



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

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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 H₂O₂ 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.

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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 Oelmüller 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 Oelmüller 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 H₂O₂ (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).

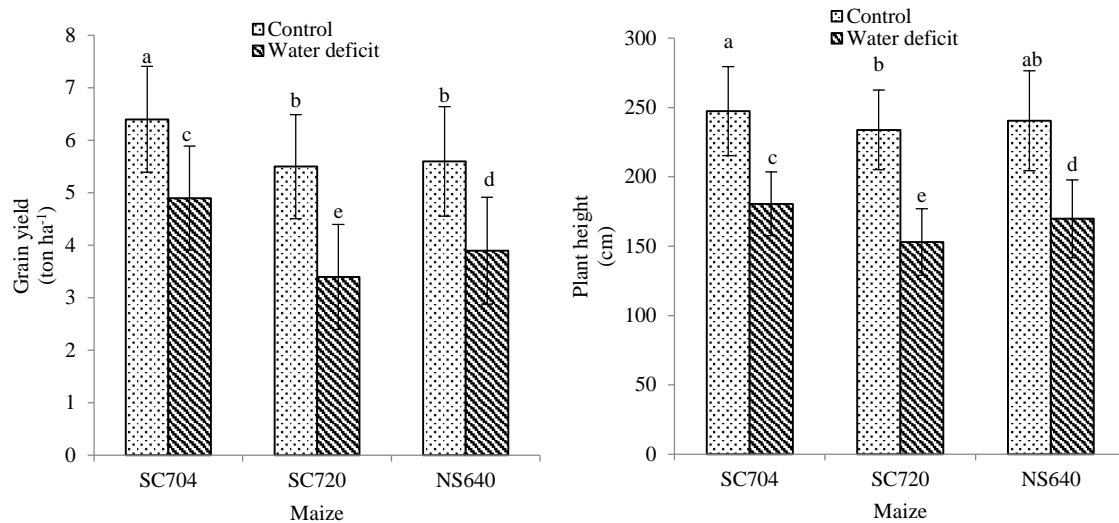


Figure 1. Grain yield and plant height (bars= \pm SE) of three maize hybrids under control and water-deficit stress conditions. Means with the same letters are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

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 H₂O₂. 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 induced 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

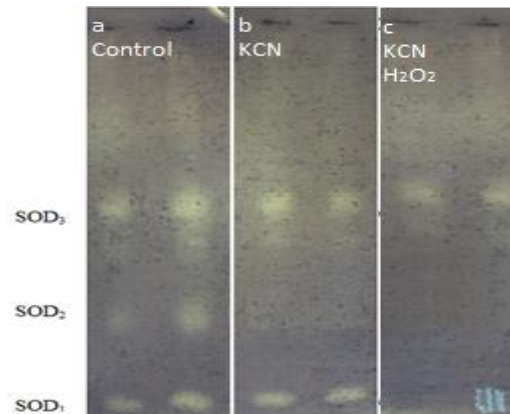


Figure 2. Detected SOD isoforms in the maize hybrids. The activity of SOD isoforms was detected by negative staining and identified based on their sensitivity to KCN and H₂O₂. Mn-SOD was resistant to both inhibitors; Cu/Zn-SOD was sensitive to both inhibitors; Fe-SOD was resistant to KCN and sensitive to H₂O₂; (a) Control (SOD₁, SOD₂, and SOD₃); (b) 2 mM KCN (SOD₁, SOD₃): Fe-SOD and Mn-SOD; (c) 2 mM KCN and 5mM H₂O₂ (SOD₃): Mn-SOD

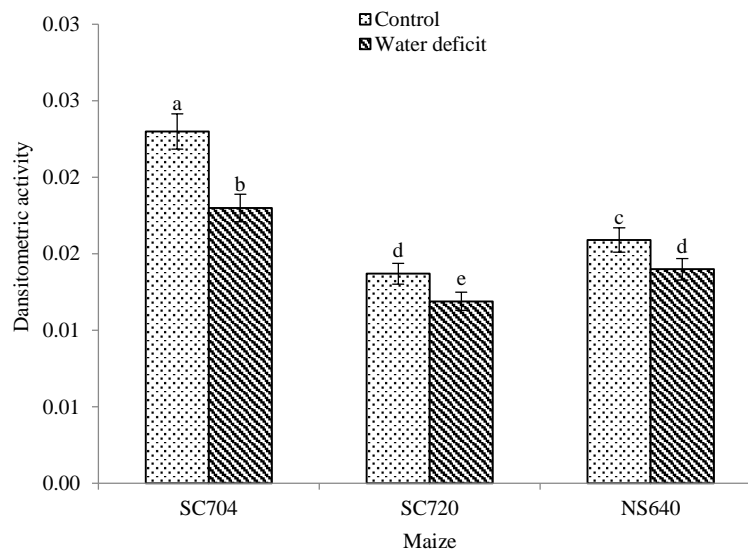


Figure 3. Cu/Zn-SOD activity (bars= ±SE) of the three maize hybrids under control and water-deficit stress conditions. Means with the same letters are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

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.

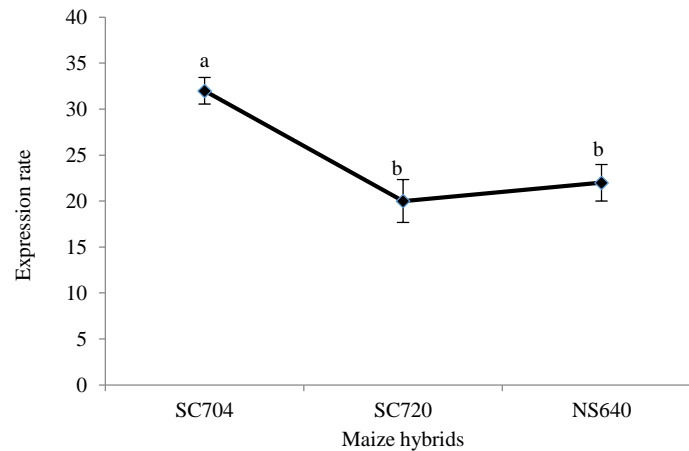


Figure 4. Expression rate (bars= \pm SE) of *Cu/Zn-SOD* in three maize hybrids. Means with the same letters are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

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

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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 کم آبی باعث کاهش عملکرد دانه و ارتفاع بوته هیبریدهای ذرت شد. آنالیز الکتروفورزی برای Cu/Zn-SOD بر اساس حساسیت به مهارکننده پتاسیم سیانید و هیدروژن پراکسید روی ژل پلی آکرلامید هشت درصد انجام گرفت. فعالیت Cu/Zn-SOD تحت تنش کم آبی در هیبریدهای ذرت افزایش یافت. بیان نسبی ژن *Cu/Zn-SOD* به روش Real-time PCR در هیبریدهای ذرت نشان داد که تنش کم آبی 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 کم آبی را در ذرت کاهش دهد.

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