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Contrasting the expression pattern change of polyamine oxidase genes and photosynthetic efficiency of maize (*Zea mays* L.) genotypes under drought stress

Hadis Pakdel, Seyedeh Batool Hassani, Ali Akbar Ghotbi-Ravandi* and Françoise Bernard

Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

**Corresponding author (Email, a_ghotbi@sbu.ac.ir)*

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The aim of this study was to contrast the effects of drought stress on polyamine oxidases gene expression and activity as well as photosynthetic efficiency in relatively tolerant (Karoon) and sensitive (260) maize genotype.d Reduction in leaf relative water content as a result of drought led to increase in root growth, but diminished shoot growth indices. Under drought stress, activity of antioxidant enzyme, catalase, significantly increased in both genotypes, whereas significant higher activity of superoxide dismutase and peroxidase was only observed in Karoon genotype. Expression of polyamine oxidase (PAO) genes (*zmPAO1, zmPAO2, zmPAO3, zmPAO4, zmPAO5, zmPAO6*) and activity of enzymatic polyamine oxidation was increased in both genotypes under drought stress. The enhancement in PAO gene expression and enzyme activity was more prominent in Karoon cultivar compared to 260. Chlorophyll *a* fluorescence and fast induction kinetics were negatively influenced by drought stress. These parameters were more affected in 260 cultivar compared with Karoon. Our results suggest that under drought stress, higher activity of polyamine oxidase pathway in back-conversion of Spermine and spermidine to putrescine (protectant of photosynthetic apparatus) as well as higher antioxidant enzymes activity in Karoon cultivar, may play a role in higher efficiency of photosynthetic process in this cultivar.

Keywords. Antioxidant enzymes; chlorophyll *a* fluorescence; drought stress; polyamine oxidase; *Zea mays*

1. Introduction

Drought stress is by far one of the most important abiotic factors that impairs growth and yield of plants (Shao *et al.* 2009). Approximately 25% of world's arable lands suffer chronically from drought stress, leading to significant decrease in agricultural products (Fathti *et al.* 2016). Drought imposes deleterious effects on several morphological, biochemical, physiological and molecular processes of plant (Toscano *et al.* 2016). Stunted growth, decline in photosynthesis and respiration rate, impairment of nutrient uptake, disturbance in normal metabolism, protein aggregation and oxidative stress are among the deleterious effects of drought stress in plants (Carvalho *et al.* 2019; Ghotbi-Ravandi *et al.* 2019). Adaptation to drought stress is complex and consists of several response mechanisms which are crucial for survival and growth of plants (Carvalho *et al.* 2017; Merwad *et al.* 2018). Under drought stress, plants exhibit a wide range of biochemical responses at cellular levels, such as over-production of abscisic acid (ABA), accumulation of compatible solutes (soluble sugars, free amino acids) and amines (polyamines) (Seki *et al.* 2007; Zhou and Yu 2010).

Polyamines (PAs) are small nitrogenous organic poly-cations formed by substitution of aliphatic hydrocarbons with two or more amino groups. The

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common PAs found in all living cells are diamine putrescine (Put), triamine spermidine (Spd), and tetramine spermine (Spm) (Kusano *et al.* 2008). PAs play a key role in several physiological functions of plants, such as cell division, developmental processes, DNA replication, senescence as well as stress response (Igarashi and Kashiwagi 2010; Reis *et al.* 2016). Accumulation of PAs confers protective mechanisms under environmental stresses (Zhou and Yu 2010; An *et al.* 2012). The biosynthetic pathway of PAs begins with formation of Put from either decarboxylation of arginine or decarboxylation of ornithine. Put is converted to Spd and Spm by successive addition of aminpropyl groups from s-adenosylmethionine. PAs catabolic pathways are catalyzed by diamine oxidase (high affinity for Put) and polyamine oxidases (PAOs). PAOs catalyze the partial or full back conversion of Spm to Spd, and Spd to Put. In maize, six members compose the PAOs gene family including *ZmPAO1-6*. The maize PAO family is orthologous to *Arabadopsis thaliana* (*AtPAO1-5*) and *Oryza sativa* (*OsPAO1-7*). Based on phylogenetic analysis, maize PAO proteins are grouped into 3 clades: clade I (*ZmPAO1*), clade II (*ZmPAO5*) and clade III (*ZmPAO2, ZmPAO3, ZmPAO4, ZmPAO6*) (Ono *et al.* 2012; Jasso-Robles *et al.* 2016).

Due to activity of PAOs in rapid inter-conversion of in polyamine cycle, the PAs pool is dynamic, changing according to cell demands (Alcázar *et al.* 2011; Szalai *et al.* 2017). There is extensive evidence that PAs pool undergoes a drastic change in response to different environmental stresses (Do *et al.* 2013; Liu *et al.* 2015) Accumulation of Put and higher rate of Put/(Spd and Spm) under stress condition has been reported in several plant species (Shu *et al.* 2012).

Conjugation of PAs and specifically Put with photosystem II (PSII) proteins may result in stability of conformational structure and function under environmental stresses (Hamdani *et al.* 2011). Photochemical reactions related to PSII are highly vulnerable to drought stress (Ghotbi-Ravandi *et al.* 2016). Inhibition of PSII activity leads to imbalance between generation and utilization of electrons and results in generation of reactive oxygen species (ROS) (Ghotbi-Ravandi *et al.* 2019). Formation of ROS in plant cells can damage membranes, proteins, nucleic acids, and pigments. Plants developed strategies to alleviate toxic effects of ROS, many of which involve antioxidant enzyme activities (Azizollahi *et al.* 2019).

Maize (*Zea mays* L.) is one of the most important crops, grown widely throughout world in tropical, subtropical temperate climate zones. Maize presently is responsible for providing approximately half of calories consumed worldwide (Liang *et al.* 2018). Maize production is seriously constrained by water shortage and increase in frequency and severity of drought pose a serious threat to sustainable maize production (Wu *et al.* 2019). In Iran, since maize cultivation is mostly extended in arid and semi-arid regions, encountering drought stress during the growth period is inevitable (Ghotbi-Ravandi *et al.* 2014). Hence, it is necessary to study the impact of drought exposure on growth and yield of maize. Furthermore, understanding the physiological and molecular aspects of plant tolerance to drought is a fundamental part for crop improvement programs.

In present study, we aimed to examine the effects of drought stress on expression of genes involve in polyamine metabolism as well as responses of photosynthesis and antioxidant enzymes in maize genotypes and compare these responses in genotypes differing in tolerance to drought stress.

2. Materials and methods

2.1 *Plant material, growing condition and drought treatment*

Two maize (*Zea mays* L.) genotypes differing in response to drought stress were used in this study. Seeds of tolerant (Karoon) and susceptible (260) cultivars were obtained from Safi Abad Agricultural and Natural Resources Research and Education Center and Seed and Plant Improvement Institute of Iran (SPII), respectively. Maize plants were grown in mixture of peat moss and perlite in 70% of soil water holding capacity (WHC) under controlled condition (25 ± 2°C day/ 20 ± 2°C night, 16 h photoperiod with light intensity of 200 µmol m^{-2} s^{-1} and 40–50% relative humidity). After 2 weeks of growth (4-leaf stage), drought stress was induced by withholding water until 20% of WHC was reached in drought treated plants (15 days). Normal irrigation (70% WHC) was continued for control group. Sampling from control and drought treated plants were performed simultaneously from youngest fully expanded leaves.

2.2 *Growth indices and relative water content (RWC) measurement*

At harvest, the length of shoot and root was measured. Harvested plants were dried in oven (72 h at 50°C) and dry weight was determined.

To calculate RWC, fresh weight (FW) of fully expanded leaves was determined immediately after harvesting. Turgid weight (TW) of leaves was obtained after 16 h rehydration in distilled water. Samples then were oven-dried at 50°C to reach constant weight (DW). RWC was calculated by the equation: RWC = (FW-DW)/(TW-DW).100

2.3 *Enzymatic activity assays*

Fresh leaves (250 mg) were homogenized in phosphate buffer (50 mM, pH 7) containing EDTA (1 mM) and 1% polyvinylpyrrolidone (PVP). After centrifugation, the resulting supernatants were used for antioxidant enzymes assay. Protein content of each sample was determined according to Bradford (1976). Catalase (CAT, EC: 1. 11. 1. 6) activity was determined spectrophotometrically (CARY 300, Agilent, Santa Clara, California, USA) by monitoring the decrease in absorbance at 240 nm as a result of H_2O_2 degradation for 3 minutes (Aebi 1974). Peroxidase (POX, EC: 1. 11. 1. 7) activity was assayed via increase in absorbance at 470 nm as a consequence of guaiacol oxidation (Wu *et al.* 2003). Superoxide dismutase (SOD, EC: 1. 15. 1. 1) activity was assessed by monitoring the inhibition of photochemical reduction of NBT (nitro blue tetrazolium) at 560 nm according to Giannopolitis and Ries (Giannoplitis and Ries 1997).

The polyamine oxidase activity (PAO, EC: 1.5.3.11) was determined by peroxidase/guaiacol method according to Kaur-Sawhney *et al.* (1981). The procedure involved measurement of oxidation rate of guaiacol by H_2O_2 with Spm as substrate. Fresh samples (100 mg) were homogenized in sodium phosphate buffer (100 mM, pH 6) and centrifuged at 12000 rpm for 10 min at 4° C. The protein concentration of supernatants was determined according to Bradford (1976). Reaction mixture was prepared with 100 µl supernatant, phosphate buffer (100 µl), guaiacol (50 µl), peroxidase (50 µl, 1 mg/ml) and Spm (50 µl, 0.5 mg/ml). The absorbance of the reaction mixture was determined within 60 min at 470 nm.

2.4 *Measurement of chlorophyll a fluorescence transient (JIP-test) and fast fluorescence induction kinetics*

Chlorophyll *a* fluorescence measurements were performed on attached leaves in room temperature using plant efficiency analyzer (Handy PEA, Hansatech instruments Ltd, Lynn, UK). Samples were dark adapted for 30 min prior to experiment. The fluorescence rise after illumination was recorded and analyzed with biolyzer 4HP software (Bioenergitic laboratory, University of Geneva, Switzerland). Variation of fluorescence intensity during 1 second after illumination is known as Chlorophyll *a* fluorescent transient curve which comprises of four separate steps including $F_{0,}$ (fluorescence intensity at 10 µsec), F_J (fluorescence intensity at 2 ms), F_I (fluorescence intensity at 20 ms) and F_M (maximal fluorescence) (Strasser and Strasser 1995; Strasser *et al.* 2004). Definition of fluorescence kinetic parameters extracted from JIP test is presented in supplementary table 1.

2.5 *RNA extraction, cDNA synthesis and quantitative real-time PCR*

Total RNA was extracted from youngest fully expanded leaves using RNX-Plus solution (CinnaGen Company, Iran) according to manufacturer's protocol followed by DNase I (RQ1 RNase-Free DNaseI, Promega) treatment to eliminate genomic DNA contamination. Synthesis of cDNA was performed using iScriptc DNA Synthesis Kit (Bio-Rad Inc., Hercules, California, USA). The specific primers for MEP (membrane protein PB1A10.07c) and *ZmPAO1-6* were designed via primer 3 software (supplementary table 2). The Real-Time PCR was performed via iQ thermocycler (Bio-Rad Inc., Hercules, California, USA) using iQsybergreen supermix kit (Bio-Rad, USA). The MEP gene was used as internal control for comparative analysis (Manoli *et al.* 2010). The REST 2009© software (Pfaffl *et al.* 2002) was used to analyze the relative expression ratio and the significance of the changes in the gene expression levels.

2.6 *Statistical analysis*

The experiment was designed based on complete randomize design (CRD) with three independent replications. Data are presented as mean ± SD (standard deviation). The analysis of variance (ANOVA) and Duncan's multiple range test was performed by SAS software (SAS Institute Inc., Cary, NC, USA) to determine the significance ($P \leq 0.05$) of variations in groups.

3. Results

3.1 *Growth and water status of cultivars under drought stress*

The imposed drought stress significantly ($P \leq 0.05$) reduced water status of both genotypes (figure 1). Shoot length and dry matter significantly ($P \leq 0.05$) decreased under drought in both genotypes (figure 2). These reductions were more prominent in 260 cultivar compared to Karoon. On the other hand, drought stress led to significant ($P \leq 0.05$) increase in both root dry weight and length (figure 2). In Karoon cultivar, drought-induced increase in root length was significantly higher than 260 cultivar. Under drought stress, root biomass was increased 2.8- and 2.2-fold in Karoon and 260 cultivars, respectively, compared to control groups.

3.2 *Alteration in polyamine oxidases gene expression and polyamine oxidase enzyme activity in response to drought*

Quantitive real-time PCR results revealed that *ZmPAO1*, *ZmPAO2, ZmPAO4,* and *ZmPAO6* transcripts were significantly expressed ($P \leq 0.05$) in response to drought stress in both genotypes (figure 3). However, increased expression of *ZmPAO3* and *ZmPAO5* was only observed in tolerant Karoon cultivar. Under drought stress, transcript level of *ZmPAO1, ZmPAO2,* and *ZmPAO3, ZmPAO5* and *ZmPAO6* was significantly higher in tolerant Karoon compared to 260 cultivar (figure 3). Enzymatic polyamine oxidation activity significantly ($P \leq 0.05$) increased in both genotypes in response to drought stress compared to control (figure 4). Under drought, significantly higher enzymatic activity was observed in Karoon cultivar compared to 260.

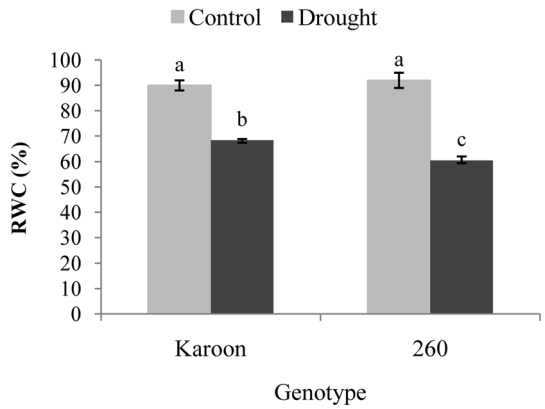

Figure 1. Effects of drought stress on leaf relative water content (RWC) in Karoon and 260 maize cultivars. Values are the mean ± SD of three independent replicates. Different letters indicate significant difference ($P \leq 0.05$).

3.3 *Effects of drought stress on chlorophyll a fluorescence transient and kinetics*

Chlorophyll *a* fluorescence (OJIP-test) is a highly sensitive method for evaluation of the PSII photochemistry as well as electron transport efficiency and has been widely used to probe the integrity and activity of the photosynthetic apparatus. The effects of drought on shape of chlorophyll fluorescence transient curve are presented in figure 5. The fluorescence transient curve of Karoon cultivar exhibited a slight increase in J and I step compared to control group. However, in 260 cultivar, considerable increase in J, I, and P step was observed under drought stress (figure 5). Calculated parameters from chlorophyll *a* fluorescence are presented in figure 6. Under drought stress, the maximum quantum yield (F_V/F_M), yield of primary photochemical reactions (φP_O), Exiton transfer to electron transport chain (Ψ_O), electron transport yield (φE_O) and performance index (PI_{abs}) significantly ($P \leq 0.05$) decreased in both genotypes (figure 6). However, the drought-induce decrease in these parameters were significantly lower in 260 cultivar, compare to Karoon. Drought treatment also resulted in significant increase in thermal dissipation of absorbed light (φD_O) (figure 6).

3.4 *Antioxidant enzymes activity in response to drought*

Drought stress resulted in significant ($P \leq 0.05$) increase in specific activity of CAT, SOD, and POX enzymes in Karoon genotype (figure 7). Under drought, activity of CAT was higher in 260 cultivar, whereas activity of SOD and POX did not significantly affected by drought (figure 7)

4. Discussion

The aptitude to adapt with drought stress relies on several different mechanisms. One of the most important strategies is the ability to maintain high RWC under drought stress. In the present study, both maize genotypes underwent a significant water loss as a result

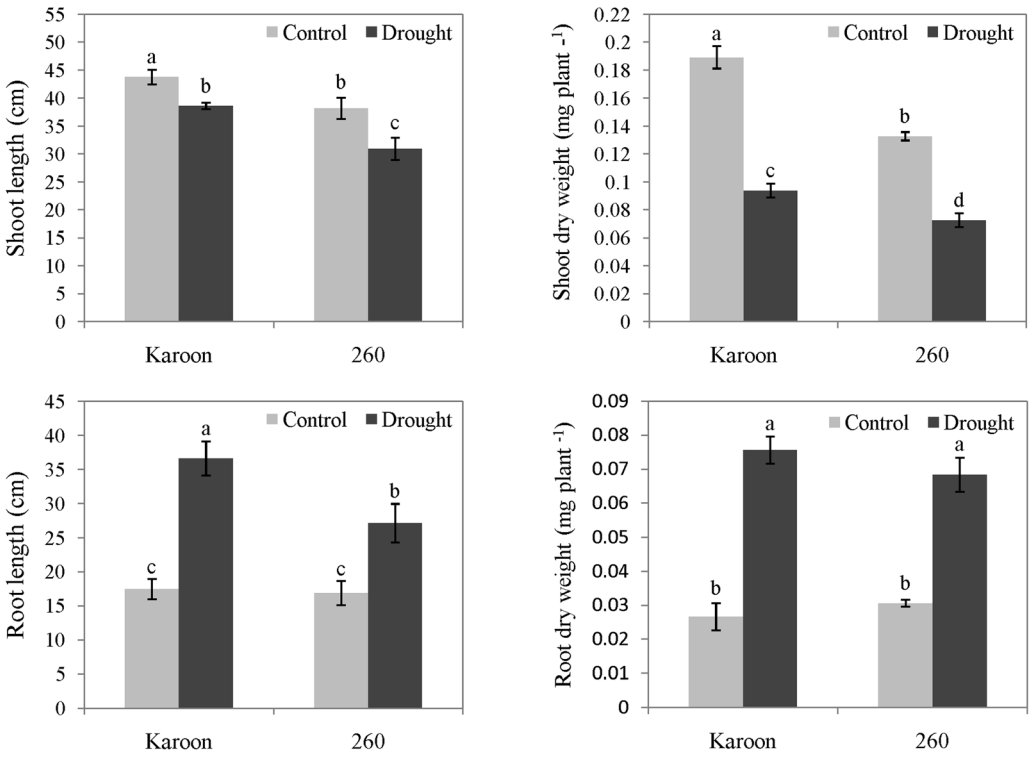

Figure 2. Effects of drought stress on root and shoot length and dry weight in Karoon and 260 maize cultivars. Values are the mean ± SD of three independent replicates. Different letters indicate significant difference ($P \leq 0.05$).

of withholding water. Karoon cultivar was more successful in maintaining higher RWC compared to 260 (figure 1). In the early stages of drought, shoot water content is affected prior to root. As shoot water content begins to decrease, turgor pressure gradually declines. Since the cell expansion and growth are turgor-dependent processes, they are extremely sensitive to dehydration. At this time, photosynthetic carbon fixation is not yet affected by drought (Ghotbi-Ravandi *et al.* 2014). Inhibition of shoot growth reduces the utilization of photosynthetic assimilates by the shoot and greater proportion of carbon and energy can be allocated to root system and support root growth. Induction of root growth is response to drought is more efficient in C4 plant such as maize, since the photosynthetic processes are less affected by drought compared to C3 plants. In the present study, significant increase in root biomass and length of the both genotypes was followed with significant decrease of these traits in shoot (figure 2). Plant ability to develop deeper root system to extract water from deeper soil layers has a great importance in balancing leaf water content (Henry *et al.* 2012). Higher RWC in Karoon compared to 260 genotype can be contributed to the higher rate of root growth induction under drought. The significance of deep root system in order to uptake water from deeper soil layers under drought stress have been reported in various crops such as sorghum, rice, chickpea and wheat (Kashiwagi *et al.* 2006; Wasson *et al.* 2012; Rostamza *et al.* 2013; Steele *et al.* 2013).

As drought stress progress, CO_2 absorption reduces due to partial or complete stomatal closure. Stomatal limitation and suppression of CO_2 fixation in Calvin cycle lead to decrease in $NADP^+$ pool. Depletion of $NADP^+$ as a main electron acceptor in photosystem I (PSI) results in diversion of electron flow from ferrodoxin to O_2 and generation of ROS. Furthermore, electron leakage from reduced plastoquinone to O_2 as well as direct formation of singlet oxygen $^1O_2$ by PSII, contribute to higher rate of ROS formation in drought stressed plants (Ghotbi-Ravandi *et al.* 2014). Overproduction of ROS can lead to disruption of redox homeostasis and oxidative damage in plant cells (Azizollahi *et al.* 2019). The magnitude of tolerance to drought stress is highly correlated with of ROS scavenging capacity of plant cells. Enhanced antioxidant enzymatic activity is one of protective mechanisms adopted by plant to eliminate stress-induced ROS. In the present study, drought stress triggered the enhancement of antioxidant enzyme activity. CAT activity significantly increased in both genotypes compared to control group in response to drought stress

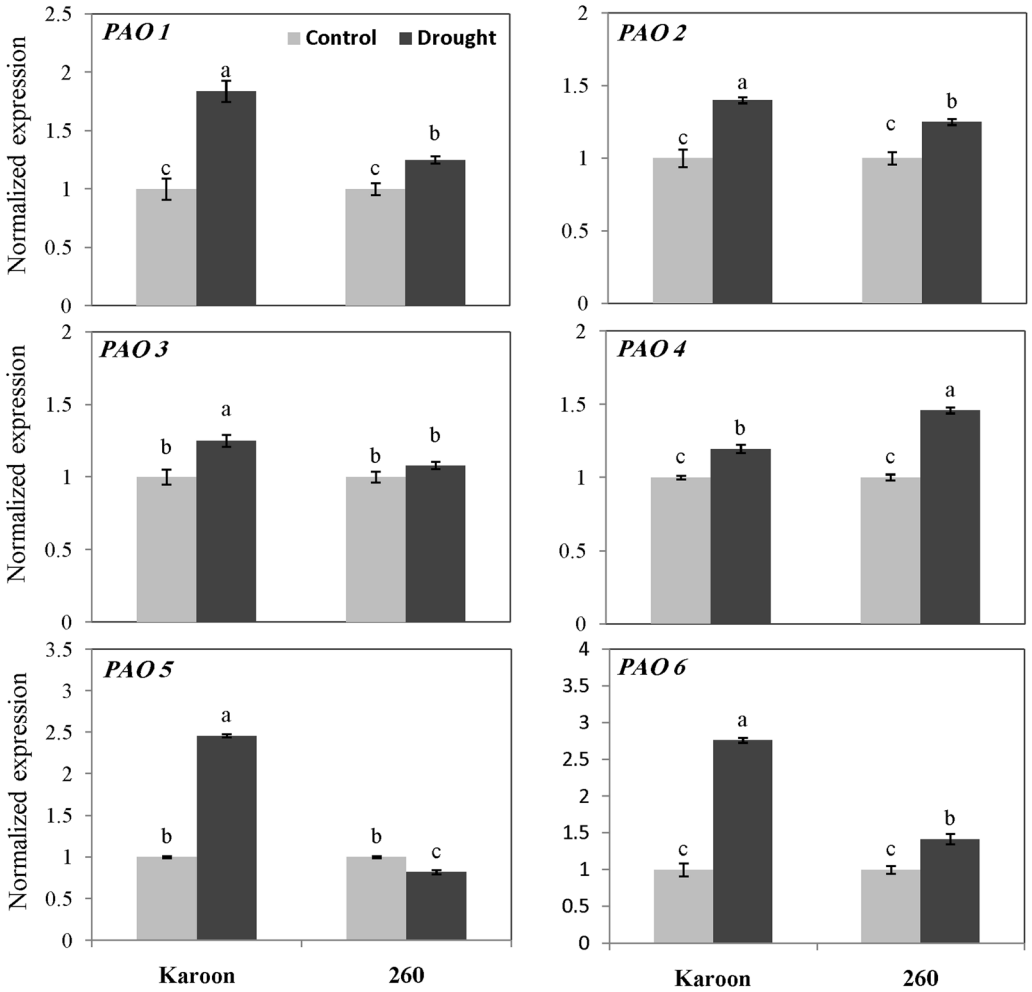

Figure 3. Changes in expression patterns of polyamine oxidase genes (*PAO1*, *PAO 2, PAO 3, PAO 4, PAO5, PAO6*) in response to drought stress in Karoon and 260 maize cultivars. Relative mRNA level was calculated with respect to that of MEP as reference gene. Values are the mean ± SD of three independent replicates. Different letters indicate significant difference ($P \leq 0.05$).

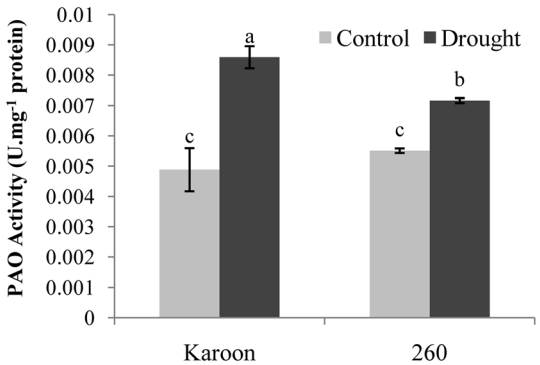

Figure 4. Effects of drought stress on activity of activity of polyamine oxidase in Karoon and 260 maize cultivars. Values are the mean ± SD of three independent replicates. Different letters indicate significant difference ($P \leq 0.05$).

(figure 7). Whereas, increased activity of SOD and POX enzymes in response to drought more prominent in tolerant Karoon genotype (figure 7). These results are in accordance with previous reports on the elevation of antioxidant enzymes activity associated with drought response in barley (Ghotbi-Ravandi *et al.* 2019), maize (Chugh *et al.* 2011), rice (Sharma and Dubey 2005), sesame (Fazeli *et al.* 2007) and oilseed rape (Abedi *et al.* 2010).

Extensive changes in PAs pool are prevalent in plants under abiotic stresses. Numerous studies have reported that stress tolerance is highly correlated to capacity of plants to accumulate PAs upon stress exposure (Liu *et al.* 2015). Stabilization of membranes, scavenging of ROS, regulation of certain ion transporters as well as modulation of DNA, RNA and protein turnover are among the adaptive functions of PAs in response to drought stress (Groppa and Benavides 2008; Hamdani *et al.* 2011). However, high content of PAs and specially Spm and Spd under stress condition may result in

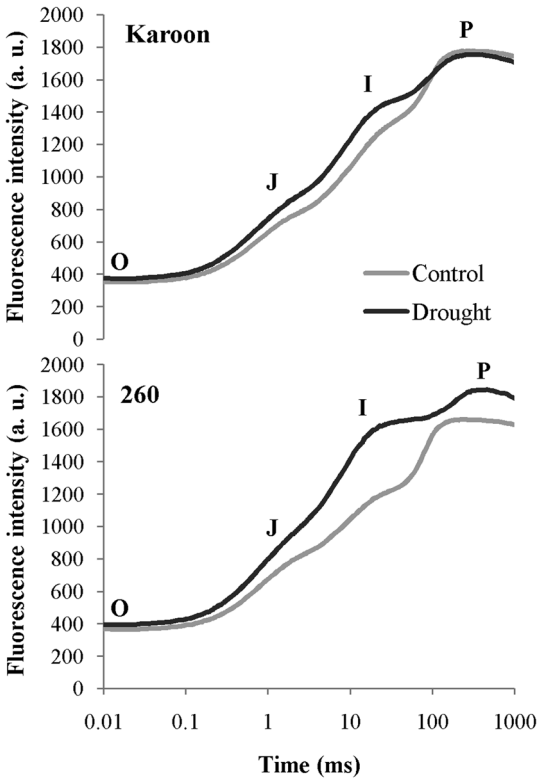

Figure 5. Effects of drought stress on fast fluorescence induction curve (logarithmic time scale) of Karoon and 260 maize cultivars.

plant injury (Shu *et al.* 2012). Usually, accumulation of PAs is accompanied by PA oxidation. PAOs are FAD containing enzymes that catalyze the oxidation and sequential back conversion of Spm and Spd to produce Spd and Put, respectively. Conversion of Spm and Spd to Put and accumulation of Put in response to stresses may alleviate the possible adverse effects of Spm and Spd in plants. Szalai *et al.* (2017) applied different concentrations of Put, Spd and Spm in wheat and maize plants. They reported that while Put induced no negative changes in either wheat or maize, these plants were sensitive to higher concentrations of Spd and specially Spm. Deleterious effects of Spd and especially Spm manifested as growth inhibition and oxidative stress. In Arabidopsis, Spd treatment led to root growth inhibition and changes in plant morphology (Tassoni *et al.* 2000). Similarly, application of exogenous Spd induced programmed cell death in maize (Tisi *et al.* 2011). However, transgenic wheat plants expressing an oat arginine decarboxylase (Put biosynthetic enzyme) cDNA and exhibited higher Put level (300–400 nmol g^{-1} FW), were phenotypically normal and fertile (Bassie *et al.* 2008). In the present study, drought stress led to increase in PAO genes expression compared to well-watered condition in both genotypes. Expression of *zmPAO1*, *zmPAO2*, *zmPAO3*, *zmPAO5* and *zmPAO5* genes were significantly higher in tolerant Karoon genotype compared to 260 genotype (figure 3). Consistent with gene expression analysis, Polyamine oxidation capacity of PAO enzymes was significantly increased upon drought treatment and this elevation in enzymatic activity was higher in tolerant Karoon genotype compared to 260 (figure 4). These results suggest that under drought, composition of polyamine pool may alter to accumulate Put rather than Spd and Spm, due to higher rate of back-conversion of Spd and Spm to Put, as final product of PAO activity. This process was more prominent in drought tolerant Karoon cultivar and can be considered as one of the tolerance mechanisms employ by tolerant cultivars. Consistent with these findings, Ebeed *et al.* (2017) reported that drought stress caused an increase in Put content accompanied with reduction in Spd and Spm content in wheat plants. Similarly, increased expression of PAOs genes in back-conversion pathway during dehydration has been reported in Arabidopsis (Alcázar *et al.* 2011).

Protective role of PAs, specifically Put, against impact of environmental stresses on photosynthetic machinery has been well documented (Ioannidis *et al.* 2012; Shu *et al.* 2012; Shu *et al.* 2014). Put may interact with PSII reaction center proteins (D1, D2 and CP43), LHCII (light harvesting complex II) antenna complex, PSI reaction center as well as subunits of ATP synthase (Shu *et al.* 2012; Shu *et al.* 2015). Shu *et al.* (2015) reported that Put can alleviate alteration of transcription and translation of D1 and D2 proteins induced by salt stress in cucumber. Furthermore, Due to its poly-cationic nature, Put can form electrostatic interactions with photosynthetic proteins and enhance the stability and photochemical efficiency of photosynthetic apparatus under stress condition (Hamdani *et al.* 2011; Shu *et al.* 2015).

In the present study, poly-phasic chlorophyll *a* fluorescence transient was used to determine the effects of drought stress on PSII structure and function. The OJIP transient evaluates the successive reduction of electron acceptors in PSII and photosynthetic electron transfer chain (Govindjee 1995). Our results revealed that drought stress altered the shape of fluorescence transient curve, resulted in increase in J and I steps in Karoon as well as J, I, and P steps in 260 cultivar (figure 5). The J step represents the redox state of Q_A (quinone A), as the first electron acceptor from PSII. On the other hand, J step describes the accumulation of $Q_A^- Q_B$ (Lazar 1999; Shahbazi *et al.* 2007). Fluorescence intensity at J phase of 260 cultivar exhibited a

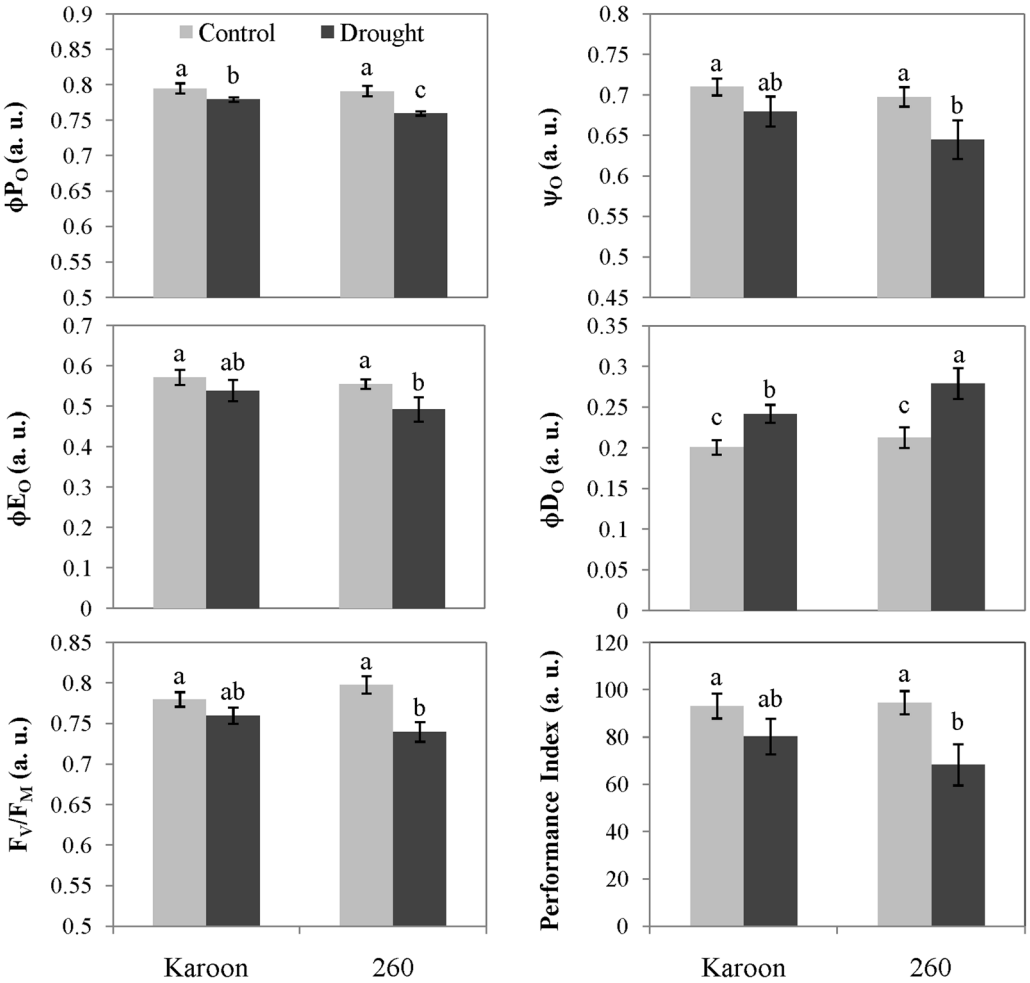

Figure 6. Selected chlorophyll *a* fluorescence parameters extracted OJIP transient curve in Karoon and 260 maize cultivars in response to drought stress. Values are the mean ± SD of three independent replicates. Different letters indicate significant difference ($P \leq 0.05$).

distinct rise in response to drought compare to small increase in Karoon cultivar. Exiton transfer to electron transport chain (ΨO) is a fraction of electron transported beyond Q_A per exiton trapped by PSII reaction center (Oukarroum *et al.* 2007). Based on our results, Ψ_O decreased in both genotypes in response to drought stress (figure 6). Rise in fluorescence intensity at J step accompanied with decrease in Ψ_O can be attributed to decrease in Q_A re-oxidation efficiency due to inhibition of electron transport beyond Q_A or reduction in size of Q_A pool (Oukarroum *et al.* 2007; Mehta *et al.* 2010). Karoon cultivar exhibited a minor increase in I step of chlorophyll *a* fluorescence transient, whereas in 260 cultivar, drought induced a distinct raise in fluorescence intensity. The I step reflects accumulation of $Q_A^- Q_B^-$ (Strasser and Strasser 1995; Lazar 1999). Increase in I step accompanied by significant reduction in values of φE_O (yield of electron transport) (figure 6) suggests a disorder in kinetics of oxidation/reduction of plastoquinone pool and inhibit electron transfer downstream Q_B (Gomes *et al.* 2012). Increased fluorescence intensity at P step was only observed in 260 cultivar, under drought stress condition (figure 5). The P phase describes re-reduction of plastocyanine and PSI ($P700^+$). The rise in P step can be contributed to disorder in acceptor side of PSI (Toth *et al.* 2007). Similar to our findings, Gomes *et al.* (2012) reported that drought stress caused an increase in all steps of fluorescence transient curve in passion fruit. Drought stress also resulted in raise in chlorophyll *a* fluorescence curve in Acer (Banks 2018). Another research conducted by Kalaji *et al.* (2011) revealed that similar to drought, salinity stress caused an increase in all steps of chlorophyll *a* fluorescence transient in Syrian barley landraces.

Maximum quantum yield of PSII (F_V/F_M) is one of the most sensitive parameters to environmental stresses. The F_V/F_M exhibited a significant reduction in 260

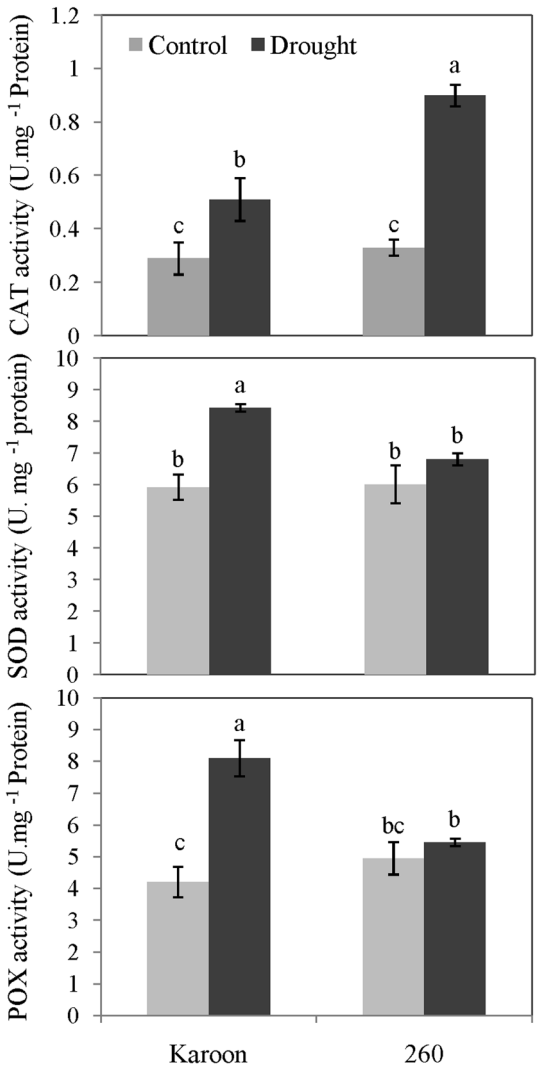

Figure 7. Effects of drought stress on specific activity of catalase (CAT), superoxide dismutase (SOD) and peroxidase (POX) in Karoon and 260 maize cultivars in response to drought stress. Values are the mean ± SD of three independent replicates. Different letters indicate significant difference ($P \leq 0.05$).

cultivar under drought stress (figure 6). Under controlled condition, the value of F_V/F_M is near 0.8–0.83 in most plant (Bjorkman and Demmig 1987). In the present study, reduction in F_V/F_M observed in both maize genotypes under water deprivation might be due to damaged and photochemically inactive reaction centers (Basu *et al.* 1998). Disassociation of LHCII from PSII core and low energy transfer rate from LHCII to PSII reaction center may also contribute to reduced rates of F_V/F_M (Kalaji *et al.* 2011). Similar to these findings, decrease in F_V/F_M under drought stress has been reported in barley (Ghotbi-Ravandi *et al.* 2016) and passion fruit (Gomes *et al.* 2012). Decrease in quantum yield is often accompanied by increase in non-photochemical quenching processes including thermal dissipation. In the present study, the thermal dissipation yield (φD_O) significantly increased in both genotypes as the quantum yield declined (figure 6). Under drought stress, increase in non-photochemical quenching also affects the yield of primary photochemistry reactions (φP_O). Loss of absorbed light energy as heat instead of engaging electrons in photochemical reactions leads to decrease rate of primary photochemical reactions. In the present study, the φP_O values significantly decreased in both genotypes under drought stress. Similar to our finding, decrease in φP_O as well as increase in φD_O in response to drought stress has been reported in barley genotypes (Ghotbi-Ravandi *et al.* 2016).

Performance index (PI_{ABS}) is a complex multiparametric parameter and represents energy conversion from absorbed light by PSII and successive reduction of electron acceptors in photosynthetic electron transport chain (Mehta *et al.* 2010). PI_{ABS} is a combination of efficiency of light absorption, energy flow to PSII reaction centers and yield of electron transport in intersystem electron carriers (Mehta *et al.* 2010). PI_{ABS} has previously been proven to be a very sensitive parameter to environmental stresses and can be used as an indicator of plant viability under different unfavorable conditions (Zushi *et al.* 2012). Decrease in PI_{ABS} was reported by Ghotbi-Ravandi *et al.* (2016) in wild and cultivated barley genotypes as result of drought stress. Zivcak *et al.* (2008) demonstrated the PI_{ABS} continuously decreases from beginning of dehydration in all seven winter wheat genotypes. Consistent with these findings, in the present study, drought exposure led to significant decrease in PI_{ABS} parameter in 260 genotypes, whereas in Karoon cultivar, the reduction in PI_{ABS} was not significantly different from control group (figure 6). This observation suggests that structure and function of PSII and activity of electron transport chain are more vulnerable in 260 cultivar compared to Karoon. Higher efficiency of photosynthetic electron transport chain in Karoon is consistent with higher PAOs gene expression and enzyme activity in this cultivar, since the end product of PA oxidation pathway, Put, plays a protective role of photosynthetic apparatus. Furthermore, higher efficiency of antioxidant enzymes activity in Karoon genotype may contribute to higher efficiency of photosynthetic machinery, since the activity of these enzymes alleviates the deleterious effects of ROS on pigments and photosynthetic membranes and protein complexes.

5. Conclusions

Our results suggest that PAOs may be involved in response of maize genotypes to drought stress. Under drought, higher expression of PAO genes and enzymatic PA oxidation activity as well as elevated activity of antioxidant enzymes may contribute to higher efficiency of photosynthetic light reactions and overall tolerance of Karoon cultivar compared to 260.

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Corresponding editor: MANCHIKATLA VENKAT RAJAM