

## Physiological Response of Sea Buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson) to Water-Use Strategies

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**ABSTRACT** We investigated the response of Sea Buckthorn to drought in a nursery experiment that has been studied for the first time in the world for Iranian Sea Buckthorn. Biomass and physiological differences in response to drought were compared between four *Elaeagnus rhamnoides* seedlings inhabited in Qazvin Province origin seeds of Iran. The experimental design included four water regimes including 2, 4, 8 and 12 days irrigation and three blocks. Water Use Efficiency (WUE), Relative Water Content (RWC), Water Potential (WP), Water Saturation Deficit (WSD), Root and shoot weight of fresh leaves were determined at the end of the watering treatment (four months). We found that drought tolerance was highly related to the plant physiology in *E. rhamnoides*. With the extension of drought stress from 2 to 12 days, *E. rhamnoides* seedlings WUE was increased; between one and second treatment, also between third and fourth treatments we observed significant difference. RWC gradually was declined with decreasing water supplies. WP was decreased, while drought was increased from first to last treatment. WSD gradually was increased by accelerating drought in all treatments. WSD values did not differ significantly between treatments three and four. Significant differences at 0.05 levels were not observed between 8 and 12 days-irrigated in both of R and S weight, but in all treatments was decreased toward drought. Our results provided new clue and new insight to study the drought-tolerant mechanism for the study species.

**Key words:** *Ecophysiology, Hippophae, Stress, Toleration*

### 1 INTRODUCTION

Sea Buckthorn (*Hippophae rhamnoides* L.) from Elaeagnaceae family has become a crop of interest for the food processing industry. Accepted name in Theplantlist.org of this species is *Elaeagnus rhamnoides* (L.) A. Nelson. The exact number of species in the

genus *Hippophae* is still unclear however, there are considered to be seven species and *Hippophae rhamnoides* has nine subspecies (Lu R, Ahani H, 2013). Iranian Sea Buckthorn in semi-arid distribution of native species, with good biological characteristics and economic benefits in small part of the country, for the first

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time has been studied. In brief, the drought avoidance and drought tolerance mechanisms include water conservation (small leaves, limited leaf area and stomatal closure), effective water conservation (extensive, deep or dense root systems), turgor maintenance (osmotic adaptation and low elastic modulus) and synthesis of protective solutes and desiccation tolerant enzymes (García-Sánchez *et al.*, 2010). Previous study showed, using chlorophyll and photosynthesis measurements under drought conditions that the biochemical capacity of physiology for increasing photosynthesis to survival (Susiluoto and Berninger, 2007).

However, up to now, there still exist controversies over their drought-resistance mechanisms and adaptive strategies, and few researches have been reported on the effects of precipitation changes and water stress on Sea Buckthorn (Li *et al.*, 2005; Zhang, 2005; Li *et al.*, 2007; Guo *et al.*, 2007). Differences in drought adaptation among populations of *Eucalyptus microtheca* of Australia were demonstrated and attributed to differences in morphological and physiological responses to water availability. The effect of watering and interaction between watering and population were highly significant (Gibson *et al.*, 1995). Drought depressed the growth, RWC, net assimilation rate, stomatal conductance and transpiration rate of *Adansonia* seedlings but increased their water use efficiencies. *Adansonia* species are able to withstand drought by reducing water loss through stomatal closure and their ability to store water within roots (Randriamanana *et al.*, 2012).

Stressful environments have a more negative impact on females than on males in shoot height on Sea Buckthorn, whereas females had higher specific leaf area, stomatal length and stomatal index than males along the altitudinal gradient (Li *et al.*, 2007). The controlled

experiment was to compare the ability of *Hippophae rhamnoides* L. to acclimate to a water deficit by architectural plasticity and growth responses. Their changes in branching pattern parameters, and in dry matter accumulation and allocation, were recorded after 2 years of exposure to four different water supply levels. Their branching patterns showed that *H. rhamnoides* tended to expand horizontally, with more, shorter, thinner branches and a larger branch angle. Root to shoot ratio of 315, 227, 167 and 115 mm artificial precipitation were 0.42, 0.43, 0.62 and 0.71 respectively. Leaf Area and Special Leaf Area were 1710, 800, 391, 95 cm<sup>2</sup> and 38, 34, 32, 16 cm<sup>2</sup> g<sup>-1</sup>. WSD were decreased 0.42, 0.46, 0.51 and 0.54 g g<sup>-1</sup>. (Guo *et al.*, 2007). An increase of belowground proportion was observed indicating higher root/shoot ratio of *H. rhamnoides* under drought stress conditions, and drought further increased N and P use efficiencies and intrinsic water use efficiency (Fang *et al.*, 2012). Compared with *Salix paraqpleisia* under medium drought stress 40% field capacity (FC), *H. rhamnoides* showed less change of morphology characteristics, higher P use efficiency, and water use efficiency (WUE) due to better N sources. However, *S. paraqpleisia* showed higher N use efficiency and WUE under severe drought stress condition (20%FC) than *H. rhamnoides*. The results suggested that the expanding dry valley could seriously influence endemic species in the ecotone, and N-fixing plant such as *H. rhamnoides* could adapt to moderate drought stress better than non-N-fixing plant (Fang *et al.*, 2012).

For longer time scales, plant structural modifications and growth pattern adjustments maybe important indicators of the consequences of water deficit. Purpose of this study was to investigate different morphological and physiological responses of this species. Investigation on the underlying physiological responses has not been reported in the literature

previously in Iran. This study investigated the water relations of *Elaeagnus rhamnoides* to simulated drought, and to explore the relationship between physiological responses and types of irrigation, in order to further evaluate the drought-resistant capacity and uncover underlying physiological mechanism of *Elaeagnus rhamnoides*. With increasing aridity and growing population, water will become an even scarcer commodity in the near future (Passioura, 2002). Therefore, in this study, an artificially controlled water gradient experiment was carried out, based on four levels of water supply. The hypothesis of this study is tolerating of this species in dry conditions. Will physiological system accelerate the rate of resistant at which drought stress treatment develops?

## 2 MATERIALS AND METHODS

### 2.1 Plant material and experimental design

Seeds were obtained from the Qazvin origin of Iran and sown on plastic pots (70 cm<sup>3</sup>). After seed germination, the seedlings were put into plastic pots (1600 cm<sup>3</sup>) and grown for about 4 month. Eighty four healthy seedlings of uniform height were chosen and each seedling was transferred to 84 pots (Fang *et al.*, 2012). Soil of pots had texture of containing 67% sand, 23% silt 10% clay and 1.04 organic matters. Bulk density, EC and pH were 1.42 g cm<sup>-3</sup>, 6.61 dS m<sup>-1</sup> and 7.4 respectively due to soil laboratory experiment of Research Center of Khorasan Razavi province. Our experiments were conducted in a nursery under semi-controlled environmental conditions for a whole growing season. The seedlings were grown in a natural environment for the rest of the experiment in the field of Torogh nursery of Mashhad. In the well-watered treatment I, 21 pots of each population were watered to 100% of field capacity by supplying an amount of water by weighting whole pot of each treatment equal to transpiration losses every other day (Saxton *et*

*al.*, 1986). In the water-stressed treatment II keeping drought in the soil; 21 pots of seedlings were maintained by watering every four days. In the water-stressed treatment III (keeping severe drought in the soil) 21 pots were irrigated by watering every eight days and in the water-stressed treatment IV (keeping severe drought in the soil) 21 pots were affected by watering every twelve days. Four-month-old seedlings were examined with four different water regimes corresponding to 2, 4, 8, 12, days duration for four month. We selected five seedlings of each treatment randomly for water relations (Pourhashemi *et al.*, 2012). The experimental design was a randomly complete block design that included four treatments with seven replicate plants in each treatment in three blocks.

### 2.2 Water use efficiency

For the gas exchange measurements, net photosynthesis rate and stomatal conductance, was measured using the ADC-LCA4. We selected five seedlings of each treatment for this step. Transpiration efficiency or Physiological water use efficiency (WUE) was calculated by dividing net photosynthetic rate by Evapotranspiration (E) according to (Wu *et al.*, 2008; Farajia *et al.*, 2009).

### 2.3 Relative water content

The soil-related parameters were measured in the laboratory of Khorasan Razavi research center, and relative water content were recorded too (determined with weighing method, each treatment repeated seven times). Measurements were performed on the youngest fully expanded leaves. The midday relative water content (RWC) was calculated after measuring the fresh, turgid and dry weights (dw) of leaf discs. Measurements were performed as follows: discs from adjacent leaves were immediately weighed to obtain a fresh weight. The turgid weight was obtained by weighing discs after placing them in water overnight in the darkness. The leaves were

subsequently dried at 80 °C for 24 h and reweighed to obtain their dry weights. The RWC was calculated as  $[(M_f - M_d) / (M_t - M_d)] \times 100$ , according to (Morgan, 1984; Díaz-López *et al.*, 2012), where  $M_f$  is the fresh weight,  $M_t$  is the turgid weight and  $M_d$  is the dry weight.

## 2.4 Water potential

All measurements were made with a single mature leaf from the mid-stem region of each of the seven replicate plants. Pre-dawn and mid-day leaf water potentials were measured every other day using a pressure chamber (Figure 2). The sample leaves were cut at the base with a sharp blade and placed in the chamber with the petioles protruding through the opening of the chamber. The pre-dawn leaf water potential ( $\Psi_w$ ) was measured at the end of the experiment using a pressure chamber (PMS instrument, Corvallis).

## 2.5 Water Saturation Deficit

WSD was calculated as formula (Čatský, 1969; Guo *et al.*, 2007):  $100 - \text{RWC}$ .

## 2.6 Growth traits

At the end of the experiment, biomass samples divided into shoot (leaves and stem) and root were weighed then measured freshly (Beadle *et al.*, 1993). These traits have been shown by R for Root and S for Shoot in this paper.

## 2.7 Statistical analysis

We used Shapiro-Wilk and Bartlett's test for normality test and equal variances of data respectively. Because of non-laboratory experiment for pots, two-way analysis of variance procedure was used with the randomized complete block design. Pearson's correlation coefficients were calculated to determine the relationships between variables for different treatments. Statistical analyses were done with the SAS and Mini Tab software package. Differences between means were

separated using Tukey's Studentized Range (HSD) Test at 0.05 level of probability.

# 3 RESULTS

## 3.1 Potential points

Important potential points including permanent wilting point (PWP) and field capacity (FC) of pots soil were drawn (Figure 1). These points used for calculating amount of water for irrigation each pot of each treatment.

## 3.2 Water use efficiency

With the extension of drought stress from 2 to 12 days, can be seen from figure 3 *Elaeagnus rhamnoides* seedlings WUE increased. Nevertheless, WUE only showed a little differences with respect to the control for the water regime corresponding to same field capacity (Table 1).

## 3.3 Relative water content

Most of the RWC gradually declined with decreasing water supplies (Table 1). However, significant differences between drought-stressed treatments were observed first and second. In addition between third and fourth regime we did not see significant difference ( $P < 0.05$ ).

## 3.4 Water potential

WP decreased, while drought increased from first to last regime, all of treatments has been shown by significantly different in the table 1 ( $P < 0.05$ ). Compared to the control treatment, the second onward to last drought treatments decreased  $\Psi_w$ .

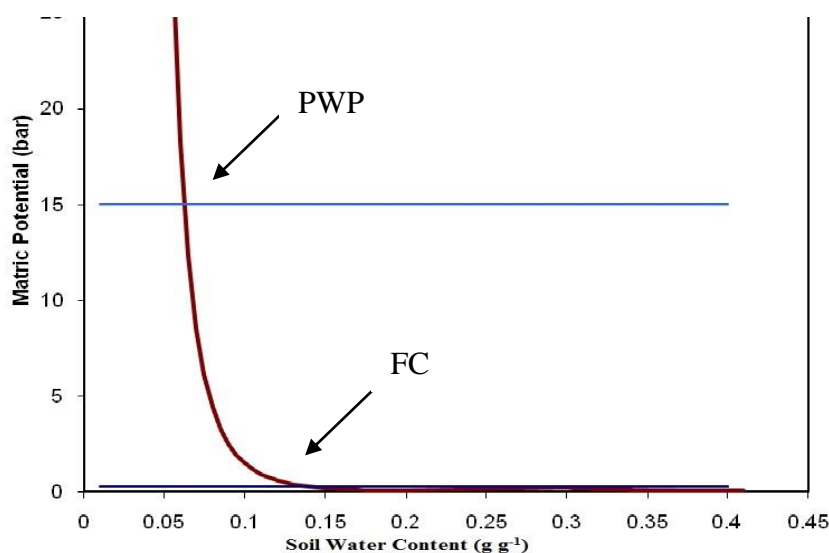
## 3.5 Water Saturation Deficit

WSD increased gradually with both time and increasing soil water deficit. At this sampling day, the WSD values did not differ significantly between the three and four treatments; only between one and second in this index we observed three group by Tukey test.

### 3.6 Growth traits

Root (R) and shoot (S) weight also tended to decrease progressively with the drought treatments and time. At the end of the experiment, significant differences at 0.05 levels were not observed between 8 and 12 days-irrigated in both of R and S weight. In R between first and second treatments were not significant difference but in S between 2 days versus 4 days watering regime we saw significant difference. In addition, the growth reduction in the drought stress treatment (12 days) compared with the wellwatered (2 days)

seedlings was higher in the S than in the R, i.e., 95.5%, and 65.8%, respectively. Consequently, R was significantly higher in the drought-stressed plants than in those given an adequate water supply. Weight was decreased by all of the drought treatments with respect to the well-watered plants; the values in the treatments were not similar to each other. Reasons for procedure will be explained in discussion part.



**Figure 1** PWP and FC of soil in the present study

**Table1** Mean with standard error of study characteristics. Changes in irrigation treatments with WUE, RWC, WP, R and S of *Elaeagnus rhamnoides* under drought stress

Treatments	WUE ( $\mu\text{mol mmol}^{-1}$ )	RWC (%)	WP (bar)	WSD (%)	Root (g)	Shoot (g)
2 days	5.64 $\pm$ 0.19 <sup>a</sup>	70.89 $\pm$ 1.59 <sup>a</sup>	12.80 $\pm$ 0.06 <sup>a</sup>	29.11 $\pm$ 1.59 <sup>a</sup>	2.31 $\pm$ 0.07 <sup>a</sup>	3.75 $\pm$ 0.01 <sup>a</sup>
4 days	6.23 $\pm$ 0.13 <sup>a</sup>	62.19 $\pm$ 1.99 <sup>b</sup>	15.62 $\pm$ 0.17 <sup>b</sup>	37.81 $\pm$ 1.99 <sup>b</sup>	1.89 $\pm$ 0.02 <sup>b</sup>	3.42 $\pm$ 0.03 <sup>b</sup>
8 days	7.15 $\pm$ 0.09 <sup>b</sup>	29.14 $\pm$ 0.17 <sup>c</sup>	19.15 $\pm$ 0.18 <sup>c</sup>	70.86 $\pm$ 0.17 <sup>c</sup>	0.812 $\pm$ 0.01 <sup>c</sup>	0.31 $\pm$ 0.00 <sup>c</sup>
12 days	7.57 $\pm$ 0.11 <sup>b</sup>	26.33 $\pm$ 1.74 <sup>c</sup>	24.46 $\pm$ 0.21 <sup>d</sup>	73.67 $\pm$ 1.74 <sup>c</sup>	0.79 $\pm$ 0.01 <sup>c</sup>	0.17 $\pm$ 0.00 <sup>c</sup>

### 3.7 Statistical analysis

Table 1 shows the Pearson's correlation coefficients at the end of the drought-stress period for the physiological and biomass variables of *Elaeagnus rhamnoides* under four watering regimes. WUE positively correlated with the WP only and negatively correlated with R and S.

Data in the same column followed by different small letters are significantly different at 0.05 levels, same with the following table.

S were correlated with all of variables in first treatment except R. RWC were positively correlated with WP but negatively correlated with S. WP was negatively correlated with S, R and WSD in first treatment.

In second treatment there were robust significant negative correlations between WUE and WP, WSD and S; also positively correlation with RWC measured. WP was positively correlated with S. The RWC was negatively correlated with R. WSD was positively correlated with R in 2days treatment. As can be seen from Table 2 there were significant negative correlations between WUE

and WP. RWC had negatively correlation with R but positively correlated with S in third watering regime. WP was positively correlated with R and negatively with S. The R was negatively correlated with S. The WSD was positively correlated with R but negatively correlated with S.

In fourth treatment there were significant positive correlations between WUE with WP, R and S measured solely. RWC was also positively correlated with R. Also, WP was positively correlated with S but negatively correlated with the R. WSD had negatively correlation with R in 12 days treatment.

Between traits of different watering regime, based on Tukey's HSD (honest significant difference) can be seen from table 1. Means followed by different letters are significantly different ( $P < 0.05$ ) according to Tukey's multiple range tests.

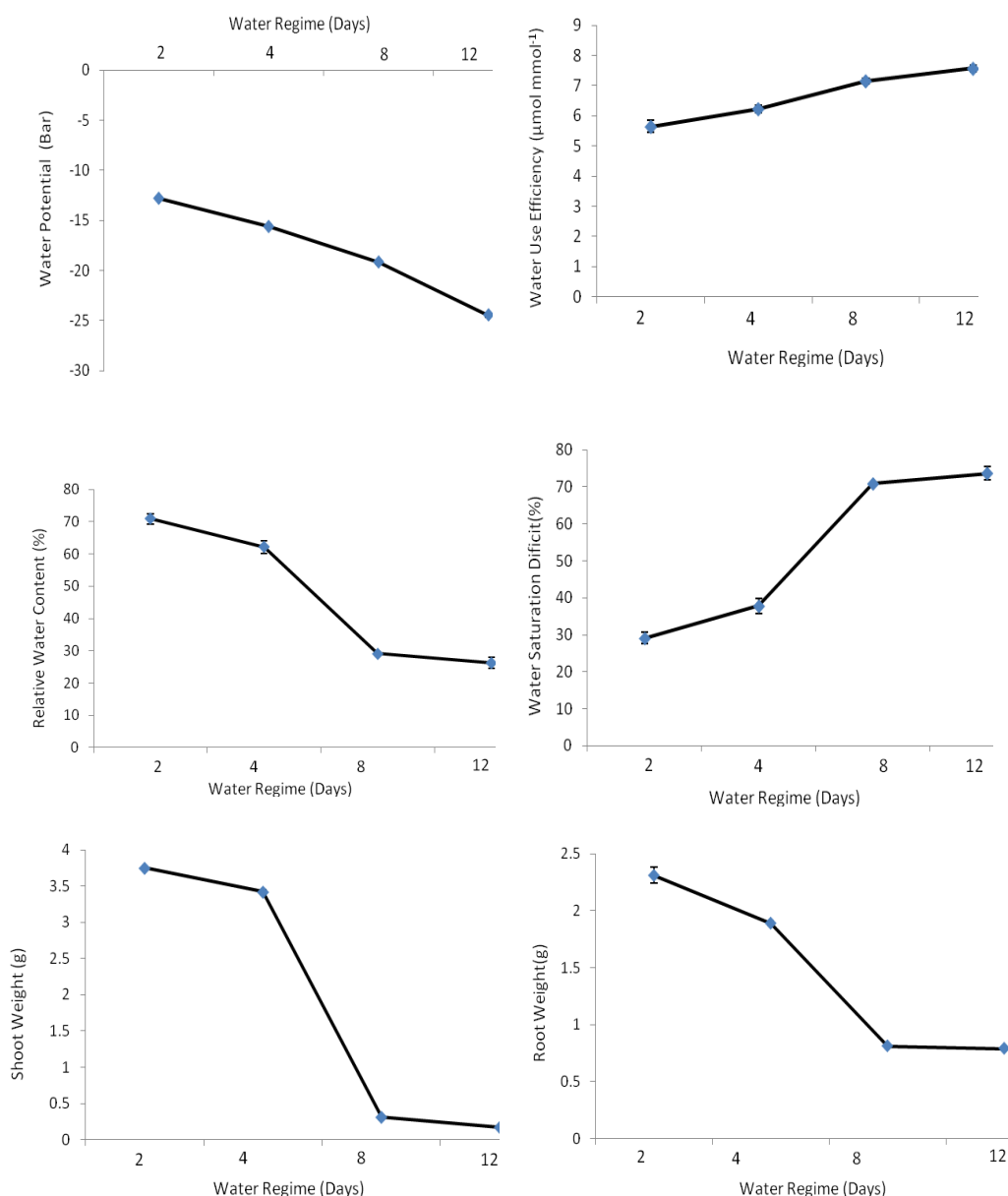


**Figure 2** Water potential measuring in seedlings leaf subjected to different treatments (Left) and seedlings after treatment (Right)

**Table 2** Correlation coefficients of physiological characters (WUE, RWC, WP and WSD) and biomass production (R and S) of *Elaeagnus rhamnoides* under four watering regimes

Water Regime (days)		WUE	RWC	WP	WSD	R
2	RWC	0.62 <sup>ns</sup>				
	WP	0.91 <sup>**</sup>	0.89 <sup>**</sup>			
	WSD	-0.62 <sup>ns</sup>	-1 <sup>**</sup>	-0.89 <sup>**</sup>		
	R	-0.94 <sup>**</sup>	-0.33 <sup>ns</sup>	-0.72 <sup>**</sup>	0.33 <sup>ns</sup>	
	S	-0.81 <sup>**</sup>	-0.99 <sup>**</sup>	-0.98 <sup>**</sup>	0.96 <sup>**</sup>	0.57 <sup>ns</sup>
4	RWC	0.71 <sup>*</sup>				
	WP	-0.79 <sup>*</sup>	-0.13 <sup>ns</sup>			
	WSD	-0.71 <sup>*</sup>	-1 <sup>**</sup>	0.14 <sup>ns</sup>		
	R	-0.51 <sup>ns</sup>	-0.97 <sup>**</sup>	-0.11 <sup>ns</sup>	0.97 <sup>**</sup>	
	S	-0.89 <sup>**</sup>	-0.31 <sup>ns</sup>	0.98 <sup>**</sup>	0.31 <sup>ns</sup>	0.06 <sup>ns</sup>
8	RWC	-0.21 <sup>ns</sup>				
	WP	-0.86 <sup>**</sup>	-0.31 <sup>ns</sup>			
	WSD	0.21 <sup>ns</sup>	-1 <sup>**</sup>	-0.31 <sup>ns</sup>		
	R	-0.52 <sup>ns</sup>	-0.72 <sup>*</sup>	0.88 <sup>**</sup>	0.72 <sup>*</sup>	
	S	0.49 <sup>ns</sup>	0.74 <sup>*</sup>	-0.86 <sup>**</sup>	-0.74 <sup>*</sup>	-0.99 <sup>**</sup>
12	RWC	-0.67 <sup>ns</sup>				
	WP	0.99 <sup>**</sup>	-0.63 <sup>ns</sup>			
	WSD	0.67 <sup>ns</sup>	-1 <sup>**</sup>	0.63 <sup>ns</sup>		
	R	-0.73 <sup>*</sup>	0.99 <sup>**</sup>	-0.70 <sup>*</sup>	-0.99 <sup>**</sup>	
	S	0.83 <sup>**</sup>	-0.14 <sup>ns</sup>	0.86 <sup>**</sup>	0.14 <sup>ns</sup>	-0.24 <sup>ns</sup>

P&lt;0.05\*, P&lt;0.01\*\*, ns: no significant



**Figure 3** Variations of indices subjected to different water regime treatments

#### 4 DISCUSSION

In the present study, the leaf water relation parameter data suggest that *E. rhamnoides* could be considered a semi drought-tolerant plant because it was able to maintain an adequate plant water status under mild stress. The data in this study indicate that the WUE, RWC, WP, WSD, R and S are effective tools

for rapid estimation of physiological indices in Sea Buckthorn during the growing season; except destructive for sample pots. Once general relationships are established for assessment of physiological changes over time and delineating the effects of management practices such as irrigation. As can be seen from table 1, with the drought process, all of



indices except WUE had undergone significant changes in 2 to 4 days watering regime. Therefore between physiological and morphological index same WUE in compare R and S can be seen differently.

There were significant correlations between some traits in first and third watering regime more than others watering regime. These relations show us the 2 days watering regime has been matched with all of the indices. Correlation showed which traits can be deleted for future study. In our study WSD and RWC had most correlation. WUE was not suitable for comparing correlation between first and second watering regime.

WP had significant changes with the downward trend during four types drought stress. WUE in 4, 8 and 12 days drought were 1.1, 1.26 and 1.34 times than the first treatment; while RWC in drought 4, 8 and 12 days were 0.87, 0.41 and 0.37 times than the first watering regime. On the contrary, WP in drought 4, 8 and 12 days were -1.22, -1.49 and -1.91 in minus upward trend. WSD in 4, 8 and 12 days drought were 1.29, 2.43 and 2.53 times than the first treatment; while R and S in drought 4, 8 and 12 days were 0.81, 0.35 and 0.34 times and 0.91, 0.08 and 0.04 times than the first watering regime respectively. Compared with the 2 day, all of treatments changed. The highest shoot and root dry weights were observed in the first treatment.

Differences in drought adaptation among populations of *Eucalyptus microtheca* were demonstrated and attributed to differences in morphological and physiological responses to water availability (Gibson *et al.*, 1995). This result supports our study species. Since survival strategies are adaptations to the environmental conditions in which the seedlings have evolved. Our seedlings water relations may differ in another condition. Study on *Leucaena leucocephala* showed that the seedlings had a good ability to keep water as its Water Leaf

Content (LWC), and did not decrease significantly until 12 days drought. The WUE increased (Chen *et al.*, 2012), also in our research results showed that seedlings could maintain a reasonable physiological activity by keeping water, increasing WUE and WSD and reducing WP, biomass and RWC when subjected to drought stress.

Four-month-old *Jatropha curcas* seedlings, maintained a good water status regardless of the drought stress treatment because all water regimes affected the leaf relative water content, whereas the leaf water potential was only reduced in the water-stressed plants from the 0% and 25% FC treatments. Drought treatments reduced leaf, stem and root growth. However, the decrease in growth was higher in the aerial part of the plant than in the root (Díaz-López *et al.*, 2012), similar of our result on Sea Buckthorn. Hence, we conclude that the strong control of transpirational water loss by reducing both A (Photosynthesis) and biomass from aerial parts could be involved in the ability of our study plant same as above research to resist drought conditions. This was accompanied by a reduced allocation to roots, results that are in accordance with *Adansonia digitata*'s responses to drought (Cuni Sanchez *et al.*, 2011; De Smedt *et al.*, 2012). Apart from developing deep fine roots to access underground water (like most drought-tolerant species), *Adansonia* seedlings develop thick taproots enabling them to store water (Wickens and Lowe, 2008; De Smedt *et al.*, 2012), similar of our result on Sea Buckthorn for decreasing of RWC and flexibility of morphological traits. *Adansonia grandidieri* and *A. rubrostipa* which both originate from drier environments allocated more resources to such root development in comparison with *A. madagascariensis*. Seedlings from drier provenances have higher water content and invest more biomass in their root system but less in foliage (Cuni Sanchez *et al.*, 2011; De Smedt *et al.*, 2012). WUE in

*Eucalyptus camaldulensis* was decreased with ascending drought stress (Gindaba et al., 2005). WUE of *Populus davidiana* significantly increased with the increase of drought stress (Zhang et al., 2004), same as the present study. Water use efficiency significantly increased in *Catharanthus roseus* under water stress. Drought stress biomass yield (Abdul Jaleel et al., 2008). An efficient osmotic adjustment in drought-treated *Jatropha curcas* seedlings at lower  $\Psi_w$  values ( $-1$  MPa) than were observed in our experiment, which produced  $\Psi_w$  values ranging between  $-0.36$  and  $-0.70$  MPa. On the other hand,  $\Psi_w$  tended to decline with the drought treatments, but the leaf water relative content was not affected. These plant–water relationship parameters suggest an elastic adjustment process in non-irrigated *J. curcas* seedlings [Silva et al., 2010]. Well-watered *Jatropha curcas* seedlings produced  $1.49 \pm 0.31$  g dry biomass day<sup>-1</sup>. Under medium stress (40% plant available water) they produced at lower rate ( $0.64 \pm 0.18$  g day<sup>-1</sup>) (Achten et al., 2010). The highest shoot and root weights were observed in the well-watered treatment. These parameters gradually declined with decreasing water supplies. Generally, the root and shoot ratio was significantly higher in the drought-stressed plants than in those given an adequate water supply. Under drought in *Adansonia digitata* L. resulted in a midday leaf water potential of  $-1$  MPa followed by significant leaf loss water content (Van den Bilcke<sub>1</sub> et al., 2013). The reduction in irrigation in the tamarind seedlings was reflected in a decrease in WUE (Van den Bilcke<sub>2</sub> et al., 2013), This idea is supported by the fact that treated seedlings, regardless of the drought treatment, showed slowly declines in leaf RWC.

WUE of *Hippophae rhamnoides* significantly increased with the increase of drought stress (Fang et al., 2012). Drought increased the root/shoot ratio, long-term water use efficiency, declined the net photosynthesis

rate, total biomass on Sea Buckthorn. Under drought conditions, leaf relative water content (RWC) decreased in humid population greatly, but not in arid population. Gas exchange traits, were less responsive to drought in high population than those in low population (Gang, 2007). This research shows the RWC and gas exchange factor not follow regularly by drought. Sea Buckthorn anatomical structures have to adapt to drought. Drought conditions, the Sea Buckthorn leaf thickness is reduced, fence tissue development is stronger than the spongy tissue, is one of the strategies of the Sea Buckthorn adapt to arid environment. Sea Buckthorns female were used to drought adaptability more than the male plants (Liu, 2005). Artificially controlled water gradient experiments of *H. rhamnoides* were carried out with both shrubs, based on four levels of water supply (normal precipitation, slight drought, drought, and extreme drought). The results showed significant impacts of drought on net photosynthesis rate, biomass accumulation, and biomass allocation in both species. Water use efficiency varied with different species, scales, and water stress intensities. This idea is supported by treated seedlings, regardless of the drought treatment, showed declines in RWC, biomass and WP.

WUE at the leaf scale (WUE<sub>i</sub>) was highest under moderate water stress, while the WUE at the community scale (WUE<sub>b</sub>) decreased with increasing water stress (Guo et al., 2010), whereas in our study WUE was increased by drought. Sea Buckthorn uses water resources more efficiently under favorable water conditions, and *Caragana intermedia* shows competitive advantages under drought conditions (Guo et al., 2010).

The highland population of Sea Buckthorn in Tibet experienced a greater inhibition in plant growth, lower root nodule biomass and root mass/foilage area ratio, and higher LWC loss paralleling with higher enhancement of abscisic

acid level in response to drought, as compared with lowland population. WUE in drought condition had not significant difference in compare of control treatment. LP showed effective adaptation strategies such as improvement of water economy (Yang *et al.*, 2010). However, the higher reduction observed in leaves than in roots caused an increase in the root-to-shoot ratio. This redistribution of photo assimilates in favour of the roots instead of the shoots could be an adaptive mechanism to reduce the evaporative surface area.

In any woody and shrubby plants, the leaf relative water content reaches 40–50% and occasionally as low as 20% during severe drought, which is accompanied by leaf senescence (Galle *et al.*, 2007). In compare with other researches we conclude physiological traits of each provenance of Sea Buckthorn is changable. Our data suggest that the drought tolerance of our seedlings could be based on morphological and physiological responses that minimise transpiration in the plant.

## 5 CONCLUSION

In conclusion, *E. rhamnoides* has a mild ability to maintain water in leaf texture, and drought can increase WUE and WSD, reduce the RWC, WP, R and S rate during drought stress. All the physiological characteristics underlie the mild drought resistance of this species, which could explain the reason why it was selected as a pioneer species for ecological restoration of the degraded ecosystems in the Iran for future. Thereupon for parsimony of water in duration less than 8 days this species can be efficiency irrigation. Therefore the existence of a large number of species and varieties of *Hippophae* *sp* growing in many diverse habitats enables the selection of species and seed sources for almost any environmental condition, including severe drought. The results of the present study showed that growth of this species was affected due to water stress. However, the species of this

species had the ability to survive under water stress but they behaved differently in terms of enduring such a similar level of water stress. Water availability is a major limitation to plant productivity and is one of the major factors regulating the distribution of plant species. Our results provide strong evidence for adaptive differentiation between seedlings. However, changes in water potential and water use efficiency, relative water content under drought were rather modest.

Currently, over 35% of world's land surface is considered to be arid or semi-arid, receiving precipitation that is inadequate for most agricultural uses. Agricultural regions affected by drought can experience yield losses up to 50% compared to other unaffected areas (FAO, 2013). These differences in drought responses maybe used as criteria for genotype selection in arid and semi-arid regions. From a practical point of view, because the mechanism for coping with drought conditions has a strong effect on growth, decreasing greatly the aerial part biomass, and because the intrinsic water use efficiency is decreased from third treatment onward, we can conclude that the this crop could be used in semiarid environments to avoid soil degradation. Physiological traits of each provenance of Sea Buckthorn differ even by changing in elevation of one region; hence study on seedling tolerate of every seed origin before plantation is very important. Further research is required on the types of stress such as wastewater, salinity and drought by this species.

Nevertheless, in the near future, for plantation the real water requirements of Sea Buckthorn on field to obtain a good yield (production and quality) should be evaluated in semi arid conditions with high evaporative demand in the summer, when the rainfall is scarce, comparing it with other plants and studying its economic viability to determine its possible use in arid habitat.

## 6 ACKNOWLEDGEMENT

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## پاسخ فیزیولوژیکی سنجد تلخ (*Elaeagnus rhamnoides* (L.) A. Nelson) به رهیافت‌های مصرف آب

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**چکیده** برای ارزیابی تأثیر تنش خشکی روی مورفولوژی نهال سنجد تلخ (*Elaeagnus rhamnoides*) ایران، آزمایشی برای اولین بار در دنیا در نهالستان انجام شد. تفاوت زیست‌توده و صفات فیزیولوژی در نهال‌های مبدأ بذر قزوین در چهار تیمار بررسی شد. در قالب طرح بلوک‌های کامل تصادفی در سه تکرار و چهار تیمار آبیاری ۲، ۴، ۸ و ۱۲ روزه انجام شد. کارایی مصرف آب، محتوی نسبی آب، پتانسیل آب، کمبود آب اشباع، وزن تر ریشه و شاخه بعد از چهار ماه اندازه‌گیری شد. نتایج نشان دادند که تحمل گیاه به خشکی با صفات فیزیولوژی ارتباط زیادی دارد. با افزایش دور آبیاری از دو به دوازده روز و از هشت به دوازده روز، کارایی مصرف آب نهال‌های سنجد تلخ افزایش معنی‌داری نشان داد. محتوی نسبی آب با کاهش آبیاری به تدریج کم شد و پتانسیل آب برگ نیز کاهش نشان داد. کمبود آب با افزایش تنش خشکی زیاد شد ولی تفاوت معنی‌داری بین تیمار سوم و چهارم دیده نشد. علی‌رغم کاهش وزن زیست‌توده با افزایش تنش خشکی، بین تیمار آبیاری هشت روز و دوازده روز در وزن ریشه و شاخه تفاوت معنی‌داری دیده نشد. نتایج این تحقیق گام نخست و نگاهی جدید برای بررسی مکانیزم تحمل این گیاه به خشکی را نشان می‌دهد.

**کلمات کلیدی:** اکوفیزیولوژی، تحمل، تنش، سنجد پر خار