Research Article

Alleviation of Adverse Effects of Water Stress on *Zea mays* (Cv Azam) by Exogenous Application of $CaCl_2$

Nadia Mubarik[1], Aqib Iqbal[1*], Iqbal Munir[1] and Muhammad Arif[2]

[1]*Laboratory of Biochemistry and Molecular Biology, Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, PO Box 25130, Pakistan;* [2]*Department of Agronomy, The University of Agriculture Peshawar, PO Box 25130, Pakistan.*

Abstract | An experiment was conducted to study the effect of foliar application of $CaCl_2$ on alleviation of the adverse effects of water stress on maize (CV. Azam). The experiment was conducted in completely randomized design (CRD) using pots in a glass house and comprised of 2 irrigations (irrigated and water stress) and three concentrations of $CaCl_2$ (5, 10 and 15 mM $CaCl_2.2H_2O$) as foliar spray on maize seedlings 20 days after emergence (DAE). Water was sprayed as untreated control on maize seedlings 20 DAE. After foliar application, the plants were regularly irrigated upto one week and water stress was imposed by withholding water from half of the pots for 20 days. Data were recorded on the relative water content (RWC), proline, sugar content and transcripts abundance of *Lhcb2* gene in the selected leaves. Application of $CaCl_2$ increased the RWC, proline and sugar content as well as the *Lhcb2* expression in the leaves under both irrigation conditions; however, the effect was more prominent under water stress. In the control plants, water stress decreased RWC (-57%) and *Lhcb2* transcript abundance (-44.37%) but increased proline (9.02 fold) and sugar content (3.42 fold) after 20 days. Significantly higher RWC (82.00 and 55.67%), proline (4.13 and 13.29 $nmol{\cdot}g^{-1}$ FW) and sugar content (51.19 and 102.00 $mg{\cdot}g^{-1}$ FW) under irrigated and water stressed conditions, respectively, was noted when 10 mM $CaCl_2$ was applied as foliar spray. Furthermore, 10 mM $CaCl_2$ application enhanced the *Lhcb2* transcripts by 18 and 62% under irrigated and water stress conditions, respectively. These results demonstrate the importance of $CaCl_2$ foliar application on the improvement in the water relations and biochemical adaptation, resulting in improvement in the performance of plants under water deficit conditions. These results also demonstrated that senescence, which is a type of cell death program that could be inappropriately activated during drought, was also delayed in the supplemented seedlings as highlighted by a high RWC and expression of the *Lhcb2* gene.

Received | FApril 05, 2017; **Accepted** | February 20, 2018; **Published** | May 08, 2018
***Correspondence** | Aqib Iqbal, Laboratory of Biochemistry and Molecular Biology, Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, PO Box 25130, Pakistan; **Email:** aqib72@aup.edu.pk
Citation | Mubarik, M., A. Iqbal, I. Munir and M. Arif. 2017. Alleviation of adverse effects of water stress on *Zea mays* (Cv Azam) by exogenous application of $CaCl_2$. *Sarhad Journal of Agriculture,* 34(2): 327-333.
DOI | http://dx.doi.org/10.17582/journal.sja/2018/34.2.327.333
Keywords | Maize, Calcium priming, Lhcb2 expression, Sugar, Proline, Drought alleviation

Introduction

Water stress is one of the major abiotic stress affecting plant development and growth in Pakistan. Plants experience water stress in different ways i.e. either plants are incapable to uptake water by roots or when rate of transpiration is higher and these situations frequently overlap in arid- and semi-arid environments. The plants ability to tolerate water stress is observed in almost all species of plants to variable degree (Ashraf et al., 2015). Plants experience various changes in morphology, biochemistry and gene expression to adapt to water stressed environments (Suralta and Yamauchi, 2008). Calcium is known as a second messenger molecule in plant signaling processes and its application improves water conservation and increases the hydrophobicity of cellular membrane under drought stress (Bush, 1995). Calcium seems to have a vital role in numerous defense mechanisms induced by drought, and calcium signaling is involved in the attainment of drought tolerance (Cousson, 2009). $CaCl_2$ has been revealed to mitigate the detrimental effects of water stress on plants (Xu et al., 2013). Improved tolerance in plants to abiotic stress has been reported by the use of $CaCl_2$ as foliar spray. Maize is considered as an important crop in Pakistan where population is rapidly increasing and has already experienced the shortage of the available food supplies (Afzal et al., 2013). Water stress is the problem in maize cultivating areas as it is much prone to drought stress. This experiment was planned in a way that whether foliar application of $CaCl_2$, before the imposition of water stress, could ameliorate the negative effects on maize seedlings. Plant water relations, proline, sugar content and *Lhcb2* gene expression were taken into consideration to determine the response of maize seedlings to water stress.

Materials and Methods

Plant material and experimental plan

To evaluate the water stress tolerance of *Zea mays* seedlings (CV Azam) primed with different concentrations of Ca^{2+} (as $CaCl_2$), a research was performed in glasshouse at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, Pakistan during 2012-2014. The experiment was arranged according to completely randomized design with three replicates for each treatment and each replication had five pots. Seeds were planted in plastic pots (diameter 22.86 cm and depth 19.0 cm) filled with 5.5 kg of clay loam soil from New Development Farm, Malakandher, The University of Agriculture Peshawar and FYM (Farm Yard Manure (1:1 w/w mixture). Thinning was done 10 days after emergence and three seedlings of uniform size were maintained in each pot. The seedlings were regularly irrigated for 10 days. Twenty days old seedlings were applied with foliar spray of distilled water or 5, 10, 15mM $CaCl_2$ in two doses at 2 days interval. The seedlings were then washed with tap water after two days and regularly irrigated with 2L of tap water upto one week. Water stress was imposed on 31 days old seedlings by withholding water for 20 days from half of the pots. The third and fourth leaf from the top of the plant at the start of the experiment were selected and used for analysis. Data was collected after 0, 10 and 20 days of stress imposition from irrigated and drought stressed seedlings.

Relative water content (RWC)

Leaf samples from irrigated and water stressed seedlings were taken and weighed with analytical balance to determine fresh weight (FW). Then samples were submerged in ddH_2O and were kept for 24 hours in dark at 4°C. The leaf samples were blotted dry on filter paper and were weighed to determine the turgid weight (TW). The samples were kept at 70°C in oven for 48 hours, and then dry weight (DW) was calculated. RWC (Relative water content) was calculated using the following formula (Barz and Weatherley, 1962).

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

Proline content

Proline content was determined by a method recommended by Bates et al. (1973) with minor modifications from irrigated and water stressed seedlings. Frozen plant material (~20 mg) was homogenized in 1ml of 3 % aqueous sulpho-salicylic acid. Then it was centrifuged at 9,000 rpm for 5 min to remove the debris. Glacial acetic acid (1 ml) was added to 250 µl extract and 750 µl (Methanol 67: distilled water 33) in a test tube. Afterward, 1 ml of acid ninhydrin solution was added to it and the mixture was kept in water bath at 100 °C for 45 min. Dark pink color appeared and the reaction was ended in an ice bath. Lastly, to the reaction mixture, 5ml toluene was added and was vortexed for 2 min each. Absorbance was measured by spectrophotometer at 520 nm. From a standard curve the amount of proline was measured in the range of 0.3-1 µM of L-proline.

Sugar content

Total sugar concentration in the irrigated and water stressed maize seedlings was measured by the method of Dubious et al. (1956). Plant material (20 mg) was homogenized in 4ml extraction buffer (Methanol 12: Chloroform5: distilled water 1) and centrifuged to remove the debris at 9000 rpm for 5 min. Supernatant was taken and the pellet was re-extracted with 4 ml extraction buffer using the above mentioned procedure. The supernatant was pooled into fresh test tube. Chloroform (2 ml) and distilled water (3 ml) were added to supernatant to get two layers. Then 1ml sample was taken from upper layer of sample tube and 1 ml 5% phenol was added to it. Then, 5 ml H_2SO_4 (conc) was mixed to the sample tube and was shaken for 10 minutes. By spectrophotometer, optical density was measured at 490 nm. From standard curve the amount of sugar was with standards of D-Glucose (0, 0.1, 0.5, 1, 5, 10 μM).

RNA extraction and PCR analysis of Lhcb2 expression

Semi-quantitative Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR) analysis were used to estimate the expression of *Lhcb2* gene (Gen Bank accession number X68682) under control and drought stress condition after $CaCl_2.2H_2O$ application. Total RNA was extracted from leaves (3rd and 4th) of *Zea mays* seedlings under both irrigated and water stress conditions by RNA extraction kit (Promega Corporation, USA). The cDNA first strand was synthesized using the OligodT20 primer and the Moloney Murine Leukemia Virus, reverse transcriptase (MMLV reverse transcriptase) with 5 μg total RNA as template according to manufacturer's protocol (Thermo Scientific®). These products were used as templates for subsequent PCR reactions using gene specific 5'-ATGCGCCGCACCGTCAAGAG-3' forward and 5'-GCAAGGCCGTACATGTGTACTAC-3' reverse primers. The PCR conditions were 95°C for 3min, 94°C, 55°C and 72°C for 1 minute each and final elongation at 72°C for 10 minutes. The PCR products (~720 bp) were separated by 1% agarose gel electrophoresis and the bands were quantified by comparing the intensities of β-actin with respective gene under each treatment. The data was recorded thrice and data was presented as mean with standard deviation. Actin-2 with sequence 5′-GAGCTCTCCAGAACCGAA- 3′ forward and 5′ -ATCAAGGGCAACGTAGGCA- 3′ reverse primers was used as a loading control.

Statistical analysis

Obtained data was exposed to analysis of variance according to CRD (completely randomized design) and by attaining significant differences, LSD (least significant difference) test was applied for comparison of treatment means.

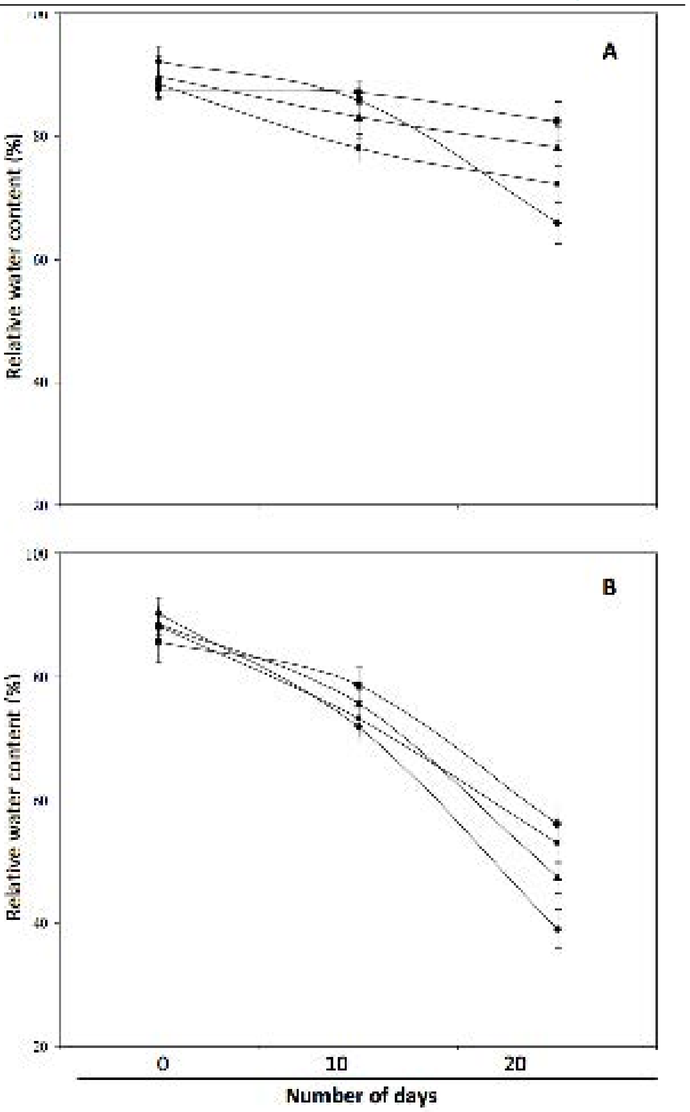

Figure 1: *Mean RWC of the selected leaves of maize seedlings supplemented with tap water (♦) or 5 (■), 10 (●) and 15 mM (▲) of $CaCl_2$ after 0, 10 and 20 days of irrigation* **(A)** *or water stress* **(B)** *conditions.*

Results and Discussion

Relative water content (RWC) of maize seedlings under different treatments

Water stress caused a marked decrease in relative water content of the non-supplemented maize seedlings from 90.37 to 39.11% (57% decrease). Under similar supplementation, the RWC of the well irrigated seedlings decreased by only 17% after 20 days (Figure 1). At the start of the experiment, the RWC (relative water content) of the maize seedlings ranged between 85.67–91.67% and there was no significant effect of Ca^{2+}application (Figure 1). There was a decrease in RWC of the seedlings regularly irrigated or exposed to water stress, after 10 days of treatments (Figure 1). Moreover, Ca^{2+} as foliar spray had no significant effect on the RWC under irrigated conditions, but

Table 1: *Proline content (nmol. g^{-1} FW) of maize seedlings on day 0, 10th and 20th under irrigated and water stressed conditions supplemented with different concentrations of $CaCl_2.2H_2O$.*

Treatment	Proline content (Irrigated)			Proline content (Water Stressed)		
	Day 0	Day 10	Day 20	Day 0	Day 10	Day 20
NS	0.961 k	1.331 jk	2.831 gh	0.985 k	5.741 d	8.841 c
5mM $CaCl_2$	1.371 ijk	1.551 ijk	3.341 fg	1.431 ijk	5.191 de	18.691 a
10mM $CaCl_2$	1.631 ijk	2.181 hij	4.311 ef	2.051 h-k	6.141 d	13.291 b
15mM $CaCl_2$	2.340 g-j	3.421 fg	5.751 d	2.451 ghi	8.571 c	9.121 c

Mean values followed by at least one similar alphabet are not different statistically at 1% level of probability ($\alpha_{0.01}$); **NS:** *Non-supplemented.*

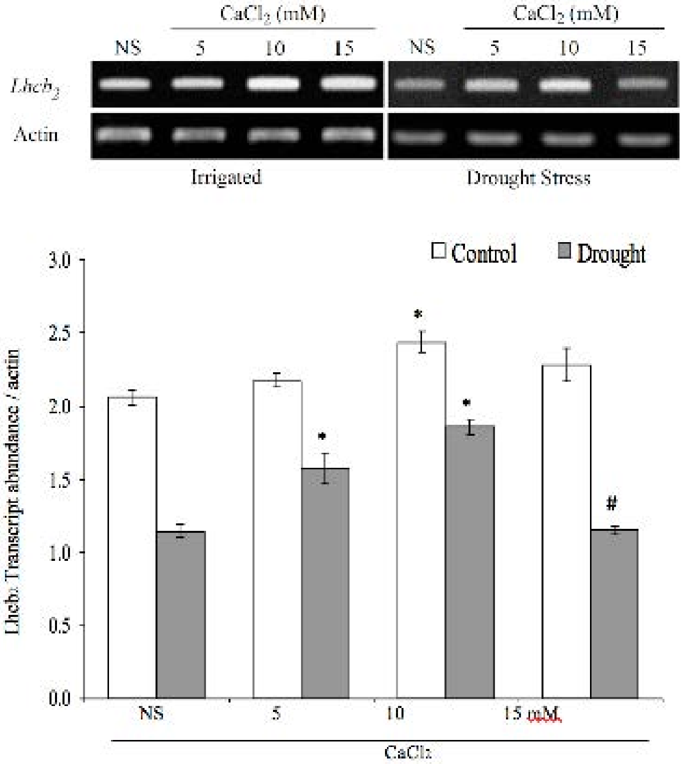

Figure 2: *Agarose gel analysis of Lhcb2 gene transcripts under irrigated and water stress conditions in maize after application of $CaCl_2.2H_2O$ (A). Relative expression of Lhcb2 gene with respect to control seedlings under irrigated (white bars) and drought stress (grey bars) conditions (B).*

the applied seedlings sustained significantly higher relative water content under water stressed conditions. The maximum RWC after days under irrigated and water stressed conditions (86.25±3.29 and 78.63±2.97%, respectively) was maintained by the seedlings supplemented with 10mM $CaCl_2$ (Figure 1). The decrease in RWC of the maize seedlings was more marked under both conditions on day 20 (Figure 1). Likewise, Ca^{2+} application as foliar spray significantly increased the relative water content of the maize seedlings under both irrigated and water stressed conditions. Additionally, high relative water content under both irrigated and water stressed conditions (76.25±3.24 and 56.15±2.94%, respectively) was obtained in maize seedlings applied with 10mM $CaCl_2$ on day 20. Therefore, maize seedlings supplemented with 10mM $CaCl_2$ as foliar spray had approximately 1% and 44% increase in the RWC under the irrigated and water stressed conditions, respectively (Figure 1).

Proline content of maize seedlings under different treatments

Data showing proline content in maize seedlings under irrigated and water stressed conditions is shown in Table 1. In the non-supplemented seedlings, mean values of proline content under irrigated condition were 0.96, 1.33 and 2.83 nmol.g^{-1} FW on day 0, 10 and 20, respectively. When maize seedlings were exposed to water stress, significant increase in proline content was noted. Consequently, the proline content was 0.98, 5.74 and 8.84 nmol.g^{-1} FW on day 0, 10 and 20 respectively, in water stressed condition. Thus in the non-supplemented seedlings, an increase of 2.95 and 9.02 fold was noticed in the proline content after 20 days in irrigated and water stressed conditions, respectively (Figure 2). Maximum proline content was recorded in seedlings applied with 15mM $CaCl_2$ (2.34, 3.42 and 5.75 nmol. g^{-1} FW on day 0, 10 and 20, respectively) under irrigated condition. Maximum mean value of proline content under drought stress was observed in 15mM $CaCl_2$ (2.45 and 8.57 nmol. g^{-1} FW on day 0 and 10, respectively), and 5mM $CaCl_2$ (18.69 nmol. g^{-1} FW on day 20). There was an increase of approximately 19.07, 13.56 and 9.31 folds proline content in 5, 10 and 15mM $CaCl_2$, respectively, under water stressed condition (Table 1).

Sugar content of maize seedlings under different treatments

Water stress imposition increased the sugar content in the non-supplemented seedlings to 14.66, 58.21 and 62.51 mg.g^{-1} FW on day 0, 10 and 20, respec

Table 2: *Sugar content (mg.g^{-1}) of maize seedlings on day 0, 10 and 20 under irrigated and water stressed conditions supplemented with different concentrations (5, 10, 15mM) of $CaCl_2 . 2H_2O$.*

Treatment	**Sugar content (Irrigated)**			**Sugar content (Water Stressed)**		
	Day 0	**Day 10**	**Day 20**	**Day 0**	**Day 10**	**Day 20**
NS*	12.93 k	22.18 ij	35.76f g	14.66 jk	58.21 cde	62.51 cd
5mM $CaCl_2$	13.93 jk	24.90 hi	41.08 f	12.95 k	64.53 c	116.62 a
10mM $CaCl_2$	14.13 jk	32.05 gh	50.32 e	11.72 k	66.45 c	107.19 b
15mM $CaCl_2$	11.65 k	51.67 e	54.85 de	11.80 k	51.63 e	66.19 c

Mean values followed by atleast one similar alphabet are not different statistically at 1% level of probability ($\alpha_{0.01}$); **NS:** *Non-supplemented.*

tively, compared with 12.93, 22.18 and 35.76 mg.g^{-1} FW on day 0, 10 and 20, respectively, when regularly irrigated. Thus, sugar content was increased by appro-ximately 176.57 % in irrigated and 326.40 % under water stressed condition (Table 2). A significant increase in sugar content was observed under water stress as compared to irrigated condition when seedlings were applied with different concentration of $CaCl_2$ before water stress imposition. Increase in sugar content was noticed under irrigated condition, and maximum sugar content was 51.67 and 54.85 mg.g^{-1} FW in seedlings supplemented with 15mM $CaCl_2$, on day 10 and 20, respectively. Likewise, an increase in sugar content was noticed with the increase of water stress duration. Maximum mean values of sugar content were noted on day 20, which were 116.62 and 107.19 mg.g^{-1} FW when seedlings were sprayed with 5 and 10 mM $CaCl_2$, respectively, under water stressed condition. On day 10, maximum sugar content (66.45 mg.g^{-1} FW) was observed in maize seedlings primed with 10 mM $CaCl_2$ (Table 2).

Response of Lhcb2 expression to drought and Ca^{2+} application

Light harvesting chlorophyll a/b binding protein (LHCP) family in plants plays essential role in light capture and photo-protection in the photosystem. *Lhcb2* is one of the major proteins of the LHCP complex in the photosystem-II. Exposure to drought stress resulted in approximately 50% reduction in the transcripts of this gene (Figure 2). There was an increase in the expression of this gene with the application of $CaCl_2$ under both the watering conditions. Furthermore, this increase in the transcripts of *Lhcb2* gene after $CaCl_2$ application was more pronounced under water stress conditions. Maximum 16 and 62% increase in the expression of *Lhcb2* with respect to the control seedlings was noted after application of 10mM $CaCl_2$ under both control and drought stress conditions, respectively. However, further increasing the concentration of $CaCl_2$ resulted in down-regulation of the expression of *Lhcb2* under both the conditions (Figure 2).

Sustained agricultural production is risking due to the scarcity of water resulting from less precipitation and increased evapo-transpiration. Drought stress affects the water status, metabolism, growth and development, free proline accumulation and sugar content in tissues (Nayek et al., 1983). Water deficit also result in a decrease in uptake of mineral elements and therefore, nutrient management is an important and essential component of improved production under water scarcity conditions. Further, plants can remobilize mineral sequestered in cellular stores or older tissues. During this experiment, the results demonstrated that foliar spray of $CaCl_2$ prior to imposition of drought stress partially alleviated the adverse effects.

Osmotic adjustment is mechanism of plant tolerance to drought and heat stress (Ludlow et al., 1990). The foliar spray of Ca^{2+} was more effective in enhancing the water status of the plants at both the vegetative and reproductive stage (Nayek et al., 1983). The foliar spray of 10 mM $CaCl_2$ increased relative water content (RWC) and other parameters in both tall fescue and Kentucky bluegrass under heat stress indicating heat tolerance (Jiang and Huang, 2000). In the present study, 10 mM $CaCl_2$ pre-treated maize seedlings showed maximum RWC under both control and water stressed conditions, indicating enhanced water relations under water stress conditions.

Under water stress conditions, free proline accumulation in the leaves has significance in plant adaptation during stress. Significant increase in free proline content was more pronounced in drought stressed plants during plant development. Ca^{2+} pre-treatment in stressed plants, inhibited the increase in free proline (Nayek et al., 1983). The drought tolerance of

Zoysia grass was enhanced to some degree by the supplementation of 5 and 10 mM $CaCl_2$ under water stress conditions, indicated by decrease in proline content (Xu et al., 2013). Our data indicates the same that proline content increased in irrigated and water stressed conditions but it was more pronounced under water stressed condition. Pretreatment with 5 and 15 mM $CaCl_2$ decreased the proline content under water stress conditions, on day 10 and 20, respectively. This might be due to increase in the amount of proline degrading enzyme and decrease the proline synthesizing enzyme (Jaleel et al., 2007).

Increase in sugar content plays a role in the regulation of inner osmolarity and defence to the biomolecules and membranes (Sinniah et al., 1998). Proline and soluble sugar levels were increased in potato leaves under water stress conditions (Farhad et al., 2011). In this experiment, similar results were obtained. Increase in sugar content was noticed in seedlings supplemented with 5 and 10 mM $CaCl_2$ under water stress conditions on different days which is due to its regulation of osmotic potential and osmotic adjustment. Sugar and proline content act as compatible solutes or osmo-protectants which permit osmotic adjustment of plant cells when exposed to drought stress, free radical scavenging, defense from photo-inhibition and metabolic detoxification (Orthen et al., 1994). Foliar application of $CaCl_2$ increased the RWC, proline and sugars accumulation may have a contribution in the mitigation of water stress.

The light harvesting chlorophyll a/b binding (*Lhcb*) protein is the major chloroplast proteins in plants, which plays pivotal role in photosynthesis and adaptation to environmental stresses (Anderson et al., 2003). During this experiment, there was a decrease in the *Lhcb2* expression in the irrigated seedlings with age after exposure to drought stress. Reduced Ca^{2+} accumulation in the leaves resulted in early senescence phenotypes (Ma and Berkowitz, 2011). During senescence the content of different photosynthetic enzymes decreases, carbohydrates accumulates and proteolytic activities increases which is followed by chlorophyll degradation (Parrott et al., 2005). The degraded products of different macromolecules like nucleic acids, proteins and chlorophyll macromolecules in the senescent leaves are remobilized to support the growth of younger leaves and fruits (Cao et al., 2003). Similarly, there is a decrease in antioxidants with age as well as after exposure to stresses, resulting in an increase in ROS production that also induces senescence (Zimmermann and Zentgraf, 2005). Delayed leaf senescence is a manifestation of enhanced capacity of the plants to survive drought stress conditions. Further, significant increase in *Lhcb2* transcripts were noted after Ca^{2+} foliar spray under both water stress and irrigated conditions as well as with age in leaves. This proved the key role of exogenous Ca^{2+} in delaying senescence and improvement of the photosynthetic performance under both irrigated and water stress conditions. These results suggested that foliar spray of Ca^{2+} delayed senescence and ameliorated the adverse effects of water stress in maize seedlings under both conditions.

Acknowledgement

All the acknowledgement goes to Higher Education Commission and Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar. This research work was fully sponsored by Higher Education Commission, Islamabad, Pakistan under 5000 Indigenous Scholarships program.

Author's Contribution

Nadia Mubarik: Conducted the experiments, collected and arranged the data and wrote the manuscript.
Aqib Iqbal1: Planned and approved the experiment, analysed the data, arranged facilities and resources for the study.
Iqbal Munir: Provided facilities and resources for the study, reviewed the final manuscript.
Muhammad Arif: Analysed the dat and critically reviewed the manuscript.

References

Afzal, S., N. Akbar, Z. Ahmad, Q. Maqsood, M.A. Iqbal and M.R. Aslam. 2013. Role of seed priming with zinc in improving the hybrid maize (*Zea mays* L.) yield. Am.-Eur. J. Agric. Environ. Sci. 13 (3): 301-306.

Andersson, J., M. Wentworth, R.G. Walters, C.A. Howard, A.V. Ruban, P. Horton and S. Jansson. 2003. Absence of the Lhcb1 and Lhcb2 proteins of the light-harvesting complex of photosystem II - effects on photosynthesis, grana stacking and fitness. Plant J. 35: 350–361. https://doi.org/10.1046/j.1365-313X.2003.01811.x

Ashraf, M.A., R. Rasheed, I. Hussain, M. Iqbal, M.Z. Haider, S. Parveen and M.A. Sajid. 2015. Hydrogen peroxide modulates antioxidant system and nutrient relation in maize (*Zea mays* L.) under water-deficit conditions. Archives Agron. Soil Sci. 61 (4): 507-523. https://doi.org/10.1080/03650340.2014.938644

Barz, H.D. and P.E. Weatherley. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian J. Biol. Sci.. 15: 413-428. https://doi.org/10.1071/BI9620413

Bates, L. S., R.P. Waldren, I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. Plant Soil. 39: 205–207. https://doi.org/10.1007/BF00018060

Bush, D.S. 1995. Calcium regulation in plant cells and its role in signaling. Ann. Rev. Plant Physiol. Plant Mol. Biol. 46: 95-122. https://doi.org/10.1146/annurev.pp.46.060195.000523

Cao, J., F. Jiang, Sodmergen and K. Cui. 2003. Time-course of programmed cell death during leaf senescence in *Eucommia ulmoides*. J. Plant Res. 116 (1): 7-12.

Cousson, A. 2009. Involvement of phospholipase C-independent calcium-mediated abscisic acid signaling during Arabidopsis response to drought. Biol. Plant. 53: 53-62. https://doi.org/10.1007/s10535-009-0008-0

Dubious, M., K.A. Gille, J.K. Hamilliton, P.A. Rebers and F. Smith. 1956. Colorimetric methods of detection of sugars and related substances. Anal. Chem. 28: 350–356. https://doi.org/10.1021/ac60111a017

Farhad, M.S., A.M. Babak, Z.M. Reza, R.S. M. Hassan, Afshin and Tavakoli. 2011. Response of proline, soluble sugars, photosynthetic pigments and antioxidant enzymes in potato (*Solanum tuberosum* L.) to different irrigation regimes in greenhouse condition. Australian J. Crop Sci. 0 01/2276909481.

Jaleel, C.A., P. Manivannan, B. Sankar, A. Kishorekumar, R. Gopi, R. Somasundaram and R. Panneerselvam. 2007. Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: Effects on oxidative stress, proline metabolism and indole alkaloid accumulation. Colloids Surf B: Biointerfaces. 60 (1): 110-116. https://doi.org/10.1016/j.colsurfb.2007.06.006

Jiang, Y. and B. Huang. 2000. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. J. Exp. Bot. 52 (355): 341-349. https://doi.org/10.1093/jexbot/52.355.341

Ludlow, M.M., F.J. Santamaria and S. Fukai. 1990. Contribution of osmotic adjustment to grain yield of *Sorghum bicolor* (L.) Moench under water-limited conditions. II. Post-anthesis water stress. Australian J. Agric. Res. 41: 67–78. https://doi.org/10.1071/AR9900067

Ma, R., M. Zhang, B. Li, G. Du, J. Wang and J. Chen. 2005. The effects of exogenous Ca^{2+} on endogenous polyamines levels and drought-resistant traits of spring wheat grown under arid conditions. J. Arid. Environ. 63: 177-190. https://doi.org/10.1016/j.jaridenv.2005.01.021

Nayek, B., A.K. Biswas and M.A. Choudhury. 1983. Effect of calcium on water-stress- induced biochemical changes and yield of field-grown rice. Biol. Plant. 25 (2): 117-123. https://doi.org/10.1007/BF02902121

Orthen, B., M. Popp and N. Smirnoff. 1994. Hydroxyl radical scavenging properties of cyclitols. Proceedings of the Royal Society of Edinburgh. Section B. 269-272. https://doi.org/10.1017/S0269727000014226

Parrott, D., Yang, L., Shama, L. and Fischer, A.M. 2005. Senescence is accelerated and several proteases are induced by carbon 'feast' conditions in barley (*Hordeum vulgare* L.) leaves. Planta. 222: 989–1000. https://doi.org/10.1007/s00425-005-0042-x

Sinniah, U.R., R. H. Ellis and P. John. 1998. Irrigation and seed quality development in rapid-recycling *Brassica*: soluble carbohydrates and heat stable proteins. Ann. Bot. 82: 647-655. https://doi.org/10.1006/anbo.1998.0738

Suralta, R.R. and A. Yamauchi. 2008. Root growth, aerenchyma development, and oxygen transport in rice genotypes subjected to drought and water logging. Environ. Exp. Bot. 64: 75–82. https://doi.org/10.1016/j.envexpbot.2008.01.004

Xu, C., X. Li and L. Zhang. 2013. The Effect of calcium chloride on growth, photosynthesis, and antioxidant responses of *Zoysia japonica* under drought conditions. PLoS One, 8 (7): e68214. https://doi.org/10.1371/journal.pone.0068214

Zimmermann, P. and U. Zentgraf. 2005. The correlation between oxidative stress and leaf senescence during plant development. Cell Mol. Biol. Lett. 10: 515–534.