J. Agr. Sci. Tech. (2013) Vol. 15: 593-602

Effects of Drought Stress on the Lipid Peroxidation and Antioxidant Enzyme Activities in Two Canola (*Brassica napus* L.) Cultivars

M. Mirzaee [1], A. Moieni [1*], and F. Ghanati [2]

ABSTRACT

Drought is one of the most important abiotic stresses affecting plant growth and development. In the present study, the changes in lipid peroxidation rate and antioxidant enzyme activities were determined at different concentrations of PolyEthylene Glycol (PEG) 6000 (0, 5, 10, and 15% (w/v)) for two canola cultivars (SLM046 and Hyola 308). In order to produce water deficit, 12 days old canola seedlings were treated with PEG 6,000 in half strength Hoagland solution for 24 hours. PEG treatments increased the content of Malondialdehyde (MDA), a product of lipid peroxidation, in roots and shoots of both cultivars; but for Hyola 308 cultivar, the rate of increase of MDA was higher than SLM046 cultivar. In addition, drought did not have any significant effect on MDA content in roots of SLM046 cultivar. On the other hand, water stress increased Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT) and Ascorbate peroxidase (APX) antioxidant enzyme activities of both shoots and roots of the studied cultivars; but activity of these antioxidants in SLM046 cultivar was obviously higher than in Hyola 308 cultivar. These results showed a higher water stress tolerance for SLM046 cultivar.

Keywords: Antioxidant, MDA, Osmotic stress, PEG 6000.

INTRODUCTION

After cereals, oil seeds are the second source of food, and canola is the third source of oil seeds crop in the world after palm oil and soybean (FAO, 2011). In Iran, a large amount of vegetable oil for human consumption is imported and, hence, cultivation and appropriate management of oil seeds to enhance yield is very important. Canola (*Brassica napus* L.) species is considered as a relatively moderately drought-sensitive species within which there is certain variability towards drought tolerance (Omidi, 2010). With 240 mm annual average rainfall, Iran is considered as an arid and semi-arid country. Water scarcity, high evapotranspiration, and other factors may cause several limitations in crop production; therefore, research on the effects of drought stress and selection of appropriate cultivars is encouraged (Moradshahi *et al*., 2004; Tohidi-Moghadam *et al*., 2009; Youssefi *et al*., 2011).

Drought is considered as one of the most important environmental stresses limiting plant growth and crop productivity (Terzi and Kadioglu, 2006). Up to 45% of the world agricultural lands are subject to continuous or frequent drought stress, wherein 38% of the world human population resides (Ashraf and Foolad, 2007). In addition, water-stressed plants could be more sensitive to other biotic or abiotic

[1] Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

[*] Corresponding author; e-mail: moieni_a@modares.ac.ir

[2] Department of Plant Biology, Faculty of Plant Science, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

stresses such as pathogen attack and weeds, which limit plant productivity (Caruso *et al*., 2008). Drought can be defined as the absence of adequate soil moisture necessary for a plant to grow normally and complete its life cycle (Manivannan *et al*., 2008).

Osmotic solutions are used to impose water stress reproducibly under controlled conditions. PEG molecules with a $M_r \geq 6{,}000$ (PEG 6,000) are inert, non-ionic, and virtually impermeable chains that have frequently been used to induce drought and maintain a uniform water potential throughout the experimental period (Van den Berg and Zeng, 2006). Increasing evidences suggest that water stress induces oxidative stress in various plants (Manivannan *et al*., 2008), including both free radical (O_{2*}^-, superoxide radicals; OH_*, hydroxyl radical; HO_{2*}, perhydroxy radical and RO_*, alkoxy radicals) and non-radical (molecular) forms (H_2O_2, hydrogen peroxide and $^1O_2$, singlet oxygen) (Gill and Tuteja, 2010), which can destroy proteins, lipids, carbohydrates, and nucleic acids (Bian and jiang, 2009). To survive under such unfavorable growth conditions, plants have developed unique defense mechanisms and processes for acclimation that increases their tolerance to detrimental conditions (Xu *et al*., 2008).

One of the defense mechanisms against different stresses is the antioxidant enzymes production. Numerous studies have determined that the activity of antioxidant enzymes is correlated with plant tolerance to abiotic stresses, such as responses to drought stress in wheat (Shao *et al*., 2007; Abdullah and Ghamdi, 2009; Hasheminasab *et al*., 2012), alfalfa (Wang *et al*., 2009), rice (Sharma and Dubey, 2005; Qin *et al*., 2010) chickpea (Mohammadi *et al*., 2011), and ornamental plants such as marigold (Sedghi *et al*., 2012), responses to salt stress in maize (Benavente *et al*., 2004; Molazem and Azimi, 2011) and wheat (Esfandiari *et al*., 2007; Kahrizi *et al*., 2012), responses to cold stress in barley (Radyuk *et al*., 2012) and strawberry (Luo *et al*., 2011).

In addition, the degree of damage by Reactive oxygen species (ROS) depends on the balance between the product of ROS and its removal by this antioxidant scavenging mechanism (Azooz *et al*., 2009). On the other hand, it has been reported that membrane of plant cells are subject to rapid damage with increase in water stress. This leakage of membrane is caused by an uncontrolled enhancement of free radical, which cause lipid peroxidation. Damage to fatty acids of membrane could produce small hydrocarbon fragments including Malondialdehyde (MDA) (Moussa and Aziz, 2008). MDA is the final product of plant cell membrane lipid peroxidation and is one important sign of membrane system injury (Cunhua *et al*., 2010).

Therefore, in the present study, relative significance of antioxidant enzyme activities and lipid peroxidation have been examined in seedlings of drought-tolerant and drought-sensitive canola cultivars (SLM046 and Hyola 308, respectively). Finally, the varietal differences in response to drought and the differences between shoots and roots of seedlings have been determined.

MATERIALS AND METHODS

Plant Material and Drought Stress Induction

Homogenous seeds of two cultivars of canola (*Brassica napus* L.), namely, SLM046 (drought-tolerant) and Hyola 308 (drought-sensitive), were obtained from the Seed and Plant Improvement Research Institute, Karaj, Iran, and used for the experiments. These two cultivars were selected among thirteen cultivars (Talaie, OKP, PF, Zarfam, Opera, Elite, Licord, SLM046, Hyola 401, Hyola 308, RGS003, Hyola 60 and Modena) based on germination percentage and speed in different PEG 6,000 concentrations (0, -0.3, -0.5, -0.7 and -0.9 MPa) (data are not shown). The seeds were surface sterilized with a 2,5% (v/v) sodium hypochlorite (NaOCl) solution for 20 minutes, rinsed 4-8 times with distilled water to remove NaOCl, and were then germinated in

petri dishes on two wet layers of filter paper in a controlled incubator at 25ºC in darkness. After 6 days, seedlings were transferred to the containers with half strength Hoagland solution. The containers were placed in a controlled growth chamber under conditions of 16-hours photoperiod; 410 mol m^{-2} s^{-1} PAR. The temperature and relative humidity were, respectively, 23±2ºC and 70%. After 6 days acclimatization, drought stress was imposed by adding 0 (control), 5 (-0.05 MPa), 10 (-0.15 MPa) and 15% (-0.3 MPa) (w/v) of PEG 6,000 (At first, we used different concentrations of PEG i.e. 0, 5, 10, 15, 20, 25% PEG, then, three PEG concentrations were selected based on plant viability. Both cultivars died in the concentrations higher than 15% PEG.) to the half strength Hoagland solution. Each treatment was replicated three times. Shoots and roots were harvested after 24 hours.

Estimation of Lipid Peroxidation

Lipid peroxidation was determined by measuring MDA content (Vos *et al*., 1991). To this end, 0.2 g of frozen shoot and root samples were homogenized in 3 mL of TCA solution (10% w/v) and the aliquots of filtrates were heated in 0.25% TBA for 30 minutes and then cooled in ice bath. The absorbance of solution was recorded at 532 nm followed by correlation for the nonspecific absorbance at 600 nm. The amount of MDA was determined according to extinction coefficient of 155 mM^{-1} cm^{-1}.

Estimation of Antioxidant Enzymes

Shoot and root samples (0.2 g) were homogenized in 3 mL HEPES-KOH buffer (pH 7.8) with 0.1 mM EDTA. The homogenate was centrifuged at 15,000 rpm for 15 minutes at 4ºC. The supernatant was used as a source of SOD enzyme. Superoxide dismutase (SOD) activity was measured by a photochemical method (Giannopolitis and Reis, 1977). The reaction mixture (3 mL) contained 0.1 mM EDTA, 50 mM HEPES-KOH buffer (pH 7.8), 50 mM Na_2CO_3 (pH 10.2), 12 mM L-methionine, 75 NBT, 300 µL enzyme extract and 1 µM riboflavin. The absorbance was read at 560 nm and one unit activity of SOD was defined as the rate of enzyme required to result in a 50% inhibition of rate of NBT reduction. APX activity was determined by the method of Nakano and Asada (1981). Samples of 0.2 g were homogenized in 1 mL of 50 mM Na-phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 5 mM ascorbate, 5 mM DTT, 100 Mm NaCl and 2% (w/v) PVP. The homogenate was centrifuged at 15,000 rpm for 15 minutes at 4ºC. The supernatant was used as a source of enzyme. The reaction was initiated by adding H_2O_2 to a solution with final concentration of 44 µM. The decrease in absorbance was monitored at 290 nm. The rate of APX was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1} and correction was done for the non-enzymatic oxidation of ascorbic acid that was obtained prior to addition H_2O_2. CAT activity was measured by the method of Cakmak and Horsrt (1991). The reaction mixture consisted of 2.6 ml of 25 mM Na-phosphate buffer (pH 6.8), 400 µL of 10 mM H_2O_2, and 40 µL of enzyme. The decomposition of H_2O_2 was followed by the decline of absorbance at 240 nm. Activity of POD was determined in a reaction mixture, which consisted of suitable amount of 28 mM guaiacol, 5 mM H_2O_2, 25 mM Na-phosphate buffer (pH 6.8) and enzyme (Ghanati *et al*., 2002). Soluble protein content was estimated by the method of Bradford (1976), and BSA was used as a standard.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) and differences between the means were compared by least-significant difference Least-significant difference (LSD). $P \leq 0.05$ were considered as significant.

RESULTS

Effect of Drought on Lipid Peroxidation

The lipid peroxidation levels in roots and shoots of two canola cultivars were determined as the content of MDA. The level of MDA showed variation with PEG treatments. In the shoots of SLM046 (Figure 1-a), the level of MDA gradually increased with increasing PEG concentration, but stress did not have any significant effect on MDA content in the roots of this cultivar (Figure 1-b). In contrast, drought stress caused significant rise in MDA content in the shoots and roots of Hyola 308 cultivar (Figures 1-a and -b).

Effect of Drought on Antioxidant Enzymes

In the present study, antioxidant enzyme activities were enhanced with increase in the PEG concentration.

As shown in Figures 2-a and -b, PEG treatments increased the activity of POD in shoots and roots of both cultivars. But, the POD activity of SLM046 shoots and roots was higher than that of Hyola 308. However, in the control conditions and in both cultivars, POD activity in roots was higher than in shoots.

We also observed that drought resulted in higher SOD activity in shoots and roots of both cultivars. The increases in SOD activity in the 15% PEG treatment were 30 and 14% in shoots of SLM046 and Hyola 308, respectively, as compared to their controls. In brief, SOD activity in SLM046 was higher than Hyola 308, after PEG treatments (Figures 3-a and -b).

Drought stress induced CAT activity in both tissues of the two studied cultivars. This enhancement was higher in SLM046 than Hyola 308 (Figures 4-a and -b). In the shoots of SLM046, 5 and 10% PEG

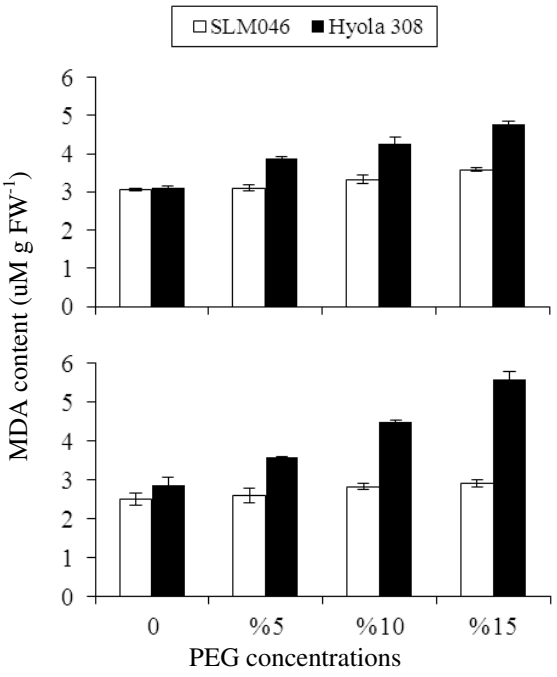

Figure 1. Effect of different PEG concentrations on MDA content in shoots (LSD 0.05= 0.025) (a) and roots (LSD 0.05= 0.045) (b) of SLM046 and Hyola 308 cultivars. Values represent the mean of three replicates. Vertical bars indicate ±SE.

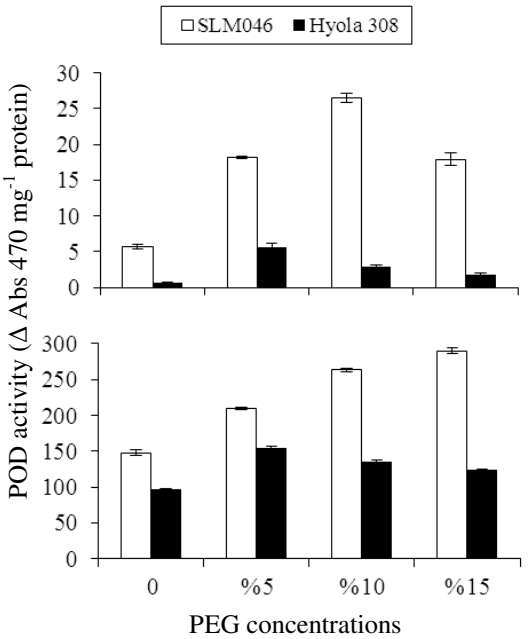

Figure 2. Effect of different PEG concentrations on POD activity in shoots (LSD 0.05= 0.76) (a) and roots (LSD 0.05= 0.04) (b) of SLM046 and Hyola 308. Values represent the mean of three replicates. Vertical bars indicate ±SE.

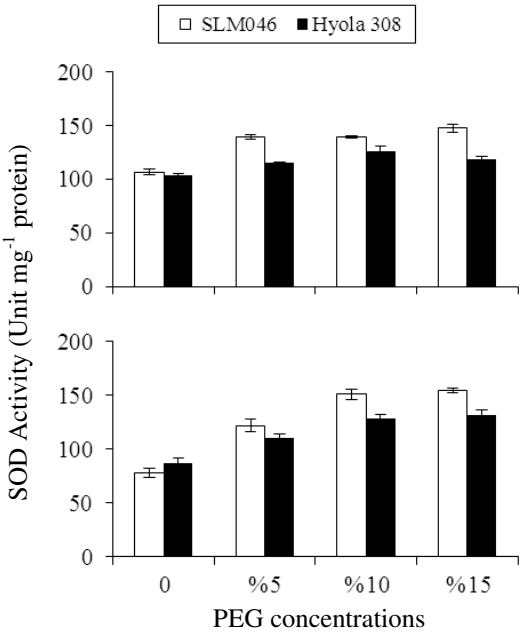

Figure 3. Effect of different PEG concentrations on SOD activity in shoots (LSD 0.05= 6.22) (a) and roots (LSD 0.05= 10.86) (b) of SLM046 and Hyola 308 cultivars. Values represent the mean of three replicates. Vertical bars indicate ±SE.

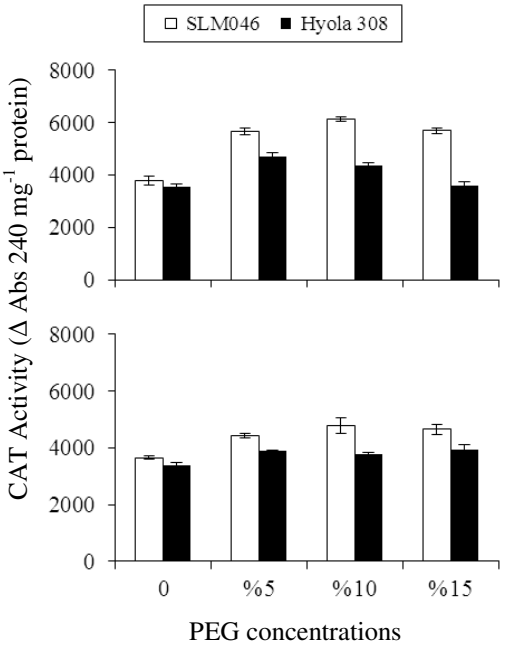

Figure 4. Effect of different PEG concentrations on CAT activity in shoots (LSD 0.05= 116.41) (a) and roots (LSD 0.05= 336.37) (b) of SLM046 and Hyola 308 cultivars. Values represent the mean of three replicates. Vertical bars indicate ±SE.

significantly increased CAT activity compared to the control, but there was no significant difference between 5 and 15% PEG treatments. On the other hand, the decrease of water potential in 5% PEG increased CAT activity compared to the control in shoots of Hyola 308, but it did not show significant difference with its activity in 10% PEG. In addition, the increase in PEG concentration to 15% decreased the CAT activity in Hyola 308 and there was no significant difference with the control. In the roots of the two cultivars, 5% PEG treatment significantly increased CAT activity, while it did not have significant difference with activity of CAT in 10 and 15% PEG treatments.

PEG treatment resulted in a significant increase in APX activity in shoots and roots of both cultivars of canola (Figures 5-a and -b). Enhancement of this enzyme in SLM046 was higher than in Hyola 308 under the same conditions. As compared to the

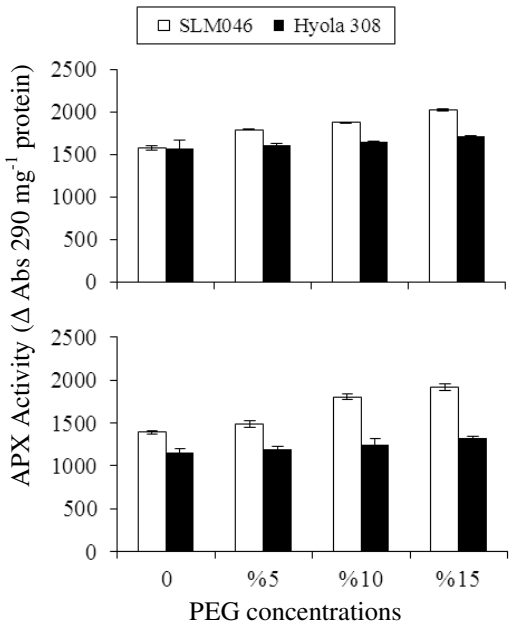

Figure 5. Effect of different PEG concentrations on APX activity in shoots (LSD 0.05= 0.0009) (a) and roots (LSD 0.05= 0.034) (b) of SLM046 and Hyola 308 cultivars. Values represent the mean of three replicates. Vertical bars indicate ± SE.

control, APX activity in both tissues of SLM046 was continuously enhanced with increasing PEG concentration. In shoot of this cultivar, APX activity was raised 13.72, 19.11, and 28.47% in 5, 10, and 15% PEG treatments, respectively. But, in Hyola 308, the significant increase in APX activity was only observed in 10 and 15% PEG treatments in shoots and 15% PEG induced stress in roots. The rates of increased APX activity in shoots of Hyola 308 were 2.89, 4.84, and 9.45% at 5, 10, and 15% levels of PEG, respectively.

DISCUSSION

Water deficit is one of the most important abiotic stresses limiting crop growth and productivity. Oxidative stress is regarded as a major detrimental factor in plants exposed to a variety of abiotic stresses including drought (Sharma and Dubey, 2005). Plants have evolved a wide range of defense mechanisms to survive continuous assault by an arsenal of biotic attacks as well as constantly changing weather and other environmental conditions (Shao *et al*., 2006). Drought, due to its osmotic effect in natural and agricultural habitats can induce a wide number of responses such as growth inhibition and synthesis of some non-toxic compounds to enhance the osmotic potential of the cell and thus allow metabolic processes to continue to increase of some antioxidant enzyme activities (Turkan *et al*., 2005). In the present research, we observed a significant difference in the MDA content and antioxidant enzyme activities between SLM046 and Hyola 308 cultivars under PEG stress condition.

Lipid peroxidation has been associated with damages provoked by some environmental stresses (Abdul Jaleel *et al*., 2008). The rise in MDA content under different stress conditions showed that drought could induce membrane lipid peroxidation by means of ROS (Moussa and Aziz, 2008). In this sense, low concentration of MDA have been associated with drought tolerance in tomato (Sanchez-Rodriguez *et al*., 2010), cowpea (Nair *et al*., 2008), maize (Bai *et al*., 2006; Moussa and Abdel-Aziz, 2008), and chickpea (Mohammadi *et al*., 2011). In our experiment, SLM046, with low concentration of MDA in different PEG concentrations, showed more tolerance to drought stress.

To cope with detrimental effects of oxidative stresses under extremely adverse conditions, plants have developed an antioxidant defense system that includes the antioxidant enzymes SOD, APX, POD, and CAT. The levels of antioxidant enzymes are higher in tolerant species than in sensitive ones under various environmental stresses (Wang *et al*., 2009). Accordingly, we observed higher SOD activity in SLM046 compared to Hyola 308 cultivar under drought stresses, which suggest that the drought-tolerant canola cultivar possesses a better reactive oxygen species scavenging ability.

These results are in agreement with prior reports revealing the increased SOD activity in drought-tolerant cultivar of bean (Turkan *et al*., 2005), sesame (Fazeli *et al*., 2007), alfalfa (Wang *et al*., 2009), and horsegram (Bhardwaj and Yadav, 2012). In addition, Abedi and Pakniyat (2010) and Tohidi-Moghadam *et al*., (2009) showed that SOD activity was increased in some other canola cultivars (Licord, Zarfam, and RGS003), which might lead to their higher protection against drought stress. SOD converts the toxic O_2^- radicals to H_2O_2 which must be scavenged to O_2 and water by the antioxidant enzymes such as CAT, POD, and APX (Ozkur *et al*., 2009).

Increase in POD activity under various stress conditions has been linked with protection from oxidative damage, lignifications, and cross-linking of cell wall to prevent from such adverse conditions (Moussa and Abdel-Aziz, 2008). In our study, drought induced POD activity in roots and shoots of both cultivars. However, activity of this enzyme in SLM046 cultivar was higher than Hyola 308 in the control and stress conditions, suggesting a better

antioxidant system for removing H_2O_2 by POD. The increased POD activity during water stress has been reported in *Arabidopsis thaliana* (Jung, 2004), wheat (Shao *et al*., 2007; Hasheminasab *et al*., 2012), and other canola cultivars (Abedi and Pakniyat, 2010).

APX scavenges peroxidase by converting ascorbic acid to dehydroascorbate (Ozkur *et al*., 2009). In our experiment, drought stress increased APX activity in SLM046 cultivar higher than in Hyola 308. Higher activity of APX in SLM046 suggests a more effective H_2O_2 removal in this cultivar. Similar results were also reported in Okapi canola cultivar compared to RGS cultivar (Omidi, 2010) and other plant species such as wheat (Abdullah and Ghamdi, 2009), olive tree (Sofo *et al*., 2008), and Kentuky blugrass (Bian and Jiang, 2009).

Catalase is an antioxidant enzyme that scavenges H_2O_2 in cells (Shao *et al*., 2007). High activity of CAT indicated drought tolerance in *Catharanthus roseus* (Abdul Jaleel *et al*., 2008), tomato (Sanchez-Rodriguez *et al*., 2010), alfalfa (Wang *et al*., 2009), peanut (Akcay *et al*., 2010) and some of the canola cultivars (Omidi, 2010; Tohidi-Moghadam *et al*., 2009). Abedi and Pakniyat (2010) reported that CAT activity was decreased in all studied canola cultivars, except in Licord and Zarfam as tolerant cultivars. Therefore, they concluded that the reduction of CAT activity was supposedly due to the inhibition of enzyme synthesis, change in the assembly of enzyme subunits, or protein degradation under drought stress. In the present research, CAT activity in SLM046 was higher than its activity in Hyola 308 in different PEG concentrations. The high activity of CAT in SLM046 cultivar during drought stresses demonstrated more ability of this cultivar to decompose H_2O_2 in stress conditions.

From the results of this experiment, it can be concluded that low concentration of MDA and higher antioxidant activity in drought stress conditions lead to higher water stress tolerance of SLM046 cultivar.

REFERENCES

1. Abdullah, A. and Ghamdi, A. L. A. 2009. Evaluation of Oxidative Stress in Two Wheat (*Triticum aestivum*) Cultivars in Response to Drought. *Int. J. Agric. Biol*., **11:** 7-12.
2. Abdul Jaleel, C., Sankar, B., Murali, P. V., Gomathinayagam, M., Lakshmanan, G. M. A., and Panneerselvam, R. 2008. Water Deficit Stress Effects on Reactive Oxygen Metabolism in *Catharanthus roseus*; Impacts on Ajmalicine Accumulation. *Colloids Surf*., **62:** 105-111.
3. Abedi, T. and Pakniyat, H. 2010. Antioxidant Enzyme Changes in Response to Drought Stress in Ten Cultivars of Oilseed Rape (*Brassica napus* L.). *Czech J. Genet. Plant Breed*., **46(1):** 27-34.
4. Akcay, U.C., Ercan, O., Kavas, M., Yildiz, L., Oktem, H.A. and Yucel, M. 2010. Drought-induced Oxidative Damage and Antioxidant Responses in Peanut (*Arachis hypogaea* L.) Seedlings. *Plant Growth Regul*., **61(1):** 21-28.
5. Ashraf, M. and Foolad, M. R. 2007. Roles of Glycine Betaine and Proline in Improving Plant Abiotic Stress Resistance. *Environ. Exp. Bot*., **59:** 206–216.
6. Azooz, M. M., Ismail, A. M. and A. Elhamd, M. F. 2009. Growth, Lipid Peroxidation and Antioxidant Enzyme Activities as a Selection Criterion for Salt Tolerance of Maize Cultivars Grown under Salinity Stress. *Int. J. Agric. Biol*., **11:** 21-26.
7. Bai, L. P., Sui, F. G., Ge, T. D., Sun, Z. H., Lu, Y. Y., and Zhou, G. S. 2006. Effect of Soil Drought Stress on Leaf Water Status, Membrane Permeability and Enzymatic Antioxidant System of Maize. *Pedosphere*, **16:** 326-332.
8. Benavente, M. L., Kernodle, S. P., Margis-Pinhero, M., and Scandalios, J. G. 2004. Salt-induced Antioxidant Metabolism Defenses in Maize (*Zea mays* L.) Seedling. *Redox Rep*., **9(1):** 29-36.
9. Bhardwaj, J. and Yadav, S. K. 2012. Comparative Study on Biochemical Parameters and Antioxidant Enzymes in Drought Tolerant and Sensitive Variety of Horsegram (*Macrotyloma uniflorum*) under Drought Stress. *Am. J. Plant Physiol*., **7:** 17-29.

10. Bian, S. and Jiang, Y. 2009. Reactive Oxygen Species, Antioxidant Enzyme Activities and Gene Expression Patterns in Leaves and Roots of Kentucky Bluegrass in Response to Drought Stress and Recovery. *Sci. Hort.*, **120:** 264-270.
11. Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein-dye Binding. *Anal. Biochem.*, **72:** 248-254.
12. Cakmak, I. and Horst, W. 1991. Effect of Aluminum on Lipid Peroxidation, Superoxide Dismutase, Catalase and Peroxidase Activities in Root Tip of Soybean (*Glysin max*). *Plant Physiol.*, **83:** 463-468.
13. Caruso, A., Chefdor, F., Carpin, S., Depierreux, C., M. Delmotte, F., Kahlem, G., and Morabito, D. 2008. Physiological characterization and identification of genes differentially expressed in response to drought induced by PEG 6000 in *Populus canadensis* leaves. *J. Plant Physiol.*, **165:** 932-941.
14. Cunhua, S., Wei, D., Xiangling, C., Xinna, X., Yahong, Z., Dong , S. and Jianjie, S. 2010. The Effects of Drought Stress on the Activity of Acid Phosphatase and Its Protective Enzymes in Pigweed Leaves. *Afr. J. Biotechnol.*, **9:** 825-833.
15. De Vos, C. H., Schat, M., De Waal, R., Vooij, S. and Ernst W. 1991. Increased to Copper-induced Damage of the Root Plasma Membrane in Copper Tolerant *Silene cucubalus*. *Plant Physiol.*, **82:** 523-528.
16. Esfandiari, E., Shekari, F., Shekari, F. and Esfandiari, M. 2007. The Effect of Salt Stress on Antioxidant Enzymes Activity and Lipid Peroxidation on the Wheat Seedling. *Not. Bot. Hort. Agrobot. Cluj.*, **35:** 48-56.
17. Food and Agriculture Organization (F.A.O). 2011. Crop Production Statistics. http:// www.Fao.org.
18. Fazeli, F., Ghorbanali, M. and Niknam, V. 2007. Effect of Drought on Biomass, Protein Content, Lipid Peroxidation and Antioxidant Enzymes in Two Sesame Cultivars. *Biol. Plantarum.*, **51:** 98-103.
19. Ghanati, F., Morita, A. and Yokota, H. 2002. Induction of Suberin and Increase of Lignin Content by Excess Boron in Tabacco Cell. *Soil Sci. Plant Nutr.*, **48(3):** 357-364.
20. Giannopolitis, C. and Ries, S. K. 1977. Superoxide Dismutases: I. Occurrence in Higher Plant. *Plant Physiol.*, **59:** 309-314.
21. Gill, S. S. and Tuteja, N. 2010. Reactive Oxygen Species and Antioxidant Machinery in Abiotic Stress Tolerance in Crop Plants. . *Plant Physiol. Biochem.*, **48:** 909-930.
22. Hasheminasab, H., Assad, M. T., Aliakbari, A. and Sahhafi, R. 2012. Influence of Drought Stress on Oxidative Damage and Antioxidant Defense Systems in Tolerant and Susceptible Wheat Genotypes. *J. Agr. Sci.*, **4(8):** 20-30.
23. Kahrizi, S., Sedighi, M. and Sofalian, O. 2012. Effect of Salt Stress on Proline and Activity of Antioxidant Enzymes in Ten Durum Wheat Cultivars. *Ann. Biol. Res.*, **3(8):** 3870-3874.
24. Luo, Y., Tang, H. and Zhang, Y. 2011. Production of Reactive Oxygen Species and Antioxidant Metabolism about Strawberry Leaves to Low Temperature. *J. Agr. Sci.*, **3(2):** 89-96.
25. Jung, S. 2004. Variation in Antioxidant Metabolism of Young and Mature Leaves of *Arabidopsis thaliana* Subjected to Drought. *Plant Sci.*, **166:** 459-466.
26. Manivannan, P., Abdul Jaleel, C., Somasundaram, R. and Panneerselvam, R. 2008. Osmoregulation and Antioxidant Metabolism in Drought-stressed *Helianthus annuus* under Triadimefon Drenching. *C. R. Biol.*, **331:** 418–425.
27. Mohammadi, A., Habibi, D, Rihami, M. and Mafakheri, S. 2011. Effect of Drought Stress on Antioxidant Enzymes Activity of Some Chickpea Cultivars. *Am-Euras. J. Agric. Environ. Sci.*, **11(6):** 782-785.
28. Molazem, D. and Azimi, J. 2011. Proline Reaction, Peroxide Activity and Antioxiant Enzymes in Varieties of Maize (*Zea mays* L.) under Different Levels of Salinity. *Aust. J. Basic Appl. Sci.*, **5(10):** 1248-1253.
29. Moradshahi, A., Eskandari, B. S. and Kholdebarin, B. 2004. Some Physiological Responses of Canola (*Brassica napus* L.) to Water Deficit Stress under Laboratory Conditions. *Iran. J. Sci. Technol.*, **28:** 43-50.
30. Moussa, H. and Abdel-Aziz, S. M. 2008. Comparative Response of Drought Tolerant and Drought Sensitive Maize Genotypes to Water Stress. *Aust. J. Crop Sci.*, **1:** 31-36.
31. Nair, A. S., Abraham, T. K and Jaya, D. S. 2008. Studies on the Changes in Lipid Peroxidation and Antioxidants in Drought

Stress Induced Cowpea (*Vigna unguiculata* L.) Varieties. *J. Environ. Biol*., **29:** 689-691.
32. Nakano, Y. and Asada, K. 1981. Hydrogen Peroxide is Scavenged by Ascorbate Specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol*., **22:** 867–880.
33. Omidi, H. 2010. Changes of Proline Content and Activity of Antioxidative Enzymes in Two Canola Genotype under Drought Stress. *Am. J. Plant Physiol*., **5(6):** 338-349.
34. Ozkur, O., Ozdemir, F., Bor, M. and Turkan, I. 2009. Physiochemical and Antioxidant Responses of the Perennial Xerophyte *Capparis ovata* Desf. to Drought. *Environ. Exper. Bot*., **66:** 487–492.
35. Qin, J., Wang, X., Hu, F. and Li, H. 2010. Growth and Physiological Performance Responses to Drought Stress under Non-flooded Rice Cultivation with Straw Mulching. *Plant Soil Environ*., **56 (2):** 51-59.
36. Radyuk, M. S., Domanskaya, I. N., Shcherbakov, R. A. and Shalygo, N. V. 2010. Effect of Low Above-zero Temperature on the Content of Low-molecular Antioxidants and Activities of Antioxidant Enzymes in Green Barley Leaves. *Russ. J. Plant Physiol*., **56 (2):** 175-180.
37. Sanchez-Rodriguez, E., Rubio-Wilhelmi, M., Cervilla, L. M., Blasco, B., Rios, J. J., Rosales, M. A., Romero, L. and Ruiz, J. M. 2010. Genotypic Differences in Some Physiological Parameters Symptomatic for Oxidative Stress under Moderate Drought in Tomato Plants. *Plant Sci*., **178:** 30-40.
38. Sedghi, M., Sharifi, R. S., Pirzad, A. R. and Balaneji, B. A. 2012. Phytohormonal Regulation of Antioxidant Systems in Petals of Drought Stressed Pot Marigold (*Calendula* officinalis L.). *J. Agr. Sci. Tech*., **14:** 869-878.
39. Shao, H. B., Liang, Z. S. and Shao, M. A. 2006. Osmotic Regulation of 10 Wheat (*Triticum aestivum* L.) Genotypes at Soil Water Deficits. *Colloids Surf*., **47:** 32-139.
40. Shao, H. B., Chu, L. Y., Wu, G., Zhang, J. H., Lu, Z. H. and Hu, Y. C. 2007. Changes of Some Anti-oxidative Physiological Indices under Soil Water Deficits among 10 Wheat (*Triticum aestivum* L.) Genotypes at Tillering Stage. *Colloids Surf*., **54:** 143-149.
41. Sharma, P. and Dubey, R. S. 2005. Drought Induces Oxidative Stress and Enhances the Activities of Antioxidant Enzymes in Growing Rice Seedlings. *Plant Growth Regul*., **46:** 209-221.
42. Sofo, A., Manfreda, S., Dichio, B. and Xiloyannis, C. 2008. The Olive Tree: A Paradigm for Drought Tolerance Mediterranean Climates. *Hydrol. Earth Syst. Sci*., **12:** 293-301.
43. Terzi, R. and Kadioglu, A. 2006. Drought Stress Tolerance and the Antioxidant Enzyme System in *Ctenanthe setosa*. *Acta Biol. Cracov. Ser. Bot*., **48:** 89-96.
44. Tohidi-Moghadam, H. R., Shirani-Rad, A. H., Nour-Mohammadi, G., Habibi, D. and Mashhadi-Akbar-Boojar, M. (2009). Effect of Super Absorbent Application on Antioxidant Enzyme Activities in Canola (*Brassica napus* L.) Cultivars under Water Stress Conditions. *Am. J. Agric. Biol. Sci*., **4 (3):** 215-223.
45. Turkan, I., Bor, M., Ozdemir, F. and Koca, H. 2005. Differential Responses of Lipid Peroxidation and Antioxidants in the Leaves of Drought-tolerant *P. acutifolius* Gray and Drought-sensitive *P. vulgaris* L. Subjected to Polyethylene Glycol Mediated Water Stress. *Plant Sci*., **168:** 223-231.
46. Van den Berg, L. and Zeng, Y. J. 2006. Response of South African Indigenous Grass Species to Drought Stress Induced by Polyethylene Glycol (PEG) 6000. *S. Af. J. Bot*., **72:** 284-286.
47. Wang, W. B., Kim, Y. H., Lee, H. S., Kim, K. Y., Deng, X. P. and Kwak, S. S. 2009. Analysis of Antioxidant Enzyme Activity during Germination of Alfalfa under Salt and Drought Stress. *Plant Physiol. Biochem*., **47:** 570-577.
48. Xu, J., Zhang, Y., Guan, Z., Wei, W., Han, L. and Chai, T. 2008. Expression and Function of Two Dehydrins under Environmental Stresses in *Brassica juncea* L. *Mol. Breed*., **21:** 431-438.
49. Youssefi, A., Nshanian, A. and Aziz, M. 2011. Evaluation of Influences of Drought Stress in Terminal Growth duration on Yield and Yield Components of Different Spring *Brassica* Oilseed Species. *Am-Euras. J. Agric. Environ. Sci*., **11(3):** 406-410.

اثرات تنش خشکی روی پراکسیداسیون لیپید و فعالیت آنزیم های آنتی اکسیدانت در دو رقم کلزا (*Brassica napus* L.)

م. میرزایی، ا. معینی، و ف. قناتی

چکیده

خشکی، یکی از مهم ترین تنش های غیر زنده است که رشد و نمو گیاه را تحت تأثیر قرار می دهد. در پژوهش حاضر، تغییرات میزان پراکسیداسیون لیپید و فعالیت آنزیم های آنتی اکسیدانت در غلظت های مختلف PEG 6000 (۰، ۵، ۱۰ و ۱۵٪ (w/v)) در دو رقم کلزا (SLM046 و Hyola 308) تعیین شد. به منظور ایجاد کمبود آب، گیاهچه های ۱۲ روزه کلزا با PEG 6000 در محلول یک دوم هوگلند برای ۲۴ ساعت تیمار شدند. تیمارهای PEG، سبب افزایش مقدار MDA (محصول پراکسیداسیون لیپید) در ریشه ها و اندام های هوایی هر دو رقم شد؛ اما در رقم Hyola 308، میزان افزایش MDA بیشتر از رقم SLM046 بود. بعلاوه، خشکی هیچگونه اثر معنی داری بر مقدار MDA در ریشه های رقم SLM046 نداشت. از سوی دیگر، تنش خشکی فعالیت آنزیم های آنتی اکسیدانت SOD، POD، CAT و APX را در ریشه ها و اندام های هوایی رقم های مورد مطالعه افزایش داد؛ اما فعالیت این آنتی اکسیدانت ها در رقم SLM046 به طور قابل توجهی بالاتر از رقم Hyola 308 بود. این نتایج تحمل بیشتر رقم SLM046 را به تنش خشکی نشان داد.