EFFECT OF WATER (DROUGHT) STRESS ON WATER RELATIONS OF
HALOXYLON APHYLLUM AND H. PERSICUM

N. ARABZADEH¹** AND S. F. EMADIAN²

¹Agricultural and Natural Resources Research Center, Kerman, I. R. of Iran
²Faculty of Natural Resources of Sari, Department of Forestry, Sari, I. R. of Iran
Email: arabzadeh_r_m@yahoo.com

Abstract – Root and shoot osmotic and elastic properties of two species of Haloxylon aphyllum and H. persicum in response to water stress were studied. The main goal of the study was to determine if the tissues of two species utilize osmotic adjustment and/or enhance their elastic property during prolonged water stress condition. The results showed that although tissue elasticity of both species of haloxylon was enhanced due to application of repeated water stress cycles, this condition also increased the tissue osmotic potential. Therefore, this study concluded that haloxylon species utilize enhancement of elasticity rather than osmotic adjustment to maintain positive turgor and withstand drought.

Keywords – Haloxylon, water relations, water stress, drought resistance, osmotic and elastic properties

1. INTRODUCTION

Haloxylon aphyllum and H. persicum are arid and semi-arid species that grow in areas with less than 200 mm annual rainfall like Kerman, Kashan, Qhum and several other arid areas in Iran. Haloxylon plants are important multi-purpose species that are used in the stabilization of sand dunes in some desert zones of Iran. Haloxylon afforestation projects are also very important for the wildlife ecosystem of some Iranian desert areas as they play an effective role in maintaining biological balance in such areas. However, unfortunately haloxylons plantation of Iranian desert areas started turning yellowish in color and eventually wilting at approximately age five. As a result, mortality rate also increased significantly. This situation provided the main incentive for researchers to perform this study with the main objective in mind to investigate the effects of water stress on water relations and drought resistance in haloxylon seedlings.

Drought resistance is stated as physiological and biochemical characteristics which cause a plant to survive limited soil water content. According to Turner [1], there are 3 types of drought resistance in plants: 1) Drought escape; 2) drought tolerance at high tissue water potential; and 3) drought tolerance at low tissue water potential. In drought escape, the plant completes its life cycle before the drought season starts, while in the second type of drought tolerance the plant maintains high tissue turgor potential despite experiencing a period of prolonged drought condition. The latter also refers to plant tolerance to water stress in spite of a reduction in tissue water potential. Some other plant species can also maintain their turgidity by means of osmotic adjustment or enhancement of cell wall elasticity in water stress condition and so can withstand prolonged periods of drought. In such plants turgidity maintenance takes place at high tissue water potential. Hennessy and Dougherty [2], Kandiko et al. [3], & Osonubi and Davies [4], showed turgidity maintenance in Pinus taeda, Tsuga heterophylla and English oak by osmotic adjustment. Turgidity maintenance by mechanism of cell wall elasticity enhancement was reported by Robichaux and
Canfield [5], & Parker and Pallardy [6], in the case of Dubautia ciliotala and Juglans nigra, respectively. Hsiao et al. [7], stated that variation in the mineral content of symplast causes osmotic adjustment. Increase of minerals in symplast is the result of organic matters synthesis in leaves or greater absorption of minerals by plant roots [8]. If osmotic potential reduces without any variation in the relative content of plant water, then osmotic adjustment is active. However, if water potential decreases due to reduction in the relative water content of the plant, then the reduction of osmotic potential is inactive [8].

Drought tolerance at high tissue water potential has been studied intensively in pine species by Youngman [9], Grim [10], & Emadian [11]. They studied different mechanisms of drought resistance such as continuity of plant water absorption by means of more massive and longer root production, reduction of water vapor conductivity from the cuticle and stomata of plant leaves, decrease in evaporation, osmotic adjustment and enhancement of tissue elasticity.

Water stress application on water relation has been studied in different plants. More recently, Hugo et al. [12] & Zgallai et al. [13] demonstrated that water stress caused an osmotic adjustment and a reduction in water potential in barley and tomato. It was also shown that in wheat water stress application reduced diurnal leaf water potential and leaf osmotic potential and thus enabled the plant to enhance its resistance to drought [14]. Moreover, Warwick and Thukten [15] reported that root/shoot ratio in acacia was associated with their tolerance to arid environments and the regulation of water relationship parameters, particularly osmotic potential. Water stress application improved the drought resistance of Zea mays by increasing water potential and turgor potential and decreasing the osmotic potential of the root and shoot tissues [16]. Wang [17] and Wang and Haung [18] also reported that water stress application increased drought resistance in Haloxylon ammodendron and Lolium and Poa species, respectively. Drought imposition rates can have a significant effect on the results of studies on drought resistance [19]. In another study it was found that water stress and moderate irradiance caused osmotic adjustment and increased growth in Fagopyrum esculentum [20]. A change in leaf water relations was noticed after 13 days of water stress application in Quercus. This was basically found to be associated with a significant decrease in leaf water potential at turgor loss point ($\Psi_{TLP}$) and a significant increase in bulk modulus of elasticity ($e$). It was believed that these two factors, together with a decrease in the tissue osmotic potential, contributed to the maintenance of turgor in the plant tissues [21].

2. MATERIALS AND METHODS

a) Seedling establishment

Seeds of Haloxylon aphyllum and H. persicum were obtained from the Research Institute of Repetec in Turkmenistan. The seeds were planted in a small plastic pot with suitable soil medium. The seedlings were raised in the Research Center of Agriculture and Natural Resources of Kerman in an open area for about a year, before they were transferred to a greenhouse for a one month-long acclimation period.

b) Water stress application

At the end of the acclimation period, water stress was applied to the seedlings. The seedlings were kept in the greenhouse during the treatment period. The treatment was initiated by selecting a sufficient number of good and healthy seedlings. Then 50% of the seedlings were used for water stress treatment and the rest were used as control, which were watered once every two days. The water stress treated seedlings received six cycles of water stress. Each cycle lasted 7-14 days. Predawn water potential of root and shoot tissues ($\Psi_w$) of water stress treated as well as control seedlings, were measured once every two days using Scholander [22] pressure chamber method. A separate set of seedlings were used for predawn water potential measurement. For each of the measurements 5 seedlings were sacrificed. Following the water
potential measurement, the remaining seedlings were watered to full saturation of the soil media in order to help the water stress treated seedlings replenish the lost water again.

At the end of each water stress cycle predawn water potential of shoot tissues of *H. aphyllum* and *H. persicum* were reduced to -16.2 and -14.2 bars, respectively and water potential of their root tissues were lowered to -14.0 and -12.1 bars, respectively.

c) Performing pressure–volume (P-V) curves

The effect of water stress application on osmotic and elastic properties of the roots and shoots of *H. aphyllum* and *H. persicum* was determined by analyzing their P–V curves. To obtain the curve, a 10-12 cm-long seedling was severed from the collar position under water in a deep tray filled with water. Prior to cutting, the seedlings were placed in a small bucket of water with a lid on and were kept in a refrigerator for at least 15 hours in order to decrease evapotranspiration and also help the seedlings reach full saturation. Next, the severed seedlings were dried with a piece of soft and absorbent cloth and then weighed. This was considered the tissue turgid weight of the root and shoot (W₀). Then the tissue was placed in a pressure chamber (Fig. 1) in such a way that the end cut of the tissue was protruding from the tight lid of the chamber. At this stage, the pressure of the chamber was slowly increased by opening a valve which led compressed air into the chamber until exuded sap appeared from the cut surface (Fig. 2). The valve was then closed and the pressure recorded as P₀. Then small pre-weighed (W₁) tygon tubing filled with small pieces of tissue paper was placed on the protruding cut surface of the tissue and simultaneously an increment pressure of 3 bars was added to the previous pressure. The increased pressure help the exuded sap collect in the tube. After about 5 minutes the tube was removed in order to weigh it again (W₂) and simultaneously the pressure was slowly reduced until the exuding sap began to disappear from the cut surface of the tissue. This pressure was recorded and considered as the first balancing pressure (P₁). The weight of the exuded sap under the first balancing pressure (Wₑ₁) was calculated by subtracting W₂ from W₁. After completion of this stage, another increment of 3 bars was added to the first balancing pressure and the procedure described above was followed in order to obtain the second pair of balancing pressure (P₂) and weight of the exuded sap (Wₑ₂). This procedure was repeated until a total of about 16 pairs of consecutive balancing pressures and their respective weight of exuded sap were obtained. This information was then used to plot the P-V curve for the experimented tissue.

---

**Fig. 1.** Pressure chamber system (Nitrogen gas operated instruments, Model 600, 2002)
Fig. 2. Scheme of how to insert a limb amputated plant pressure chamber system

d) Analyzing P-V curves

Each P-V curve has two distinct portions; a curvature portion consisting of nearly 5-8 points and a straight portion consisting of about 8-11 points. The straight portion of the curve was used to determine water potential (\(\Psi_w\)), osmotic potential (\(\Psi_s\)) and turgor (\(\Psi_p\)). This was achieved by performing a regression line for those points, which could be expressed as

\[
\frac{1}{\Psi_{st}} = \frac{1}{\Psi_{st0}} - m \sum w_i
\]

\[\text{[4]},\]

where, \(1/\Psi_{st0}\) is the inverse of initial osmotic potential at full tissue turgidity; \(m\) is the slope of regression curve which is affected by shoot or root size and osmotic characteristics of the tissue as well as the rate of the tissue water conductivity; \(1/\Psi_{st}\) is the inverse of \(\Psi_s\) for the \(r\)th pair of P-V data; and \(\sum w_i\) is the cumulative weight of the exuded sap. If the above equation holds true over the range P-V curve data, then \(\Psi_s\) at each point could be calculated by inserting each \(p_i\) and \(\sum w_i\) pair, in the equation.

The extension of the regression line intercepts the vertical axis at point B, which can be used to determine the inverse of osmotic potential \(\left(\frac{1}{\Psi_{st0}}\right)\) at tissue full turgidity of the tissue [23]. The tissue turgor potential (\(\Psi_p\)) at each point of the P–V curve was obtained by subtraction of the \(\Psi_s\) and \(\Psi_w\) values associated with the same point.

On the other hand, the extension of the regression line intercepts the horizontal axis at point E, which can be used to determine the volume of exuded sap of the tissue at extreme pressure \(\left(\frac{1}{\Psi_{st0}} / m\right)\) (Fig. 3).
This was considered as the symplastic weight of water ($W_s$). Thus, the ratio of the active osmotic weight of water ($W_{\Psi_a}$) of the tissue was calculated as: \[ \frac{W_s}{W_o - W_d} \] [24].

![Fig. 3. Pressure – Volume curve of a Haloxylon shoot and calculation of $E$ & $B$](image)

Horizontal axis is the weight of exuded sap ($w_e$) and vertical axis is the inverse of equilibrium pressure $\left( \frac{1}{\Psi_w} \right)$. Point $E$ is the intercept of osmotic line with $w_e$ axis; it shows the exuded sap from the tissue under the extreme pressure which also gives symplastic water ($w_s$). Point $B$ is the intercept of osmotic line with the axis $\frac{1}{\Psi_w}$ which shows shoot osmotic potential at full saturation. Horizontal axis is reciprocal of water potential $\frac{1}{\Psi_w}$ (bar) and vertical axis is $w_e$ (g).

**e) Weighted-average bulk modulus of elasticity ($\overline{\epsilon}$)**

The points associated with the curvature portion of the P–V curve were used to determine the tissue weighted-average bulk modulus of elasticity ($\overline{\epsilon}$) [23]. To obtain this parameter, it was necessary to first calculate the tissue $\Psi_p$ at the points associated with the curvature portion of the P–V curve. This was done by utilizing the osmotic line, which provided the means to estimate osmotic, turgor and water potentials at the desired points. Finally, the slope of this regression line was used as follows to calculate $\overline{\epsilon}$ (Fig. 4):

$$ \overline{\epsilon} = \left( \frac{d\Psi_p}{dw_e} \right) w_s $$

For analysis of the P-V curve, t-student was used to compare the effect of water stress application on water relation parameters in the tissue [25].
3. RESULTS AND DISCUSSION

Water stress application significantly modified the water relation parameters of the root and shoot tissues of both of the haloxylon species. At the end of each water stress cycle the predawn water potential of the shoot tissues of *H. aphyllum* and *H. persicum* were reduced to -16.5 (Fig. 5) and -14.1 (Fig. 6) bars, respectively and the water potential of their root tissues were lowered to -14.0 (Fig. 7) and -12.2 (Fig. 8) bars, respectively.

The shoot tissues of the water-stressed seedlings of *H. aphyllum* and *H. persicum* had $\frac{d\psi_p}{d\psi_w}$ values of -32.5 and -28.0 bars, respectively, which were significantly higher and thus, more elastic than the control tissues (with $\frac{d\psi_p}{d\psi_w}$ values of -44.8 and -39.5 bars, respectively) (Tables 1, 2). A similar result was obtained with respect to the root tissues. Water-stressed roots of *H. aphyllum* and *H. persicum* were significantly more elastic (with $\frac{d\psi_p}{d\psi_w}$ values of -30.7 and -26.2 bars, respectively) than the tissues in control seedlings (with $\frac{d\psi_p}{d\psi_w}$ values of -42.6 and -37.3 bars, respectively) (Tables 3, 4). A more elastic tissue in water-stressed seedlings of the haloxylon species implies that these species would able to maintain turgor under water stress condition.
Fig. 6. Relationship between shoot water potential ($\Psi_w$) and time of *Haloxylon persicum* after watering. Pre-dawn shoot water potential was measured every other day during a two-week period of water.

Fig. 7. Relationship between root water potential ($\Psi_w$) and time of *Haloxylon aphyllum* after watering. Pre-dawn root water potential was measured every other day during a two-week period of water.

Fig. 8. Relationship between root water potential ($\Psi_w$) and time of *Haloxylon persicum* after watering. Pre-dawn root water potential was measured every other day during a two-week period of water.
Table 1. Comparison of water relation parameters in water stress treated and control shoot tissues of *Haloxylon aphyllum* seedlings

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>$\Psi_{po}$ (Bar)</th>
<th>$\Psi_{so}$ (Bar)</th>
<th>$\Psi_{sTLP}$ (Bar)</th>
<th>$\varepsilon$ (Bar)</th>
<th>$%\Psi_s$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STRESSED</td>
<td>11.20</td>
<td>-16.50</td>
<td>-27.60</td>
<td>-32.50**</td>
<td>16.00</td>
</tr>
<tr>
<td>CONTROL</td>
<td>19.60**</td>
<td>-27.30**</td>
<td>-34.90**</td>
<td>-44.80</td>
<td>22.80**</td>
</tr>
</tbody>
</table>

On the contrary, water stress applications significantly raised the osmotic potential values of roots and shoot tissues in both species of the haloxylon at full saturation ($\Psi_{so}$) as well as at turgor loss point ($\Psi_{sTLP}$) relative to the values in the same tissues of the control seedlings (Tables 1-4). For instance, $\Psi_{so}$ values of water stressed shoots of *H. aphyllum* and *H. persicum* were of -16.5 and -14.7 bars, respectively, which were significantly higher than the $\Psi_{so}$ values of the shoot tissues in the control seedlings (-27.3 and -24.1 bars, respectively). Likewise, osmotic potential values of water stress treated roots of *H. aphyllum* and *H. persicum* were of -14.3 and -12.5 bars, respectively, which were significantly higher than the osmotic potential values of similar tissues of the control seedlings (-25.1 and -21.9 bars, respectively). The lower osmotic potential (more negative) values in the control tissues of the two haloxylon species were associated with higher tissue turgor potential values, while the opposite was true with the water stressed tissues (Tables 1-4). For example, turgor potentials in the shoots of the control seedlings for the two haloxylon species were 19.6 and 15.8 bars, respectively, which were significantly higher than the $\Psi_p$ values obtained in the shoot tissues of the water stressed seedlings of the two species (11.2 and 10.1 bars, respectively). Similarly, root turgor potential in control seedlings of the two haloxylon species were 16.8 and 14.9 bars, respectively, which were significantly higher than the values observed in the root tissues of the water stressed seedlings (10.4 and 9.3 bars, respectively). A similar response to water stress was observed in both drought-tolerant and drought-sensitive wheat cultivars by Gupta et al. [14], who found that water-stressed plants showed significantly lower turgor potential than the control plants.

Table 2. Comparison of water relation parameters in water stress treated and control shoot tissues of *Haloxylon persicum* seedlings

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>$\Psi_{po}$ (Bar)</th>
<th>$\Psi_{so}$ (Bar)</th>
<th>$\Psi_{sTLP}$ (Bar)</th>
<th>$\varepsilon$ (Bar)</th>
<th>$%\Psi_s$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STRESSED</td>
<td>10.10</td>
<td>-14.70</td>
<td>-23.10</td>
<td>-28.00**</td>
<td>13.00</td>
</tr>
</tbody>
</table>

The reason for the relatively higher level of turgor values noted in the tissues of the control seedlings than those in the water stressed ones is not known, however, this could be related to the sufficient soil moisture content in the control seedlings that should enable them to absorb more water and nutrients than the roots of the water-stressed seedlings, and that was probably why the tissues of these seedlings also showed a relatively lower osmotic potential than the tissues of the waters-stressed seedlings.

Table 3. Comparison of water relation parameters in water stress treated and control root tissues of *Haloxylon aphyllum* seedlings

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>$\Psi_{po}$ (Bar)</th>
<th>$\Psi_{so}$ (Bar)</th>
<th>$\Psi_{sTLP}$ (Bar)</th>
<th>$\varepsilon$ (Bar)</th>
<th>$%\Psi_s$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STRESSED</td>
<td>10.40</td>
<td>-14.30</td>
<td>-25.80</td>
<td>-30.70**</td>
<td>13.00</td>
</tr>
<tr>
<td>CONTROL</td>
<td>16.80**</td>
<td>-25.10**</td>
<td>-32.90**</td>
<td>-42.60</td>
<td>19.60**</td>
</tr>
</tbody>
</table>
Overall, it appears that water stressed haloxylon species are able to withstand drought condition and maintain positive turgor via enhancement of tissue elasticity. On the contrary, the seedlings that are not grown in water stress condition seem to benefit from a lower osmotic potential to maintain a high level of positive turgor. Such a trade-off between elasticity and osmotic adjustment under two contrasting water relation conditions was also reported in *Pinus taeda* [26].

Table 4. Comparison of water relation parameters in water stress treated and control root tissues of *Haloxylon persicum* seedlings

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>$\Psi_p$ (Bar)</th>
<th>$\Psi_o$ (Bar)</th>
<th>$\Psi_{TLP}$ (Bar)</th>
<th>$\varepsilon$ (Bar)</th>
<th>$W\Psi_s$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STRESSED</td>
<td>09.30</td>
<td>-12.50</td>
<td>-21.30</td>
<td>-26.20**</td>
<td>10.30</td>
</tr>
<tr>
<td>CONTROL</td>
<td>14.90**</td>
<td>-21.90**</td>
<td>-28.00**</td>
<td>-37.30**</td>
<td>16.20**</td>
</tr>
</tbody>
</table>

The ability of the plant to withstand and survive drought condition by maintaining positive turgor via enhancement of tissue or osmotic adjustment was reported in various plants such as *olive* [27], *Quercus crispula* and *Q. serrata* [21], *Pinus banksiana*, *Picea mariana* and *Eucalyptus grandis* [28], *sunflower* [29], *white spruce* [30], *grapevine* [31], *Ziziphus mauritiana* [32], *Pseudotsuga menziesii* [33], and *Dubautia ciliotala* [5].

The higher level of turgor in the tissues of control seedlings was associated with a higher percentage of the tissue symplastic water. The opposite was true with regard to the water-stressed tissues (Tables 1-4). For instance, the higher percentage of symplastic water ($W_s$) in the shoot tissues of the control seedlings (22.8 percent and 19.4 percent, respectively) for the two haloxylon species was associated with a higher level of turgor (19.6 and 15.8 bars, respectively), while the lower ratio of $W_s$ in the shoot tissues of the water-stressed seedlings (16.0 percent and 13.0 percent, respectively) was associated with a relatively lower level of turgor (11.2 and 10.1 bars, respectively). Similar results were obtained with regard to the root tissues (Tables 1-4). A relatively higher level of $\Psi_p$ (16.8 and 14.9 bars, respectively) in the root tissues of control seedlings was associated with a relatively higher ratio of $W_s$ (19.6 percent and 16.2 percent, respectively), and a relatively lower level turgor (10.4 and 9.3 bars, respectively) in the root tissues of water-stressed seedlings was associated with a relatively lower ratio of $W_s$ (13.0 percent and 10.3 percent, respectively).

4. CONCLUSIONS AND RECOMMENDATIONS

The results of this research show that both *H. aphyllum* and *H. persicum* can tolerate drought and maintain positive turgor mainly via mechanism of enhancement of tissue elasticity rather than mechanism of osmotic adjustment. In practice, this would imply that in order to enhance seedling survival in the field at the early stage of plantation establishment, the seedlings should get acclimated to drought condition by receiving 5 to 8 cycles of a 1 to 2 week-long water deficit where they are grown, whether in the greenhouse or nursery bed before they are transplanted into the field.

REFERENCES


