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FOLIAR-APPLIED CALCIUM INDUCES DROUGHT STRESS TOLERANCE IN MAIZE BY MANIPULATING OSMOLYTE ACCUMULATION AND ANTIOXIDATIVE RESPONSES

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Abstract

Influence of drought stress and foliar applied calcium (Ca^{2+}) on growth, water status, osmolyte accumulation and antioxidative defense system were evaluated in two maize hybrids, i.e. drought-tolerant Dekalb-6525 (DK-6525) and drought-sensitive Yousafwala Hybrid (YH). Drought stress caused substantial reduction in shoot dry matter (DM) production through disturbance in relative water content (RWC), protein metabolism and accelerating malondialdehyde (MDA) accumulation and disproportioning antioxidant system. However, the accumulation of total free amino acids (TFA), glycinebetaine (GB) and activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) were significantly increased by drought treatment. Foliar treatment of Ca^{2+} led to increase DM (49%), RWC (18%), accumulation of TFA (15%) and GB (25%) as well as the activities of SOD (37%), CAT (24%) and APX (49%) along with a decrease in MDA content (24%) in both hybrids under water-deficit conditions. Cultivar DK-6525 maintained relatively higher growth rate, water status, and osmolyte content and antioxidant activities than YH, irrespective of calcium supply and watering regimes. The results of the study suggested that optimal supply of Ca^{2+} is effective to make plants vigorous to thrive under moisture-deficit conditions.

Key words: Foliar calcium; Maize hybrid; Drought stress; Water status; Antioxidant system; Osmolyte accumulation.

Introduction

Drought is a serious threat to agriculture that harmfully affects plant productivity and survival (Monclus *et al.,* 2006). It affects normal root growth, nutrient uptake and mobility in soil which may involve buildup of mineral elements in plant tissues and therefore, alters various physiological and anti-oxidative plant responses (Luo *et al.,* 2011). Various biological practices have been pragmatic to mitigate adverse effects of drought on normal functioning of plants. For example, plant researchers have used the following practices in an effort to maintain plant growth under water deficit conditions i.e., plant growth regulators (Wang *et al.,* 2012), calcium ion (Upadhyaya *et al.,* 2011; Xu *et al.,* 2013) and other substances (Ishibashi *et al.,* 2011).

Essentially, calcium ion (Ca^{2+}) has occurred as an imperative secondary messenger in plants signaling networks (Cacho *et al.,* 2013). Various environmental stimuli are believed to prompt the increase of cytosolic Ca^{2+} to activate different biological and downstream responses (Zhu *et al.,* 2013) that causes plant adjustment to harmful environmental conditions (Shao *et al.,* 2008; Upadhyaya *et al.,* 2011) by manipulation of antioxidant defense system and reduction of membrane lipid peroxidation (LPO) that aid the plants to survive in stress conditions (Nayyar and Kaushal, 2002). Further, calcium acts as a regulator of plant cell metabolism and is involved in signaling anti-drought responses (Jaleel *et al.,* 2007; Shao *et al.,* 2008). Exogenous treatment of Ca^{2+}has been reported to confer improved tolerance to drought stress (Xu *et al.,* 2013) and modify stress induced reactive oxygen species metabolism, growth performance, photosynthetic efficacy and nitrogen assimilation (Zhu *et al.,* 2013). However, calcium is a relatively immobile in plants and their uptake reduces in above-ground portions of plants (shoots and leaves) as well as in roots under drought conditions due to decline in transpiration rate (Brown *et al.,* 2006). However, continuous supply of calcium is required by plants for vigorous leaf and root development and overall canopy growth (Del Amor and Marcelis, 2003). In such circumstances, foliar fertilization can be a substitute and effective means to improve the nutritive status of plants due to its comparatively rapid absorption and the independence of the soil moisture availability and root activity (Romheld and El-Fouly, 1999).

Maize is the third most important cereal with the leading global production at 829 million tons annually. Maize grain yields in developing tropical countries of Asia average 5.67 t/ha vs. temperate developed country of North-America 11.78 ton/ha (Anonymous, 2014). In both environments water limitation is the most significant abiotic stress, limiting and threatening maize grain production. Average annual yield losses by drought are supposed to be around 15% of well-watered yield potential on a worldwide basis, a number that compares to 120 million tons of grain (Edmeades, 2013).

To best of our knowledge, there is a little evidence available in the literature on the effect of drought stress and Ca supplies on accumulation of compatible osmolytes and activation of antioxidant system in maize genotypes differing in drought-resistance. We assumed that higher accumulation of compatible osmolytes and increased antioxidant activities in maize leaf tissues are the leading constituents of differential resistance mechanism in two cultivars varying in drought-resistance supplied with Ca^{2+} under water-deficit conditions. By this objective in notice, we planned a soil culture experiment to expose the response of two maize hybrids in terms of plant growth, leaf water content, osmolyte accumulation, antioxidant defense system and lipid peroxidation under drought stress and foliar sprays of calcium.

Materials and Methods

Plant materials and growth conditions: A soil culture experiment under drought stress was performed at the Department of Crop Physiology / Agronomy, University of Agriculture Faisalabad (31.25˚ N and 73.09˚ E) Punjab, Pakistan under a wire-house / rain-out shelter in February 2014. The experiment was laid out in a completely randomized design (CRD) with factorial arrangements using three replications. Two maize hybrids "Dekalb-6525 (DK-6525)" and "Yousafwala Hybrid (YH)" categorized as drought tolerant and sensitive in our previous studies were obtained from Monsanto Pakistan Agritech (Pvt.) Ltd. and Maize and Millet Research Institute, Yousafwala, Sahiwal-Pakistan, respectively (Naeem, 2016).

The seeds of both hybrids were treated with a recommended dose of insecticide (Imidacloprid) and fungicide (Topsin-M 70 WP) to avoid the damage from insects and soil-borne diseases. For the pot (soil culture) experiment, the soil collected from Crop Physiology / Agronomy Research Farm, University of Agriculture, Faisalabad, Pakistan was sun-dried, ground, sieved and mixed well in order to avoid any plant residue. The pysico-chemical features of the soil were analyzed by the method of Jackson and Barak (2005) with the following results: soil textural class = sandy loam; pH = 7.6; saturation percentage = 27%; organic matter = 1.19%; available nitrogen (N) = 610 mg kg^{-1}; available phosphorus (P) = 15.2 mg kg^{-1}; available potassium (K) = 80 mg kg^{-1} and calcium (Ca^{2+}) = 3.26 mEq L^{-1}. In each pot, recommended full dose of phosphorus (P), potassium (K) and 1/8th nitrogen (N) in the form of di-ammonium phosphate, sulphate of potash and urea, respectively, were applied at planting, while the remaining N doses were applied in three equal splits at V5 (5 leaf- 4 weeks of seeding), V12 (12 leaf- 7 weeks of seeding) and V14 (14 leaf- 8 weeks of seeding) stages of maize (Ritchie *et al.*, 1993).

Drought stress and calcium treatment: Five healthy seeds of maize hybrids (DK-6525 and YH) were grown in 12 L plastic pots (30.5 × 24 cm) containing 10 kg soil. After emergence, two plants per pot were retained. Plants were grown normally until V5, then one set of pots was maintained at 100% water-holding capacity (WHC) and the other set at 30% WHC on gravimetric basis (Nachabe 1998). Foliar sprays of calcium (40 mg L^{-1}) containing 0.1% Tween-20 were applied first at V6 (6 leaf- 5 weeks of seeding) and second at VT (tasseling-10 weeks of seeding) stages of maize (Ritchie *et al.*, 1993). A compression sprayer of 1 L capacity was used for this purpose to confirm uniform distribution of Ca^{2+} on all leaves. The calcium solution was prepared using calcium chloride di-hydrate [$CaCl_2.2H_2O$] (Sigma-Aldrich, USA) and nutrient spraying was done in morning (8:00-10:00 a.m.) during dry and sunny day. The equal amount of distilled water was used as foliar spray for comparison. The plants were harvested after two weeks of foliar Ca^{2+} supplies. The fully grown leaves from different experimental units were used for determining leaf water status, osmolyte content, activities of antioxidants and lipid peroxidation.

Estimation of plant growth and tissue water status: Plant growth in terms of shoot dry matter (DM) production was recorded after drying the samples in an air-forced oven at 70°C till constant weight. For estimation of tissue water status, leaf relative water content (RWC) was recorded. For this purpose, fresh leaf samples of 0.5g were placed in water for 6 h and then their turgid weight recorded using a digital electrical balance (Chyo, MK-500C), followed by drying in an air-forced oven at 70 ± 2 °C till constant weight (Cornic1994).

Estimation of osmolyte content: The randomly collected fresh leaf samples (0.5 g) for each treatment were chopped and then used for the determination of total soluble proteins (TSP) and total free amino acids (TFA) by the subsequent procedures published by Hamilton & Van Slyke (1943) and Riazi *et al.* (1985) respectively, and the results were expressed in mg g^{-1} DW. Glycinebetaine content was estimated by the method of Grieve and Grattan (1983).

Estimation of antioxidant activities and lipid peroxidation: Fresh leaf samples (0.5 g) were homogenized in a pestle and mortar on ice by a medium composed of 5 ml extraction buffer (50 m*M* phosphate buffer pH 7.0 and 1 m*M* dithiothreitol). The homogenate was then centrifuged at 12,000 × *g* for 20 min at 4 °C, and the supernatant was used for the analysis of the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX).

The SOD (EC 1.15.1.1) activity was determined by measuring the potential of the enzyme to impede the photochemical reduction of nitroblue tetrazolium (NBT) by photochemically-generated superoxide radicals by following the method of Giannopolitis and Ries (1977). The CAT (EC 1.11.1.6) and POD (EC 1.11.1.7) activities were examined by the method outlined by Chance and Mehley (1955). The activity of CAT was measured by the conversion rate of hydrogen peroxide (H_2O_2) to water and oxygen molecules; however, POD activity was inspected by computing peroxidation of H_2O_2 with guaiacol as an electron donor. The APX (EC 1.11.1.11) was measured by the decline in absorbance due to the formation of ascorbic acid in reaction mixture following the method of Cakmak (1994).

Lipid peroxidation was determined following the method of Heath and Packer (1968) using thiobarbituric acid reaction as index of malondialdehyde content. The absorbance of the TBA reactive substances (TBARS) was measured by the differences in absorbance at 532 to 600 nm using the extinction coefficient of 155 mM^{-1} cm^{-1} and results was presented in nmol g^{-1} DW.

Estimation of leaf calcium concentration: Leaf samples were collected for calcium analysis from below and opposite ear leaf and dried in an air-forced oven at ±70°C for 48 h and then crushed with a plant grinding mill. Wet digestion of dried plant material was carried out and Ca^{2+} concentrations in maize leaf tissues were determined using a Flame-photometer (Jenway PFP-7) according to Wolf (1982).

Statistical analyses: Data collected from different experiments of this project were subjected to Fisher's analysis of variance and a computer program of Statistix-8.1 used for analysis. Tukey's HSD (honest significant difference) tests at 0.05 probability level were used to evaluate the differences among treatment means.

Results

Plant growth: Plant growth was evaluated by computing dry matter (DM) production of shoots. Drought treatment notably ($p \leq 0.001$) reduced the shoot DM accumulation (29%) of both maize cultivars as compared to normal water supply (Fig. 1). Foliar calcium supplies significantly ($p \leq 0.001$) improved the plant growth (49%) of both genotypes under water-deficit conditions. Cultivar DK-6525 maintained considerably ($p \leq 0.01$) higher shoot DM than that of YH (Fig. 1; Table 1).

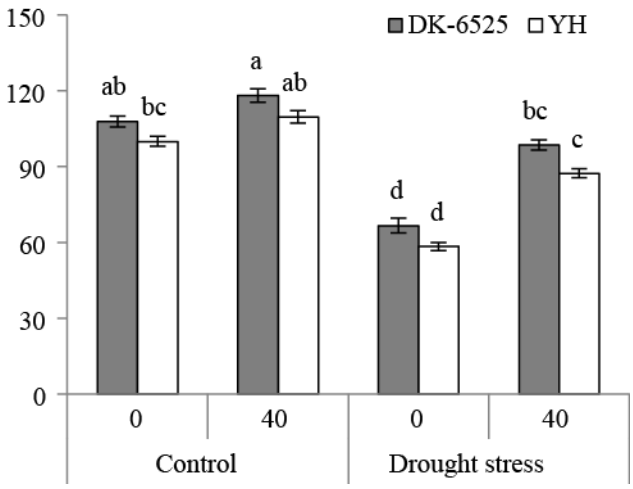

Fig. 1. Differential effects of drought stress and foliar Ca^{2+} supplies on shoot dry matter production in two maize hybrids; DK-6525 and YH represent genotype Dekalb-6525 and Yousafwala Hybrid respectively. Control: 100% water-holding capacity (WHC); Drought stress: 30% WHC. Columns not sharing a letter in common showed significant difference between eight treatments ($p \leq 0.05$)

Plant water status: Leaf relative water content (RWC) is known as an efficient indicator of plant tissue water status. Drought stress significantly ($p \leq 0.001$) reduced the leaf RWC of both cultivars by 17% as compared to normal conditions (Fig. 2a). Foliar treatment of Ca^{2+} (40 mg L^{-1}) was established to be useful for raising the RWC (18%) of both genotypes under drought stress. Drought tolerant cultivar DK-6525 showed a pronounced ($p \leq 0.01$) increase in leaf RWC (14%) compared with that in drought sensitive YH (Fig. 2a).

Osmolyte accumulation: Accumulations of low molecular weight organic osmolytes such as total free amino acids (TFA) and glycinebetaine (GB) in leaves suggest that osmoregulation may increase drought-tolerance potential of maize. Accumulation of TFA (Fig. 2d) and GB (2b) was more distinct in DK-6525 than in YH under water deficit conditions (Fig. 2). The contents of TFA and GB were significantly enhanced by 15% and 25% respectively, in water stressed plants supplied with calcium. Synthesis of total soluble proteins (TSP) was significantly ($p \leq 0.001$) reduced in both genotypes by drought treatment (Table 1; Fig. 2c). Application of Ca^{2+}also notably ($p \leq 0.05$) reduced the accumulation of TSP (11%) in both cultivars as compared to control (Fig. 2c).

Table 1. Mean square values from analysis of variance (ANOVA)of two maize hybrids (DK-6525 and YH) differing in drought tolerance tested for morpho-physiological and biochemical attributes at two watering-regimes (30% and 100% water-holding capacity) and two levels of foliar Ca^{2+} concentrations

SOV [a] / Parameters [c]	Shoot DM g plant^{-1}	Leaf RWC %	GB nmol g^{-1} DW	TSP mg g^{-1} DW	TFA mg g^{-1} DW	SOD	CAT	POD	APX ABA digested g^{-1}FW h^{-1}	MDA content nmol g^{-1} DW	Leaf Ca^{2+} content mg g^{-1} DW
						Units min^{-1}g^{-1}FW basis					
Water regimes (W)	5852.75***	914.96***	27.24***	61.07***	3.03***	62065.50***	64221.80***	87873.10***	38.92***	4.99***	4.84**
Hybrids (H)	485.52**	582.63**	1.63***	0.03	0.04*	966.70**	1237.00**	217.91	6.48***	0.14*	0.001
Calcium (Ca)	2448.13***	1245.17***	1.55***	5.20*	0.13**	10544.11***	3044.30***	517.70*	4.32***	0.48**	6.38***
W × H	3.38	0.96	0.48***	0.65	0.0001	594.91*	189.80	3.20	5.61***	0.02	0.02
W × Ca	627.25**	55.12	0.93***	3.12	0.04*	6629.40***	1651.71***	584.31*	4.08***	0.30**	0.33
H × Ca	5.04	2.04	0.04	0.14	0.0001	103.80	76.01	153.60	0.03	0.03	0.05
W × H × Ca	2.04	0.14	0.04	0.000035	0.0002	707.31*	1.91	0.50	0.0000013	0.005	0.01
CV [b]	4.96	5.68	4.88	11.58	8.02	5.37	5.11	7.28	10.37	11.55	10.34

*, **, *** Significant at $P \leq 0.05$, 0.01 and 0.001, respectively [a] SOV- Source of variation [b] CV-Coefficient of variation [c] DM = Dry matter; RWC = Relative water content; GB = Glycinebetaine; TSP = Total soluble proteins; TFA = Total free amino acids; SOD = Superoxide dismutase; CAT = Catalase; POD = Peroxidase; APX = Ascorbate peroxidase; MDA = Malondialdehyde

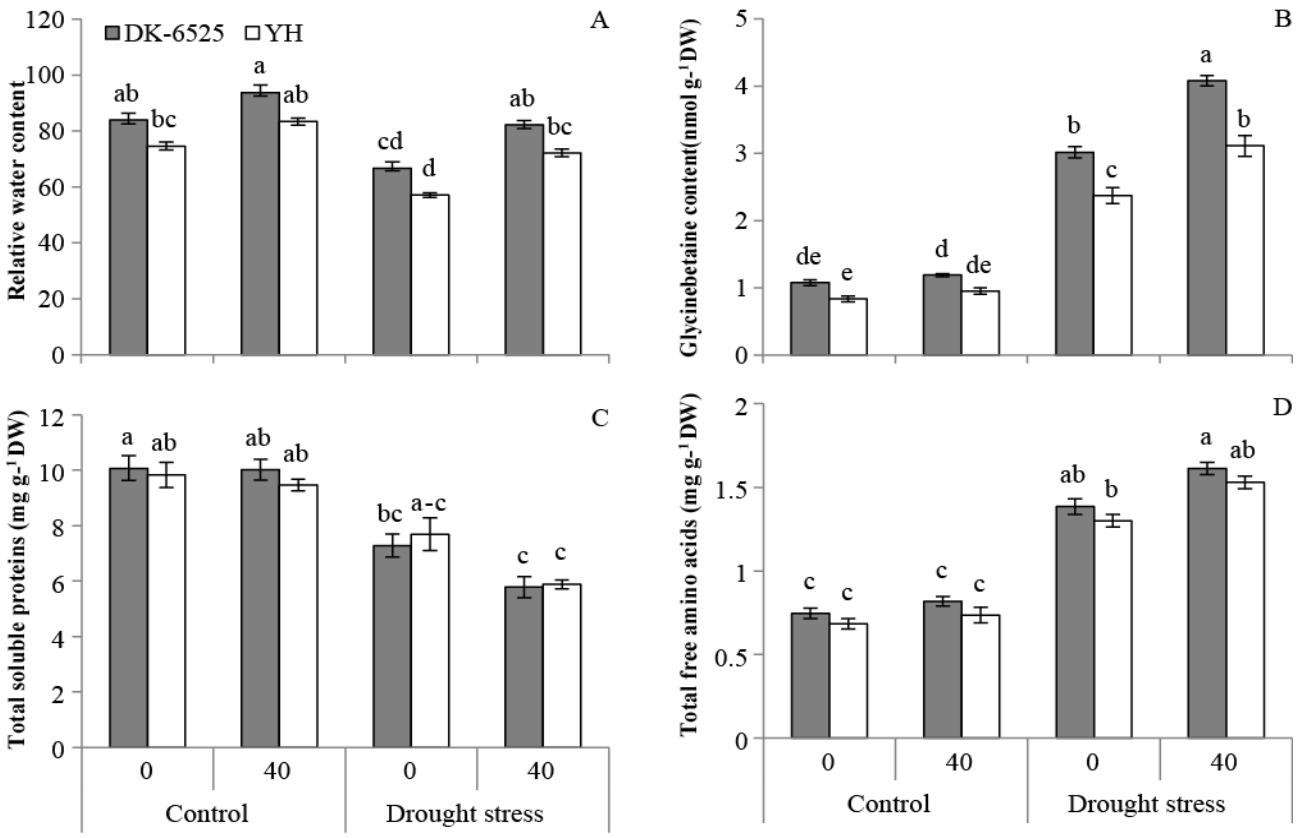

Fig. 2. Differential effects of drought stress and foliar Ca^{2+} supplies leaf water status and osmolytes accumulation in two maize hybrids; DK-6525 and YH represent genotype Dekalb-6525 and Yousafwala Hybrid respectively. Control: 100% water-holding capacity (WHC); Drought stress: 30% WHC. Columns not sharing a letter in common showed significant difference between eight treatments ($p \leq 0.05$)

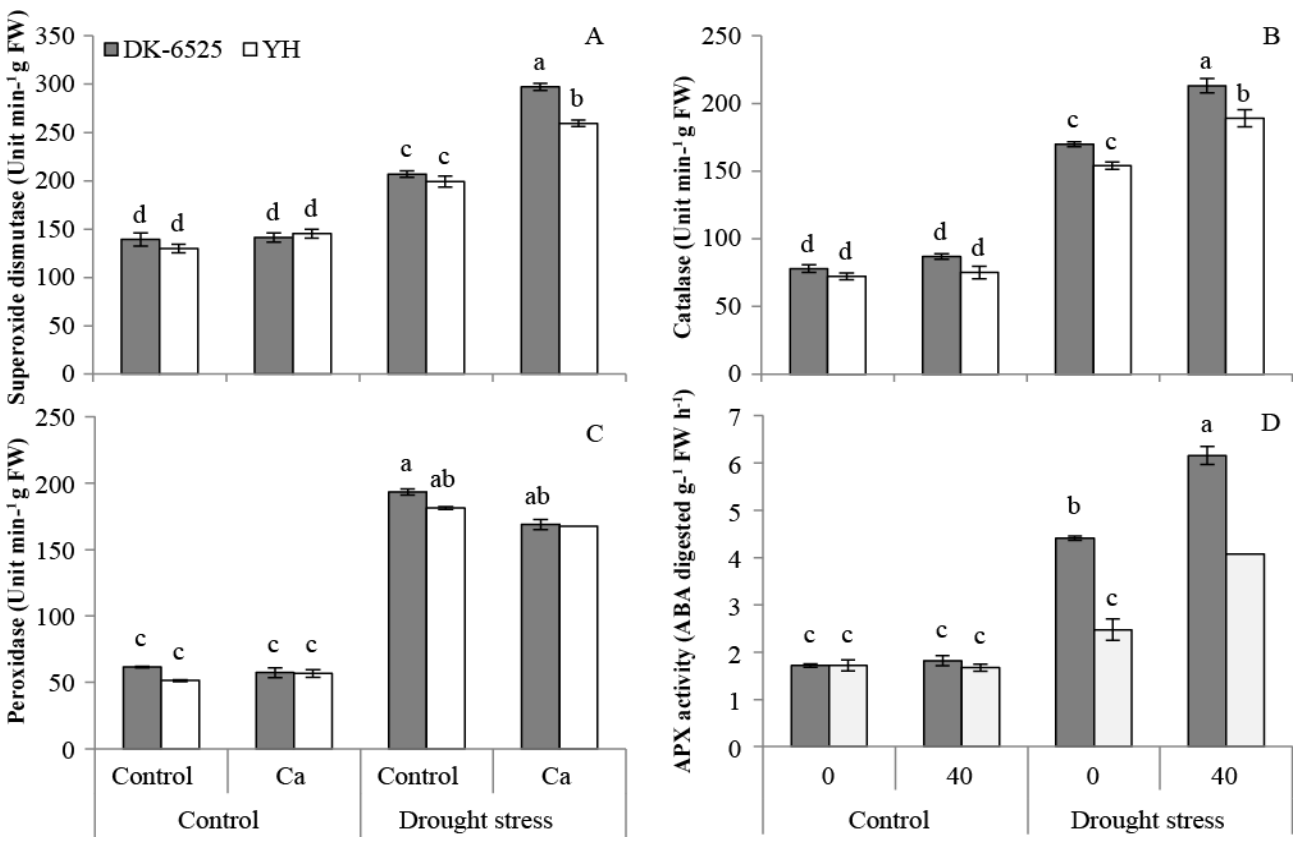

Fig. 3. Differential effects of drought stress and foliar Ca^{2+} supplies on antioxidant activities in two maize hybrids; DK-6525 and YH represent genotype Dekalb-6525 and Yousafwala Hybrid respectively. Control: 100% water-holding capacity (WHC); Drought stress: 30% WHC. Columns not sharing a letter in common showed significant difference between eight treatments ($p \leq 0.05$).

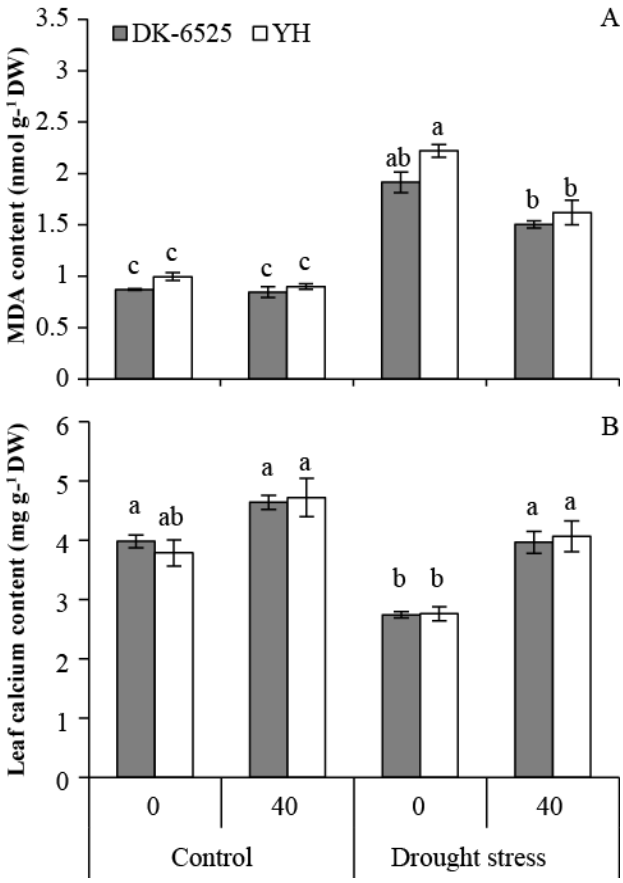

Fig. 4. Differential effects of drought stress and foliar Ca^{2+} supplies on malondialdehyde (MDA) contents and leaf Ca^{2+} content in two maize hybrids; DK-6525 and YH represent genotype Dekalb-6525 and Yousafwala Hybrid respectively. Control: 100% water-holding capacity (WHC); Drought stress: 30% WHC. Columns not sharing a letter in common showed significant difference between eight treatments ($p \leq 0.05$)

Antioxidant activities: Activity of antioxidants was greatly ($p \leq 0.001$) increased in response to water-deficit conditions (Fig. 3). Calcium application also caused a noticeable ($p \leq 0.001$) increase in the activity of SOD (Fig. 3a), CAT (3b) and APX (3d), regardless of watering regimes. The activity of POD was significantly increased in both genotypes by drought treatment irrespective of Ca^{2+}applications. A significant interaction (W × Ca) was recorded for all of the antioxidants (Table 1). Considerable ($p \leq 0.001$) increase in SOD (37%), CAT (24%) and APX (49%) activity was recorded in both cultivars exposed to Ca^{2+} under water-deficit conditions (Fig. 3). Cultivar DK-6525 showed significant or a slight ($P \leq 0.01$) increase in all of the antioxidants except POD as compared to YH, regardless of Ca^{2+}supplies and watering-regimes (Table 1). In drought-sensitive YH, the SOD (Fig. 3a) and APX (3d) activities decreased under water-deficit conditions irrespective to Ca^{2+}supplies.

Lipid peroxidation: Malondialdehyde contents, an effective indicator of lipid peroxidation, markedly ($P \leq 0.001$) increased under water-deficit conditions (Table 1; Fig. 4a). Application of Ca^{2+} considerably ($p \leq 0.01$) reduced MDA accumulation (19%) in both cultivars under normal and water-deficit conditions. Drought-tolerant YH showed more marked ($p \leq 0.05$) increase in MDA content than that in drought-sensitive cultivar DK-6525, irrespective of watering-regimes (Fig. 4a). The interactive effect of W × Ca was significant ($p \leq 0.01$) and greatly lower MDA accumulation (24%) was recorded in water-stressed plants treated with calcium (Table 1; Fig. 4a).

Leaf calcium concentration: Drought stress markedly ($p \leq 0.01$) reduced the uptake of Ca^{+2} by 21% as compared to normal conditions regardless of cultivars and Ca^{2+} supply (Fig. 4b). Foliar treatment of Ca^{2+} markedly ($p \leq 0.001$) increased its concentrations in maize leaf tissues as compared to untreated-control (Fig. 4b). However, both cultivars did not change significantly ($p \geq 0.05$) in terms of leaf Ca^{2+}concentrations (Table 1).

Discussion

Water and nutrient limitations are the causes that significantly limit growth of maize under arid/semi-arid conditions of the world. Plants absorb calcium from the soil solution, where mass flow and root interception are the primary mechanisms of Ca^{2+}transport to the root surfaces (Havlin *et al.,* 1999). Leaf calcium (Ca^{2+}) content declined dramatically under water-deficit conditions (Fig. 4b) because of their reduced mobility from soil to roots by mass flow (Brown *et al.,* 2006). The subsequent lower Ca^{2+} concentrations can further decrease the plant-resistance to drought stress and bring considerable inhibition in growth of maize (Fig. 1). Maintaining an adequate plant tissue $Ca^{2+}$is, thus, critical for plant drought-tolerance. Therefore, to counteract this situation, we decided to supply Ca^{2+}as foliar spray after the onset of drought.

Plant growth and leaf water balance of crops are adversely influenced by drought stress (Zhang *et al.,* 2014). Nonetheless, DM accumulation and leaf RWC are recognized as two promising events of plant growth performance and tissue water balance under water-limitations (Harb *et al.,* 2010). Reduction in leaf RWC implies a fall of turgor potential that results in inadequate water availability for the cell expansion processes in plants (Zhang *et al.,* 2011). Critical alterations in water balance lead to molecular impairment, growth retardation and even death of plant tissues (Anami *et al.,* 2009; Harb *et al.,* 2010). In the present study, foliar supplied Ca^{2+} considerably improved RWC of both normal and drought-stressed plants (Fig. 2a) that ultimately helped the plants to maintain cell growth which is primarily a turgor-driven process (Ashraf *et al.,* 2011; Zhang *et al.,* 2011). Calcium applied improvement in RWC and plant growth under water-deficit conditions has been reported in *Camellia sinensis* (Upadhyaya *et al.,* 2011). Our data showed that drought stress produced more marked decline in leaf RWC and shoot DM production in the drought-sensitive cultivar (YH) than in the resistant one (DK-6525) which might have been due to their differential responses to drought-stress and is comparable to what has already been observed in maize (Zhang *et al.,* 2014).

The tolerance efficiency in plants under moisture-deficit conditions is linked with the accumulation of compatible osmolytes such as endogenous-GB and TFA which react actively to alleviate the detrimental effects of drought stress on crop production (Ashraf *et al.,* 2011; Zhang *et al.,* 2014). Accumulation of osmoprotectants (GB and TFA) causes a reduction in cellular osmotic potential which helps conserve tissue water balance and an increase in turgor potential to enhance the physiological performance of plants during drought stress (Harb *et al.,* 2010; Anjum *et al.,* 2011). In the current study, a positive effect of exogenous Ca^{2+}could be viewed on accumulation of TFA (Fig. 2d) and GB (Fig. 2b) in both cultivars under water-deficit conditions. Overproduction of these osmolytes due to application of Ca^{2+} (Fig. 2b, d) might increase the defense from abiotic stress as has been supported by previous studies on *Zea mays* (Nayyar 2003) and *Brassica napus* (Alam, 2013).The reduction in TSP in drought stressed plants (Fig. 4) was probably caused by the reduced rate of protein biosynthesis and increased rate of protein breakdown (Krasensky and Jonak, 2012). It might have been due to disintegration of proteins by proteolytic activities (Parida and Das, 2004), subsequently low molecular weight amino acids increased in both maize genotypes required for osmotic adjustment (Javed *et al.,* 2014). Our data showed that exogenous treatment of Ca^{2+}caused a dramatic reduction in TSP and enhanced the accumulation compatible osmolytes in both cultivars which in turn improved their resistance to drought stress. The higher accumulation of compatible osmolytes like TFA and GB in cultivar DK-6525 suggests its greater genotypic tolerance to drought stress than YH as these biomolecules help maintain plant structures, and scavenge hydroxyl radicals under stress conditions (Guo *et al.,* 2010; Zhang *et al.,* 2014).

Oxidative damage is a primary reaction of plant cells to environmental stresses and an optimum supply of Ca^{2+} has been shown to hamper the adverse effects of such stresses through maintenance of structural and functional integrity of biological membranes as revealed by reduced MDA accumulation and improved antioxidant defense system. It has been reported that plant antioxidant system activates in response to Ca^{2+} supplies under stress conditions (Zorrig *et al.,* 2012), while data on maize is limited. The up-regulation of antioxidants is a pervasive response, which results in decreasing MDA accumulation and conserving macromolecular configurations or functions (Ashraf, 2009; Zhang *et al.,* 2014). In our study, foliar sprays of Ca^{2+} substantially reduced the MDA content through up-regulation of antioxidant activities in both cultivars. The increased SOD activity in plants supplied with Ca^{2+} under water stress is correlated with enhanced defense from oxidative damage (Miller *et al.,* 2010). The H_2O_2 is the product of SOD activity and still toxic until it is converted into H_2O in subsequent reactions. The activity of H_2O_2 catalyzing enzymes, CAT (Fig. 3b) and APX (3d) substantially increased in water-stressed plants supplied with Ca^{2+} and it might have acted simultaneously to eliminate H_2O_2 at a highest rate (Siddiqui *et al.,* 2011) that ultimately protected the plants from oxidative damage. The reduction in lipid peroxidation and up-regulation of these enzymes in Ca^{2+} applied *Zoysia japonica* have also been reported under stress conditions (Xu *et al.,* 2013). However, increased POD activity was reported under water stress that is associated with defense from oxidative damage, lignification and cross-linking of the cell wall (Dalal & Khanna-Chopra, 2001). We also observed higher POD activity in untreated water-stressed maize plants (Fig. 3c). It has been documented that stress-tolerant cultivars have greater ability to survive under abiotic stresses by prompting antioxidant defense systems (Demiral & Türkan, 2005) and similar has been reported in our case as well. Considerably higher antioxidant activities were recorded in plants exposed to water-deficit conditions which can be viewed as an approach for plants to protect cellular membranes, proteins and metabolic apparatus, from the adverse effects of drought (Harb *et al.,* 2010).

Conclusion

On the basis of these results, it can be concluded that exogenously applied Ca^{2+} induced physiological and biochemical changes and adapting capability in two maize hybrids varying in drought tolerance. The results of the current study advocate that drought stress caused a substantial reduction in plant growth through decline in nutrient uptake and disturbance in tissue water content by increasing lipid peroxidation and disproportioning antioxidant activities. Significantly improved DM production, water content, osmolyte accumulation, antioxidant activities as well as reduced lipid peroxidation were found to be the main contributors to a drought-tolerant genotype to succeed under water-deficit conditions. Additionally, plant physiological performance could be adjusted by foliar Ca^{2+} supply under water-limited conditions. Thus, we recommend that the acclimatization for drought stress by foliar application of Ca^{2+} should be rational to a drought-sensitive genotype under water-deficit conditions to increase its potential to grow vigorously under drought-prone situations.

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Conflict of Interest: All authors disclose that there is no conflict of interest.

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