Journal of Plant Physiology and Breeding

2019, 9(2): 77-84 ISSN: 2008-5168

A key response of grain yield and superoxide dismutase in maize (*Zea mays* L.) to water deficit stress

Sajjad Moharramnejad[1*] and Mostafa Valizadeh[2]

Received: April 4, 2019 Accepted: December 18, 2019
[1]Crop and Horticultural Research Department, Ardabil Agricultural and Natural Resources Research and Education Center, AREEO, Moghan, Iran.
[2]Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
*Corresponding author; Email: sm.chakherlo@yahoo.com

Abstract
To assess the grain yield, plant height and superoxide dismutase (Cu/Zn-SOD) activity, and its expression responses of maize to water deficit stress, a field experiment using a split-plot design based on randomized complete block design was performed with three maize hybrids, SC704, SC720 and NS640, under control and water-deficit stress conditions at the Research Station of University of Tabriz, Iran. The results indicated that water deficit stress reduced grain yield and plant height. Electrophoretic analysis for Cu/Zn-SOD based on sensitivity to KCN and H_2O_2 inhibitors was carried out using 8% slab polyacrylamide gels. The gene expression of *Cu/Zn-SOD* by using Real-time PCR in maize hybrids showed that water deficit stress increased *Cu/Zn-SOD* expression. SC704 with higher grain yield, plant height and Cu/Zn-SOD activity ranked as a drought-tolerant hybrid in this study. It can be concluded that the increase in *Cu/Zn-SOD* expression may decrease damage caused by water-deficit stress in maize.

Keywords: Antioxidant enzyme; Electrophoresis; Gene; Grain yield; Isoform; Plant height.

Citation: Moharramnejad S and Valizadeh M, 2019. A key response of grain yield and superoxide dismutase in maize (*Zea mays* L.) to water deficit stress. Journal of Plant Physiology and Breeding 9(2): 77-84.

Introduction

The economic importance of maize (*Zea mays* L.) has continuously risen in the world. This is probably due to the increased worldwide distribution, compatibility of maize to various environments and breeding of cultivars with high performance (Herrmann 2013).

Water is the main growth-restricting factor for plants, especially in arid and semiarid areas where plants are often exposed to periods of water deficit stress, which is one of the main reasons for the crop loss worldwide (Anjum *et al.* 2017). Drought responses in plants are complex, and it is well documented that drought stress damages multiple physiological and metabolic processes (Witt *et al.* 2012).

Oxidative stress which is induced by drought stress has adverse effects on nucleic acids, proteins, carbohydrates and membrane lipids (Kumar *et al.* 2012). A key antioxidant enzyme in the oxidative defense, is superoxide dismutase (SOD) which detoxifies reactive oxygen species (ROS) which are induced by drought stress (Huang *et al.* 2012). SOD, based on its metal cofactors can be classified into three structures including iron (Fe-SOD), manganese (Mn-SOD) and copper/zinc (Cu/Zn-SOD). Cu/Zn-SOD is mostly found in the chloroplast and cytoplasm (Alscher *et al.* 2002). The chloroplast is also the site of ROS production in plants (Tripathy and Oelmuller 2012). ROS has a role in the regulation of the oxidative defense system as a regulator in the signal transduction processes related to stress (Tripathy and Oelmuller 2012). Thus, they have a positive role in signal transduction processes and a damaging toxic role as antioxidants in plants.

Gene expression changes due to environmental stresses are common reactions in plant cell metabolism (Huang *et al.* 2012). The chloroplast precursor Cu/Zn-SOD which is encoded by the *CSD2* nuclear gene in *Arabidopsis thaliana* (Kliebenstein *et al.* 1998), is synthesized and transported into chloroplast by an N-terminal transit peptide (Li and Chiu 2010). *Cu/Zn-SOD* genes in the chloroplast from different plants have been separated and their role in increasing plant resistance to oxidative damage has been elucidated (Apostolova *et al.* 2012; Zhang *et al.* 2015). According to several reports, the antioxidant function is associated with tolerance to abiotic stresses including drought stress (Yang *et al.* 2015; Anjum *et al.* 2017). Water deficit stress increased isoforms of SOD during per-and post-flowering stages in the maize plant (Moharramnejad *et al.* 2019).

The current study was carried out with a hypothesis that water deficit-induced alteration in Cu/Zn-SOD activity could influence the grain yield of maize hybrids under water deficit situations. Therefore, this research was accomplished to assess the impact of water-deficit stress on plant height, grain yield and Cu/Zn-SOD activity in maize hybrids.

Material and Methods

Plant materials and field experiment

A field trial was performed during the 2016 growing season at the Research Station of the University of Tabriz, Tabriz, Iran. A split-plot design based on randomized complete block design with two factors including two water deficit conditions (normal irrigation as the control and irrigation interruption during 27 days before flowering) and three maize hybrids (SC704, SC720, NS640) was carried out. Irrigation conditions were arranged in main plots and maize hybrids were allocated in subplots. Seeds were planted on April 30, 2016. The experimental plots were two rows of 3-m long with the between row spacing of 0.75 m and the within row spacing of 0.50 m between hills. Initially, three seeds were planted in a hill but thinned to two plants three weeks after sowing. The final population density was 60,000 plants per hectare. The soil of the experimental site was sandy-loam (sand 49.8%, clay 18.5%). Nitrogen fertilizer was used at the rate of 60 kg N ha^{-1} at planting. An additional 60 kg N ha^{-1} was used as top dressing four weeks after planting.

Antioxidant enzymes

The crude extracts of fresh leaves were prepared in a tris-HCl extraction buffer [Tris 50 mM, pH 7.5, ascorbic acid (50 mM), sucrose 5%, sodium metabisulfite (20 mM), PEG (2%), 2-mercaptoethanol (0.1%)] before use (Valizadeh *et al.* 2013), with a ratio of 0.5 mg μl^{-1} (1W: 2V). The extracts were centrifuged at 10,000 *g* for 10 min at 4 °C using small Eppendorf tubes. The enzyme extracts were absorbed on a filter paper and loaded onto 7.5% horizontal slab polyacrylamide gels (0.6×15×12 cm) with the use of the TBE (Tris-Borate-EDTA) electrode buffer (pH = 8.8). Electrophoresis was performed at 4 °C for 3 h. Two slices prepared from the slab gels were stained after electrophoresis for measuring the enzyme activity.

The staining SOD isoforms were analyzed based on the sensitivity to 2 mM KCN or 5 mM H_2O_2 (Wendel and Weeden 2012).

RNA detection

Total RNA was isolated from the fresh leaves of maize hybrids by using the RNeasy Kit (QIAGEN GmbH, Germany). The quality and quantity of isolated RNA were checked by gel electrophoresis and a spectrophotometer. To purify RNA from DNA contamination, RQ1 RNase-free DNase (Promega, USA) was used. Then, 2 µg of the RNA was taken to synthesize the complementary DNA (cDNA) by Power cDNA Synthesis Kit (iNtRON Biotechnology, Korea). Primers for the *Cu/Zn-SOD* gene as forward 5'TGTTGCAAATGCTGAGGGCATAGC3' and reverse 5'CCAACAACACCACATGCCAGTCTT3' were used (Shiriga *et al.* 2014). The following PCR mix with a total volume of 20 µL was prepared: 0.5 µL cDNA, 0.4 µM gene-specific forward primer, gene-specific reverse primer (0.4 µM), 1X Taq DNA polymerase master mix red (Amplicon, Denmark) and free-nuclease water. The amplification of the gene was as follows: 35 cycles of initial denaturation at 95 °C for 2 min, denaturation at 95 °C for 30 sec, annealing at the specific temperature for *Cu/Zn-SOD* for 30 sec, extension at 72 °C for 1 min and the final extension at 72 °C for 2 min (Harb *et al.* 2015).

Statistical analyses

Image analysis was carried out by the MCID software to detect the optical density × area for Cu/Zn-SOD activity. Analysis of relative expression by the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen 2001) using the maize *18S RNA* gene as the constitutive control was conducted. Data analyses were performed by SPSS 21.0 software and differences among treatment means were determined by Duncan's multiple range test at $p \leq 0.05$.

Results and Discussion

Grain yield and plant height

Water deficit stress decreased the grain yield of the three maize hybrids (SC704, SC720, NS640) 23.92, 39.32 and 32.45 percent, respectively. Furthermore, water-deficit stress decreased the plant height of the hybrids about 27.03, 34.63 and 29.42 percent for SC704, SC720 and NS640, respectively. Therefore, SC704 was more tolerant than other hybrids in this study in terms of grain yield and plant height (Figure 1). Moharramnejad *et al.* (2019) reported a 40-55% yield reduction in maize when water-deficit stress was imposed at the silk emergence stage. Anjum *et al.* (2017) also reported a marked decrease in grain yield and plant height in maize when it was subjected to the short-term drought. In another study on maize plants, water deficit conditions had an adverse effect on plant height and grain yield of the maize plant (Talaat *et al.* 2015). Reduction in grain yield and plant growth in these maize hybrids caused by water stress could be attributed to some key physio-biochemical processes regulating plant growth; e.g., change in photosynthesis, nutrient uptake/accumulation, osmolyte accumulation, enzyme activity, etc. (Ge *et al.* 2012).

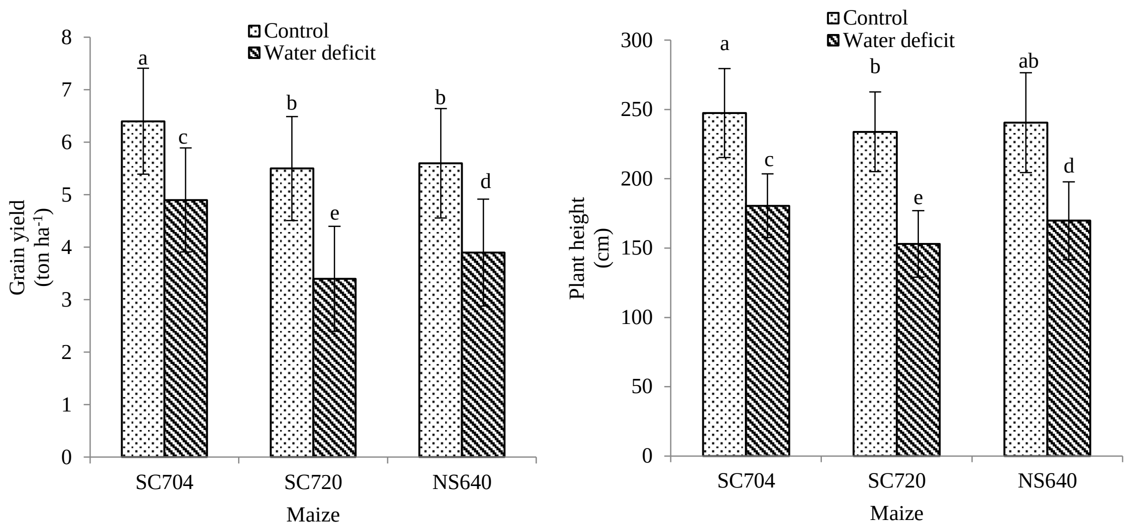

Figure 1. Grain yield and plant height (bars= ±SE) of three maize hybrids under control and water-deficit stress conditions. Means with the same letters are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

Cu/Zn-SOD activity and its expression

We detected the activity of SODs, which were three isoforms on the polyacrylamide gels (Figure 2). Fe-SOD is resistant to KCN and sensitive to H_2O_2. On the other hand, Cu/Zn-SOD is sensitive and Mn-SOD is resistant to both inhibitors (Figure 2). Analysis of variance indicated the significant effects of water deficit stress and maize hybrids on Cu/Zn-SOD activity (data are not shown). Water deficit stress increased Cu/Zn-SOD activity in all of the maize hybrids; however, the SOD activity of SC704 was higher under water-deficit stress compared to other hybrids (Figure 3).

The plants' ability to defend against oxidative stress partially depends on the inducted SOD activity and eventually on the up-regulation of other antioxidant enzymes (Anjum *et al.* 2016a). Moharramnejad *et al.* (2016) reported an increase in the SOD activity in maize seedlings under osmotic stress. According to Anjum *et al.* (2016b), the drought-tolerant maize hybrids had high levels of SOD activity, and also could scavenge superoxide better than the drought-sensitive hybrids under drought stress. Ashraf *et al.* (2015) reported a high level of SOD in drought-tolerant maize. In another study, Mn-SOD activity was increased in maize under drought stress (Moharramnejad *et al.* 2016). Sytykiewicz (2014) also reported the increased Cu/Zn-SOD activity under drought stress in maize. According to Moharramnejad *et al.* (2019) SOD isoform activity and organic osmolytes such as glycine betaine and proline were associated with grain yield of the maize lines under drought stress.

The expression of only one isoform was evaluated in this study. Water deficit stress significantly increased *Cu/Zn-SOD* expression. SC704 had a higher level of *Cu/Zn-SOD* expression (about 32% increase) than the other two hybrids (Figure 4). These results showed that maize hybrids with higher levels of *Cu/Zn-SOD* expression could reduce the adverse effects of water-deficit stress. Cu/Zn-SOD can safeguard PS II from superoxide induced oxidative by water

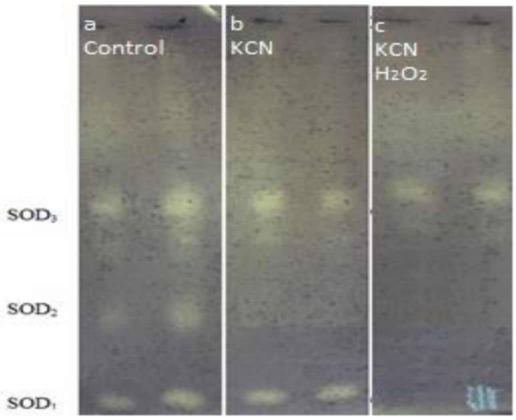

Figure 2. Detected SOD isoforms in the maize hybrids. The activity of SOD isoforms was detected by negative staining and identified based on their sensitivity to KCN and H_2O_2. Mn-SOD was resistant to both inhibitors; Cu/Zn-SOD was sensitive to both inhibitors; Fe-SOD was resistant to KCN and sensitive to H_2O_2; (a) Control (SOD_1, SOD_2, and SOD_3); (b) 2 mM KCN (SOD_1, SOD_3): Fe-SOD and Mn-SOD; (c) 2 mM KCN and 5mM H_2O_2 (SOD_3): Mn-SOD

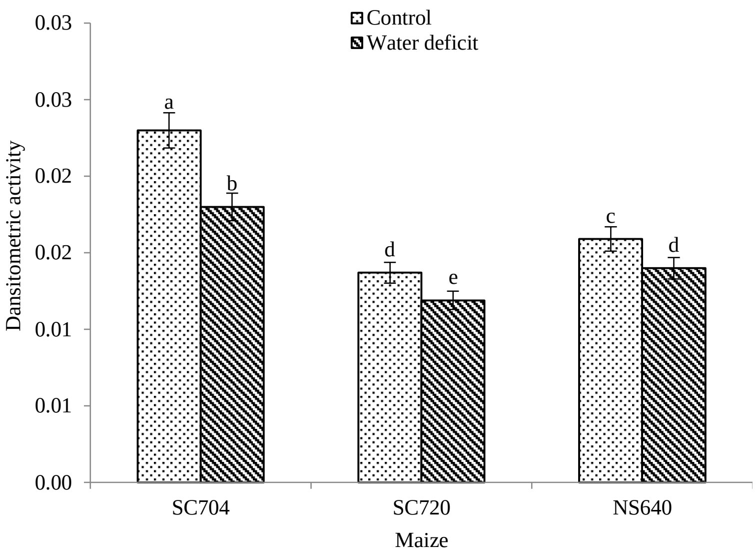

Figure 3. Cu/Zn-SOD activity (bars= ±SE) of the three maize hybrids under control and water-deficit stress conditions. Means with the same letters are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

stress (Huo *et al.* 2016). Drought stress enhanced SOD expression, which was higher in the drought-tolerant cultivars of maize (Xu *et al.* 2011). Shiriga *et al.* (2014) studied the expression of SOD in various maize hybrids under drought stress and observed the increased *Cu/Zn-SOD* gene expression under drought stress.

Conclusion

Water-deficit stress significantly decreased grain yield and plant height for about 32 and 30.4 percent, respectively, but Cu/Zn-SOD activity and its expression increased in all maize hybrids. In this study, Cu/Zn-SOD activity was associated with its expression due to the intricate regulation mechanisms of gene expression. The increase in Cu/Zn-SOD activity may be regarded as one mechanism in the maize plant to decrease ROS damage under water-deficit stress conditions.

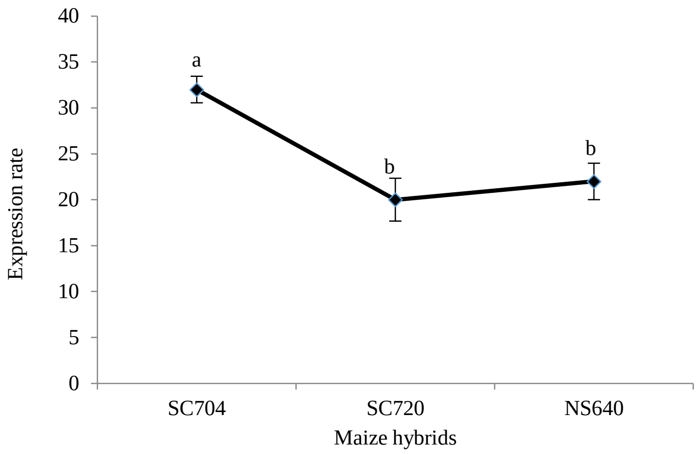

Figure 4. Expression rate (bars= ±SE) of *Cu/Zn-SOD* in three maize hybrids. Means with the same letters are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

References

Alscher RG, Erturk N and Heath LS, 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Journal of Experimental Botany 53(372): 1331-1341.

Anjum SA, Ashraf U, Tanveer M, Khan I, Hussain S, Shahzad B, Zohaib A, Abbas F, Saleem MF, Ali I and Wang LC, 2017. Drought-induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids. Frontiers in Plant Science 8: 69. doi: 10.3389/fpls.2017.00069.

Anjum SA, Tanveer M, Ashraf U, Hussain S, Shahzad B, Khan I and Wang L, 2016a. Effect of progressive drought stress on growth, leaf gas exchange, and antioxidant production in two maize cultivars. Environmental Science and Pollution Research 23(1): 17132-17141.

Anjum SA, Tanveer M, Hussain S, Shahzad B, Ashraf U, Fahad S, Hassan W, Jan S, Khan I, Saleem MF, Wang L, Mahmood A, Samad RA and Tung SA, 2016b. Osmoregulation and antioxidant production in maize under combined cadmium and arsenic stress. Environmental Science and Pollution Research 23(12): 11864-11875.

Apostolova E, Rashkova M, Anachkov N, Denev I, Toneva V, Minkov I and Yahubyan G, 2012. Molecular cloning and characterization of cDNAs of the superoxide dismutase gene family in the resurrection plant *Haberlea rhodopensis*. Plant Physiology and Biochemistry 55: 85-92.

Ashraf MA, Rasheed R, Hussain I, Iqbal M, Haider MZ, Parveen S and Sajid MA, 2015. Hydrogen peroxide modulates antioxidant system and nutrient relation in maize *(Zea mays* L.) under water-deficit conditions. Archives of Agronomy and Soil Science 61(4): 507-523.

Ge T, Sui F, Bai L, Tong C and Sun N, 2012. Effects of water stress on growth, biomass partitioning, and water-use efficiency in summer maize (*Zea mays* L.) throughout the growth cycle. Acta Physiologiae Plantarum 34: 1043-1053.

Harb A, Awad D and Samarah N, 2015. Gene expression and activity of antioxidant enzymes in barley (*Hordeum vulgare* L.) under controlled severe drought. Journal of Plant Interactions 10(1): 109-116.

Herrmann A, 2013. Biogas production from maize: current state, challenges and prospects. 2. Agronomic and environmental aspects. BioEnergy Research 6: 372-387.

Huang C-H, Kuo W-Y, Weiss C and Jinn T-L, 2012. Copper chaperone-dependent and-independent activation of three copper-zinc superoxide dismutase homologs localized in different cellular compartments in *Arabidopsis*. Plant Physiology 158(2): 737-746.

Huo Y, Wang M, Wei Y and Xia Z, 2016. Overexpression of the maize *psbA* gene enhances drought tolerance through regulating antioxidant system, photosynthetic capability, and stress defense gene expression in tobacco. Frontiers in Plant Science 6: 1223. DOI: 10.3389/fpls.2015.01223

Kliebenstein DJ, Monde RA and Last RL, 1998. Superoxide dismutase in *Arabidopsis*: an eclectic enzyme family with disparate regulation and protein localization. Plant Physiology 118(2): 637-650.

Kumar S, Gupta D and Nayyar H, 2012. Comparative response of maize and rice genotypes to heat stress: status of oxidative stress and antioxidants. Acta Physiologiae Plantarum 34: 75-86.

Li H-M and Chiu C-C, 2010. Protein transport into chloroplasts. Annual Review of Plant Biology 61: 157-180.

Livak KJ and Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. Methods 25(4): 402-408.

Moharramnejad S, Sofalian O, Valizadeh M, Asgari A, Shiri MR and Ashraf M, 2019. Response of maize to field drought stress: oxidative defense system, osmolytes' accumulation and photosynthetic pigments. Pakistan Journal of Botany 51(3): 799-807.

Moharramnejad S, Valizadeh M, Sofalian O, Shiri MR and Asgari A, 2016. Effect of water deficit stress on agronomic traits and superoxide dismutase (Mn-SOD) activity in three maize (*Zea mays* L.) hybrids. Cereal Research 6(4): 521-531 In Persian with English abstract).

Shiriga K, Sharma R, Kumar K, Yadav SK, Hossain F and Thirunavukkarasu N, 2014. Expression pattern of superoxide dismutase under drought stress in maize. International Journal of Innovative Research in Science, Engineering and Technology 3(4): 11333-11337.

Sytykiewicz H, 2014. Differential expression of superoxide dismutase genes in aphid-stressed maize (*Zea mays* L.) seedlings. PLoS One 9: 4. e94847. doi.org/10.1371/journal.pone.0094847.

Talaat NB, Shawky BT and Ibrahim AS, 2015. Alleviation of drought-induced oxidative stress in maize (*Zea mays* L.) plants by dual application of 24-epibrassinolide and spermine. Environmental and Experimental Botany 113: 47-58.

Tripathy BC and Oelmuller R, 2012. Reactive oxygen species generation and signaling in plants. Plant Signaling and Behavior 7(12): 1621-1633.

Valizadeh M, Moharamnejad S, Ahmadi M and Mohammadzadeh Jalaly H, 2013. Changes in activity profile of some antioxidant enzymes in alfalfa half-sib families under salt stress. Journal of Agricultural Science and Technology 15(4): 801-809.

Wendel JF and Weeden NF, 2012. Visualization and interpretation of plant isozymes. In Soltis DE *et al.* (eds.). Isozymes in Plant Biology. Pp. 5-45. Springer, Dordrecht, Netherlands.

Witt S, Galicia L, Lisec J, Cairns J, Tiessen A, Araus JL, Palacios-Rojas N and Fernie AR, 2012. Metabolic and phenotypic responses of greenhouse-grown maize hybrids to experimentally controlled drought stress. Molecular Plant 5(2): 401-417.

Xu L, Han L and Huang B, 2011. Antioxidant enzyme activities and gene expression patterns in leaves of Kentucky bluegrass in response to drought and post-drought recovery. Journal of the American Society for Horticultural Science 136(4): 247-255.

Yang L, Fountain JC, Wang H, Ni X, Ji P, Lee RD, Kemerait RC, Scully B and Guo B, 2015. Stress sensitivity is associated with differential accumulation of reactive oxygen and nitrogen species in maize genotypes with contrasting levels of drought tolerance. International Journal of Molecular Sciences 16(10): 24791-24819.

Zhang X, Wan Q, Liu F, Zhang K, Sun A, Luo B, Sun L and Wan Y, 2015. Molecular analysis of the chloroplast Cu/Zn-SOD gene (AhCSD2) in peanut. The Crop Journal 3(3): 246-257.

پاسخ کلیدی عملکرد دانه و سوپراکسید دیسموتاز در ذرت (*Zea mays* L.) به تنش کم آبی

سجاد محرم‌نژاد[1] و مصطفی ولیزاده[2]

۱- بخش تحقیقات علوم زراعی و باغی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان اردبیل (مغان)، سازمان تحقیقات، آموزش و ترویج کشاورزی، مغان.

۲- گروه به‌نژادی و بیوتکنولوژی گیاهی، دانشکده کشاورزی، دانشگاه تبریز، تبریز.

مسئول مکاتبه؛ Email: sm.chakherlo@yahoo.com

چکیده

به منظور اندازه‌گیری عملکرد دانه، ارتفاع بوته، فعالیت و بیان ژن آنزیم سوپراکسید دیسموتاز (Cu/Zn-SOD) تحت تنش کم آبی، آزمایشی به صورت کرت‌های خرد شده بر پایه بلوک‌های کامل تصادفی با سه هیبرید ذرت (سینگل کراس ۷۰۴، سینگل کراس، سینگل کراس ۶۴۰) تحت شرایط مزرعه در ایستگاه تحقیقاتی دانشگاه تبریز اجرا شد. نتایج نشان داد که تنش کم‌آبی باعث کاهش عملکرد دانه و ارتفاع بوته هیبریدهای ذرت شد. آنالیز الکتروفورزی برای Cu/Zn-SOD براساس حساسیت به مهارکننده پتاسیم سیانید و هیدروژن پراکسید روی ژل پلی آکریلامید هشت درصد انجام گرفت. فعالیتCu/Zn-SOD تحت تنش کم‌آبی در هیبریدهای ذرت افزایش یافت. بیان نسبی ژن *Cu/Zn-SOD* به روش Real-time PCR در هیبریدهای ذرت نشان داد که تنش کم‌آبی باعث افزایش بیان نسبی *Cu/Zn-SOD* شد. بر این اساس، سینگل کراس ۷۰۴ با بیشترین عملکرد دانه، ارتفاع بوته و فعالیت Cu/Zn-SOD به عنوان هیبرید متحمل به خشکی شناسایی شد. بر اساس نتایج حاصل چنین می‌توان نتیجه گرفت که افزایش بیان نسبی *Cu/Zn-SOD* می‌تواند صدمات ناشی از تنش کم‌آبی را در ذرت کاهش دهد.

واژه‌های کلیدی: ارتفاع بوته؛ الکتروفورز؛ آنزیم آنتی اکسیدان؛ ایزوفرم؛ ژن؛ عملکرد دانه.