A Study on the Impact of Drought Stress on Changes of Free Proline in Two Species of *Haloxylon persicum* and *Haloxylon aphyllum*

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**ABSTRACT**

For the purpose of studying the changes of free proline as one of the important metabolites in plants under drought stress, the seeds of two types of *Haloxylon persicum* and *Haloxylon aphyllum* were planted in vases. Then their resulting twigs were taken care for one year and after one month compatibility with greenhouse environment, they underwent drought stress operations. The study of changes of this osmolyte in the branchlet and roots of the twigs of these two types of haloxylons were programmed within the format of a Completely Randomized Design with two treatments of species and fifteen treatments of tension (avoiding irrigating the twigs). The two species of *Haloxylon persicum* and *Haloxylon aphyllum* and the levels of 0 (control), 2, 4 ... and 28 days of no-irrigation were determined as treatments of the experiment. The free proline was measured by using Bates method. The analysis of data was done through the method of two sides’ variance analysis and averages were compared by using Duncan’s test. The study of the data of branchlet showed that the impact of drought stress on the increase of the rate of proline in both types of haloxylons was meaningful with a 99% possibility, however, no meaningful changes was observed between these two species. The analysis of the data of the both species roots confirmed a very meaningful impact of the factor of tension and the meaningful impact of the factor of species on changes of free proline. The study of the impact of drought stress on changes of the quantity of total proline showed that the changes of this feature is in a full similarity with the changes of the proline of the root and following the same model. The increase of the rate of proline of branchlet, root and total proline was in agreement with tension intensity.

**Key words:** Haloxylon, drought stress, free proline, resistance to dryness

**INTRODUCTION**

Drought stress is considered a very important factor in the accumulation of proline in the cells of the plants under tension (Ditmarova *et al.*, 2010; Behnamnia *et al.*, 2009; Mohamed *et al.*, 2007; Ennajeh *et al.*, 2006; Javadi *et al.*, 2006; Kavi-Kishor *et al.*, 2005; Yamada *et al.*, 2005; Thiery *et al.*, 2004; De Ronde *et al.*, 2000a, b; Yoshiha *et al.*, 1997; Dallmier and Stewart, 1992; Sells and Keppe, 1981; Quarrie, 1980). Proline is one of the important sources of energy (Kohl *et al.*, 1991; Walton *et al.*, 1991) which is accumulated in response to the shortage of water and low water potential of the growth environment as compared with other amino acids in a greater rate in plants under drought stress (Heuer, 1999; Kuznetsov and Shevyakova, 1999; Gzik, 1996; Williamson and Slocum, 1992; Ranney *et al.*, 1991; Rhodes *et al.*, 1986). Proline as an adjusting
and signaling molecule, by activating the multifold responses in line with the process of compatibility (Peng et al., 2000; Hare et al., 1999; Harrak et al., 1999; Girousse et al., 1996; Berteli et al., 1995; Chen and Kao, 1995; Buhl and Stewart, 1983; Hanson et al., 1979; Waldren and Teare, 1974; Bardziki et al., 1971), regulates the osmotic active nitrogen in line with the stability of the cellular membrane (Ashraf and Harris, 2004) and makes possible the resistance of plant against dryness (Van Heerden and De Villiers, 1996; Oregan et al., 1993; Van Rensburg et al., 1993; Singh et al., 1972).

Proline is among the natural combinations of organic plants which acts as compatible materials (Claussen, 2005; Yoshiha et al., 1992) or counteracting materials of the impact of drought stress (Samaras et al., 1993). Despite synthesis of proline from glutamate or ornithine (in regular conditions of growth), Glutamate is considered to be the pre-maker of its accumulation in the condition of drought stress (Sairam and Tyagi, 2004; Kuznetsov and Shevyakova, 1999; Lutts et al., 1996; Paleg and Aspinall, 1981). The accumulation of proline, in addition to protecting the enzymes of cells against damaging impacts of drought stress (Zaifihejad et al., 1997), is effective on inducing or activating the enzymes of its own biosynthesis too (Kavi-Kishor et al., 2005; Rhodes, 2001) and its measuring will be an important scale to determine the endurance of plant against drought stress (Cicek and Cakirlar, 2002).

However, some researchers believe that the accumulation of free proline in response to drought stress is undeniable, but they consider its solidarity with the adjustment of osmosis and maintenance of cellular turgidity is slight and insignificant (Delauney and Verma, 1993). It is taken for granted that the accumulation of this osmotic material in the conditions of drought stress [depending on the type of plant species and intensity of tension (Kavi-Kishor et al., 2005)] is of specific significance from the viewpoint of protecting the cells of plant against damaging effects (Kuznetsov and Shevyakova, 1999; Berteli et al., 1995; Samaras et al., 1995; Csonka, 1989). Furthermore, it protects the protein enzymes against rinsing resulting from osmotic tension (Bandurska, 1983). Proline makes possible the adjustment of osmosis [reduction of osmotic potential (Mohamed et al., 2007) and increase of water potential (Ditmarova et al., 2010)] and makes possible the maintaining of cells turgidity (Singh et al., 1972).

Drought stress is the factor for the consumption of glucose and leads to the accumulation of free ammonium (NH$_4^+$ and H$_2$N$^-$) and poisonous state of cells of plants in the early periods of tension but the activating state of the process of de-poisoning of cells along with the intensification of tension (Rabe, 1990) and reduction of the activity of the enzyme of proline dehydrogenase (Dalmier and Stewart, 1992) leading to accumulation of combinations containing nitrogen (including proline) which in xerophyte plants, it has a considerable impact on osmotic adjustment of the liquid part of cytoplasm of cells (Renard and Guerrier, 1997) and makes possible the continuation of the survival of plant in drought short periods (Sanchez et al., 1998; Kuznetsov and Shevyakova, 1997; Taylor, 1996).

The motivation of the synthesis of proline from glutamic acid, the reduction of its export through rinsing vessels, the reduction and prevention from its oxidation (Sells and Koepppe, 1981) during tension (Stewart, 1997; Kiyo et al., 1996; Boggess et al., 1976) and the destruction and disturbance in the process of synthesis of proteins (Harrak et al., 1999; Roosens et al., 1999; Girousse et al., 1996; Hanson et al., 1979; Waldren and Teare, 1974) are recognized as four main factors for the increase of accumulation and accumulation of proline in drought stress (Lutts et al., 1996).
MATERIALS AND METHODS

In order to study the effect of drought stress on changes of the quantity of proline of branchlet, roots and also the changes of the total proline in two types of *H. persicum* and *H. aphyllum*, their seeds were planted in plastic vases. They were taken into care for one year at the Center of Agriculture and Natural Resources Researches of Kerman (Center of Kerman Province located at the south east of Iran). The plastic vases were selected in a five-liter capacity to provide possibility for a better and greater growth of the roots of twigs. Their soil consisted of wind sands, clay and leaf-soil in ratio of 2, 1 and 1 accordingly. The aim of including leaf-soil in the mentioned combination was to supply nutrition necessary for twigs during the experiment period. At this status, in order to prevent from unwanted accumulation of water in vases, fine holes were created at their bottom.

After one year, the twigs were transferred to the central greenhouse and for the purpose of their compatibility with the condition of greenhouse; the treatments of drought operations were conducted one month after the transfer of twigs into greenhouse. For this purpose, a sufficient number of good and healthy twigs were selected for testing. Half of them were considered for applying drought treatments and the rest as the control that were irrigated every two days.

In this research, for each of the two types of haloxylon, 2 to 28 days of drought tension (avoiding twig irrigation) were applied regularly and with the time span of two days. So, totally 15 drought stress treatments (including control and 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28 days lack of irrigation) and 2 treatments of species (*H. persicum* and *H. aphyllum*) were determined as test treatments. In each test (measuring the quantity of proline), branchlets and roots of 5 twigs were cut (repetition of measuring in each tension treatment).

For measuring the content of free proline, the method of Bates *et al.* (1973) was used. The obtained data was reviewed within the format of a Completely Randomized Design and by using the method of two-side variance analysis. Comparison of averages was done by using Duncan’s test. The quantity of each data in the tables of average comparison was the result of at least five measuring.

RESULTS

The analysis of the variance of proline changes in the branchlet of both types of haloxylon showed that drought stress with a possibility of 99% in both species was effective on changes of the rate of proline but no meaningful changes were observed between the two species (Table 1). The above-mentioned model was prevailing in respect of changes of proline of roots in both types of haloxylon but the difference resulting from the application of tension between the two types was observable and meaningful at the level of 5% (Table 2).

The study and comparison of the averages of the impact of drought stress on changes of proline of both types of haloxylon branchlets (Table 3) showed that it is possible to classify the mentioned

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Table 1: Analysis of variance of free proline changes in the branchlet of *H. persicum* and *H. aphyllum*

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (A)</td>
<td>2-1=1</td>
<td>90.271</td>
<td>90.271</td>
<td>0.947</td>
</tr>
<tr>
<td>Stress (B)</td>
<td>15-1=14</td>
<td>1970.8</td>
<td>1407.178</td>
<td>14.760**</td>
</tr>
<tr>
<td>Interaction (AB)</td>
<td>1×14=14</td>
<td>440.321</td>
<td>31.451</td>
<td>0.33</td>
</tr>
<tr>
<td>Sum</td>
<td>29</td>
<td>32231.09</td>
<td>697.923</td>
<td>-</td>
</tr>
<tr>
<td>Error</td>
<td>120</td>
<td>11440.12</td>
<td>95.334</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>31671.2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**p<0.01
impacts in three categories of treatment A (0 to 2 days lack of irrigation), B (4 to 10 days of lack of irrigation) and C (12 to 28 days of lack of irrigation), such that the difference between each category with the previous category and after that at the level of 1% became meaningful. The difference between treatments in the first category was not meaningful. In the second category, the difference of each treatment with the previous treatment and after that was meaningful at the level of 5% and in the third category at the level of 1%. The difference between the treatments of tension with the control treatment was started from the treatment of 4 days lack of irrigation at the probability level of 95%. It continued from the treatment of 6 days lack of irrigation onwards at the level of 1% meaningfulness (reliability level of 99%). The intensity of the impact of drought stress on changes of the rate of the proline of branchlet in both types of haloxylon was different in different categories. It was such that the least impact was related to the category A and the highest rate of impact was related to the category C. The intensity of the impact of tension in category C from the treatment of 20 days lack of irrigation onwards in both types of haloxylon was accelerated. The study of the impact of the type of species in changes of the rate of proline of branchlet indicates the lack of a meaningful difference among these species (Table 3).

The impact of drought stress on the average of changes of proline of root in both types of *H. persicum* and *H. aphyllum,* despite a similarity with the model of branchlet, with a slight different was associated in the levels of treatments of tension and treatments of species
Table 4: The impact of the rate of drought stress on average changes of free proline (milligram per gram wet weight) of root of *H. persicum* and *H. aphylhum*

<table>
<thead>
<tr>
<th>Stress intensity (days of no-irrigation)</th>
<th><em>H. aphylhum</em></th>
<th><em>H. persicum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.82±0.0641</td>
<td>1.22±0.0312</td>
</tr>
<tr>
<td>2</td>
<td>1.94±0.0244</td>
<td>1.33±0.0291</td>
</tr>
<tr>
<td>4</td>
<td>2.63±0.0323</td>
<td>2.10±0.0320</td>
</tr>
<tr>
<td>6</td>
<td>3.24±0.0291</td>
<td>2.81±0.0301</td>
</tr>
<tr>
<td>8</td>
<td>4.11±0.0322</td>
<td>3.61±0.0319</td>
</tr>
<tr>
<td>10</td>
<td>5.14±0.0344</td>
<td>4.59±0.0330</td>
</tr>
<tr>
<td>12</td>
<td>6.45±0.0607</td>
<td>5.38±0.0315</td>
</tr>
<tr>
<td>14</td>
<td>8.11±0.0629</td>
<td>5.18±0.0340</td>
</tr>
<tr>
<td>16</td>
<td>10.68±0.0650</td>
<td>7.53±0.0328</td>
</tr>
<tr>
<td>18</td>
<td>13.37±0.0400</td>
<td>9.29±0.0317</td>
</tr>
<tr>
<td>20</td>
<td>16.40±0.0332</td>
<td>11.61±0.0345</td>
</tr>
<tr>
<td>22</td>
<td>19.72±0.0662</td>
<td>14.05±0.0330</td>
</tr>
<tr>
<td>24</td>
<td>23.10±0.0872</td>
<td>17.12±0.0305</td>
</tr>
<tr>
<td>26</td>
<td>26.60±0.0595</td>
<td>20.46±0.0372</td>
</tr>
<tr>
<td>28</td>
<td>31.71±0.0644</td>
<td>23.89±0.0364</td>
</tr>
</tbody>
</table>

*The common figure shows the meaningfulness at the level of 5%, two common figures shows the lack of meaningfulness and uncommon figures means its meaningfulness at the level of 1%.

(Table 4). It is such that the impact of drought stress on changes of the proline of root can be placed in both types of haloxylon in three categories of treatment A (0 to 2 days lack of irrigation), B (4 to 12 days lack of irrigation) and C (14 to 28 days lack of irrigation). The difference between each category of treatment with the previous category and after that was meaningful with a possibility of 99%. The difference between treatments in the category of A was not meaningful but difference in each treatment with the previous treatment and after itself in the category B became meaningful with 95% probability. In category C, differences between treatments were observable and meaningful with the possibility of 98% too. The difference between treatments of tension with the control treatment like the branchlet started from the treatment of 4 days lack of irradiation at the level of 5% meaningfulness and continued from the treatment of 6 days lack of irrigation onwards at the level of 1% probability. The intensity of the impact of drought stress on changes of the rate of proline of root of both types of haloxylon in all three categories was different from each other. It was such that the minimum impact was related to category A and its maximum impact was related to category C. The intensity of the tension in category C from treatment of 14 days lack of irrigation onwards in both species of *H. persicum* and *H. aphylhum* accelerated. The study of the influence of the type of the species in the changes of the rate of proline of roots indicated a meaningful difference (level of 5%) between the two types of haloxylon. This difference was started from treatment of 14 days of lack of irrigation (in category C) and continued up to the treatment of 28 days of lack of irrigation (Table 4). The study of the impact of drought stress on changes of total proline (branchlet+root) by using the comparison of averages in both types of haloxylon (Table 5) confirms that there is a full similarity between this impact and the impact of drought stress on changes of proline of the root. It is such that the impact of tension on changes of total proline can be classified in three categories of treatment A (0 to 2 days of lack of irrigation), B (4 days to 12 days of lack of irrigation) and C (14 to 28 days of the lack of irrigation) in which the difference between each category with the previous category and after that was meaningful at the level of 1%. The difference between treatments in the first category (A) was not meaningful. In the
second category (B), the difference of each treatment with the previous treatment and after itself became meaningful at the level of 5% and in the category C at the level of 1%. The difference between treatments of tension and control treatment started from the treatment of 4 days of lack of irrigation at the level of probability of 95% and continued from the treatment of 6 days of lack of irrigation onwards at the level of 1% meaningfulness. The intensity of the impact of drought stress on changes of the rate of total proline of both types of H. persicum and H. aphyllum in different categories was different. It was such that the least impact related to category A and the greatest impact to category C. The intensity of the impact of drought stress on category C from treatment of 14 days of lack of irrigation onwards in both types of haloxylon accelerated. The study of the impact of the type of species on changes of the quantity of total proline indicated the meaningful difference at the level of 5% between the two species of H. persicum and H. aphyllum. This difference started from the treatment of 14 days of lack of irrigation (in category C) and continued up to the treatment of 28 days of lack of irrigation (Table 5).

DISCUSSION
In general, the process of compatibility of plants is divided into unfavorable factors such as drought stress into two stages. In the first stage, the non-exclusive protective mechanisms are developing in response to the factor of tension quickly and prepare the survival of plants for a short term and make the start of development of specific compatibility protective mechanisms. Usually at the first stage of compatibility, the rate of local proline does not reach to a maximum rate, so the increase of its quantity at this stage of reflection to tension is not meaningful (Kuznetsov and Shevyakova, 1999; Gzik, 1996) but at the second stage, when specific mechanisms are developing due to agreement against tension, the thickness of free proline is maximized and in these conditions, in addition to a protective role (Yamada et al., 2005) which they undertake, their impact in adjusting inter-cell osmosis increases (Kuznetsov and Shevyakova, 1999).

Synthesis and increase of proline as a result of drought stress is firstly a fast response in line with the compatibility of plants with new humid conditions and secondly it regulates the osmotic
potential of the plants under tension (Bardzik et al., 1971; Berteli et al., 1995; Blum and Ebercon, 1976; Boggess et al., 1976; Girousse et al., 1996; Levitt, 1980; Sojka et al., 1981).

Comparing the rate of proline of root and branchlet of two types of haloxylon (Table 6) showed that the rate of check (control) proline in the roots of *H. persicum* and *H. aphyllum* was 3.9 and 2.8 times of the quantity of this material in the branchlet of these species, respectively. In the highest rate of tension intensity (28 days of lack of irrigation) the rate of proline of the root of *H. persicum* was 1.8 times of branchlet and the rate of proline of the root of *H. aphyllum* was 1.25 times of branchlet.

Comparing the rate of proline of the branchlet of check (control) treatment (zero tension) and the treatment of 28 days of lack of irrigation (the highest intensity of tension) in the *H. aphyllum* species (Table 6) showed that the rate of proline of treatment of 28 days of lack of irrigation was 22 times of the check treatment. This issue was also true in the case of the root of *H. aphyllum*. It was such that the rate of proline of the mentioned treatment was 17.4 times of the check treatment, which like the branchlet indicates the intensity of increase of rate of proline of the root in harmony with the increase of the intensity of dryness. The study and comparison of the rate of proline of branchlet and the root of the check treatment and the treatment of 28 days of lack of irrigation in the *H. persicum* species (Table 6) was also similar to the type of *H. aphyllum*. It was such that this rate in the branchlet of the treatment of 28 days of lack of irrigation was 42.7 times of the check treatment and in the root of this treatment was 19.4 times of the check treatment. So, it can be claimed that in proportion with the intensity of tension in branchlet and root of both types of haloxylon, a great rate of proline is produced which possibly can be evaluated as the factor of keeping a physiologic balance of these species by having impact on the osmotic adjustment of cells.

The study on the results of impact of tension on the changes of the rate of proline showed that the rate of proline of branchlet and the root of *H. aphyllum* in all treatments of drought stress was greater than their parallel rate in the type of *H. persicum*. This can be possible interpreted in line with adjusting greater osmosis of the *H. aphyllum* in unfavorable humid conditions.

The osmotic adjustment is one of the important mechanisms in plants under drought stress which makes the endurance of plants towards dryness moves up (McNeil et al., 1999). When the potential of the water of soil is going down, the absorption of water by roots is reduced, consequently, the Relative Water Content (RWC) and potential of leaf-water also reduces. Plants which are resistant against dryness, keep the pressure of their turgidity up by using different mechanisms. This work is done by increasing combinations such as proline which its accumulation and thickness are the highest frequent reaction being observed when plant tissues are void of water (Paleg and Aspinall, 1981).
The results of this research showed that in the root and branchlet of both types of haloxylon, a great content of proline was accumulated in drought condition. For example, twelve days after tension (treatment of 12 days of lack of irrigation), the content of proline was 3.3 times of check treatment in the H. aphyllum and 5.4 times of the check treatment in H. persicum. Twenty eight days after tension (treatment of 28 days of lack of irrigation), the content of proline was 22 times of the check treatment in H. aphyllum and 42 times of this treatment in the type of H. persicum (Table 5). The previous researches have shown that with the reduction of the potential of leaf-water as a result of drought tension, the increase of the production of proline (up to 11 micromole in a day in gram wet weight of the plant) in the conditions of deduction of water, moves up the osmosis of plant syrup and increases the durability of plant towards conditions of dryness (Huang and Cavalieri, 1979).

In general, the results of this research showed that the rate of proline in all treatments of the tension of branchlet and root of both types of H. persicum and H. aphyllum increased and there was a positive linear relation between the content of proline and the rate of drought tension. The results of this research apparently is compatible with the results of the studies being done on accumulation of proline on other plants (Somal and Yapa, 1998; Sanchez et al., 1998; DeLauney and Verma, 1999). There are also reports that proline due to playing the role of osmosis supplies the survival of plants under tension condition (Stewart and Lee, 1974). In this study, also in line with previous studies (Sanchez et al., 1998; DeLauney and Verma, 1993), in both types of haloxylon under tension, in proportion with the increase of rate of tension, the rate of free proline was added. It was such that after application of drought stress of 28 days of lack of irrigation, the rate of proline of branchlet and root of both types of haloxylon showed a very meaningful difference with the control treatment. The start of difference in the average of changes of rate of proline of root and the total Proline at the level of 5% difference between two types of H. persicum and H. aphyllum from the treatment of 14 days of lack of irrigation onwards can be interpreted and evaluated in line with the higher intensity of osmotic adjustment in the Haloxylon aphyllum and its greater resistance in facing more intensive tensions of drought.

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