

Full Length Research Paper

Drought stress and its intensity, the factor of strategies selection for drought tolerance in *Haloxylon aphyllum*

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Osmotic parameters of *Haloxylon aphyllum* were studied after inducing dryness. Water relations parameters with improving resistance to dryness of this species through inducing severe dryness and clarification of physiologic mechanisms of this plant in response to a low water and dryness were among the objectives of this study. For this purpose, the method of pressure chamber was employed. By this method, the pressure-volume curve was drawn and the parameters of water relations of the plant were obtained from analyzing them. A relatively mild dryness was induced to plants through a lack of irrigation. After two weeks, *Haloxylon* water potential reached -16.5 bars. A severe dryness was also induced to that but after four weeks of no irrigation, it was reduced to -27.2 bars. Relatively mild and severe dryness were repeated for six and 11 periods respectively. In both series of experiment, the control water potential that were being watered every two days once, remained fixed at about -12.7 bars. Based on the results, although the relatively mild dryness increased the elasticity of plant textures, but it had not a meaningful impact on its osmotic potential. Although the use of a relatively severe dryness decreased both osmotic potential and osmotic adjustment, but at the same time, it increased the elasticity too.

Key words: *Haloxylon*, water relations, water stress, drought resistance, osmotic potential, elasticity.

INTRODUCTION

Plants use two mechanisms which are: 1) tolerating dryness and 2) escaping from it in confronting with dryness (Turner, 1979; May and Milthorpe, 1962). *Haloxylon aphyllum* is among species which with the help of endurance mechanism is able to spend dry periods. These mechanisms have been studied in some of the species like various kinds of pine (Emadian, 1988; Grime, 1979; Bilan et al., 1979:1978; Youngman, 1965).

Haloxylon species like many other species, by osmotic

adjusting or increasing the elasticity of cellular wall in the condition of water tension maintain their turgescence better and consequently tolerate better drought periods (Emadian, 1988).

H. aphyllum like other multi-functional plants of *haloxylon* type is of great value and importance in protecting and supporting breakable ecosystems like deserts.

It is such that as a live windbreaker prevents soil

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erosion (Tokasi et al., 2007; Safarnejad, 2005; Jafari et al., 2004), and as a correcting element increases the organic materials of soil and in a long term improves the structure of soil (Jafari et al., 2004), and increases the plant enrichment of the area under coverage (Bakhshi and Biroudian, 2008). In addition for those who live in deserts, it is an important source for the provision of fuel and fodder for cattle (Tokasi et al., 2007). As it has a broad emission spectrum in different soils from the viewpoint of texture (Javanshir et al., 1996), it can be noticed much more.

However, unfortunately, despite the high importance of haloxylon in biologic stabilization of sand lands, most of the planted plants in desert regions are facing the problem of dryness. Based on the confirmation of authorities and those in charge of desertification projects, after transferring the plants of *H. aphyllum* planted in vase to a natural plantation bed and despite observing principles related with post-plantation stages, after sometimes, a high percentage of transferred plants were afflicted with dryness and they had to be replanted. Taking action to replant instead of dried plants imposes relatively extravagant costs to the executive system of natural resources. According to the existing documents in the File Keeping Department of Agriculture Ministry of Iran, in average in each period, more than 10% of the vases with *H. aphyllum* were fading and became dried in the fields, and required replanting. In order to help reduce mortality percentage of planted plants in main beds of plantation, it seemed that placing one-year plants of *H. aphyllum* which were exposed to periodic dryness and possibility of their compatibility with unfavorable condition resulting reduction of rainfall and periodic droughts could be tested as an appropriate approach.

The main goal in conducting this research was firstly the study the changes of water relations parameters in order to improve resistance to dryness of *H. aphyllum* through induction of periodic dryness and also clarification of physiological mechanisms of this species in response to water shortage and dryness.

MATERIALS AND METHODS

The identified seeds of *H. aphyllum* were planted in plastic vases and were taken into care for one year. Plastic vases with an approximate capacity of three liters were selected in order to pave way for a better and a greater growth of the plants roots. The soil consisted of wind sand, soil and leaf-soil in proportions of 2, 1 and 1 respectively. Supply of necessary nutrition for plants during the experiment was done by including leaf-soil in the mentioned combination. At the same time, in order to prevent unwanted accumulation of water in vases, some fine holes were made in their bottom. After one year, the plants were transferred to a greenhouse.

Upon completion of one-month period of plants compatibility in greenhouse, the treatments of dryness induction was applied. For this purpose, a sufficient number of good and healthy plants were selected for the experiment. Half of them were considered for induction of dryness and the rest were selected as the controls that

were being watered every two days once. In this research, two series of experiments were conducted. The plants of the experiment of the first series received 6 periods of 7 to 14 days of dryness. During this period, the water potential (before sunrise) of their plant was measured every two days once by using the pressure chamber and according to Scholander method (Scholander et al., 1965). At this state, the water potential of haloxylon plants at the end of each period was reduced to -16.5 bars. The experiment second series plants, in addition to the mentioned dry periods, received five periods of dryness of 14 to 28 days too. In this series of tests, water potential of *H. aphyllum* plant was reduced at the end of each period to -27.2 bars. Induction of stress in mild (14-7 days) and severe (28-14 days) stresses were 6 and 6+5 (11) periods (replications) respectively. So every place (points) on the curve 3 is resultant 6 stress periods, and on the curve 4 is resultant 11 stress periods (repetitions).

It is worth mentioning that in order to measure the water potential of plants, separate plants were considered and in each measuring, 5 plants were cut and used. Plants were watered fully at the end of dryness period. Their before sunrise water potential in the day after irrigation was increased by -5.3 to -4.3 bars. Also, the control plants water potential was increased from -12.7 bars in both series of test to about -9.7 to -8.7 bars.

The impact of dryness induction on the elasticity and osmotic parameters of *H. aphyllum* plants became possible through an analysis of pressure-volume curves. These curves were prepared by using *Scholander method* (Figure 1).

The horizontal axis is the exit liquid volume (W_e) and its vertical axis is balancing pressure or (Ψ_w)¹. Point E, is the place of intersection of osmotic line with W_e axis showing the rate of water which is exited under the infinite pressure from plant known as simplistic water (W_s). Point B is the place of conjunction of osmotic line with the axis of (Ψ_w)⁻¹ showing that Ψ_s of plant is in the condition of full turgescence.

As Figure 1 shows each pressure-volume curve has two outstanding parts: 1) curve parts which encompasses about 5 to 8 points; 2) direct part which includes 8 to 11 points. The direct part of curve was used to estimate Ψ_w , Ψ_p , & Ψ_s . According to the recommendation of Cutler et al. (1979), this action was performed by drawing a regression curve on at least 7 to 8 points of the last spots of pressure-volume curves. The overall equation of the curve is as follows:

$$(\Psi_{st})^{-1} = (\Psi_{s0})^{-1} - m \sum W_i$$

In the relations, (Ψ_{s0})⁻¹ is the inverse of primary osmotic potential of plant at full turgescence condition; m is the slope of regression which is under the influence of plant size and osmotic feature of texture and rate of exchange of plant water, (Ψ_{st})⁻¹ is the opposite of Ψ_s for t times of a pair from the data of pressure-volume and $\sum W_i$ is its corresponding accumulated quantity of exited liquid. With this assumption that the mentioned relation to be fully true in the considered range, by placing each pair of P_i and $\sum W_i$, it is possible to get the Ψ_s . The continuation of this line crosses the vertical axis in point B which specifies the inverse of osmosis potential in full turgescence (Ψ_{s0})⁻¹ (Tyree and Jarvis, 1982). Subsequently, the turgor potential (Ψ_p) of the plant in each point of pressure-volume curve was obtained through difference of Ψ_w and Ψ_s related to the same point. On the other hand, the correlation line cut off the horizontal axis in point E, which determines the volume of exited liquid from plant in an infinite pressure [$(\Psi_{s0})^{-1} m^{-1}$] (Figure 1). Active osmotic water of plant ($W\Psi_s$) was calculated in form of $W_s (W_0 - W_d)^{-1}$ and its inactive osmotic water in form of $1 - W_s (W_0 - W_d)^{-1}$ (Cutler et al., 1979).

The existing data in the part of curvature of pressure-volume curve was used to estimate the average of the absolute value of elasticity ($\bar{\epsilon}$) of *H. aphyllum* (Tyree and Jarvis, 1982). To achieve

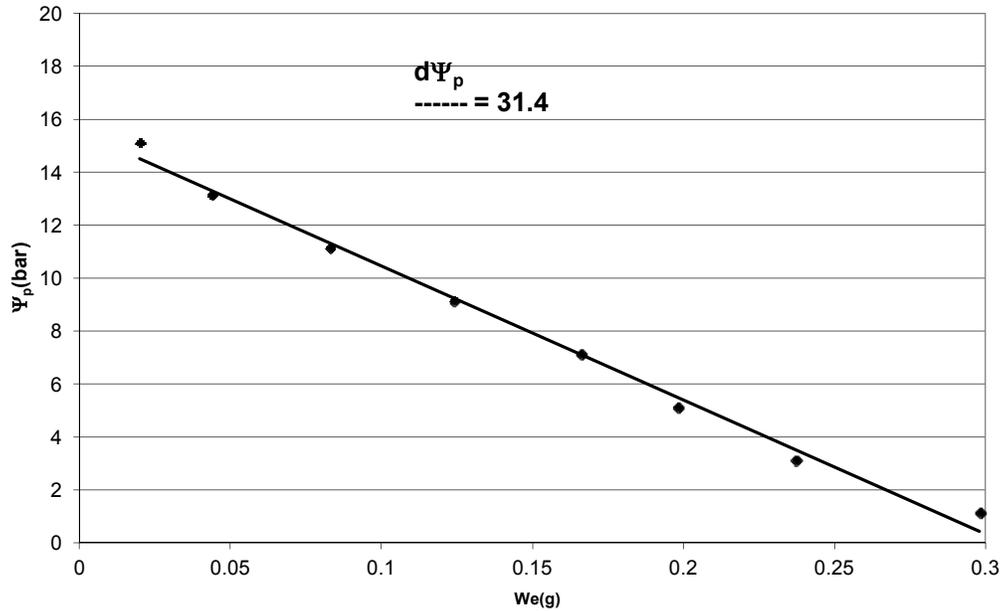


Figure 1. Pressure-volume curve of a plant of *Haloxylon aphyllum*.

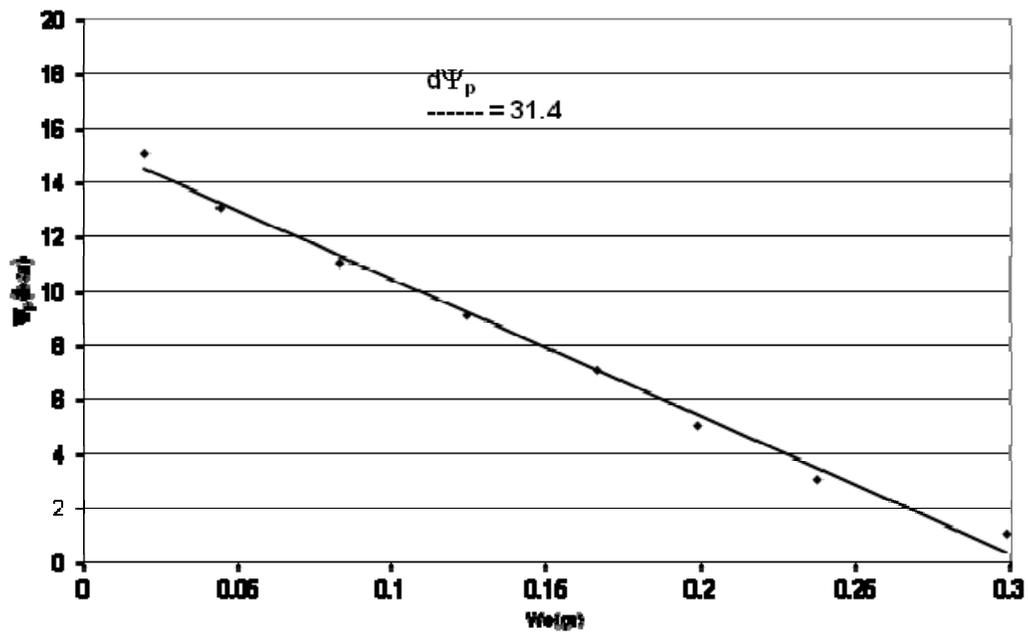


Figure 2. Calculating the rate of elasticity changes of cellular wall in *Haloxylon aphyllum* textures by using the curvature part of pressure-volume curve. Horizontal axis is exitted volume (W_e), and vertical axis is swollen potential. In order to calculate the average of absolute value of the elasticity ($\bar{\mathcal{E}}$), the curve slope of $d\Psi_p (dW_e)^{-1}$ of plant was used.

this parameter, it was necessary that firstly Ψ_p of the plant in the part of curvature of the pressure-volume curve be calculated. This action was performed by using the osmotic line and method of estimation of osmotic, turgor and water potentials. Then the obtained turgor potentials [(Ψ_p) s] in the mentioned limit with the

volume of corresponding condensed exitted liquid was drawn in coordinates sheet and their regression equation was calculated (Figure 2). The ($\bar{\mathcal{E}}$) was also calculated from the product of the slope of line [$(d\Psi_p) (dW_e)^{-1}$] in W_s .

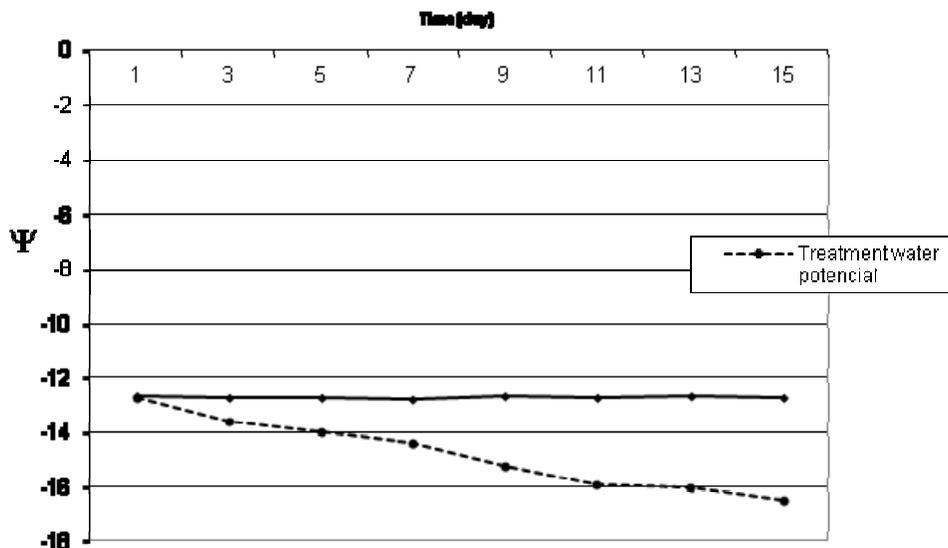


Figure 3. Relationship between plant water potential (Ψ_w) and time in *Haloxylon aphyllum* after watering. Pre-dawn plant water potential was measured every other day during two-week no-watering period.

Table 1. Comparing the result of water relations in *Haloxylon aphyllum* under mild dryness induction with control plants.

Treatment	Ψ_{wo} (Bar)	Ψ_{po} (Bar)	Ψ_{so} (Bar)	Ψ_{wTLP} (Bar)	Ψ_{sTLP} (Bar)	ϵ (Bar)	$W\Psi_s$ (%)
STRESSED ¹	-2.50**	12.30**	-14.80	-20.90	-23.10	44.8**	14.00**
CONTROL ²	-5.90	07.30	-13.20	-22.50	-21.40	30.50	28.80

¹Seedlings, in addition to six early dry periods, five-day dry period 7 to 14 also received. The average water potential (before sunrise) at the end of their term limit had been reduced to -16.5 bars. Value of each parameter treated seedlings is related to seven seedlings. ²Both seedlings were irrigated once a day. Amount of each parameter of control seedlings is related to five seedlings.

$$\bar{\epsilon} = W_s [(d\Psi_p) (dW_e)^{-1}]$$

The used statistical method was t- student (Snedecor and Cochran, 1980).

RESULTS

Results of mild dryness induction

At the end of each period of mild dryness induction, water potential (Ψ_w) of *H. aphyllum* reduced about -16.5 bars; whereas Ψ_w of control remained at a higher range, that is in average -12.7 bars (Figure 3).

Parameters of active osmotic water ($W\Psi_s$) and pressure potential at the condition of moisture full saturation (Ψ_{po}) reduced and increased significantly and reached from 28.8 to 14% and was promoted from 7.3 to 12.3 bars, respectively. Their elasticity modulus ($\bar{\epsilon}$) and water potential (Ψ_{wo}) had a highly significant increase

(level 99%). Thus they accelerated from 30.5 to 44.8% and from -5.9 to -2.5 bars but the osmotic potential of tension treatments in full turgescence did not show a meaningful difference as compared with control (Table 1).

Results of severe dryness induction

After applying severe dryness induction, the water potential of *H. aphyllum* diminished in average up to about -27.2 bars, whereas the water potential of controls more or less remained fixed at the level of mild dryness induction (-12.7 bars) (Figure 4).

Water potential and pressure potential in fully saturation moisture condition increased significantly ($P < 1\%$), so that with the promotion to a higher level, it reached to the level of -2 and 18.8 bars respectively. At the same time, like the mild dryness tension, very meaningful increase of elasticity ($\bar{\epsilon}$) and very meaningful reduction of osmotic active water ($W\Psi_s$) were observed.

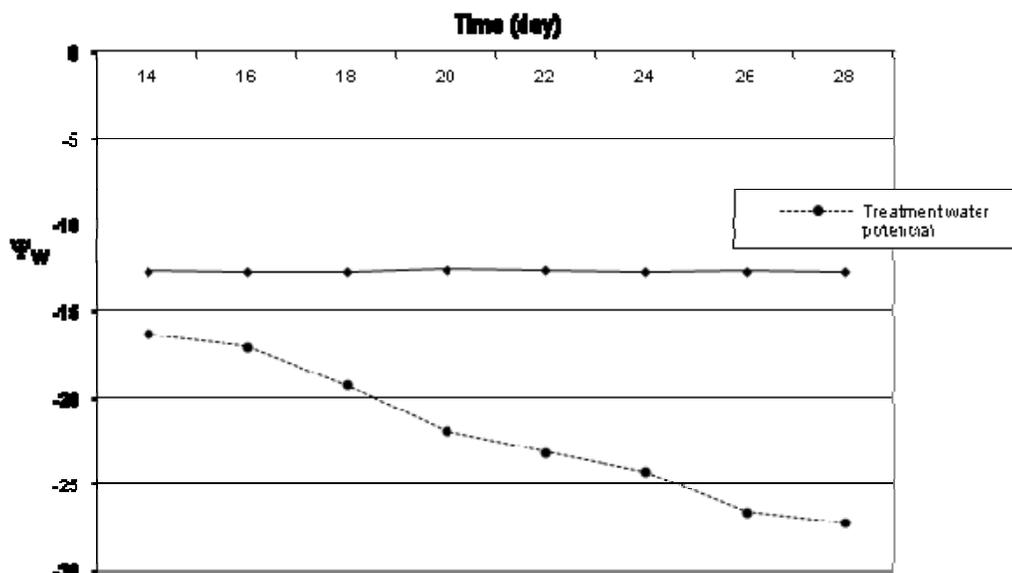


Figure 4. Relationship between plant water potential (Ψ_w) and time in *Haloxylon aphyllum* after watering. Pre-dawn plant water potential was measured every other day during a four-week period of no-watering.

However, concurrent with these changes, the osmotic potential in the condition of moisture full saturation (Ψ_{s0}) and dryness threshold (Ψ_{stlp}) reduced significantly ($P < 1\%$) and decreased from -13.5 to -20.8 bars and from -20.9 to -30.6 bars respectively.

DISCUSSION

Primarily it seemed that the reaction of parameters of water relations (like Ψ_{w0} , Ψ_{s0} , Ψ_{p0} , Ψ_{wtlp} , $\bar{\varepsilon}$) of *Haloxylon aphyllum* in mild dryness condition was in conflict with their corresponding parameters in the control treatment (Table 1), because measured absolute value of elasticity ($\bar{\varepsilon}$) of under the treatment plants was 44.8 bars that was more elastic than $\bar{\varepsilon}$ of control plants (30.5 bars) significantly. On the other, the measured osmotic active water ($W\Psi_s$) in treated and controlled plants was 14 and 28.8% respectively, and this difference was meaningful in the level of 99%. Possibly, the concept of this with regard to the relation of ($\bar{\varepsilon}$) is that the more elastic plant needs a less water to maintain its turgescence. In addition, in the condition of full turgescence, the water potential of plants which were given dryness was higher than water potential of control plants.

Plant physiologists believe that many plants of dry regions are able to maintain their turgescence through mechanism of elasticity and consequently increase resistance to dryness. This feature is true for *Pinus taeda* (Emadian and Newton, 1989), *Pseudotsuga menziesii* (Joly and Zaerr, 1987), *Dubautia ciliolata* (Robichaux and

Canfield, 1985), and *Juglans nigra* (Parker and Pallardy, 1985). On the other, though the elasticity of treated plants increased, but their osmotic potential was without any meaningful change. In other words, in condition of mild dryness induction, no meaningful difference was observed at any statistical level between Ψ_{s0} and Ψ_{stlp} in control and dryness tension treatments (Table 1). With regard to mentioned condition, it seems that in the mild dryness condition, *H. aphyllum* through increase of elasticity is able to maintain its turgescence and continues its growth. Apparently, this will be possible only in lieu of an exchange with reduction of osmotic adjustment.

Water relations parameters reaction in severe stress (28-14 days without irrigation) took place in continuing mild dryness condition, and osmotic adjustment was activated (Table 2) so that in addition to increase cell wall elasticity of shoot tissues (such as effect of periodic mild stress) osmotic potential of the tissue significantly decreased compared to control tissue, and continued the life of stressed-plants. After induction, severe periodic dryness on plants (*Haloxylon aphyllum*), the increase of water and turgor potentials (300 and 250%, respectively) and reduction of osmotic potential (more than 50%) in the condition of moisture saturation and at the dryness threshold was considerable.

The results showed that the induction of periodic mild dryness was not able to have an impact on the increase of resistance to dryness in *Haloxylon aphyllum*. It only changed the strategy of plant in confronting with stresses resulting from drought. Instead of that, periodic induction of severe dryness could make ideal changes in osmotic parameters, and not only caused the viable and

Table 2. Comparing the result of water relations in *Haloxylon aphyllum* under severe dryness induction with control plants.

Treatment	Ψ_{wo} (Bar)	Ψ_{po} (Bar)	Ψ_{so} (Bar)	Ψ_{wTLP} (Bar)	Ψ_{sTLP} (Bar)	ϵ (Bar)	$W\Psi_s$ (%)
MDI	-2.50	12.30	-14.80	-20.90	-23.10	44.80	14.00
SDI	-2.00	18.80	-20.80	-20.30	-30.60	44.50	14.50

Table 3. Comparing the result of water relations in *Haloxylon aphyllum* under two water regimes: mild dryness induction and severe dryness induction.

Treatment	Ψ_{wo} (Bar)	Ψ_{po} (Bar)	Ψ_{so} (Bar)	Ψ_{wTLP} (Bar)	Ψ_{sTLP} (Bar)	ϵ (Bar)	$W\Psi_s$ (%)
Stressed	-2.00**	18.80**	-20.80**	-20.30**	-30.60**	44.50**	14.50**
Control	-5.80	07.50	-13.50	-29.70	-20.90	31.80	26.50

¹Seedlings, in addition to six early dry periods, five-day dry period 14 to 28 also received. The average water potential (before sunrise) at the end of their term limit had been reduced to -27.2 bars. Value of each parameter treated seedlings is related to seven seedlings. ²Both seedlings were irrigated once a day. Amount of each parameter of control seedlings is related to five seedlings.

freshness of plant but also enabled plants to bear the severe condition of dryness and could maintain their water potential at a very high level. Comparing the parameters of water relations of *H. aphyllum* in mild dryness with severe dryness condition (Table 3) confirm the importance of the mentioned subject matter.

Mild dryness induction and severe dryness induction

Though under severe and longer dryness induction, cellular wall elasticity of *H. aphyllum* was maintained, however, osmotic adjustment became active for maintaining cellular turgescence. Therefore plants remained alive and continued their physiological and biochemical activities. Reduction of osmotic potential in the condition of saturation (by 6 bars) and reduce osmotic potential at threshold of wilt and dryness (by 7.5 bars) along with compatibility of plants to frequent dryness tension may enable them (compared to plants that were not exposed to any intensity of drought) tolerate drought periods resulting from factors affecting moisture shortage, and may be protected from the deleterious effects of drought periods in the same range of drought that seedlings similar to control wilt.

This idea can be put forth as the symbol of increase of resistance to dryness in *H. aphyllum* (which had received and accustomed to severe periodic dryness stresses) and to be used as a base for future research. Anyway, a decisive comment on this issue demands further studies and supplementary studies.

Conclusion

The results of this research showed that *H. aphyllum*

tolerates dryness well. If dryness be mild and its period be short, this species will be able to maintain its turgescence and continue its life by increasing elasticity mechanism. Apparently, this action is possible only in lieu of an exchange with reduction of osmotic adjustment. On the other hand, if dryness be severe and its period be long, for keeping its turgescence, in addition to increase elasticity, it uses the mechanism of osmotic adjustment too. In each of the two mentioned condition, maintaining turgescence in dryness condition cause the continuation of physiologic and biochemical activities of this species and make its growth and viability possible despite excessive and long dryness. Based on the results of this research, the executive officials of forestry and production of plants departments recommend that if they use *Haloxylon aphyllum* for biologic stability of sand lands in arid and semi-arid zones, prior to transfer of plants to main field, they should place them under five to eight periods of dryness induction for at least three to four weeks.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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