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Physiologic Responses of *Haloxylon aphyllum* to Consecutive Tensions of Dryness and Study of Their Role in Improving Resistance to Dryness of Vase Twigs

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Abstract: Osmotic and elastic parameters of *Haloxylon aphyllum* branchlets after inducing dryness were studied. The study of changes of water relations parameters with the aim of improving resistance to drought of this species through inducing chronic dryness and clarification of physiologic mechanisms of this plant in response to a low water and dryness are among the objectives of this study. For this purpose, the method of pressure chamber was employed. By this method, the pressure-volume curves were drawn and through their analysis, plant water relations parameters were obtained. A relatively mild dryness was induced to twigs through a lack of irrigation. It was such that after two weeks, the potential of the water of the branchlet of *H. aphyllum* twigs reached to -16.5 bars. A very intensive dryness was also induced to that but after four weeks of prevention from irrigation; it was such that the potential of the water of *H. aphyllum* branchlet was diminished to -27.2. A relatively mild dryness was repeated for six terms and a relatively harsh dryness for eleven consecutive periods. In both series of experiment, the potential of water of control twigs were being watered every two days once, remained fixed at about -12.7 bars. Based on the results, though the relatively mild dryness induction to twigs of this type of *Haloxylon*, made the increase of elastic property of branchlet textures but it had not a meaningful impact on its osmotic potential. Although the application of a relatively intensive dryness reduced the osmotic potential of *H. aphyllum* branchlets but at the same time, it increased the elasticity of the tissues too.

Key words: *Haloxylon*, resistance to drought, water potential, osmotic potential, water relations

INTRODUCTION

Haloxylon shrubs as resistant plants are scattered in different growth areas from more or less sporadic masses in natural form in some of the desert and arid zones of Iran (Arabzadeh *et al*., 2009).

Plants show a wide range of adaptations, at different levels, to drought stress (Xoconostle-Cazares *et al*., 2010). Reproductive growth of plants is exposed to low rainfall and drought stress in vegetative parts of Iran (Danesh-Shahraki *et al*., 2008). There are few physiological processes in plants that are not impaired by water deficit (Kumari, 2010) but, stress management is able to more resistant the plants such as *Haloxylon* against the negative effects of stress (Arabzadeh, 2009). Improving drought tolerance is important for breeding (Khalily *et al*., 2010) and vigority (Arabzadeh *et al*., 2009).

Several environmental factors adversely affect plant growth. Drought stress is one of the major factors in limiting production (Omidi, 2010) and growth (Arabzadeh *et al*., 2009) particularly in arid and semi-arid locations. In such regions, germination and seedling establishment constitute are the most critical periods in the life-cycle of plants (Omidi, 2010).

Drought stress causes a wide variety of physiological and biochemical changes in plants (Omidi, 2010). Drought is one of the most important factors in the ecological field which many crops such as corn (Ngaboyisonga *et al*., 2009) and other crops such as *Haloxylon* (Arabzadeh *et al*., 1992) be able to interact with it.

Drought is the most serious threat to agriculture and is far more important globally (Ali, 2011). Growth of plants such as wheat and others is reduced depending upon degree of water stress and on the rate of stress development, thereby limiting final product (Ali, 2011).

Water stress is a major harmful factor in arid and semi-arid regions worldwide (Ali, 2011). In these regions, it is one of the severe limitations of crop growth as it has a vital role in plant growth and development at all growth stages (Gorji *et al*., 2011). Drought stress is an important factor in environmental stresses range which limits natural sources and agriculture productions (Imanparast and Hassanpanah, 2009) and controls the plant growth directly (Kufa and Burkhardt, 2011). It is responsible for production losses and is a major factor that limits the growth and reproduction of plants (Naderikharaji *et al*., 2008).

Plants absorb maximum water from the soil and minimal water loss from their leaves to cope with drought stress (Rad *et al*., 2011). For drought resistance, several morphological, physiological and biochemical mechanisms are used (Rad *et al*., 2011). Drought stress influences virtually all physiological functions that are responsible for plants growth (Kufa and Burkhardt, 2011). *Haloxylon* plants strategy to deal with drought stress and its negative effects is the production of protective substances and increase the elasticity of the tissues (Arabzadeh, 2012). Survival of these plants under drought stress is possible using osmotic adjustment too (Arabzadeh, 2011).

The term resistance to dryness indicates the physiologic and biochemical features of plant which in the condition of limitation of the soil wetness makes it viable. Plants use two mechanisms of bearing drought or escaping from it in confronting with drought (Turner, 1979; May and Milthorpe, 1962). *H. aphyllum* is among species which with the help of tolerance mechanism is able to spend drought periods. This mechanism has been studied in some of the species like various kinds of pine (Emadian, 1988; Grime, 1979; Bilan *et al*., 1978, 1977; Youngman, 1965).

The *Haloxylon* species like many other species, by adjusting the Osmosis or increasing the elastic property of the cellular wall of their own textures in the conditions of water stress, maintain their Turgescence better and consequently bear drought periods better (Emadian, 1988).

H. aphyllum like other multi-functional plants of *Haloxylon* genus is of great value and importance in protecting and supporting breakable ecosystems like desert. It is such that as a live windbreaker it prevents from erosion of soil (Tokasi *et al*., 2007; Safarnejad, 2005; Jafari *et al*., 2004) and as an improving element increases the organic materials of soil and in a long term, it 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 desert, it is an important source for the provision of fuel and fodder for cattle (Tokasi *et al*., 2007).

However, unfortunately, despite the high importance of *Haloxylon* in biologic stabilization of sand lands, most of the planted twigs in desert scenes are facing the problem of dryness. Based on the confirmation of authorities and those in charge of desertification projects, after transferring the twigs 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 twigs were afflicted with dryness and they had to be replanted (Arabzadeh *et al*., 1992; Kerman Natural Resources Office, 1990). Taking action to replant the dried twigs imposes relatively extravagant costs to the executive system of natural resources. According to the existing documents in the File Keeping Department of Ministry of Agriculture of Iran, in average in each period, more than 10% of the vases with *H. aphyllum* into the scene were fading and became dried and were in need of re-plantation (Arabzadeh *et al*., 1992). In order this action and aiming at reducing the percentage of mortality of vase based planted twigs in main beds of plantation, it seemed that placing a one-year old twig of *H. aphyllum* which are exposed to periodical dryness and possibility of their compatibility with unfavorable conditions resulting from reduction of rainfall and periodical droughts can be tested as an appropriate approach. The main motivation in conducting this research was firstly to study the changes of parameters of water relation in order to improve resistance to the dryness of *H. aphyllum* through induction of periodical dryness and also clarification of physiological mechanisms of this species in response to water shortage and dryness.

MATERIALS AND METHODS

Production and location of twigs: The identified seeds of *H. aphyllum* were planted in plastic vases and they 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 twigs roots. Their soil consisted of wind sand, soil and leaf-soil in proportions of 2, 1 and 1, respectively. By including leaf-soil in the mentioned combination, the aim was to supply necessary nutrition for twigs during the experimental period. At the same time, in order to prevent from unwanted accumulation of water in vases, some fine holes were made in their bottom. After one year, the twigs were transferred to a greenhouse.

Dryness induction: Upon completion of a one-month period of compatibility of twigs in greenhouse, the treatment of dryness induction was applied. For this purpose, a sufficient number of good and healthy twigs were selected for the experiment. Half of them were considered for induction of dryness and the rest as the control and they were being watered every two days once. In this study, two series of experiments were conducted. The twigs of the experiment of the first series received 6 periods of 7-14 days of dryness. During this period, the water potential (before sunrise) of their branchlet was measured every two days once by using the set of pressure chamber and according to Scholander method (Scholander *et al*., 1965). At this state, the water potential of *Haloxylon* branchlets at the end of each period diminished in average -16.5 bars. The twigs of the experiment of the second series, in addition to the mentioned dry periods received five periods of dryness of 14-28 days too. In this series of tests, water potential of the *H. aphyllum* branchlet was diminished at the end of each period in average to -27.2 bars. It is worth mentioning that in order to measure the water potential of branchlets, separate twigs were considered and in each measuring, the branchlets of 5 twigs were cut and used. After the end of dryness period, twigs were watered fully. It was such that their water potential (before sunrise) in the day after irrigation was increased by -5.3 to -4.3 bars. The water potential of control twigs also increased from -12.7 bars in both series of test to about -9.7 to -8.7 bars.

Preparing pressure-volume curves: The impact of dry induction on the elastic and osmotic properties of the branchlet of *H. aphyllum* twigs became possible through an analysis of pressure-volume curves. These curves were prepared by using Scholander method (Fig. 1).

Analysis of pressure-volume curves

Osmotic potential, turgor potential and water potential: As Fig. 1 also shows, each pressure-volume curve has two outstanding parts: (1) curve part which encompasses about 5 to 8 points and (2) direct part which includes 8 to 11 points. The direct part of curve was used to estimate Ψ_w, Ψ_p and Ψ_s. According to the recommendation of Cutler *et al*. (1979), this action was performed by drawing a regression curve on at least 7-8 points of the last spots of pressure-volume curves.

The overall equation of the curve is as follows:

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

In the above relations, $(\Psi_{s0})^{-1}$ is the inverse of primary osmotic potential of branchlet at full turgescence conditions; m is the slope of regression curve which is under the influence of size of branchlet and osmotic feature of texture and rate of exchange of twig water, $(\Psi_{st})^{-1}$ is the opposite of Ψ_s for t times of a pair from the data of pressure-volume and ΣW_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 ΨW_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 osmotic potential in full turgescence $(\Psi_{s0})^{-1}$ (Tyree and Jarvis, 1982). Subsequently, the turgor potential (Ψ_p) of the branchlet 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 prolongation of the mentioned regression crosses (cuts) the horizontal axis in point E which determines the volume of exited liquid from branchlet in an infinite pressure $[(\Psi_{s0})^{-1} m^{-1}]$ (Fig. 1). Active osmotic water of branchlet $(W\Psi_s)$ 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).

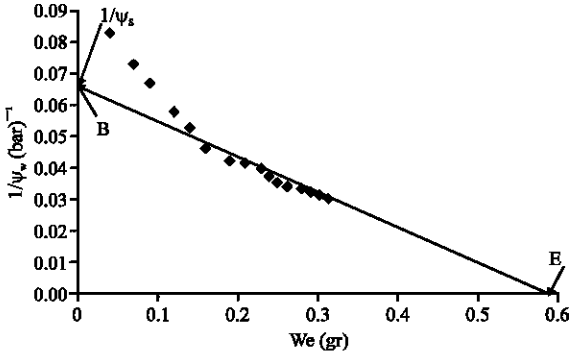

Fig. 1: Pressure-volume curve of a branchlet of a *H. aphyllum*: The horizontal axis is the exit liquid volume (W_e) and its vertical axis is balancing pressure or $(\Psi_w)^{-1}$. Point E, is the place of intersection of osmotic line with W_e axis showing the rate of water which is exited under the infinite pressure from branchlet known as symplastic water (W_s). Point B is the place of conjunction of osmotic line with the axis of $(\Psi_w)^{-1}$ showing that Ψ_s of branchlet is in the conditions of full turgescence

Average of absolute value of elastic property (bulk-moduls of elasticity or $\bar{\varepsilon}$): The existing data in the part of curvature of pressure-volume curve was used to estimate the average of the absolute value of elastic property ($\bar{\varepsilon}$) of the branchlet of *H. aphyllum* (Tyree and Jarvis, 1982). For the purpose of achieving this parameter, it was necessary that firstly Ψ_p of the branchlet in the part of curvature of the pressure-volume curve to be calculated. This action was performed by using the osmotic line and method of estimation of osmotic potential, turgor potential and water potential of branchlet. Then the obtained turgor potentials $[(\Psi_p)s]$ in the mentioned limit with the volume of corresponding

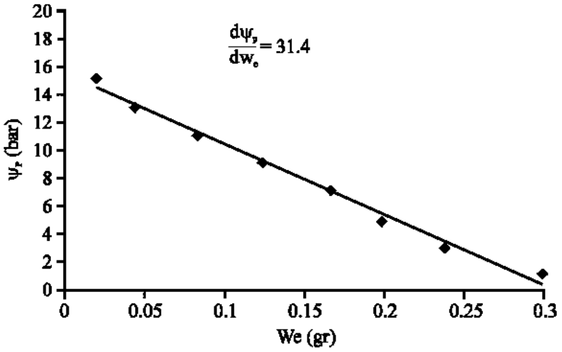

Fig. 2: Calculating bulk-moduls of elasticity ($\bar{\varepsilon}$) of *H. aphyllum* branchlet by using the curvature part of pressure-volume curve, the horizontal axis of exited volume (W_e) and its vertical axis is turgor potential

condensed exited liquid was drawn in coordinates sheet and their regression equation was calculated (Fig. 2). The ($\bar{\varepsilon}$) was also calculated from the multiplied by the slope of line $[(d\Psi_p)(dW_e)^{-1}]$ in Ws:

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

In order analyzing the pressure-volume curves and for comparing the impact of dry induction on water parameters between twigs under treatment and control, the statistical method of student's t-test was used (Snedecor and Cochran, 1980).

RESULTS

Results related to a relatively mild dry induction: At the end of each period of a relatively mild dry induction, Ψ_w of the branchlet of twigs under the treatment of *H. aphyllum* reduced in average about -16.5 bars, whereas Ψ_w of the branchlet of control twigs, remained at a higher limit, i.e., in average -12.7 bars (Fig. 3). Parameters of the active osmotic water ($W\Psi_s$) and turgor potential at the conditions of wet full saturation (Ψ_{p0}) reduced and increased very significantly and reached from 28.8-14% and promoted from 7.3-12.3 bars, respectively. The ability of the elastic of branchlet textures ($\bar{\varepsilon}$) and their water potential (Ψ_{w0}) with 99% reliability had a very meaningful increase and accelerated from 30.5-44.8% and from -5.9 to -2.5 bars, respectively but the osmotic potential of stress treatment in full turgescence did not show a meaningful difference as compared with control (Table 1).

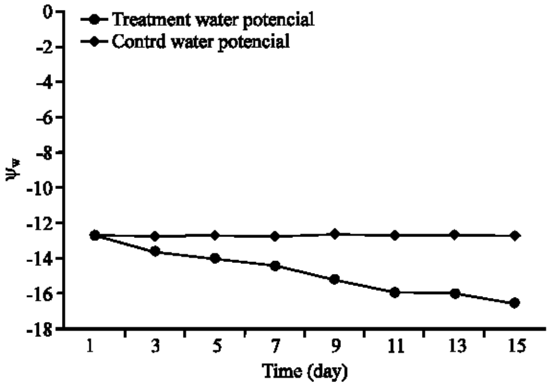

Fig. 3: Relationship between shoot (branchlet) water potential (Ψ_w) and time after seedlings watering. Pre-dawn shoot water potential was measured every other day during a two-week period of water

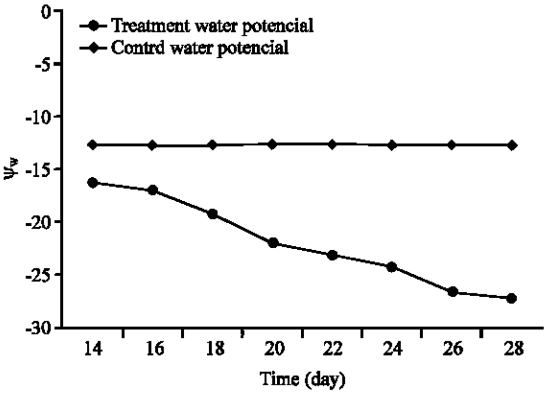

Fig. 4: Relationship between shoot (branchlet) water potential (Ψ_w) and time after seedlings watering. Pre-dawn shoot water potential was measured every other day during a four-week period of water

Table 1: Comparing the results of water relation of the branchlet of *H. aphyllum* under a relatively mild dry induction with respective control twigs

Treatment	Ψ_{wo} (Bar)	Ψ_{po} (Bar)	Ψ_{so} (Bar)	Ψ_w TLP (Bar)	Ψ_s TLP (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

[1] Seedlings, in addition to six early dry periods, five-day dry period 7-14 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. [2] Both seedlings were irrigated once a day. Amount of each parameter of control seedlings is related to five seedlings

The results related to a relatively intensive dry induction: After induction of a relatively intensive drought, the water potential of the twigs branchlets under the treatment of *Haloxylon aphyllum* diminished in average up to about -27.2 bars whereas, the water potential of the branchlet of control twigs of *H. aphyllum* remained fixed at the level of mild drought induction (-12.7 bars) more or less (Fig. 4). Water and turgor potentials in fully saturation wet condition had a very meaningful increase ($p<1\%$) and reached to -2 and 18.8 bars, respectively. At the same time, like the mild water stress, the very meaningful increase of elasticity ($\bar{\varepsilon}$) and a very meaningful reduction of osmotic active water ($W\Psi_s$) in the texture of branchlet were observed. However, concurrent with these changes, the osmotic potential in the condition of wet full saturation (Ψ_{s0}) and drought threshold (Ψ_{stlp}) diminished in a very meaningful way ($p<1\%$) and decreased from -13.5 to -20.8 bars and from -20.9 to -30.6 bars, respectively.

Table 2: Comparing the results of water relation of the branchlet of *H. aphyllum* under a relatively intensive dry induction with respective control twigs

Treatment	Ψ_{wo} (Bar)	Ψ_{po} (Bar)	Ψ_{so} (Bar)	Ψ_w TLP (Bar)	Ψ_s TLP (Bar)	ε (Bar)	$W\Psi_s$ (%)
Stressed[1]	-2.00	18.80	-20.80	-20.30	-30.60	44.50	14.50
Control[2]	-5.80	07.50	-13.50	-29.70	-20.90	31.80	26.50

[1] Seedlings, in addition to six early dry periods, five-day dry period 14-28 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. [2] Both seedlings were irrigated once a day. Amount of each parameter of control seedlings is related to five seedlings

DISCUSSION

Primarily it seemd that in the branchlet of *H. aphyllum*, the reaction of parameters of water relations like Ψ_{w0}, Ψ_{s0}, Ψ_{p0}, Ψ_{wtlp}, $\bar{\varepsilon}$ and so on against a very mild relative drought induction to be in conflict with their corresponding parameters at control treatment (Table 1), because in one side, the absolute value of elasticity ($\bar{\varepsilon}$) of twig branchlets under the treatment of *Haloxylon* were measured to be 44.8 bars, that at the statistical level of 1% in a meaningful rate was more elastic than $\bar{\varepsilon}$ of branchlets of control twigs (30.5 bars) and on the other side, the osmotic active water of branchlet ($W\Psi_s$) for treated and controlled twigs were measures to be as 14 and 28.8% and this difference in reliability level of 99% was meaningful. Possibly, the concept of this difference, with regard to the relation of ($\bar{\varepsilon}$), is that the more elastic branchlet needs a less water to maintain its own turgescence. In addition, in the conditions of full turgescence, the water potentials of twigs which were given drought induction, in a meaningful way were higher than potential of water of controlled twigs.

Plant physiologists believe that many plants of dry regions through mechanism of elastic property of their own textures are able to maintain turgescence and consequently increase resistance to drought and have continuation of growth. This feature is true for *Pinus taeda* (Emadian and Newton, 1989), *Pseudotsuga menziesii*(Joly and Zaerr, 1987), *Dubautia ciliotala* (Robichaux and Canfield, 1985), *Juglans nigra* (Parker and Pallardy, 1985). On the other side, though the elastic property of the branchlet of treated twigs increased but their osmotic potential (along with very meaningful increase of water potential) was in lack of any meaningful change. In other words, in the condition of induction of a relatively mild drought induction, no meaningful difference was observed at any statistical level between Ψs_0 and Ψs_{tlp} of control and drought stress treatments (Table 1). With regard to mentioned conditions, it seems that in the mild drought conditions, *H. aphyllum* through increase of elastic property of branchlet textures is able to maintain its turgescence and continues it growth. Apparently, this will be possible only in lieu of an exchange with reduction of osmotic adjustment. In relatively intensive stresses (14-28 days of lack of irrigation), the reaction of different parameters of water relations of *H. aphyllum* branchlet took place followed by reaction process in the relatively mild stress induction conditions and osmotic adjustment was activated (Table 2). It was such that in addition to elastic property of cellular wall of the textures of branchlet (like the impact of periodical mild drought stresses), the osmotic potential of these textures reduced as compared with controlled textures in a meaningful way and made plants under tension to continue their life in the mentioned conditions. After exposing the twigs of *H. aphyllum* to a relatively intensive periodical drought stresses, the increase of water potential and turgor potential in the rate of 300 and 250%, respectively and reduction of osmotic potential in the conditions of wet saturation and at the threshold of drought in the rate of more than 50% are considerable and subject to comment. The results showed that the induction of relatively mild and periodical drought was not able to have an impact on the increase of resistance against drought of *H. aphyllum* and it only changed the strategy of plant in confronting with stresses resulting from change. Instead of that, successive induction of relatively intensive drought could make ideal changes in osmotic parameters and not only cause the viable and freshness of plant but also it enabled twigs to bear the relatively intensive condition of drought and they could maintain their water potential at a very high level. Comparing the parameters of water relation of *H. aphyllum* in a relatively mild drought stress and those of very intensive ones (Table 3) confirm the importance of the mentioned subject matter. It is such that four important and influential parameters in elucidation of this analysis enjoyed uni-direction changes with an increase of endurance vis-à-vis drought (Ψ_{w0} increased for 0.5 bars, Ψ_{p0} increased by 6.5 bars, Ψ_{s0} decreased by 6 bars, Ψ_s of branchlet at the threshold of dryness decreased by 7.5 bars).

Table 3: Comparing the results of water relation of the branchlet of *H. aphyllum* under two water regimes: a relatively mild dry and a relatively intensive dry induction

Treatment	Ψ_{wo} (Bar)	Ψ_{po} (Bar)	Ψ_{so} (Bar)	Ψ_{w} TLP (Bar)	Ψ_{s} TLP (Bar)	ε (Bar)	$W\Psi_{s}$ (%)
RMD	-2.50	12.30	-14.80	-20.90	-23.10	44.80	14.00
RID	-2.00	18.80	-20.80	-20.30	-30.60	44.50	14.50

Though under a very relatively intensive and longer drought induction, elastic property of the textures of *H. aphyllum* was maintained, however, osmotic adjustment was made active to enable twigs maintain their turgescence, to remain alive and continue their physiologic and biochemical activities and consequently their own growth. Reduction of osmotic potential in the conditions of saturation by 6 bars and the fall of this potential at the threshold of wither and dryness by 7.5 bars along with compatibility of twigs to frequent drought stresses might enable them to bear drought periods resulting from factors affecting wet shortage as it is compared with a one-year *H. aphyllum*, the ones which were not exposed to any kind of intensive drought stress and in a limit of dryness in which similar twigs with controlled twigs face wither, it is possible to be immune against damaging impacts of drought periods. This idea as the symbol of increase of resistance to drought of *H. aphyllum* (which have received, borne and accustomed to a relatively intensive and periodical drought stresses) can be put forth and to be used as a base for future researches. Anyway, a solid comment on this issue demands further studies and supplementary investigation.

CONCLUSION

The results of this research showed that *H. aphyllum* bears dryness well. On the other hand, if dryness to be relatively mild and its period is short, by the mechanism of increase of elastic property of textures of branchlet, this species will be able to maintain their turgescence and continue its life. Apparently, this action is possible only in lieu of an exchange with reduction of osmotic adjustment. On the other hands, if dryness is relatively intensive and its period long, for keeping its turgescence, in addition to the mechanism of increase of elastic property uses the mechanism of osmotic adjustment too. In each of the two mentioned conditions, maintaining turgescence in dry conditions cause the continuation of physiologic and biochemical activities of the twigs of this species and make their growth and viability possible despite excessive and long dryness. Based on the results of this research, the executive officials of forestry and production of twigs departments are recommended that if they use *H. aphyllum* (to have a biologic stability of sand lands in arid and semi-arid zones), prior to transfer of twigs to the main scene of plantation, they should place them under 5 to 8 periods of dry induction for at least 3 to 4 weeks.

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