ECOPERSIA

2013, 1 (4), 329-337

Peroxidase, Superoxide Dismutase and Catalase Activities of the *Pistacia khinjuk* Seedlings under Drought Stress

Javad Mirzaei[1]* and Hamed Yousefzadeh[2]

[1] *Department of Forest science, Faculty of Natural resources, Ilam University, Ilam, Iran.*
[2] *Department of Forestry, Faculty of Natural resources, Tarbiat Modares University, Noor, Iran.*

Received: 15 June 2013 / Accepted: 2 December 2013 / Published Online: 15 June 2014

ABSTRACT The aims of this study were to assess the effects of drought stress on peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) activities as well as free proline content and growth parameters of *Pistacia khinjuk* seedlings under drought stress. Therefore, the one-year seedlings of *Pistacia khinjuk* subjected to water stress (25%, 50%, 75%, and 100% field capacity) for 8 months in greenhouse condition. Results showed that drought stress decreased height, collar diameter, shoot dry weight and root dry weight of *P. khinjuk* seedlings. But it had different effects on antioxidant enzyme activities in root and shoot of *P. khinjuk* seedlings. Drought stress increased CAT activity in shoot and root of seedlings and its activity was higher in 25% field capacity than other treatments. Also, the POD enzyme activity increased in root and shoot of seedlings subjected to drought stress. The SOD activity was at the lowest level in 100% FC than other treatments. The results also showed that free proline accumulation was lower in well watered seedlings and increased under drought stress.

Key words: *Catalase, Drought stress, Peroxidase, Pistacia khinjuk, Superoxide dismutase*

1 INTRODUCTION

Pistacia khinjuk Stocks is a shrub or tree of the family Anarcadiaceae growing to 3-7 m tall in arid and semi-arid regions of Iran (Marvie-Mohadjer 2005). These are adapted to summer drought typical of Mediterranean climate and grew slowly in hardy and drought conditions in Zagros forests (Al-Saghir and Porter, 2005). The natural regeneration of this species is limited due to arid condition.

Drought stress is an important environmental factor that limits plant growth in Zagros region (Mirzaei *et al*., 2011). Seedling establishment is a critical process in these regions, especially under adverse environmental conditions. Drought stress is a severe abiotic environmental factor that constraint survival (Engelbrecht *et al*., 2005), growth (Xu *et al*., 2007), nutrient relations (Richardson, *et al.*, 2004; Engelbrecht *et al*., 2007), photosynthesis (Jinying, *et al.*, 2007; Apostol, *et al.,* 2009), and respiration (Aroca *et al*., 2008) in plants.

Seedlings adapt to environmental stress by different mechanisms, including changes in morphological and developmental patterns as well as physiological and biochemical processes. Adaptation is associated with maintaining osmotic homeostasis by metabolic

* *Corresponding author: Assistant Professor, Department of Forest science, Faculty of Natural resources, Ilam University, Ilam, Iran. Tel: +98 918 843 0219, E-mail: mirzaei_j452@yahoo.com*

adjustments that lead to accumulation of metabolically compatible compounds such as enzyme activity (Zhang *et al*., 2010), soluble sugar, and proline content (Jinyou, *et al*., 2004; Rao, *et al.*, 2008; Heidari and Moaveni, 2009). So that, drought stress had significant effects on free proline content of *Zizyphs spinosus* (Jinying *et al*., 2007), *Abies fabri* (Guo *et al*., 2010), *Albizzia lebbek*, *Dalbergia sissoo*, *Leucaena leucocephala*, *Shorea robusta* and *Tectona grandis* tree seedlings (Rao, *et al*., 2008). Also, enzymatic responses of *Juniperus oxycedrus* (Alguaci *et al*., 2006) and *Carthamus tinctorius* (Hojati *et al*., 2011) are determined. But, there is a little information about the different response mechanisms of *P. khinjuk* under drought stress. Therefore, an adequate supply of water is the most important factor determining the suitability of *P. khinjuk* cultivation areas in some regions of Zagros forest. Thus, this paper seeks to further clarify adoption and responding mechanism under drought stress and provide valuable information for *P. khinjuk* planting.

2 MATERIAL AND METHOD

2.1 Greenhouse experiment

P. khinjuk seeds were collected in November 2008 and maintained at 4°C for 2 months to overcome seed dormancy. Germinated seeds were transplanted into pots with a 2:1 (v/v) mixture of autoclaved native soil and sand (Table 1). Seedlings were watered to field capacity every 3 days for 2 weeks and arranged randomized in a greenhouse condition. The diurnal period was set at 12-14 h and day/night temperatures and humidity were set at 23/17°C and 35/43%. A completely randomize design was employed to study 4 levels of irrigation (25, 50, 75, 100% of field capacity). Treatments were arranged in 4 plants with 5 replicates of each treatment.

Table 1 The soil characteristics in the greenhouse experiment

Soil parameter	**Value**
pH (1:2.5)	7.5
Electrical conductivity EC (1.5 mmhos/cm)	392.7
Total organic C (%)	0.53
Total N (g/kg)	23.1
Total K (g/kg)	53
Total P (g/kg)	0.25
Mg (g/kg)	25
Ca (g/kg)	8.1
Sand (%)	50
Silt (%)	26
Clay (%)	24

2.2 Measurements and Analysis

Root and shoot biomass were determined after oven drying at 70°C for 90 h. Basal diameter, shoot height and root length were measured at the end of experiment.

For enzyme assays, frozen leaf and root samples were ground to a fine powder with liquid nitrogen and extracted with ice-cold phosphate buffer (pH 7.0). The extracts were centrifuged at 4° C for 20 min at 13000 g and the resulting supernatants, which were hereafter referred to as crude extracts, were collected and used for measuring the enzyme activities. The total superoxide dismutase (SOD) activity was determined according to the method described by Giannopolitis and Ries (1977). The activity of peroxidase (POD) was determined using the guaiacol oxidation method described by Kar and Mishra, 1976. The catalaze (CAT) activity measured using Cakmak and Horst (1991) method. Proline content was determined by the Ninhydrin and Sulfosalisilic acid method (Bates *et al*., 1973).

2.3 Statistical analysis

Normality of the variables was checked by Kolmogrov - Smirnov test and Levene test was used to examine the equality of the variances. Differences were tested with one-way analysis (ANOVA). Also, Duncan test apply to separate the averages of the dependent variables which were significantly affected by treatments.

3 RESULTS

3.1 The effect of drought stress on growth parameters

The results of growth parameters are shown at Table 2. Height and collar diameter (CD) of seedlings were the most in control treatment (100% FC) and decreased as drought stress tend to increasing. But, the root lengths (RL) of *P. khinjuk* seedlings were lower in control treatment than drought stress conditions.

The results of measured biomass demonstrated that drought stress decreased shoot dry weight (SDW) and root dry weight (RDW) of seedlings. As shown at table 1, the SDW and RDW tend to decrease as drought stress condition increasing.

Table 2 The effect of drought stress (DS) on height, collar diameter (CD), root length (RL), shoot dry weight (SDW) and root dry weight (RDW)

DS	Height (cm)	CD (mm)	RL (cm)	SDW (gr)	RDW (gr)
25 (%FC)	5.66±1.33 [b]	2.34±.095 [c]	86.6±9.45 [ab]	0.53±.084 [b]	1.03±.11 [c]
50 (%FC)	11.35±1.35 [b]	2.73±.21 [bc]	108.3±10.1 [a]	1.35±.27 [b]	1.83±.21 [bc]
75 (%FC)	26.16±5.32 [a]	3.14±.14 [b]	114.1±6.8 [a]	2.91±.48 [a]	2.75±.42 [a]
100 (%FC)	26.75±2.35 [a]	3.94±.22 [a]	77±5.3 [b]	3.32±.26 [a]	2.4±.31 [ab]

The same letter in the same row indicates not significantly difference (*P* >0.05), according to Duncan ' s test

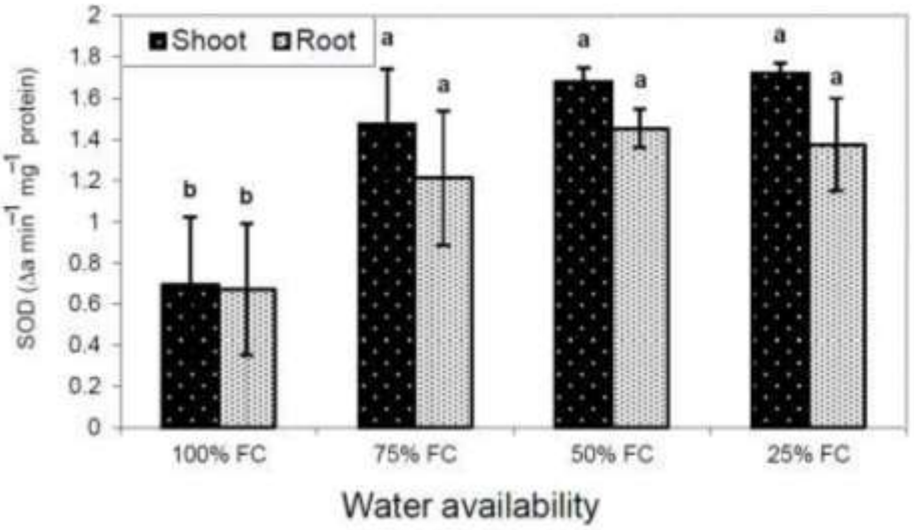

Figure 1 Comparison of the shoot and root SOD of *P. khinjuk* at different levels of drought stress; Error bars are ± SE

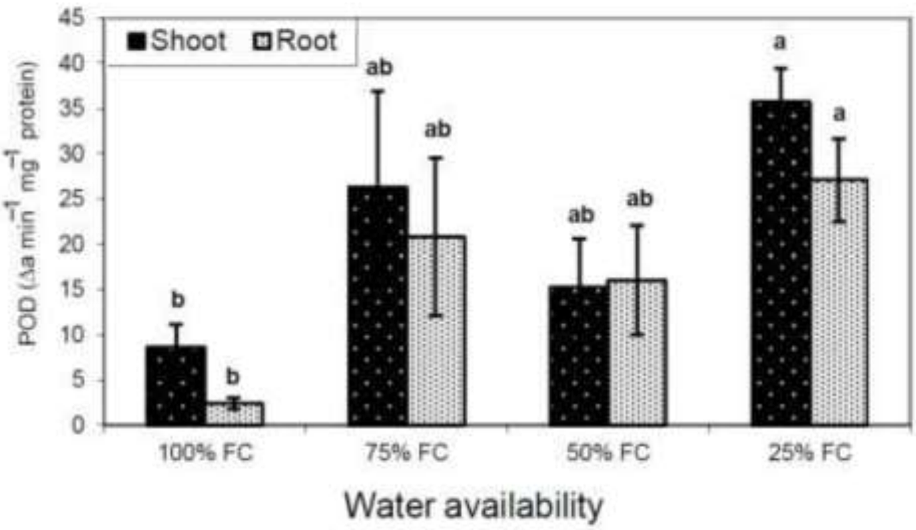

Figure 2 Comparison of the shoot and root POD of *P. khinjuk* at different levels of drought stress; Error bars are ± SE

3.2 The effect of drought stress on SOD, POD and CAT

The drought stress had different effects on SOD, POD and CAT enzyme activities. It increased the SOD in shoot of *P. khinjuk* seedlings, while drought stress had no effect on root SOD activity (Figure 1). Drought also had significant effects on POD enzyme of shoot and root. So that, the shoot and root POD was lower at control treatment (100 % FC) and increased in drought stress conditions (Figure 2). The activity of shoot CAT enzyme was higher at 25 %FC water availability, while drought hadn't significant effects on root CAT (Figure 3).

3.3 The effect of drought stress on proline content

The shoot and root proline contents were increased in plants subjected to drought stress. But it only had significant effects on free proline of shoots ($P<0.05$). The lowest amount of free proline content was seen at control treatment and it increased with increasing of drought stress (Figure 4).

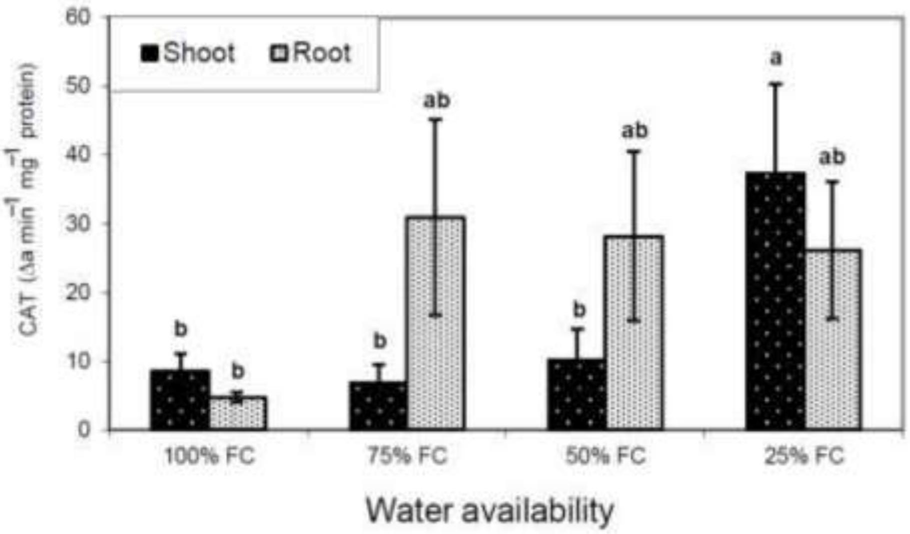

Figure 3 Comparison of the shoot and root CAT of *P. khinjuk* at different levels of drought stress; Error bars are ± SE

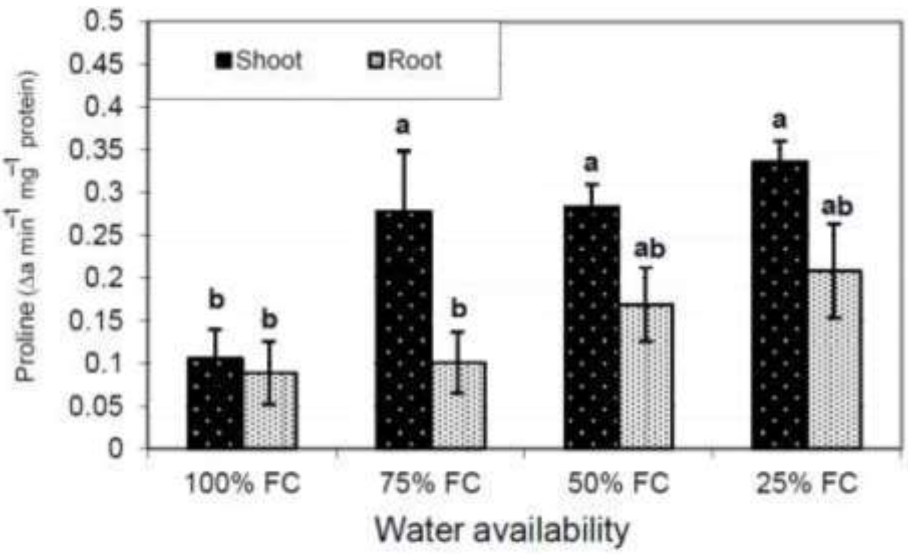

Figure 4 Comparison of the shoot and root proline of *P. khinjuk* at different levels of drought stress; Error bars are ± SE

4 DISCUSSION

P. khinjuk is an endemic and resistance species in dry and sub-dry forests in mountainous regions of Western Iran. In this study, growth and enzymatic responses of this species was assayed at different levels of water availability. The results indicated that drought stress decreased some biological parameter such as height and collar diameter of seedlings. This supported the former reports on *Sophora davidii* (Wau *et al.,* 2008), *Eucalyptus microtheca* (Li and Wang, 2003), *Zizyphs spinosus* (Jinying *et al.,* 2007) and *Poncirus trifoliate* (Wu and Xia, 2005) species. Also, this study showed that drought stress can decrease biomass of root and shoots. Similar results have been reported in some plants such as *Zizyphs spinosus* (Jinying *et al.,* 2007), and *Poncirus trifoliate* (Wu and Xia, 2005).

Although the accumulation mechanism of proline content in plant organs exposed to stress, but it is believed that engineering of the proline levels will greatly enhance the resistance capability of the plant to abiotic-stress (Cao *et al.,* 2011). High proline content in plants under stress could favor a better recovery of these plants and could also be a good defense mechanism for survival under stressful condition (Ghars *et al*., 2008). Our study showed that under drought stress, the free proline tends to increase in *P. khinjuk* organs, especially in shoot of seedlings (Figure 4). This is in accordance with observations of other studies (Smith, *et al.,* 2002; Jinyou, *et al.,* 2004; Porcel, *et al.,* 2005; Guo, *et al.,* 2010; Rao, *et al.*, 2008; Hojati, *et al.,* 2011).

According to previous studies, when the plant is subjected to oxidative condition such as water stress, the antioxidant enzymes (SOD, CAT and POD) are generated from plant tissues and its activity increased (Zhang *et al.,* 2010). They can easily convert superoxide anion radicals into O^{-2} and H_2O_2 and plant released from stressful condition. In our study, the SOD, POD and CAT activities were increased in shoots (Figure 4 and 5). Our results are confirmed by Alguaci *et al*., 2006 and Hojati *et al*., 2011 for *Juniperus oxycedrus* and *Carthamus tinctorius* respectively. They showed that there are significant correlations between plants resistance and the activities of antioxidant enzymes. This adaptation mechanism helps plants to stand in drought condition for short time. When the drought happens for long time, the induced active oxygen was greatly accumulated and the ability of the antioxidant system to cope with them decreased and damage to cellular components occurred (Sanchez, *et al.,* 1998; Suzuki and Mittler, 2006).

5 CONCLUSION

It is the first study regarding the morphological and biochemical responses of *P. khinjuk* under drought stress conditions. This study's results showed that water stress decreased all the measured morphological parameters, except root length. Moreover, the experiment results mainly presented that the proline content and the activities of POD, CAT and SOD antioxidant increased at shoot organ in drought condition. It seems that it is adaptation mechanism that *P. khinjuk* seedlings acquired in order to mitigate drought stress damages. So that, at low amount of water availability (25% FC) the seedlings survived up to end season growth, although, they have low height and collar diameter (Table 2). So, we suggest this species for reforestation in Zagros region, where there have sub-dry climate and there are amount of water availability for establishment of seedlings at first year.

6 ACKNOWLEDGMENT

We would like to thank Dr. Aria Babakhani, Dr. Mohamadi Goltapeh, Dr. Sharifi and Dr.

Akbarinia who helped us in scientific and laboratory works. Dr. Azade Salehi is thanked for help in revising the manuscript.

7 REFERENCES

Alguaci, M., Caravaca, F., Diaz-Vivancos, P., Hernandez, J.A. and Roldan, A. Effect of arbuscular mycorrhizae and induced drought stress on antioxidant enzyme and nitrate reductase activities in *Juniperus oxycedrus* L. grown in a composted sewage sludge-amended semi-arid soil. Plant Soil, 2006; 279: 209-218.

Al-Saghir, M.G. and Porter, D.M. Stomatal distribution in *Pistacia* sp. (Anacardiaceae). Int. J. Bot., 2005; 1(2): 183-187.

Apostol, K.G., Jacobs, D.F. and Dumroese, R.K. Root desiccation and drought stress responses of bare root *Quercus rubra* seedlings treated with a hydrophilic polymer root dip. Plant Soil, 2009; 315: 229-240.

Aroca, R., Alguacil, M.M., Vernieri, P. and Ruiz-Lozano, J.M. Plant responses to drought Stress and exogenous ABA application are modulated differently by mycorrhization in tomato and an ABA-deficient mutant (Sitiens). Microb. Ecol., 2008; 56:704-719.

Bates, L.S., Waldern R.P. and Teave I.D. Rapid determination of free proline for water stress studies. Plant Soil, 1973; 39: 205-207.

Cakmak, I. and Horst W. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glysin max*). Phisiol. Plant arum, 1991; 83:463-468.

Cao, H-X., Sun, C-X., Shao, H-B. and Lei, X-T. Effects of low temperature and drought on the physiological and growth changes in oil palm seedlings. African J. Biotech., 2011; 10 (14): 2630-2637.

Engelbrecht, B.M.J., Kursar, T.A. and Tyree, M.T. Drought effects on seedling survival in a tropical moist forest. Trees, 2005; 19 (3): 312-321.

Ghars, M.A., Parre, E., Debez, A., Bordenave, M., Richard, L., Leport, L., Bouchereau, A., Savoure, A. and Abdelly, C. Comparative salt tolerance analysis between *Arabidopsis thaliana* and *Thellungiella halophila*, with special emphasis on K+/Na+ selectivity and proline accumulation. J. Plant Physiol., 2008; 165: 588-599.

Giannopolitis, C.N. and Ries, S.K. Superoxide dismutase I. Occurrence in higher plants. Plant Physiol., 1977; 59: 309-331.

Guo, J., Yang, Y., Wang, G., Yang, L. and Sun, X. Ecophysiological responses of *Abies fabri* seedlings to drought stress and nitrogen supply. Physiol. Plant, 2010; 139: 335-347.

Hojati, M., Modarres-Sanavy, S.A., Karimi, M., Ghanati, F. Responses of growth and antioxidant systems in *Carthamus tinctorius* L. under water deficit stress. Acta Physiol. Plant, 2011; 33 (1): 105-112.

Jinying, L., Min, L., Yongmin, M. and Lianying, S. Effects of vesicular arbuscular mycorrhizae on the drought resistance of wild jujube (*Zizyphs spinosus* Hu) seedlings. Front. Agric. China, 2007; 1(4): 468-471.

Jinyou, D., Xiaoyang, C., Wei, L. and Qiong, G. Osmoregulation mechanism of drought stress and genetic engineering strategies for improving drought resistance in plant. Forestry Studies in China. 2004; 6 (2): 56-62.

Kar, M. and Mishra, D. Catalase, Peroxidase, and Polyphenoloxidase Activities during Rice Leaf Senescence. Plant Physiol., 1976; 57: 315-31.

Li, C. and Wang, K. Differences in drought responses of three contrasting *Eucalyptus microtheca* F. Muell. populations. Forest Ecol. Manag., 2003; 179: 377-385.

Marvie Mohajer, M. R. Silviculture. University of Tehran, Teharn., 2005; 37P.

Mirzaei, J., Akbarinia, M., Mohamadi Goltapeh, E., Sharifi, M. and Rezaei Danesh, Y. Effect of arbuscular mycorrhizae fungi on morphological and physiological characteristics of *Pistacia khinjuk* under drought stress. Iranian. J. Forest. Poplar. Res., 2011; 19 (2): 291-300.

Porcel, R., Gómez, M., Kaldenhoff, R. and Ruiz-Lozano, J.M. Impairment of NtAQP1 gene expression in tobacco plants does not affect root colonisation pattern by arbuscular mycorrhizal fungi but decreases their symbiotic efficiency under drought. Mycorrhiza, 2005; 15: 417-423.

Rao, P.B., Kaur, A. and Tewari, A. Drought resistance in seedlings of five important tree species in Tarai region of Uttarakhand. Trop. Ecolo., 2008; 49(1): 43-52.

Richardson, A.D., Aikens, M., Berlyn, G.P. and Marshall, P. Drought Stress and Paper Birch (*Betula papyrifera*) Seedlings: Effects of an organic biostimulant on plant health and stress tolerance, and detection of stress effects with instrument-based, noninvasive methods. J. Arboriculture, 2004; 30(1): 52-61.

Sanchez, F.J., Manzanares, M., Andres, E.F., Tenorio, J.L. and Ayerbe, L. Turgor maintenances, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in water stress. Field. Crop. Res., 1998; 59: 225-235.

Suzuki, N. and Mittler, R. Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction, Physiol. Plant, 2006; 126: 45-51.

Troll W. and Lindsley, J. A photometric method for the determination of praline. Biol. Chem., 1955; 215: 655-660.

Wau, F., Bao, W., Li, F. and Wu, N. Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of *Sophora davidii* seedlings. Environ. Exp. Bot., 2008; 63: 248-255.

Wu, Q.S. and Xia, R.X. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J. Plant Physiol., 2006; 163: 417-425.

Xu, H., Biswas, D.K., Li, W.D., Chen, S.B., Zhang, L., Jiang, G.M. and Li, Y.G. Photosynthesis and yield responses of ozone-polluted winter wheat to drought. Photosynthetica, 2007; 45: 582-588.

Zhang, Y., Zhong, C.L., Chen, Y., Chen, Z., Jiang, Q.B., Wu, C. and Pinyopusarerk, K. Improving drought tolerance of *Casuarina equisetifolia* seedlings by arbuscular mycorrhizas under glasshouse conditions. New Forests, 2010; 40: 261-271.

فعالیت آنزیم‌های پراکسیداز، سوپراکسید دیسموتاز و کاتالاز در نهال‌های خنجوک (*Pistacia khinjuk*) تحت تنش خشکی

جواد میرزایی[1] و حامد یوسف زاده[2]

۱- گروه علوم جنگل، دانشکده کشاورزی، دانشگاه ایلام، ایلام، ایران

۲- گروه جنگلداری، دانشکده منابع طبیعی دانشگاه تربیت مدرس، نور، ایران

تاریخ دریافت: ۲۵ خرداد ۱۳۹۲ / تاریخ پذیرش: ۱۱ آذر ۱۳۹۲ / تاریخ چاپ: ۲۵ خرداد ۱۳۹۳

چکیده مهم‌ترین هدف این تحقیق ارزیابی اثرات تنش خشکی بر فعالیت آنزیم‌های پراکسیداز (POD)، سوپراکسید دیسموتاز (SOD) و کاتالاز (CAT) و هم‌چنین رشد و محتوای پرولین نهال‌های خنجوک می‌باشد. برای این منظور، نهال‌های یک ساله خنجوک را در شرایط گلخانه‌ای و برای مدت ۸ ماه تحت تنش خشکی (۲۵، ۵۰، ۷۵ و ۱۰۰ درصد ظرفیت زراعی) قرار داده شد. نتایج نشان داد که تنش خشکی سبب کاهش رشد ارتفاعی، قطر یقه و وزن تر و خشک ریشه و اندام هوایی نهال‌های خنجوک شده است، در حالی‌که اثرات متفاوتی روی فعالیت آنتی اکسیدانت‌ها در ریشه و اندام هوایی نهال‌های خنجوک دارد. تنش خشکی سبب افزایش فعالیت آنزیم کاتالاز در ریشه و اندام هوایی نهالها گردید. بیش‌ترین میزان فعالیت این آنزیم در سطح ۲۵ درصد ظرفیت زراعی مشاهده شد. علاوه بر این، فعالیت آنزیم پراکسیداز نیز در اندام هوایی و ریشه نهال‌ها هم‌زمان با تنش خشکی افزایش پیدا کرد. فعالیت آنزیم سوپراکسیددیسموتاز نیز در تی‌مار ۱۰۰ درصد ظرفیت زراعی، کم‌ترین میزان را نشان داد. نتایج هم‌چنین نشان داد که تجمع پرولین آزاد در نهال‌هایی که به خوبی آبیاری شده‌اند کم‌ترین میزان بوده و با افزایش شدت تنش خشکی میزان آن افزایش یافته است.

کلمات کلیدی: *Pistacia khinjuk*، پراکسیداز، تنش خشکی، سوپراکسیددیسموتاز، کاتالاز