt resistance. Who also reported that increasing proline and sugar cause protecting turgor and the reduction of membrane damage of plants. So osmo regulation is an adaptation that increase the tolerance toward drought stress [28]. In barley, wheat, Broad bean and tomato during oxidative stress the accumulation of proline content with treatment salicylic acid hormone and ascorbic acid was increased and increasing proline and sugar contents and producing osmo dip in plant caused resistance against losing leaf water and increasing the plant growth rate under stress conditions [29].

It has been shown that, the concentration of sugars increased under drought stress in *Echium amoenum*. The accumulation of sugars in response to drought stress is also quite well documented [7,21]. A complex essential role of soluble sugars in plant metabolism is well known as products of hydrolytic processes, substrates in biosynthesis processes, energy production but also in a sugar sensing and signaling systems. Recently it has been claimed that, under drought stress condition, even

sugar flux may be a signal for metabolic regulation [25]. Soluble sugars may also function as a typical osmo-protectant, stabilizing cellular membranes and maintaining turgor pressure. The presence of genes functionally associated with other a biotic stresses among the drought-up-regulated genes suggested that different stresses share some common signaling pathways [30].

In this study, the protein content of plant leaf was reduced significantly under drought stress than the check. The active types of oxygen produced and accumulated under the contrary environmental conditions like drought stress. Along with it the increasing H₂O₂ increased protein oxidation in some plant species. Drought is the case of reducing the activity of rubisco and also the content of on plant [28]. Drought stress decreased the production of protein in some species of plants by decreasing cell polyzoms. Active radicals of oxygen with different conditions of amino acid in proteins caused easing effect of analyzing proteins enzymes so one of the reason of decreasing protein content in plants which are in drought stress is producing free radicals of oxygen [17]. It is reported that salicylic acid effects on producing defensive proteins and different kinds of kinases and rubisco. Also it is proved that salicylic acid inculcates the synthesis of suppressive proteins of plants proteases [31]. El-Tayeb [32] reported that the protein solution content and free amino acid in aerial organs and roots were reduced under stress conditions. So, reducing protein content or increasing analyzes or both of them can be related to increasing activity of antioxidant enzymes. In the manner that salicylic acid and ascorbic acid protect from oxidation of plant proteins by increasing the ability of antioxidant [27,33].

In recent study, drought stress increased MDA and other aldehyds, MDA was increased in 3 genotype of wheat under drought stress [27]. The observed increase of MDA in this study under drought stress condition, which results from producing active oxygen types like superoxide radicals, hydrogen peroxide and hydroxide radicals. Also, who proved that produced MDA content is different between cultivars of maize, banana and rice during drought stress; so that resistance species on drought stress can broom H₂O₂ with increasing ability of anti oxidation and by decreasing H₂O₂ produced less MDA. But the amount of produced MDA of sensitive cultivars are more [34]. Miguel [33] reported that ascorbic acid can refuse from lipids per oxidation by omitting active oxygen types and decreased MDA. In the case of salicylic acid role for solving oxidative stress, there are many

reports. Salicylic acid protects Arabidopsis against drought stress by effect on antioxidation enzyme and per oxidation of lipids [35].

One of the parameters that is measured as an index of destruction membrane is ion leakage that the amount was increased under stress condition and in this case, that per oxidation of de saturation fat acids in membrane phospholipids caused an increased electrolyte leakage. So, ion leakage can be calculated as a norm for estimating the damage of bio-membrane [36]. When resistant plant after a period of anhydrous is in a good irrigation condition, showed some signs of cell damage. One of these signs is ion leakage. Recent studies show that during cells dehydration, amphiphylc molecules enters membrane from cytoplasm humor that the existence of these molecules in membrane cause ion leakage and again in a good irrigation conditions, these molecules come out of membrane and in this case the ion leakage stop [37].

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