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Foliar applied calcium chloride confers drought tolerance in maize by modulating growth and agronomic attributes

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Abstract

Limited water availability is one of the most serious threats to crop production. Foliar application of calcium chloride has been found very effective in mitigating the harmful effects of water deficit in maize. Study was carried out to examine the role of calcium chloride in improving the growth and yield attributes of maize exposed to four different water regimes viz. normal irrigation at 6 leaves stage (V_6 as per Feekes scale), skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), normal irrigation at tasseling stage (V_T as per Feekes scale) and skipped irrigation at tasseling stage (V_T as per Feekes scale). While $CaCl_2$ was applied exogenously at 2.5 mM, 5 mM and 7.5 mM each one week after imposition of drought. Distilled water spray and untreated (control) did not receive any calcium chloride. Results showed that crop growth rate, total dry matter and leaf area of maize was decreased under drought at either crop growth stage. However, effect of water deficit and exogenous application of calcium chloride was more pronounced at 6 fully expanded leaves stage. Plant height, number of leaves per plant, biological yield, harvest index, cell membrane stability, chlorophyll contents and leaf relative water contents of maize were improved by 63%, 29%, 24%, 1.7%, 22% 7.7% and 24%, respectively, by exogenous application of $CaCl_2$ @ 7.5 mM under drought at 6 fully expended leaves stage as compared to untreated (control). Foliar application of $CaCl_2$ at 5 and 7.5 mM was advantageous under water deficit at either growth stage.

Keywords: Biomass, $CaCl_2$, cell membrane stability, crop growth stage (CGR), water deficit, *Zea mays* L.

Introduction

Food shortage is major concern globally, particularly in Asia and Africa due to rising population growth rate (Aziz *et al.,* 2015). Drought is a serious threat to food security due to its effect on productivity of crops (Monclus *et al*., 2006). It affects root development along with uptake and mobility of nutrients in soil and plants (Luo *et al*., 2011). Different metabolic processes such as photosynthesis, cell division, stomatal regulation, cell expansion are severely affected by drought which in turn reduce yield and biomass accumulation in most arable crops (Farooq *et al*., 2012). Number of strategies are being utilized to grow crops under water scarcity including foliar application of osmoprotectants, water management and development of drought tolerant varieties (Aziz *et al.,* 2018).

Drought at any development stage negatively influences maize yield (Shao *et al*., 2008; Hussain *et al*., 2013). Different approaches used in maize against drought include use of plant growth regulators, osmoprotectants and nutrients (Upadhyaya *et al*., 2011; Wang *et al*., 2012; Noreen *et al*. 2013). Development of effective strategy to improve drought tolerance in plants without reduction in yield is bigger milestone yet to achieve (Aziz *et al.*, 2018). Interest has recently emerged for foliar application of different potential cost-effective drought mitigating substances as shotgun approach to induce drought tolerance in crops (Singh *et al*., 2015; Ullah *et al.,* 2017).

Under water deficit, uptake of essential nutrients is reduced in maize (Zhao *et al*., 2015). Optimum supply of these essential nutrients is required for proper growth and copy architecture (Craine *et al*., 2013). It was reported by Brown *et al.* (2006) that calcium uptake is markedly reduced under water scarcity due to slow plant transpiration and its immobile nature. However, cytosolic Ca^{2+} is immediately increased in response to different

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environmental stimuli to activate various downstream and biological responses (Zhu *et al*., 2013) thus play an important role in adaptation of plant under stressful environment (Shao *et al*., 2008).

Calcium is the secondary messenger performing important role in signaling networks of plants (Riveras *et al*., 2015). Moreover, Ca^{2+} also regulates plant cell metabolism despite signaling for anti-drought responses (Jaleel *et al*., 2007). Continued supply of calcium is required by plants for overall canopy development (Del Amor and Marcelis, 2003). Foliar application of calcium-induced drought tolerance in plants (Xu *et al*., 2013) by excavating stress induced active oxygen species thus improves growth performance, nitrogen assimilation and photosynthetic efficiency in plants (Zhu *et al*., 2013). Lipid peroxidation of membrane is slowed down with the manipulation of antioxidant defense system (Nayyar and Kaushal, 2002).

Variation in calcium concentration of cytoplasm is associated with integration of different water stressed related signaling pathways in plants (Tuteja *et al*., 2007). However, there are few reports highlighting role of $CaCl_2$ in mitigating adverse effect of water deficit in plants (Khan *et al*., 2015). Among different vegetative and reproductive stages, variable results were reported by different researchers about most water sensitive stage of maize (Cakir *et al.*, 2004; Qasim *et al.*, 2019). Keeping in view the limited reports on the role of calcium chloride in alleviation of harmful effect of water deficit and contrasting reports on water sensitive stages of maize, the present study was carried out with the objective to optimize calcium chloride levels for improving drought stress tolerance in maize at vegetative and reproductive growth stages.

Table 1: Physico-chemical analysis of soil from experimental site (30cm depth)

Soil characteristic	Content
Soil Texture	Loam
Saturation (%)	36
EC (mS/cm)	22.52
pH	8.2
Organic Matter (%)	0.59
N (mg /kg soil)	18.3
P (mg /kg soil)	6.85
K (mg /kg soil)	163

Materials and Methods

The proposed experiment was carried out at Research Farm, Muhammad Nawaz Shareef University of Agriculture Multan (30.1575º N and 71.5249º E) during summer 2018. Experimental soil texture was loam. Table 1, shows soil physico-chemical properties of experimental site.

Experimental design and treatments: The experiment was laid out in randomized complete block design with split plot arrangement with three replicates. Different water regimes including; W_1= normal irrigation at 6 leaves stage (V_6 as per Feekes scale), W_2= skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), W_3= normal irrigation at tasseling stage (V_T as per Feekes scale), and W_4=skipped irrigation at tasseling stage (V_T as per Feekes scale) was kept in main plot. Measured quantity of water was applied by using cut throat flume. Under normal irrigation 600 mm water while under drought conditions at V_6 and V_T stages 525 mm water was applied. Foliar application of different calcium chloride levels including; T_1= untreated, T_2= distilled water spray, T_3=2.5 mM $CaCl_2$, T_4=5 mM $CaCl_2$, T_5=7.5 mM $CaCl_2$ was kept in sub-plot. Tween 20 (Polyoxyethylene sorbitan monolaurate) was used as non-ionic surfactant to improve the absorption, sticking and emulsification of spray mixture. Calcium chloride was applied one week after imposition of drought. Meteorological data during the experimental period was collected from Central Cotton Research Institute, Multan and is shown in Figure 1.

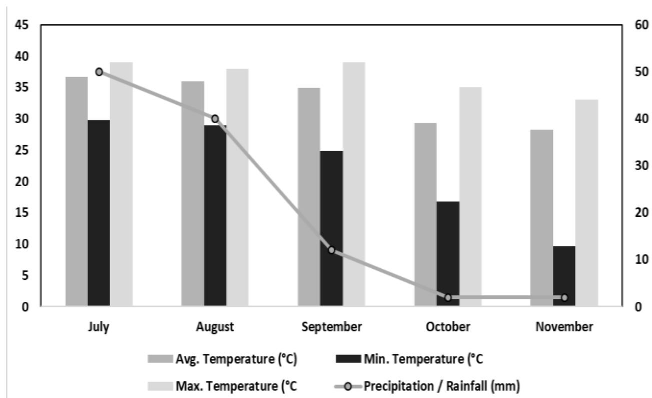

Figure 1: Meteorological data (mean monthly) during the course of studies

General agronomic practices: Maize hybrid (Monsanto 6789) was sown on both sides of beds formed at 75 cm apart. Distance between pants was maintained as 15 cm. Net plot size was kept 3m × 3m. Recommended rate of N: P: K (230:145:95 kg/ha) was applied in the form of urea, diammonium phosphate and potassium sulphate. Full phosphorus and potassium doses were applied at sowing time while nitrogen was applied in three equal splits viz. sowing, knee height and tasseling stage. Different agronomic practices were kept uniform throughout the experiment.

Recorded data

Allometric measurements: Crop growth parameters including crop growth rate, net assimilation rate, leaf area

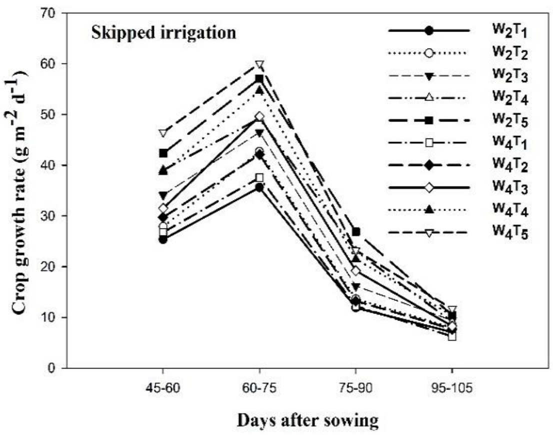

Fig. 2(a): Crop growth rate of maize as affected by foliar spray of $CaCl_2$ under drought condition imposed at different crop growth stages, T_1= untreated, T_2= distilled water spray, T_3=2.5 mM $CaCl_2$, T_4=5 mM $CaCl_2$, T_5=7.5 mM $CaCl_2$, W_1=normal irrigation at 6 leaves stage (V_6 as per Feekes scale), W_2= skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), W_3= normal irrigation at initiation of tasseling stage (V_T as per Feekes scale), and W_4=skipped irrigation at initiation of tasseling stage (V_T as per Feekes scale)

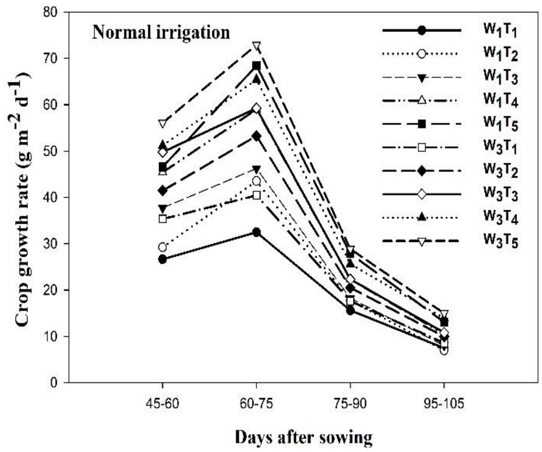

Fig. 2(b): Crop growth rate of maize as affected by foliar spray of $CaCl_2$ under normal irrigation at different crop growth stages, T_1= untreated, T_2= distilled water spray, T_3=2.5 mM $CaCl_2$, T_4=5 mM $CaCl_2$, T_5=7.5 mM $CaCl_2$, W_1=normal irrigation at 6 leaves stage (V_6 as per Feekes scale), W_2= skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), W_3= normal irrigation at initiation of tasseling stage (V_T as per Feekes scale), and W_4=skipped irrigation at initiation of tasseling stage (V_T as per Feekes scale)

duration and total dry matter were calculated at fortnight interval by method devised by Hunt (1978).

Physiological traits: Different physiological attributes including cell membrane stability index, leaf relative water contents and chlorophyll contents were computed one week after exogenous application of calcium chloride. Cell membrane stability index was measured by method given by Tuna *et al*. (2007). Chlorophyll contents were determined with the help of SPAD – 502 plus chlorophyl meter. Relative water contents of leaves were measured by following the method described by Turner and Schutte (1981)**.**

Agronomic and yield traits: Plant height at maturity and number of leaves per plant were determined by selecting ten representative plants from each plot while biological yield was determined from harvest of an area of one square meter and converted to ton ha^{-1}. Harvest index was calculated by following formula

$$HI = \frac{Grainyield}{Biologicalyield} \times 100$$

Water use efficiency was calculated with method described by Monteith *et al*. (1991) by the formula as under.

$$Water\ use\ efficiency\ = \frac{Biological\ yield\ (kg\ ha^{-1})}{Water\ use\ by\ crop\ (mm)}$$

Statistical analysis: Data was analyzed by using analysis of variance technique. Statistix 10 was utilized for data analysis. Difference among treatment means was compared by HSD Tukey's test at 5% probability level (Steel *et al*., 1997).

Results and Discussion

Allometric measurements: Crop growth rate (CGR) progressively improved till 75 Days after sowing (DAS) and then decreased (Figure 2 a, b). During 60-75 DAS, drought at 6 fully expanded leaves (V_6) had 0.7% higher CGR compared to drought at initiation of tasseling stage (V_T). Normal irrigation at V_T stage resulted in maximum CGR (56.30 g m^{-2} day^{-1}) while under normal irrigation at V_6 CGR was recorded 54.52 g m^{-2} day^{-1}. Crop growth rate was significantly affected by normal irrigation and drought at V_6 and V_T stage only during 75-90 DAS. CGR was higher under normal irrigation while lower under drought condition at either stage. Maximum CGR was 22.56 g m^{-2} day^{-1} when maize was exposed to normal irrigation at V_6 stage while lowest CGR (16.49 g m^{-2} day^{-1}) was observed under deficit irrigation at V_T stage. It may be due to more dry matter production till this period. Increase in CGR at initial stages was reported by Hussain *et al.* (2009) in case of sunflower exposed to water deficit at reproductive stage as compared to vegetative stage. Exogenous application of 7.5 mM $CaCl_2$ resulted in maximum CGR (66.6 g m^{-2} day^{-1}) till 75 DAS. It was at par with CGR recorded with 5 mM $CaCl_2$. Minimum CGR was 39 g m^{-2} day^{-1} in un-treated plots.

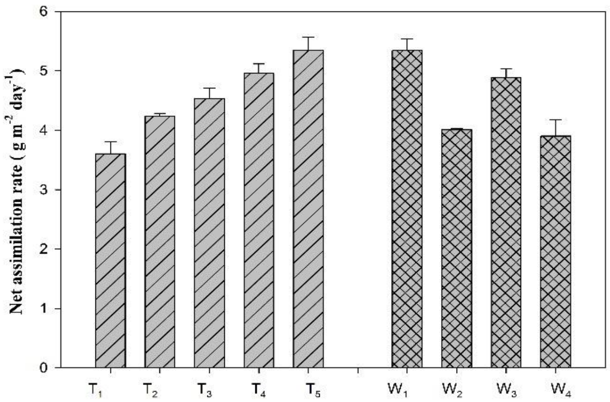

Figure 3: Net assimilation rate of maize as affected by foliar spray of $CaCl_2$ under normal or drought condition imposed at different crop growth stages, T_1= untreated, T_2= distilled water spray, T_3=2.5 mM $CaCl_2$, T_4=5 mM $CaCl_2$, T_5=7.5 mM $CaCl_2$, W_1=normal irrigation at 6 leaves stage (V_6 as per Feekes scale), W_2= skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), W_3= normal irrigation at initiation of tasseling stage (V_T as per Feekes scale), and W_4=skipped irrigation at initiation of tasseling stage (V_T as per Feekes scale)

Net assimilation rate (NAR) was more in maize under normal irrigation at either stage as compared to drought (Figure 3). NAR was 5.34 g m^{-2} day^{-1} under normal irrigation at V_6 stage, while under drought at V_6 stage it was 4 g m^{-2} day^{-1}. Drought at tasseling stage (V_t) resulted in 20% less NAR as compared to normal irrigation. NAR was significantly affected by different levels of $CaCl_2$. Maximum NAR (5.34 g m^{-2} day^{-1}) was recorded with 7.5 mM $CaCl_2$ while it was at par with NAR achieved with 5 mM $CaCl_2$. NAR was minimum in non-treated plots. Increase in NAR under normal irrigation may be due to more total dry matter than leaf area duration under normal irrigation as compared to drought condition. Tahir *et al.* (2007) reported improvement in NAR in canola with increasing irrigation.

Leaf area duration (LAD) was increased with increase in growth rate of maize. Skipping one irrigation at V_6 or V_T stage resulted in statistically similar LAD as that of normal irrigation at these stages (Figure 4). These results are different from results reported by (Tahir *et al.*, 2007) as they reported significant effect of skipping one irrigation in canola in LAD. It may be due to variation of canopy structure of both crops that resulted in variation in results. Feng *et al.* (2016) also reported that canopy structure of crop results in variation of peak values of leaf area index. Moreover, leaf area duration is dependent more on leaf area index. However, LAD was significantly affected by different $CaCl_2$ levels. Maximum LAD (425 days) was recorded with 7.5 mM $CaCl_2$ and it was statistically same as that recorded with 5 mM $CaCl_2$. Minimum LAD (251days) was recorded in un-treated maize. These results match with Noreen *et al.* (2013). Liner positive relationship was found between LAD and biological yield under normal irrigation (Figure 6 a) and drought conditions (Figure 6 b) at V_6 and V_T growth stages in maize.

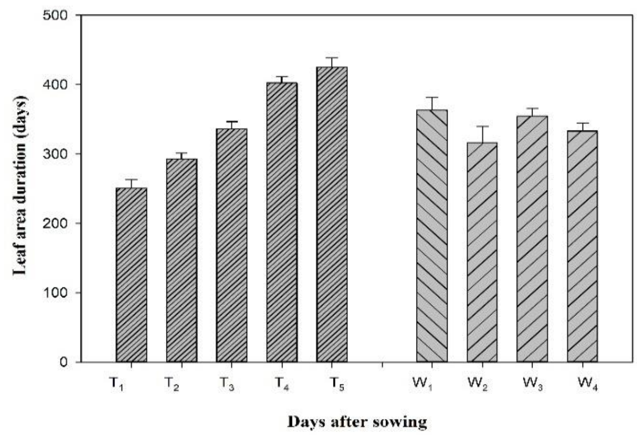

Figure 4: Leaf area duration of maize as affected by foliar spray of $CaCl_2$ under normal or drought condition imposed at different crop growth stages, T_1= untreated, T_2= distilled water spray, T_3=2.5 mM $CaCl_2$, T_4=5 mM $CaCl_2$, T_5=7.5 mM $CaCl_2$, W_1=normal irrigation at 6 leaves stage (V_6 as per Feekes scale), W_2= skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), W_3= normal irrigation at initiation of tasseling stage (V_T as per Feekes scale), and W_4=skipped irrigation at initiation of tasseling stage (V_T as per Feekes scale)

Total dry matter (TDM) progressively improved till 105 DAS under normal irrigation (Figure 5 a) and drought (Figure 5 b). TDM was higher under normal irrigation while lower under drought condition at V_6 and V_T stages. TDM (10145 kg ha^{-1}) was recorded less when maize was exposed to drought at V_6 stage due to foliar spray of 7.5mM $CaCl_2$ while it was 1093.04 kg ha^{-1} at water deficit at V_T stage in maize. TDM was minimum in non-treated plots. Results demonstrated that V_6 stage was more sensative to water deficit regarding TDM production. It may be due to cessation of cell expansion and elongation due to drought at 6 fully expanded leaves stage that resulted in slow early growth and less stem elongation and thus less total dry matter was produced. Sharma and Bhalla (1990) also validated these findings repeating less elongation in maize stem when drought was imposed at vegetative stage.

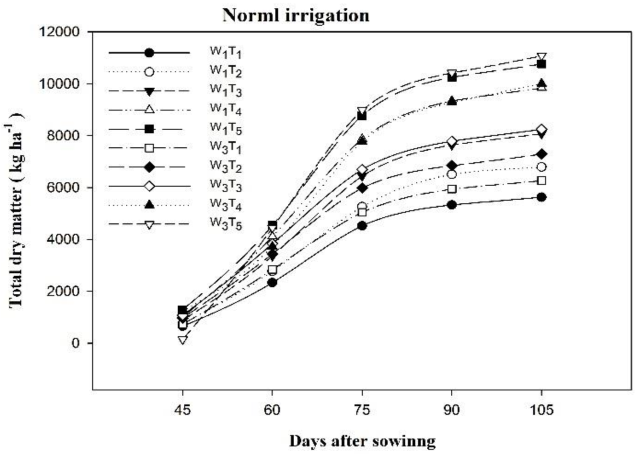

Figure 5 (a): Total dry matter (TDM) of maize as affected by foliar spray of $CaCl_2$ under normal condition imposed at different crop growth stages, T_1= untreated, T_2= distilled water spray, T_3=2.5 mM $CaCl_2$, T_4=5 mM $CaCl_2$, T_5=7.5 mM $CaCl_2$, W_1=normal irrigation at 6 leaves stage (V_6 as per Feekes scale), W_2= skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), W_3= normal irrigation at initiation of tasseling stage (V_T as per Feekes scale), and W_4=skipped irrigation at initiation of tasseling stage (V_T as per Feekes scale)

Improvement of CGR, LAD and TDM under drought due to exogenous application of $CaCl_2$ @ 7.5 mM as compared to control at either growth stage of maize may be due to defensive role of calcium in terms of Ca^{2+} signaling as part of antidrought responses. Thus, due to regulatory mechanism of calcium, maize adjusted to drought. These findings are also validated by number of researchers (Ma *et al.,* 2005; Shao *et al.,* 2008; Cousson, 2009; Upadhyaya *et al.,* 2011).

Agronomic and yield traits: Plant height (PH) in maize was significantly affected by different water regimes applied at V_6 and V_T stage. Maximum PH (165 cm) was achieved under normal irrigation at both crop growth stages while minimum PH (101.27 cm) was recorded in plots where skipped irrigation was applied at V_6 stage (Table 2). Similar findings were reported by Cakir *et al.* (2004) who recorded reduction in plant height in maize exposed to drought at V_6 and V_T stage of crop. Limited supply of water resulted in less cell expansion, thus gained less height. Different calcium chloride levels significantly affected plant height at maturity. Plant height was more where 7.5 mM $CaCl_2$ was applied. It was statistically similar to (PH) achieved with foliar spray of 5 mM $CaCl_2$. Minimum PH (140.67 cm) was achieved in non-treated plots. Aziz *et al*., (2018) also recorded significant effect of exogenous application of different osmoprotectants on plant height of cotton.

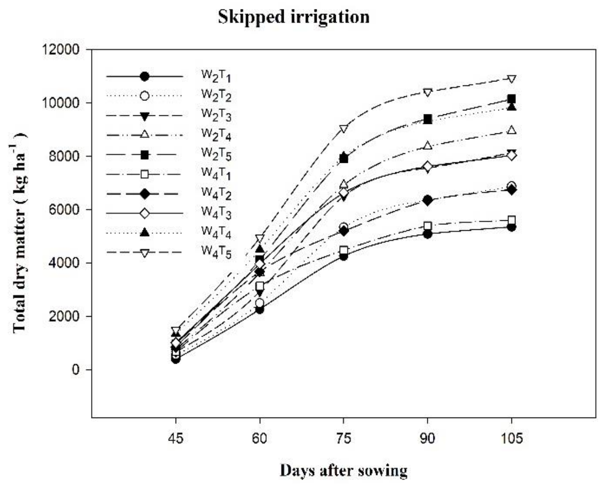

Figure 5 (b): Total dry matter (TDM) of maize as affected by foliar spray of $CaCl_2$ under drought condition imposed at different crop growth stages, T_1= untreated, T_2= distilled water spray, T_3=2.5 mM $CaCl_2$, T_4=5 mM $CaCl_2$, T_5=7.5 mM $CaCl_2$, W_1=normal irrigation at 6 leaves stage (V_6 as per Feekes scale), W_2= skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), W_3= normal irrigation at initiation of tasseling stage (V_T as per Feekes scale), and W_4=skipped irrigation at initiation of tasseling stage (V_T as per Feekes scale)

Number of leaves per plant (NLP^{-1}) were more under normal irrigation at V_6 and V_T stage as compared to skipped irrigation at respective stage (Table 2). Maximum NLP^{-1} (14) were recorded under normal irrigation at V_T stage. Minimum NLP^{-1} (10.87) were achieved in case of skipped irrigation at V_6 stage. Non-significant effect of different calcium chloride levels was recorded on NLP^{-1}. These results are in accordance with result revealed by Anjum *et al.* (2011) who recorded less NLP^{-1} under drought as compared to normal irrigated plants in maize crop.

Interaction between differnt water regimes applied at both crop growth stages (V_6 and V_T) and different calcium chloride levels was significant regarding biological yield (BY) (Table 2). Foliar application of 7.5 mM $CaCl_2$ under normal irrigation at V_T stage resulted in maximum BY (20.31t ha^{-1}) which was statistically similar to BY achieved with foliar application of similar concentration of $CaCl_2$ at

V_6 stage. Biological yield was 6.26% more under drought condition at V_T stage with foliar application of 7.5 mM $CaCl_2$ as compared to drought at V_6 stage. Minimum biological yield was achieved in un-treated plots at V_6 stage. Results depicted that V_6 stage was more water sensitive as compared to V_T stage regarding changes in biological yield. These results are differnt from result reported by Qasim *et al*. (2019) who reported that initiation of silking stage in maize was more water sensitive as compared to vegetative stage of maize. Variation in results may be due to production of less number of leaves due to water deficit at V_6 stage as compared to V_T stage.

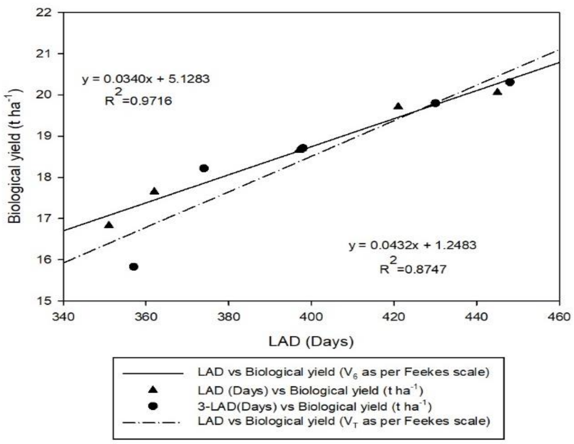

Figure 6 (a): Relationship between LAD and biological yield in maize plants as affected by foliar application of $CaCl_2$ under normal irrigation imposed at different crop growth stages

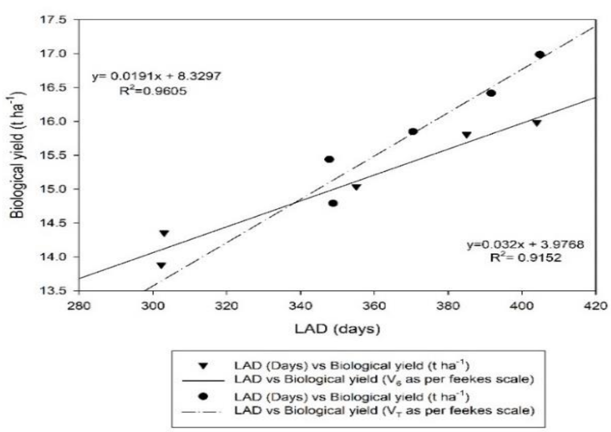

Figure 6 (b): Relationship between LAD and biological yield in maize plants as affected by foliar application of $CaCl_2$ under skipped irrigation imposed at different crop growth stages

More harvest index was recorded under normal irrigation at V_6 and V_T stage as compared to deficit irrigation at respective crop growth stages (Table 2). These results are in conformity with results reported by Khalili *et al*. (2013). Interactive effect of water regimes applied at both crop growth stages and calcium chloride levels was found significant regarding harvest index. Harvest index (49.44%) was maximum where 2.5 mM $CaCl_2$ was applied under normal irrigation at V_T stage. Minimum harvest index (47.06%) was noticed where 7.5 mM $CaCl_2$ was applied under drought at V_T stage.

Water use efficiency (WUE) in maize was significantly affected by different water regimes applied at V_6 and V_T stage (Table 2). Maximum WUE (3.16 kg ha^{-1} mm^{-1}) was achieved under normal irrigation at V_T stage while minimum WUE (2.90 kg ha^{-1} mm^{-1}) was recorded in plots where skipped irrigation was applied at both V_6 and V_T stage (Table 2). Eck *et al*. (1989) also reported increased water use efficiency when plants were subjected to water deficits on different crop growth stages. Different calcium chloride levels significantly affected WUE at different growth stages. WUE was maximum (3.22 kg ha^{-1} mm^{-1}) where 7.5 mM $CaCl_2$ was applied. Minimum WUE (2.80 kg ha^{-1} mm^{-1}) was achieved in non-treated plots.

Plant height and water use efficiency are most important parameters for measurement of agronomic efficiency. In current experiment, plant height and water use efficiency was improved by application of different $CaCl_2$ levels under drought as compared to control. Similarly, interactive effect of $CaCl_2$ with different water regimes at vegetative and reproductive stages affected biomass yield and harvest index significantly (Table 2). These results indicate the pronounced role of Ca^{2+} in mitigating the harmful effect of water deficit in maize. Similar alleviation activity was recorded in multiple plant species exposed to multiple stresses (Arshi et al., 2006; Tattini et al., 2009; Shoresh et al., 2011).

Physiological traits: Cell membranes were more stable under normal irrigation at V_6 and V_T stage. Interaction between water regimes applied at V_6 and V_T stage and different calcium chloride levels was significant regarding cell membrane stability index (Table 3). Maximum cell membrane stability index (94.06%) was recorded under normal irrigation at V_T stage where 5 mM $CaCl_2$ was applied. Under drought conditions, foliar spray of 7.5 mM $CaCl_2$ was more effective at V_6 stage while at V_T stage 5mM $CaCl_2$ was more effective which resulted in 78.79 and 84.30 cell membrane stability index, respectively. Results portrayed that at later stages of crop growth, less dose of calcium chloride is more effective as compared to early

Table 2: Effect of foliar spray of different levels of $CaCl_2$ under normal irrigation and drought imposed at different crop growth stages on agronomic, yield traits and water use efficiency of maize

Treatments	Plant height (cm)	Number of leaves per plant	Biological yield (t ha^{-1})	Harvest index%	Water-use efficiency (kg ha^{-1} mm^{-1})
T_1	140.67 c	13.25	15.87 e	48.18 c	2.80 d
T_2	141.33 c	12.75	16.41 d	48.39 bc	2.89 cd
T_3	146.17 b	13.16	17.06 c	48.87 ab	3.01 bc
T_4	150.25 a	13.50	17.93 b	49.10 a	3.14 ab
T_5	151.00 a	13.58	18.83 a	48.03 c	3.22 a
HSD	1.199	1.914	0.289	0.551	0.044
Water regimes					
W_1	165.00 a	14.00 a	18.58 b	48.96 a	3.09 a
W_2	101.27 c	10.87 b	15.01 d	48.11 b	2.90 b
W_3	164.93 a	14.53 a	19.00 a	48.70 ab	3.16 a
W_4	152.33 b	13.60 a	15.89 d	48.29 ab	2.90 b
HSD	2.610	1.563	0.385	0.686	14.240
Interaction					
W_1T_1	160.67	14.33	16.82 f	49.18 abc	2.80
W_1T_2	159.33	13.67	17.65 de	48.90 abc	2.94
W_1T_3	164.33	13.67	18.66 bc	48.78 abc	3.11
W_1T_4	170.00	13.67	19.71 a	49.35 a	3.28
W_1T_5	170.67	14.67	20.06 a	48.58 a-d	3.34
W_2T_1	95.00	12.00	13.88 l	47.68 bcd	2.72
W_2T_2	97.67	11.66	14.35 kl	47.64 cd	2.79
W_2T_3	102.33	9.33	15.04 ij	48.16 a-d	2.92
W_2T_4	105.67	10.67	15.81 ghi	49.10 abc	3.01
W_2T_5	105.67	10.67	15.98 gh	47.99 a-d	3.07
W_3T_1	160.00	13.33	17.99 cd	47.60 cd	2.99
W_3T_2	160.00	13.33	18.21 bcd	48.59 a-d	3.03
W_3T_3	165.67	16.00	18.70 b	49.44 a	3.11
W_3T_4	169.00	14.00	19.79 a	49.33 ab	3.29
W_3T_5	170.00	16.00	20.31 a	48.51 a-d	3.38
W_4T_1	147.00	13.67	14.79 jk	48.27a-d	2.69
W_4T_2	148.33	12.00	15.44 hij	48.44 a-d	2.81
W_4T_3	152.33	13.67	15.85 ghi	49.10 abc	2.89
W_4T_4	156.33	15.67	16.41 fg	48.60 abc	2.99
W_4T_5	157.67	10.00	16.98 ef	47.06 d	3.10
HSD	NS	NS	0.830	1.671	NS

Values followed by the same letters do not differ significantly at 5 % probability level, HSD= Honest Significant Difference at 5% probability level, NS= Non-significant., T_1= untreated, T_2= distilled water spray, T_3=2.5 mM $CaCl_2$, T_4=5 mM $CaCl_2$, T_5=7.5 mM $CaCl_2$, W_1=normal irrigation at 6 leaves stage (V_6 as per Feekes scale), W_2= skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), W_3= normal irrigation at initiation of tasseling stage (V_T as per Feekes scale), and W_4=skipped irrigation at initiation of tasseling stage (V_T as per Feekes scale).

vegetative stage regarding cell membrane stability index. Minimum cell membrane stability index (60.61) was achieved under un-treated control. Aziz *et al* (2018) also they reported that membrane stability index was less under drought than normal irrigation in cotton.

Chlorophyll contents (SPAD units) were significantly affected by interaction of water regimes applied at V6 and V_T stage and different calcium chloride levels (Table 3). Maximum chlorophyll contents (49 SPAD units) were recorded with foliar application of 7.5 mM $CaCl_2$ under normal irrigation at V_T stage. Chlorophyll contents recorded

Table 3: Effect of foliar spray of different levels of $CaCl_2$ under normal irrigation and drought imposed at different crop growth stages on cell membrane stability, chlorophyll content, and leaf relative water contents of maize

Treatments	Cell membrane stability index	Chlorophyll contents (SPAD value)	Leaf relative water contents (%)
T_1	76.75 c	40.44 c	58.96 c
T_2	78.82 c	41.29 c	58.90 c
T_3	83.54 b	45.32 b	71.35 b
T_4	86.94 a	46.04 b	78.29 a
T_5	86.91 a	47.46 a	80.41 a
HSD	2.819	0.882	3.010
Water regimes			
W_1	87.80 a	45.84 a	79.19 b
W_2	71.71 b	42.54 b	63.37 c
W_3	90.95 a	44.99 a	78.61 a
W_4	79.80 ab	43.08 b	64.16 c
HSD	14.240	1.258	6.095
Interaction			
W_1T_1	83.86 cde	42.63 d-g	61.88 e-h
W_1T_2	84.93 b-e	41.20 fgh	62.93 d-g
W_1T_3	88.01 abc	47.66 ab	72.39 c-e
W_1T_4	90.57 abc	48.73 a	80.27 a-c
W_1T_5	91.59 ab	48.96 a	83.46 ab
W_2T_1	60.61 g	39.33 h	50.42 h
W_2T_2	66.24 g	41.46 fgh	50.78 h
W_2T_3	74.43 f	43,66 def	67.84 def
W_2T_4	78.79 ef	43.30 def	73.30 b-e
W_2T_5	78.97 ef	45.03 bcd	74.50 b-d
W_3T_1	87.62 a-d	39.70 h	66.13 d-g
W_3T_2	88.73 abc	41.46 e-h	66.95 d-f
W_3T_3	91.32 abc	46.93 abc	79.76 bc
W_3T_4	94.06 a	47.86 a	89.12 a
W_3T_5	93.00 a	49.00 a	91.09 a
W_4T_1	74.91 f	40.10 gh	57.39 f-h
W_4T_2	75.37 f	41.13 fgh	54.95 gh
W_4T_3	80.37 def	43.03 def	65.41 d-g
W_4T_4	84.30 b-e	44.26 cdf	70.45 c-e
W_4T_5	84.05 cde	46.86 abc	72.58 b-e
HSD	22.927	2.350	11.695

HSD= Honest Significant Difference at 5% probability level, T_1= untreated, T_2= distilled water spray, T_3=2.5 mM $CaCl_2$, T_4=5 mM $CaCl_2$, T_5=7.5 mM $CaCl_2$, W_1=normal irrigation at 6 leaves stage (V_6 as per Feekes scale), W_2= skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), W_3= normal irrigation at initiation of tasseling stage (V_T as per Feekes scale), and W_4=skipped irrigation at initiation of tasseling stage (V_T as per Feekes scale), Values followed by the same letters do not differ significantly at 5 % probability level**.**

with 5 and 7.5 mM $CaCl_2$ were statistically similar under normal irrigation at V_6 and V_T growth stages in maize. Under drought condition, 7.5 mM $CaCl_2$ resulted in more SPAD units in both growth stages as compared to rest of the $CaCl_2$ levels. Minimum SPAD units (39.33 and 40.10) were recorded in un-treated plots at V_6 and V_T growth stages in maize, respectively. Moussa and Abdel-aziz (2008) also reported more chlorophyll content under normal irrigated condition as compared to water deficit. Liner positive relationship was found between SPAD units and biological yield under normal irrigation (Figure 7a) and drought condition (Figure 7 b) at V_6 and V_T growth stages in maize.

Interactive effect of water regimes applied at different crop growth stage (V_6 and V_T as per Feekes scale) and

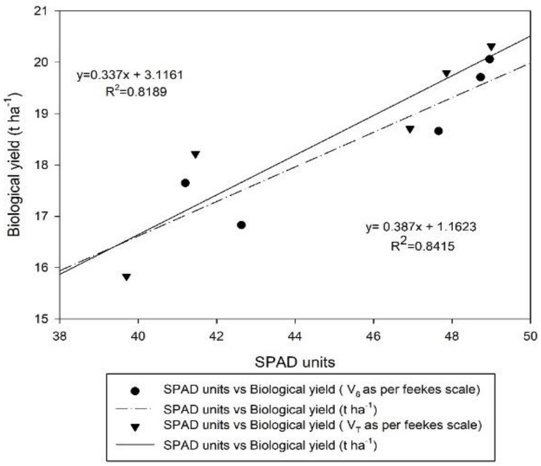

Figure 7 (a): Relationship between SPAD units and biological yield in maize plants as affected by foliar application of $CaCl_2$ under normal irrigation imposed at different crop growth stages

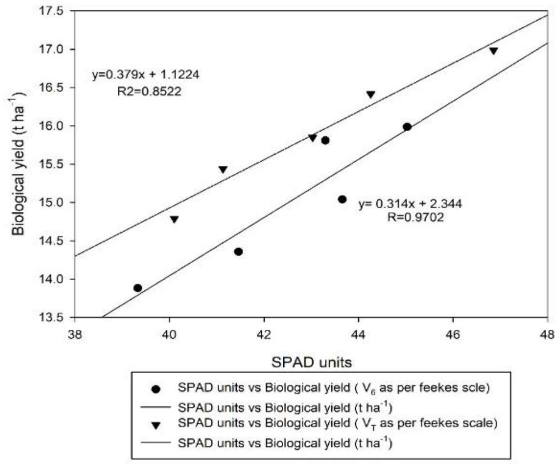

Figure 7 (b): Relationship between SPAD units and biological yield in maize plants as affected by foliar application of $CaCl_2$ under skipped irrigation imposed at different crop growth stages

Table 4. Benefit cost ratio for maize exposed to normal irrigation and drought condition at different growth stages as influenced by foliar spray of $CaCl_2$

Treatments	Total cost	Gross income	Net benefit	Net return	BCR
			US $		
W_1T_1	957.37	2263.3	1864.2	1305.9	1.36
W_1T_2	976.53	2360.9	1932.9	1384.3	1.42
W_1T_3	983.83	2488.2	2040.3	1504.4	1.53
W_1T_4	991.12	2659.0	2186.9	1667.8	1.68
W_1T_5	998.42	2664.4	2184.4	1666.0	1.67
W_2T_1	942.62	1870.3	1510.2	927.7	0.98
W_2T_2	961.79	1919.1	1534.9	957.3	0.99
W_2T_3	969.08	2041.1	1637.5	1072.0	1.11
W_2T_4	976.38	2119.7	1701.0	1143.3	1.17
W_2T_5	983.67	2125.1	1698.6	1141.4	1.16
W_3T_1	957.37	2341.9	1935.0	1384.5	1.45
W_3T_2	976.53	2420.5	1986.6	1444.0	1.48
W_3T_3	983.83	2528.9	2077.0	1545.1	1.57
W_3T_4	991.12	2667.1	2194.2	1676.0	1.69
W_3T_5	998.42	2691.5	2208.9	1693.1	1.70
W_4T_1	942.62	1946.2	1578.5	1003.6	1.06
W_4T_2	961.79	2049.2	1652.1	1087.4	1.13
W_4T_3	969.08	2130.5	1718.1	1161.4	1.20
W_4T_4	976.38	2184.7	1759.6	1208.3	1.24
W_4T_5	983.67	2187.4	1754.8	1203.7	1.22

1US $ =134 Pak Rupees

T_1= untreated, T_2= distilled water spray, T_3=2.5 mM $CaCl_2$, T_4=5 mM $CaCl_2$, T_5=7.5 mM $CaCl_2$, W_1=normal irrigation at 6 leaves stage (V_6 as per Feekes scale), W_2= skipped irrigation at 6 leaves stage (V_6 as per Feekes scale), W_3= normal irrigation at initiation of tasseling stage (V_T as per Feekes scale), and W_4=skipped irrigation at initiation of tasseling stage (V_T as per Feekes scale).

different $CaCl_2$ levels significantly affected leaf relative water contents (RWC) in maize (Table 3). Leaf RWC were recorded maximum under normal irrigated condition and drought conditions where 7.5 mM $CaCl_2$ was applied. It was found that maize leaves maintained 2.64% more RWC due to foliar spray of 7.5 mM $CaCl_2$ under drought condition at V_6 growth stage as compared to V_T stage.

Rest of the $CaCl_2$ levels resulted in lower relative water contents as compared to foliar spray of 7.5 mM $CaCl_2$. Minimum leaf RWC (5.42%) was recorded when maize was exposed to drought at V_6 stage in un-treated plots. (Edmeades *et al*. 1997) also reported importance of plant water status at reproductive stage for maximum contribution in yield of maize.

Economic analysis elucidated positive effect of different calcium chloride levels in maize exposed to different water regimes at V_6 and V_T stages (Table 4). Different $CaCl_2$ levels elevated net return under normal irrigation and drought conditions at V_6 and V_T stages. Benefit cost ratio (BCR) was more with foliar spray of 5 and 7.5 mM $CaCl_2$ as compared to untreated plots and rest of the $CaCl_2$ levels.

Conclusion

According to study, V_6 (six fully expanded leaves) stage was more sensative to water deficit than V_T (initiation of tasseling) in terms of reduction in grain yield. Application of different calcium chloride levels improved different allometric, yield attributes in maize exposed to water deficit at 6 fully expanded leaves and initiation of tasseling stage as compared to un-treated control. Maximum improvement in yield trait was noticed with 7.5 mM $CaCl_2$. However, more ratio was recorded with 5 mM $CaCl_2$ under water deficit condition at 6 fully expanded leaves and initiation of tasseling stage in maize. Both $CaCl_2$ levels can be utilized for improving yield and related traits in maize exposed to water deficit at V_6 and V_T stage.

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