Effects of Different Water Stress on Photosynthesis and Chlorophyll Content of *Elaeagnus rhamnoides*

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Abstract

We studied the response of *Elaeagnus rhamnoides* (Sea Buckthorn) to drought stress in a nursery. Photosynthesis and chlorophyll content under drought were change rather modest. Growth and physiological differences in response to drought were compared between four Sea Buckthorn seedlings treatments inhabited in the Qazvin provenance origin seeds in Mashhad city of Iran. The experimental design included four water regimes (100% of field capacity) and three blocks. Our experiments were under semi-controlled environmental conditions for four growing season. Four-month-old seedlings were examined with four different water regimes corresponding to 2, 4, 8, 12, day's duration. At the end of watering regimes, Chlorophyll content and Photosynthetic of leaves was measured using SPAD, CCI (Chlorophyll Concentration Index) and A (Photosynthetic rate) by several devices. The A was positively correlated with SPAD and DS (Dry Stem) but negatively correlated with the CCI and DR (Dry Root) in all of treatments except 12 day irrigation. CCI was positively correlated with DR, in 2day treatment, with SPAD and DR in 8day with all of traits in 4day and 12 day but negatively correlated with others. There were significant negative correlations between SPAD with DR in first watering regime and DS in third watering regime. Between DR and DS were significant positive correlations only in 12day treatment but significant negatively correlated in 2day and 8day watering regime measured. We concluded SPAD and A were tolerated efficiently between 4day and 8day, then we can irrigate seedlings more than 4day and less than 8day treatment. Different watering treatments employ different strategy for physiological and morphological responses of this plant.

Keywords: CCI; SPAD; *Hippophae*; Iran; Biomass;Water stress

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Introduction

With increasing aridity and growing population, water will become an even scarcer commodity in the near future (Passioura, 2002). Sea Buckthorn (*Hippophae rhamnoides* L.) (Elaeagnaceae) has become a crop of interest for the food processing industry. Accepted name of this species is *Elaeagnus rhamnoides* (L.) A.Nelson (Nelson, 1935).

The seedlings of *Eucalyptus camaldulensis* Dehnh. and *Eucalyptus globulus* Labill were encountered to glasshouse. In well watered seedlings, both *Eucalyptus* species had higher A and g (stomatal conductance) as compared to another deciduous species (*Cordia africana* Lam., *Croton macrostachyus* Del., *Millettia ferruginea* Hochst.) (Gindaba *et al.*, 2004). Drought resistance mechanism for water conservation include limited leaf area and stomatal closure, extensive and deep roots, osmotic and turgor maintenance and synthesis desiccation tolerant hormones (García-Sánchez *et al.*, 2010). Results on *Adansonia* species investigation indicated that unwell watered treatment decreased the growth and A of seedlings but promoted water use efficiency (Randriamanana *et al.*, 2012).

Environment with drought conditions have a more pressure on females than on males height, on *Hippophae rhamnoides*, whereas females have more specific leaf area and stomata than males (Li *et al.*, 2007). *Hippophae rhamnoides* L., a deciduous and dioecious shrub, mainly restricted to sunny and south facing slopes (Lu, 1992). Water strategy of *H. rhamnoides* and *Caragana intermedia* were studied in China, result showed under extreme drought conditions *H. rhamnoides* was less efficiently competitive advantages than another species (Guo *et al.*, 2010).

In China, *Hippophae rhamnoides* compared with *Salix paraqplesia* under 40% field capacity, *H. rhamnoides* had less morphology changes and water use efficiency. *S. paraqplesia* showed higher water use efficiency under 20% field capacity than *H. rhamnoides*. The researchers found *H. rhamnoides* can adapt to moderate drought more than non-nitrogen fixing species (Fang *et al.*, 2012). Physiological and morphological characteristics are important indicators of the water deficit and drought stress of plants. Study on Photosynthesis, Chlorophyll and biomass on *Elaeagnus rhamnoides* seedlings has not been reported in the papers previously in Iran, but there are several papers about this subject on Sea Buckthorn in other countries. This study has investigated physiological responses of *Elaeagnus rhamnoides* to four drought stress treatments and appraises several characteristics of this plant and levels of irrigation, in order to further research the capacity of different origins Sea Buckthorn to drought tolerant.

The hypotheses were that during the drought stress Sea Buckthorn seedlings would grow less, increase their root biomass, chlorophyll and photosynthesize of third and fourth less than first and second treatment.

Materials and Methods

Plant materials and experimental design

Seeds from the Qazvin population of Iran were sown on plastic pots (70 cm^3). After germination of seeds, the seedlings were situating into plastic pots (1600 cm^3) and grown for 4 month. Eighty four uniform seedlings in order to height and leaves approximately were chosen and transferred to pots containing 7% sand, 23% silt, 10% clay and 1.04 organic matters. Bulk density, EC and pH were 1.42 g/cm^3, 6.61 ds/m and 7.4 respectively in order to soil laboratory experiment of Research Center of Khorasan Razavi province. The seedlings were grown under natural environment in the field of Torogh nursery of Mashhad. Artificially controlled water supplies treatments were carried out on four levels (normal precipitation, slight drought, drought and extreme drought) (Guo *et al.*, 2010). In the well water treatment I, 21 pots were irrigated to every other day. Second treatment by keeping drought II; 21 pots were watered every four day. In the third water stressed treatment III, 21 pots were watered every eight day and other pots (IV treatment) were affected by watering every twelve day. Four-month-old seedlings were examined with four different water regimes corresponding to 2, 4, 8, 12, day's duration for four month.

Photosynthetic rate

For the gas exchange measurements, net photosynthesis rate (A), was measured using the ADC-LCA4 gas exchange system, with a 4 cm^2 aperture plate in the leaf cuvette (Fig.I). Gas exchange measurements were done on fresh expanded leaves.

Fig. I. Measurement of seedlings traits subjected to different devices.

Chlorophyll content measurement

Chlorophyll content of leaves was measured using a SPAD (Special Product Analysis Division) Chlorophyll meter (Konica Minolta sensing Inc) and CCI (Chlorophyll Content Index) meter by CCM-200 at the end of watering regimes (Fig.I).

Biomass measurement

Growth traits at the end of experiment were measured. Seedlings were harvested in each water treatment of the pots. Biomass samples divided into shoot (leaves and stem) and root were dried to constant and weighed then measured based on the procedure by Beadle at al. (1993). Dry roots (DR) and dry shoot (DS) and presented in the results section.

Statistical analysis

Two-way analysis of variance procedure was used with the randomized complete block design (RBCD). Pearson's correlation coefficients were calculated to determine variables relationships between different treatments. Statistical analyses were carried out with the SAS and MiniTab software package. Differences between means were defined using Tukey's Studentized Range (HSD) test at 0.01 level of probability. General and stepwise regressions were calculated to determine the relationships between A as a response variable and others as predictors for different treatments in differential watering regime.

Results

Table 1 shows Pearson's correlation coefficients at the end of experiment for variables physiological characters (A, CCI, SPAD) and biomass production (DR and DS) of *Elaeagnus rhamnoides* under four watering regimes. The A was positively correlated with SPAD and DS but negatively correlated with the CCI and DR in all of treatments except 12 day irrigation. CCI was positively correlated with DR, in 2day treatment, with SPAD and DR in 8day with all of traits in 4day and 12 day but negatively correlated with others.

As shown in Table 1 there were significant negative correlations between SPAD with DR in first watering regime and DS in third watering regime. Between DR and DS were significant positive correlations only in 12day treatment. With the extension of drought stress from 2day to 12day, as shown in Table 2 *E. rhamnoides* leaf photosynthetic rate (A), CCI, SPAD, DR and DS changed and except in DR, valuables decreased significantly ($P < 0.01$). All traits decreased, while DR increased from first to second regime and then decreased to fourth regime (Table 2). Most of the traits gradually decreased with declining water supplies (Fig.II) . In addition, significant diiferences between treatments were only seen first to

Table 1
Correlation coefficients of physiological characters (A, CCI, SPAD) and biomass production (DR and DS) of *Elaeagnus rhamnoides* under four watering regimes.

Treatment		A	CCI	SPAD	DR
2Day	CCI	-0.95**			
	SPAD	0.73**	-0.91**		
	DR	-0.87**	0.98**	-0.97**	
	DS	0.98**	-0.86**	0.56ns	-0.74**
4Day	CCI	0.59*			
	SPAD	-0.74**	0.09 ns		
	DR	0.41 ns	0.98**	0.3 ns	
	DS	-0.78**	0.03 ns	0.99**	0.25 ns
8Day	CCI	-0.95**			
	SPAD	0.08 ns	0.22 ns		
	DR	-0.52 ns	0.76**	0.81**	
	DS	0.6*	-0.81**	-0.74**	-0.99**
12Day	CCI	0.7*			
	SPAD	0.86**	0.97**		
	DR	0.75**	0.99**	0.98**	
	DS	0.96**	0.87**	0.97**	0.89**

ns: no significant, P<0.05*, P<0.01**

second regime and these treatments had significantly different with third and fourth regime in A trait (P <0.01). Afterwards the growth reduction in drought stress treatment (12day) compared with the wellwatered (2day) seedlings was higher in the leaves and stems (DS) than in the roots (DR), as 90.15% and 51.02%, respectively. Thereupon, DS was significantly more in drought stressed plants than in those given an adequate water supply.

Leaf gas exchange and chlorophyll fluorescence declined gradually with increasing soil water deficit, reached to least values at the end of drought-stress period (Table 2).The A values did not differ significantly between 4day and 8day; 8day and 12day treatments. CCI tended to decrease progressively with drought stress, except for seedlings from 8day to 12 day regimes. At the end of drought stress period significant difference at 0.01 levels were observed between 12day-irrigated versus well-watered plants (2day) in all of traits except DR.

Seedlings that those grown under fourth treatment conditions showed no significant reduction in all of study traits by a significant increase in the irrigation duration with regards from the 8 day onwards. Result of regression formula showed the higher R square in 2day and 12day treatments than 4 and 8day watering regime (Fig. II).

Discussion

The data in this study indicate that the SPAD, CCM and ADC are effective tools for rapid and nondestructive estimation of chlorophyll content and photosynthetic rate in Sea Buckthorn leaves during the growing season. Once general relationships are established for assessment of

Table 2
Mean with standard error of study characteristics. Changes in irrigation treatments and Photosynthetic rate (A), CCI, SPAD, DR (Dry Root) and DS (Dry Shoot) of *Elaeagnus rhamnoides* under drought stress.

Treatments	A ($\mu molm^{-2}s^{-1}$)	CCI	SPAD	DR (g)	DS (g)
2day	9.22±0.47a	4.04±0.11a	39.32±0.11a	0.49±0.01a	1.32±0.01a
4day	5.60±0.42b	1.88±0.66ab	33.69±2.25a	0.68±0.35a	0.84±0.02b
8day	2.98±0.27bc	0.81±0.18b	28.59±2.67ab	0.25±0.02a	0.16±0.007c
12day	1.54±0.18c	0.99±0.29b	16.29±1.07b	0.24±0.01a	0.13±0.002c

Data in the same column followed by different small letters are significantly different at 0.01 levels, same with the following table.

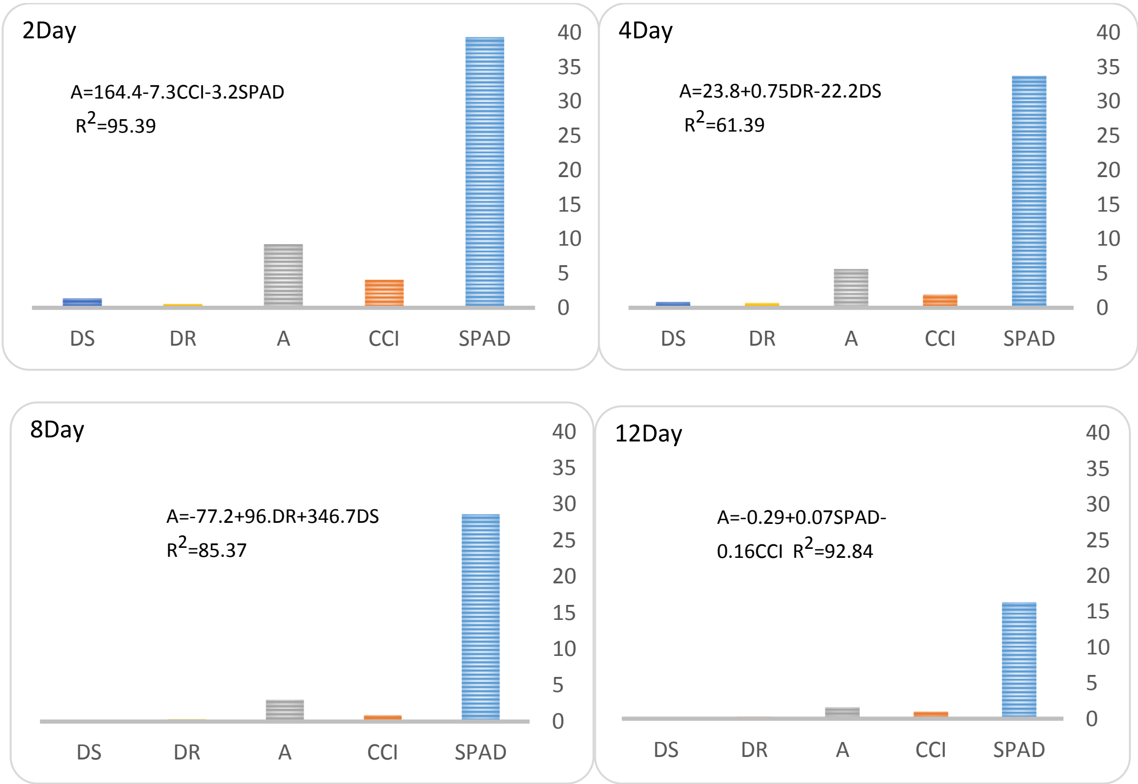

Fig. II. Comparison of characteristics means in *E. rhamnoides* L. seedlings in response to drought stress treatments by general regression formula and R square of each watering regime

physiological changes over time and delineating the effects of management practices such as irrigation. Therefore between CCI and SPAD can be seen differently sometimes. There were significant correlations between some traits in second and third watering regime less than fourth watering regime. Then first and final watering regime were suitable for comparing correlation. A, CCI, SPAD, DR and DS had significant differents with the downward trend during drought stress. A in 4, 8 and 12day drought were 0.61, 0.32 and 0.17 times than the first tratment; while CCI in drought 4 and 12 days were 0.46 and 0.24 times than the first watering regime. On the contrary, CCI in drought 8 and 12 were 0.2 and 0.24 in not significant upward trend. Compared with the 2day, all of treatments decreased. The highest shoot and root dry weights were observed in the first and second treatments, respectively. These results shows us *Hippophae rhamnoides* of Iran by progressively drought from 2day to 4day irrigation, decreased Chlorophyll content more than Photosynthesis, in addition declining in water supplies downward to 8day cause same result, but downward to 12day irrigation had not significant differences. We can be seen by increasing drought stress from 8day to 12 day cholorophyll was ascended for carring out Photosynthesis, then plants try to survival. Plant in drought conditions increased Chlorophyll content and reduced photosynthesis activity in longtime irrigation for instance in 12day in *Leucaena leucocephala* (Chen *et al*., 2012) such as our result in fourth treatment. The result of decreasing in net assimilation CO_2 for photosynthesis in all treatments by ascending of drought on *Jatropha curcas* species was same *Elaeagnus rhamnoides* (Diaz-Lopez *et al*., 2012).

Water stress affected dry biomass in different population such as result on many species for instance *Eucalyptus* that populations from northwest and central Australia had higher biomass than in those from southeastern Australia (Gibson *et al*., 1995; Tuomela, 1997). In this research strong control of transpirational water loss by reducing both A and biomass from aerial parts could be involved in the ability of *H. rhamnoides*, that are in accordance with studies on *Adansonia digitata* 's responses to drought stress. Hence, biomass depend on seedlings provenances (Cuni Sanchez *et al*., 2011; De Smedt *et al*., 2012). Previous studies showed, using chlorophyll and photosynthesis measurements under drought conditions that the biochemical capacity of physiology for increasing photosynthesis to survival (Susiluoto & Berninger, 2007).

In a study showed Sea Buckthorn female plants used to drought adaptability more than male plants (Liu, 2005). In addition *Hippophae* of China in more altitude had lower growth than less elevation (Yang *et al*., 2010). Drought increased the root/shoot ratio, long-term water use efficiency, declined the net photosynthesis rate, total biomass. Gas exchange traits, were less responsive to drought in high population than those in low population (Gang, 2007). Sea Buckthorn anatomical structures have to adapt to drought. Drought conditions, the Sea Buckthorn leaf thickness is reduced, palisade mesophyll tissue development is stronger than the spongy tissue, is one of the strategies of the Sea Buckthorn adapt to arid environment. In our study DR/DS ratio from 4day treatment to extreme drought treatment were reached from 0.82 to 1.92 and in Chinese Sea Buckthorn were 1.53 to 1.58 respectively in approximately same conditions of seedlings and irrigation (Fang *et al*., 2012). Photosynthesis increased with the soil water content. In normal condition A of male plants is higher than female Sea Buckthorn. Under drought stress female photosynthesis is more than male's. Photosynthesis of subsp. *rhamnoides* was more than subsp. *Sinensis* (Liu, 2006). Such as another results (Guo *et al*., 2010), resistance of seedlings varied with different intensities of water stress. *H. rhamnoides* uses water resources more efficiently under favorable water. Between viability of 8day and 12 day watering regim, based on Tukey s HSD (honest significant difference) can be seen no significant different. Consecuently for appraisal of tolerance more research are needed.

E. rhamnoides has a strong ability to maintain leaf water and can increase chlorophyll content, reduce the photosynthesis during drought stress. All the physiological traits underlie the drought resistance of Sea Buckthorn, which could define the reason why it was selected as a pioneer species for restoration of degraded forest of Iran and other countries that situated in semi-arid zones.

Consequently for parsimony of water supplies for efficiency irrigation of seedlings of this species can be recommended between 4day and 8day. The results of the present study showed that growth of this species was affected due to water stress. However, 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. Our results provide strong evidence for adaptive differentiation between seedlings on four drought stress levels. Currently, over 35% of world's terrestrials are considered to be arid and semi-arid. Agricultural lands affected by drought can experience yield losses up to 50% compared to others regions (FAO, 2013). Hence, Sea Buckthorn is suggested for horticulture activities because of pharmaceutical and alimentary affairs. These differences in responses to drought stress maybe used as criteria for genotype and elite selection for inbreeding. 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. Further research is required on the types of stress such as wastewater, salinity and drought by this species.

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