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Original Article

Physiological responses of *Pistacia khinjuk* (Stocks) seedlings to water stress

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Abstract

Because in recent years many attempt to planting *Pistacia khinjuk (Stock*) seedlings in blank forest area in Zagross mountain region. Other hand a little information available on the impact of drought stress on physiological functions of *Pistacia khinjuk* Stocks seedlings. Therefore seedling of *Pistacia khinjuk* were subjected to drought stress but irrigation in four different time (every third day, every fifth day, every seventh day and every ninth day). Indicators such as; photosynthesis gas exchange, stomatal conductance, chlorophyll and praline content were monitored for the duration of the irrigation treatment. The obtained results indicated that the photosynthesis deceased in every ninth day irrigation and internal stomatal CO_2 concentration from 280µmol μ^{-1} in every third day increased to 300.05µmol μ^{-1} in every ninth day. Stomatal conductance from 0.172µmol m-2 s^{-1} in every third day decreased to 0.012µmol m-2 s^{-1} in every ninth day irrigation treatment. Total Content of chlorophyll a, and chlorophyll b were varied. There were significantly differences between treatments for each parameter of chlorophyll. Variation in praline concentration between treatments was different and increasing concentration of praline occurred with increasing drought stress. Prolin might play a more complex role conferring drought resistance.

***Key words*: Water stress, Photosynthesis, Stomatal conductance, Proline, *Pistacia khinjuk* (Stocks)**

Introduction

Pistacia khinjuk (stocks) is a native species widely distributed in semi-arid and arid mountain and high-land steppes all over Iran (Sabeti,1976 and Djavanshir,1976). It is also covered 2.400.000 ha. In Zagross mountain and established pure or mixed with Oak as an open forests (Fani,2004). *Pistacia khinjuk* (stocks) is an important tree for ecological conservation and resin production in Zagross mountain region. Therefore it might become evermore important for Mountain of Zagross if climate changes will have happen. Although in recent years many attempt to planting of *pistacia khinjuk* (Stocks) seedlings in blank forest area, but a little information is available on the impact of drought stress on physiological functions of *Pistacia khinjuk* (Stocks) seedlings. Environmental factors affecting seedling development and survival (e.g. light, water, nutrients, temperature and other plants or enemies) can severely limit the recruitment dynamics of plant population (Ray and Alcantara, 2006). To understand plant population treatment it is thus necessary to analyzed how seedling response to microhabitat variability. This responses may become apparent as changes in growth rate, leaf shape or biomass allocation (Gross, 1984; Tripath and Khan 1990; Saveri, muthu and Westoby 1996; Broncano, Riba and Retana, 1998; Huante Rincon; Chpin 1998 and Guerfel, 2008). In the spectrum of responses ranging from extreme drought avoidance to extreme tolerance (Auge,1998). The tolerance strategies involve immediate physiological and biochemical responses, whereas the avoidance strategies involve long-term developmental and morphological trait (Mc Cue and Hanson, 1990).

Although there is a continuous range of responses to water stress and not all plant fit closely into one or the other category, the division of plant responses into categories or strategies can be a valuable aid to understanding the ecological consequences for a species e. g. potential for Carbon acquisition and growth during drought, metabolic costs of resistance mechanisms, long-term plant survival (Auge, *et. al*., 1998). Several studies indicate that reductions in plant growth due to water deficit are associated with a decrease in photosynthesis activities (Pagter *et al*., 2005). The objective of this study is to evaluate *Pistacia khinjuk* (Stocks) physiological response to water stress.

Materials and Methods

Study area

The study was conducted in Sanandaj (35º 16′ N, 47º 1′ E and 1415 meters elevation) in Kurdistan province of Iran. The climate semi-dry Mediterranean type with a mean annual temperature is 13 ± 1ºC and mean annual rainfall of 450-500mm. The dry period typically extends from June to November. During the dry season the mean monthly temperature ranges from 18ºC in June to 25- 30ºC in July and August (Hajmirzaei, 2006).

Plant material and experimental treatment

Two years old plastic pot seedlings similarity in height, collar diameter, morphology, media and environmental conditions were selected from Grezeh forest nursery in Sanandj. The experimental used a completely random design (CRD) with four treatments of every third day, every fifth day, every 7th day and every 9th day irrigation. Each treatment comprised of 40 seedlings.

Physiological measurements

Photosynthetic gas exchanges parameters and stomatal conductance and transpiration were measured with LCA4. All measurements carried out at 10 - 12 am. and the highest light density 1000 -1200µ mol. m-2 s^{-1}(Yang *et al*., 2001 and Ahmad *et al*., 2002). As Gruters, *et al*.,(1995) reported at 1000µ mol, photon stomatal conductance will have maximum. Leaf sample were kept in freezer and using Acetone and Arnon method for measuring chlorophyll a and b concentration (Ashraf *et al.,* 1994). Chlorophyll a and b concentration and total chlorophyll calculated as;

$\{12.7(a) – 2.69(b) \}\times V \div (1000 \times W)$

$\{22.9(b) – 4.69(a) \}\times V \div (1000 \times W)$

$\{20.3(b) + 8.02(a) \}\times V \div (1000\times W)$

Where;

A: Amount of absorption in wave length 663nm
B: Amount of absorption in wave length 645nm
V: Total volume extracted
W: Fresh leaf sample weight

Measurement of praline concentration in leaf carried out according method (Bates *et al*.,1973). Data were evaluated with SPSS and differences between individual means was identified using Duncan Multiple range test(*p* <0.5%)

Results and Discussion

Higher photosynthesis was 3.998µ mol. $m^{-2}s^{-1}$ related to every third day irrigation treatment, while the lowest value of photosynthesis with 0.146 µmol. m^{-2} s^{-1} related to every ninth day irrigation treatment which was significantly different at %5 level. Value of photosynthesis related to four treatments are shown in fig.1

The computed F value for transpiration of different treatments was 21.748 which were significantly at %5 level. The comparison showed that the every 3rd day irrigation had the highest value (4.582µ mol m^{-2} s^{-1}) which was significantly different from every ninth day irrigation treatment value which was the lowest (0.892µ mol. m^{-2} s^{-1}). Although the data analysis showed that there were not significant differences between every 3rd day and every 9th day irrigation treatment. The mean value of seedlings transpiration calculated for four treatments are given in figure -2

Stomatal conductance

The computed F value for stomatal conductance was 16.294 which were significantly different at %5 level. Every 3rd day irrigation had the highest value while the every ninth day irrigation treatment had the lowest value. Means calculated for four treatments are given in fig.3.

The highest value of internal stomatal CO_2 concentration related to every fifth day irrigation treatment (300.050µ mol μ^{-1}) while every seventh day irrigation treatment showed the lowest value. The means calculated of internal stomatal CO_2 concentration are given in figure 4.

Chlorophyll

The content of chlorophyll a in leaves of seedlings was the highest for every fifth day irrigation treatment (1.290 mg g^{-1} DW), and the lowest value was 0.709 mg g^{-1} DW) for every third day irrigation treatment. The comparison between treatments showed significantly different at 5% level. Although content of chlorophyll b was highest for every fifth day irrigation treatment (0.43 mg/g^{-1}/ DW) and the lowest value was for every third day irrigation treatment (0.282mg/g^{-1}/DW) which was significantly different at 5% level. Content of total chlorophyll and chlorophyll a/b ratio were significantly different at 5% level. Every 5th day irrigation treatment had the highest value for total chlorophyll (1.721mg/g^{-1}/DW) and chlorophyll a/b (2.9998mg/g^{-1}/DW), while every 3rd day had the lowest value were 0.991mg/g$^{-1/}$DW and 2.595mg g^{-1}/DW for total chlorophyll and chlorophyll a/b respectively. Content of chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratio are given in table- 1.

Proline concentration

The variation of praline concentration between seedlings subjected to different treatments were more pronounced. The lowest concentration was found in every fifth day irrigation treatment and the concentration of increased with drought treatment. The contribution increased to 4.104 mg g-1/DW in the most stressed seedlings. The variation of proline concentration between treatments is given in fig. 5.

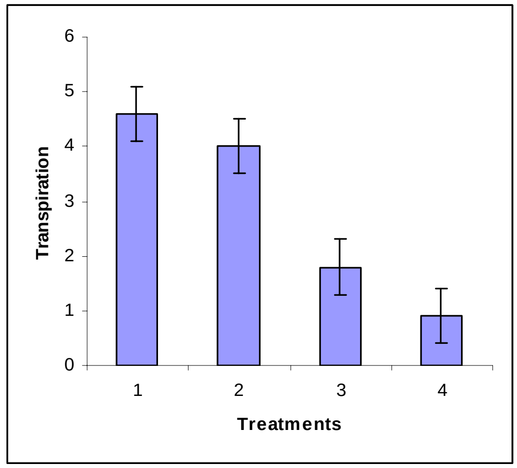

Fig. 2: Mean value of seedling transpiration in different treatment

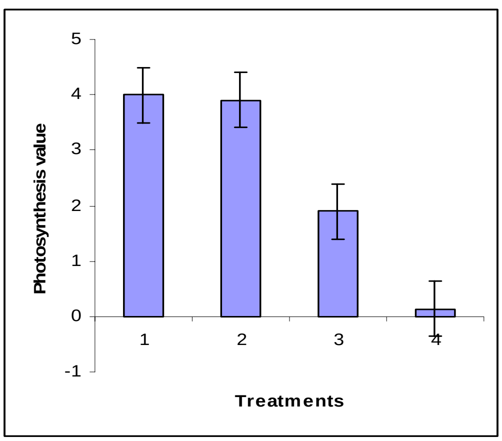

Fig.1: Photosynthesis value for treatments

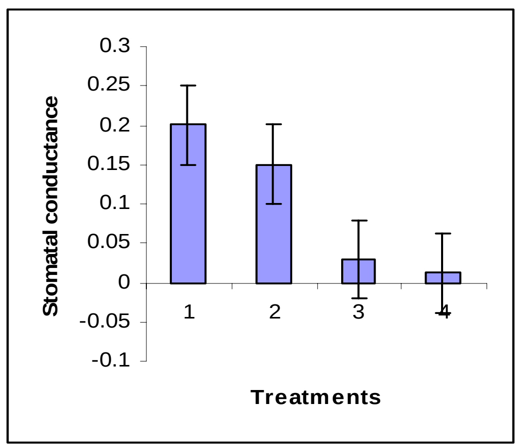

Fig.3: Mean value of seedlings stomatal conductance

Table - 1: Total chlorophyll content of irrigation treatments on *Pistacia khijuk*

Irrigation treatments	Chl. a mg/ g^{-1} DW*	Chl. b mg/ g^{-1} DW	Total Chl.mg/ g-^{1} DW	Chl. a/b ratio
Every third day	0.7.9 ± 0.04 d	0.282 ± 0.03 b	0.991 ± 0.06 d	2.595 ± 0.14 b
Every fifth day	1.29 ± 0.04 a	0.430 ± 0.03 a	1.721± 0.06 a	2.999 ± 0.14 a
Every seventh day	1.133 ± 0.04 b	0.384 ± 0.03 a	1.516 ± 0.06 b	2.960 ± 0.14 a
Every ninth day	0.873 ± 0.04 c	0.320 ± 0.03 b	1.193 ± 0.06 c	2.805±0.14 ab

Fig with different letter superscript within columns is statistically different at the 5% probability level.
*Leaf dry weight.

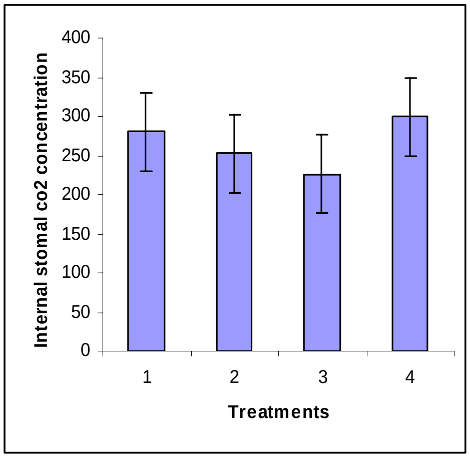

Fig. 4: Mean value of seedling internal stomatal CO_2 concentration**.**

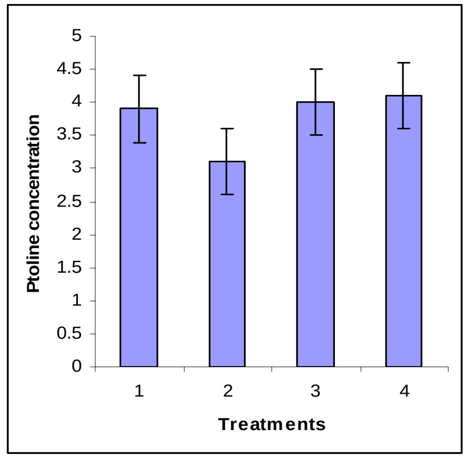

Fig. 5: Variation of Proline concentration in different irrigation treatment

Discussion

Differences in drought adaptation among seedlings of *Pistacia khijuk* (Stocks) were demonstrated to different in physiological responses to water availability significantly differences among seedlings were found in photosynthesis functions by increasing irrigation. No significantly different results was observed by increasing irrigation until the every 5th day irrigation, but in every seventh day irrigation the amount of photosynthesis function considerably was reduced. Decreasing photosynthesis may depends to low chlorophyll concentration (Castrilo and Calcagno,1984, Deborah and Bruce,1998 and Mohsenzadeh *et al*., 2005). Decreasing chlorophyll due to water stress results from a synthesis (Schuts and Fangmmeir,2001), Otherhand decreasing chlorophyll due to water stress may be to activity chlorophylls (Mihailovic *et al*., 1997) or chlorophylls and peroxides (Ashraf *et al*., 1994). The higher value for photosynthesis was found in every third day and the lower value was related to every ninth day irrigation treatment. There was significantly different between irrigation treatments, but no significantly differences were found between every third day and every 5th day irrigation.

Higher transpiration was observed in every 3rd day irrigation. However no significantly differences was found between every third and every 5th day irrigation, but other treatments had significantly different at 5% level. The decrease in stomatal conductance in *Pistacia khinjuk* (Stocks) seedlings under water stress was accompanied by decreases in transpiration rate and in CO_2 assimilation rate. Although stomatal conductance and the CO_2 assimilation rate decline significantly when plants were subjected to low water stress (every 5th day irrigation). All parameters stayed rather unaffected until plants were subjected to sever stress (every seventh day and every 9th day irrigation). According to Farquhar *et al*., (1989) a decrease in the partial pressure of CO_2 in the sub-stomatal cavities associated with a change in stomatal conductance will cause a decrease in the rate of CO_2 assimilation, which is proportionally smaller than that in stomatal conductance. Results reported by Parry *et al*.,(2002) suggested that impaired CO_2 assimilation in tobacco under water stress was due to lowered Rubisco activity. Whenever Rubisco activity was reduced by sever drought, it occurred at a stage where the CO_2 assimilation rate was already severely depressed (Pagter *et al*., 2005). This is in agreement with earlier studies concerning other plant species (Sanchez-Rodriguez *et al*., 1999, Tezara *et al*.,1999). Although stomatal closure seems to be the main determinant for decreased photosynthesis, but non-stomatal effects can not be completely neglected (Pagter *et al*., 2005).

The chlorophyll a and b and total chlorophyll content were significantly reduced by water stress. The lower amount of chlorophyll under water stress may be due to increased chlorophylls activity, although a stimulated activity under water stress might not be purely hydrolytic (Mihailovie *et al*., 1997).

The highest proline concentration was observed in every ninth day irrigation which was significantly different at %5 level. However high proline concentration was found in every third day irrigation, but like osmolality, the praline concentration did not increase significantly before the water stress level was rather high (Pagter *et al*., 2005). This is supported by observations of Thomas (1991) and Sanchez *et al*.,(1998), who reported that praline accumulated in leaf laminate of *Lolium perenne* and leaves of pea cultivars only after considerable drops in leaf water potentials. The importance of proline as an osmolyte therefore seems modest in the sense of osmotic adjustment, although the percentage was increasing with increasing water stress (pagter *et al*.,2005). Indeed, praline is primarily accumulating in the cytoplasm (Aubert *et al.,* 1999). Which makes up 10 - 20% or less of mature plant cells. Besides, praline might play a more complex role in conferring drought resistance than simply contributing to osmotic adjustment. According to Pagter *et al*., (2005) since praline only contributed 0.3 – 0.6% to total osmolality the content of other solutes must have increased considerably.

Conclusion

Water stress strongly affected on stomatal conductance of Pistacia Khinjuk (stocks) seedlings. Therefore the decrease in stomatal conductance was accompanied by decrease in transpiration rate and in Co2assimilation rate. It seems the stomatal closure to be the main determinant for decreased photosynthesis, but non-stomatal effects can not be completely neglected.

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References

Ahmad S., Nawata E., Hosokawa M., Domae Y. and Sakuratani T. 2002; Alterations in photosynthesis and some antioxidant enzymatic activities of mungbean subjected to water logging. *Plant Science* . 163: 117 – 123.

Ashraf M. Y., Azmi R. A., Khan H. A. and Ala A. S. 1994; Effect of water tress on total phenols , peroxides activity and chlorophyll content in wheat. *Acta physiologies plant-arum*. 16(3): 185 – 191.

Aubert S. Hennion F., Bouchereau A., Gout E., Bligny R. and Dorne A. J. 1999 ; Sub cellular compartmentation of praline in the leaves of the sub Antarctic Kerguelen cabbage *Pringlea antiscorbutica* R. Br. *In vivo* C-13-NMR study. *Plant Cell Environ*. 22: 255 – 259.

Auge Robert, M., Croker Xiangrong Duan, Jennifer, L., Witte Willard, T. and Green Craig, D. 1998. Foliar dehydration tolerance of twelve deciduous tree species. *Journal of Experimental Botany* ,49 (321); 753 -759.

Bates, I. S., Waldern, P. R. and Teare, D. I. 1973. Rapid determination of tree praline for water stress studies. *Plant and Soil*, 39: 205 - 207.

Broncano, M. J., Riba M. and Retana, J. 1998. seed germination and seedling performance of two Mediterranean tree species, Holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Mil.): A multifactor experimental approach. *Plant Ecology*, 138: 17 - 26.

Castrilo, M. and Calcagno, M. A. 1989. Effects of water stress and rewatering on ribulose 1,5 biphosphate carboxylase activity, chlorophyll and protein contents in two cultivars of tomato. *Journal of Horticultural Science*,64(6): 717 - 724.

Deborah, L. B. and Bruce, B. G. 1998. Photosynthetic capacity and dry mass partitioning in dwarf and semi-dwarf wheat(*Triticum aestivum*). *J. Plant Physiology*.153: 558 - 568.

Djavanshir Karim, 1976. Atlas of Woody Plants of Iran. Published by: The National Society for the Conservation of Natural Resources and Human Environment.

Fani, B. 2004. The effect of ecological factors on distribution of *Pistacia khinjuk* in Kurdistan province of Iran, Forest and rangeland Research Institute of Kurdistan province, Iran.

Farquhar, G. D., Wong, S. C., Evans J. R. and Hubick K. T. 1989. Photosynthesis and gas exchanges, in: Jones H. G., Flowers, T. J., Jones, M. B. (Eds.) Plant under stress. Cambridge University Press, Cambridge, pp. 47- 69.

Gross K. L. 1984. Effects of seed size and growth from on seedling establishment of six monocarpic perennial plants. *Journal of Ecology* ,72; 369 – 378.

Gruters, U., Fangmeire, A. and Jager, J. H. 1995. Modeling stomatal responses of spring wheat(*Triticum aestivum L. CV. Turbo*) to ozone and different levels of water supply. *Environmental pollution*. 87: 141 - 149.

Guerfel M. , Baccuri, O., Boujnah, D., Chibi, W. and Zarrouk, M. 2008; Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf stricture in the two main Tunisian olive (*Olea europaea L.*) cultivars. *Sciatica Horticulture* Vol. 119:3.

Hajmirzaee, A. 2006; Effect of drought stress on physiological functions of *Pistacia atlantica*. Msc. Thesis. Department of Forestry , Faculty of Natural Resources, University of Guilan, 93pp.

Huante, P., Rincon, E. and Chapin, F. S. 1998. Effect of changing light availability on nutrient foraging in tropical deciduous tree-seedlings. *Oikos*, 82: 449- 458.

McCue, K. F. and Hanson, A. D. 1990; Drought and salt tolerance: towards understanding and application. *TibTech*, 8: 358 - 362.

Mihailovic, N., Lazarevic M., Dzeletovic Z., Vuckovic and Durdevic M. 1997. Chlorophylls activity in wheat *Triticum aestivum L.* leaves during drought and its dependence on the nitrogen ion from applied. Plant Sci. 129: 141 – 146.

Mohsenzadeh, S., Malboobi, M.A., Razavi, K. and Farrahi-Aschtiani, S. 2006. Physiological and molecular responses of *Aeluropus lagopoides* (Poaceae) to water deficit. *Environmental and Experimental Botany*,56: 314 – 322.

Pagter Majken, Bragato Claudia and Brix Hans 2005; Tolerance and physiological responses of *phragmites australis* to water deficit. *Aquatic Botany*, 81; 285 -299.

Parry M. A. J., Andralogic P. J., Khan S., Lea P. J. and Keys A. J. 2002 ;Rubisco activity ; effects drought stress. Ann. Bot. 89: 833 – 839.

Rey Pedro J. and Alcantara Julio M. 2004; Seedling establishment in *Olea europaea*: Seed size and micro habitant affect growth and survival. *Ecoscience*, 11(1) 310-318.

Sabeti Habibollah, 1976. Forests, Trees and Shrubs of Iran. National Agriculture and Natural Resources Research Organization, Tehran Iran.

Sanchez, F. J., Manzanares M., de Andres E. F., Tenorio, J. L. and Ayerbe, L. 1998. Turgor maintenance, osmotic adjustment and soluble sugar and praline accumulation in 49 pea cultivars in response to water stress. *Field Crops Res*. 59: 225 – 235.

Sanchez-Rodriguez, J., Perez, P. and Martinez-Carrasco, R. 1999. Photosynthesis, carbohydrate levels and chlorophyll fluorescence –estimated intercellular CO_2 in water-stressed *Casuarina equistifolia* Forest. & Forest. Plant. *Cell Environ*. 22: 867 – 873.

Saverimuttu, T. and Westoby, M. 1996. Seedling survival under deep shade in relation to seed size. *Journal of Ecology*, 84: 681 -689.

Schutz, M. and Fangmeir, A. 2001; Growth and yield responses of spring wheat (*Triticum aestivum L. CV. Minaret*) to elevated CO_2 and water limitation. *Environmental Pollution*. 114: 187 – 194.

Tezara, W., Mitchell, V. J., Driscoll, S. D. and Lawlor, D. W. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401: 914 – 917.

Thomas H. 1991; Accumulation and consumption of solutes in swards of *Lolium perenne* during drought and after rewatering. *N. Phytol*. 118: 35 – 48.

Tripathi, R. S. and Khan, M. L. 1990; Effects of seed weight and micro site characteristics on germination and seedling fitness in two species of *Quercus* in a subtropical wet hill forest. *Oikos*, 57; 289 – 296.

Yang, J., Zhang, J, Wang, Z., Zhu, Q. and Wang, W. 2001; Remobilization of carbon reserves in response to water deficit during grain filling of rice. *Field crops research*. 71: 47 – 55.