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Role of Calcium Chloride in Ameliorating the Growth of *Vicia faba* Plants Under Drought Stress

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Abstract. This study was carried out to throw a beam of light on the role of $CaCl_2$ application to ameliorate the growth and phenotypic traits of broad bean plants under drought stress. Decreasing water holding capacity (WHC) significantly reduced fresh and dry biomass, leaf relative water content, shoot, root lengths and leaf area. This reduction was associated with significant decline of chlorophylls concentration (Chl. a, Chl. b), quantum yield of photosynthetic system II (Fv/Fm), nutrient elements and growth hormones content. On the contrary, there was a significant increase of malondialdehyde (MDA) and abscisic acid (ABA) contents. Moreover, superoxide dismutase(SOD) and catalase (CAT) activities were significantly increased compared to well-water plants. Irrigation with 50 mM $CaCl_2$ significantly enhanced the eliminating and scavenging of generate ROS beside increased the nutrient elements and growth hormones content of drought stressed plants in comparison to those in absence of $CaCl_2$.

Keywords: drought stress, growth hormones, quantum yield of photosynthetic system II, reactive oxygen species

Introduction

Drought stress or shortage of available water, is a severe abiotic environmental stress reduces several morphological and physiological parameters, decreases the water and nutrient absorption, suppress the synthesis and function of photosynthetic pigments and photosynthetic enzymes machinery as well as inhibits the physiological and metabolic processes resulting in an impairment of plant development, growth and yield (Ma *et al*., 2020; Sorour *et al*., 2017; Farooq *et al*., 2009). Also, the generation of reactive or toxic oxygen species (ROS), under drought stress, causes severe oxidative damage of cellular components (Gill and Tuteja, 2010). To mitigate the inhibitory effects of water deficit and oxidative damage, several glycophytes can develop complex defense mechanisms depending upon plant species, plant organs and application time of stress. These defense mechanisms include the biosynthesis and accumulation of osmolytes as soluble small organic molecular weight molecules (Mark *et al*., 2012), biosynthesis of non-enzymatic antioxidants (Ashraf *et al*., 2010) and enhancement of enzymatic antioxidants reported by (Devi *et al*., 2011) and biosynthesis of stress proteins by (Li *et al*., 2015).

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Calcium is a macro-nutrient element required for plant growth and development. It is essential for middle lamella, cell wall and plasma membranes structure (Tuteja and Mahajan, 2007) which acts as secondary messenger in the cytosol (Mohanta *et al*., 2018; Batistic and Kudla, 2012) and as counter ion for organic and inorganic anions (Mahajan and Tuteja, 2005). It is well known that the active and passive mechanisms are responsible for Ca^{2+} uptake and transportation to aerial parts.

The decreasing transpirational rate and inducting of stomatal closure under drought stress can reduce the rate of Ca^{2+} uptake and this is reported by (Liu *et al*., 2010; Pomper and Grusak, 2004; White and Broadley, 2003; White, 2001) and several reports demonstrated the role of cytosolic Ca^{2+} in physiological and biochemical processes to maintain the growth of plants under various environmental conditions. For instance, application of Ca^{2+}could stimulate the germination and growth of several plants such as sunflower (Ibrahim *et al*., 2016) and barley (Kaczmarek *et al*., 2017) subjected to water stress. On the other hand Zhang *et al*. (2017) suggested that inhibition the growth of *Camellia oleifera* suffered from Ca^{2+} toxicity could be related to disorder the activity of plasma membrane associated ATPases and tonoplastic associated H^+/Ca^{2+}antiporters

but the stimulation of *Carpinus pubescens* growth might be attributed to efflux cytosolic Ca^{2+} to apoplast or the vacuoles of sub cellular cytoplasmic organelles. Moreover, there are several reviews have been demonstrated that Ca^{2+} might induce the tolerance or defense mechanisms *via* induction of enzymatic anti-oxidants in plants under adverse environmental stresses (Mohanta *et al*., 2018; Hassan *et al*., 2017; Naeem *et al*., 2017; Ibrahim *et al*., 2016; Xu *et al*., 2013) found a marked increase of superoxide dismutase (SOD), catalase (CAT) and peroxidases (APx) activities in Ca-pretreated *Zoysia japonica* under drought stress and in foliar $CaCl_2$ sprayed maize plants respectively.

The present study was to explore the effect of drought stress on some growth and morphological parameters, change in hormone and nutrients levels, generation of ROS (as indicated by MDA content) and induction of enzymatic defense mechanism in faba bean plants. This study aimed to investigate the role of $CaCl_2$ application in ameliorating the growth under drought stress.

Materials and Methods

Faba bean seeds (*Viciafaba*, cv. Giza 2) were kindly supplied by the Giza Agricultural Research Center (GARC), Giza Egypt. Prior to germination, the seeds were surface sterilized by soaking for 2 min in 4% sodium hypochlorite solution, then washed several times with distilled water.

Treatments. Ten sterilized faba bean seeds were sown in each plastic pots (20 cm in diameter, 20 cm length with a hole at the bottom) filled with fixed amount of mixture of acid washed quartz sand and clay soil (3:1) and the pots were placed in growth chamber under controlled conditions (photoperiod, 10 h/14 h light/dark, temperature, 23±2 °C light/ 20±2 °C dark; light intensity, 32 μmol/m^2/s). The pots were arranged in complete randomized design with three replicates, two drought levels 60% and 30% along with 90% WHC (as control). These pots were divided into two groups, the first was kept under 60% and 30% WHC alone, while the second one supplemented with 50 mM $CaCl_2$ along with different drought treatment. The drought levels were maintained throughout the experiment buy irrigation with distil H_2O or 50 mM $CaCl_2$.

At 21 days, homologous plants were harvested, washed thoroughly from adhering soil particles, gently plotted, dissected to shoots and roots and quickly saved for estimation the various growth parameters, chemical analyses and enzymes assay. Other samples were dried at 60 °C to constant weight and saved for estimation of dry biomass and elements content.

Growth and phenotypic parameters. Ten shoots and roots of homologous plants (three replicates from each treatment) were taken and weighed for fresh biomass. The oven-dry biomass was determined after drying the samples in an oven at 60 °C till constant weight. Shoot and root lengths were measured as the mean of ten samples. Leaf area (mean of normal ten foliage leaves) was measured by digital planimeter (Placom KP-90). The relative water content (RWC) of leaves was determined which based on the equation: RWC = [(f.m. – d.m.)/(t.m. – d.m.)]. 100 (Silveira *et al*., 2003). Where f.m. is the fresh biomass, d.m. the dry biomass and t.m. is the turgid mass (after soaking in water for 4 h at room temperature).

Chemical analyses. ***Determination of photosynthetic pigments*.** The photosynthetic pigments chlorophyll a, b (Chl.a, Chl.b) and carotenoids (Carot.) were extracted and determined using Spectrophotometer method described by Metzner *et al*. (1965). The pigments were extracted by grinding a known fresh weight (about 0.5 g) of leaves in 85% (v/v) acetone in complete darkness, after centrifugation at an aliquot of the supernatant was taken and the absorbance was measured atthree wavelengths 665, 647 and 453 nm using spectrophotometer (IENWAY, 6305, UK). The concentration of each pigment fraction in μg/mL was calculated from the following equations:

Chl. a = 10.3E665–0.918E647
Chl. b = 19.7E647–3.87E665
Carot. = 4.2 A453–(0.0264 Chl.a + 0.426 Chl.b)

The values were then expressed as mg/g f.m.

Measurement of quantum yield of PSII (Fv/Fm). *In vivo* this measurement was monitored in fully-expanded and young leaves according to Branquinho *et al*. (1997). The measurements were performed with OS-30P pulse modulated chlorophyll fluorimeter (Opti sciences, Hudson, USA). Fluorescence was excited by illuminating leaves with a wear, red pulsed measuring light intensity (<0.1 μmol/m/s) with a peak wavelength 650 nm.

Determination of malondialdehyde (MDA) content. ***Extraction*.** Fresh plant materials (0.2 g) were homogenized in 5 mL 0.1% (w/v) trichloro acetic acid (TCA) solution on ice. The homogenates were centrifuged

at 10,000 g for 5 min at 4 °C and the supernatants were collected in clean test tubes (Heath and Packer, 1968).

Determination. One mL of 20% (w/v) TCA containing 0.5% (w/v) thiobarbituric acid (TBA) was added to a 0.5 mL aliquot of the plant extract. The mixture was kept in boiling water for 30 min and immediately cooled on ice. After centrifugation at 10,000 g for 10 min, the optical density of the supernatant was taken at 532 nm and 600 nm. The absorbance at 600 nm was subtracted from the absorbance at 532 nm and the MDA concentration was calculated using its extinction coefficient 155 mM-1/cm (Heath and Packer, 1968) and the results were expressed as µmol/g f.m.

Determination of growth regulators. The growth regulators were extracted by the method adopted by Shindy and Smith (1975). To estimate the concentration of indolacetic acid (IAA), abscisic acids (ABA) and gibberellic acid (GA_3), the plant hormone fractions and standard ones were methylated according to Vogel (1975) to be ready for GC analysis using Helwell Packered Gas Chromatography (5896). Peak identification was performed by comparing the relative retention time peak of IAA, GAs and ABA with standards.

Determination of some nutrient elements. The elements extract (K, Ca, Mg, P, Fe, Mn, Zn) was carried out according to standard method of Kimbrough and Wakakuwa (1989). The digested solutions were subjected to ICP-OES (Alileu 5100UDV, USA) for assaying the elements.

Assays of some antioxidant enzymes. ***Enzymes extraction method.*** Fresh shoot and root (0.2 g) homogenized in a mortar and pestle with 4 mL of ice-cold extraction buffer (100 mM potassium phosphate buffer pH 7, 0.1 mM EDTA). The homogenate was filtered through muslin cloth and centrifuged at 16,000 g for 15 min. The supernatant fraction was used as crude extract for enzyme activity. All operations were carried outat 4 °C (de AzevedoNeto *et al*., 2006).

Activity measurement. ***Superoxide dismutase (SOD, EC 1.15.1.1).*** Superoxide dismutase activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT), as described by Gannoplitis and Ries (1977). The reaction mixture (1.5 mL) contained 50 mM potassium phosphate buffer (pH 7.8), 0.1 µM EDTA, 13 mM methionine 75 µM NBT, 2 µM NBT, µM riboflavin and 50 µL enzyme extract. Riboflavin was added the last tubes were shaken and illuminated with a two 20-W fluorescent tubes (blanks were kept in dark). The reaction was allowed to proceed for 15 min after which lights were switched off and the tubes covered with a black cloth. Absorbance of the reaction mixture was read at 560 nm. One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT photo reduction rate and the result expressed as U/g f.m./min.

Catalase (CAT, EC 1.11.1.6). Catalase activity was measured according to the method of Beers and Sizer (1952) with minor modifications as described by de Azevedo Neto *et al*. (2006). The reaction mixture (1.5 mL) consisted of 100 mM potassium phosphate buffer (pH 7), 0.1 µM EDTA, 20 mM H_2O_2 and 50 µL enzyme extract. The decrease of H_2O_2nm and quantified by its molar extinction coefficient (36 M/cm) and the results expressed as µmol H_2O_2/g/f.m./min.

Statistical analysis. Statistical analysis of the results was done using SPSS software package version 20.0 to obtain the standard error (SE), mean and for comparison between the different groups involved in this ANOVA was used for comparison between independent samples and LSD was estimated $P \leq 0.05$.

Results and Discussion

Decreasing of soil water holding capacity resulted in a significant reduction of growth and phenotypic biomarkers of faba bean plants compared to well-watered control (Table 1). For instance, the decrease of fresh biomass in shoots and roots of severely drought stressed plants was 61% and 78% respectively, compared to well-watered control. This reduction was accompanied with significant decline of shoot and root lengths, leaf area and leaf relative water content (Table 1 and Fig. 1). Irrigation with 50 mM $CaCl_2$ resulted in a significant maintenance of growth and phenotypic traits of drought stressed plants compared to those in absence of $CaCl_2$. The increase of fresh biomassin shoots and roots of 50 mM Ca^{2+}- treated well-watered plants was 26 and 34% respectively compared to 90% WHC-treated plants in absence of Ca^{2+}. Whereas, at 30% WHC, the increase in f.m. of shoots and roots irrigated with 50 mM $CaCl_2$ was 62% and 50%, respectively, compared to those non Ca- irrigated ones, while, the increase of SL and RL was 68% and 58%, respectively (Table 1).

Table 1. Changes in fresh (f. m.) and dry (d. m.) biomass (g/plant) of shoots and roots, shoot length(SL), root length(RL) and leaf area (LA) of 21-d old faba bean plants in response to drought stress and Ca^{2+} treatment. Each value is the mean of three replicates ±SE

Treatment		Shoot		Root		SL (cm)	RL(cm)	LA(cm^2)
WHC%	Ca^{2+}mM	f.m.	d.m.	f.m.	d.m.			
90	0	11.235±0.70^{a}	0.786±0.04^{a}	6.264±0.37^{b}	0.420±0.02^{ab}	19.7±1.64^{ab}	12.3±0.82^{ab}	15.69±0.98^{b}
60	0	7.440±0.59^{b}	0.437±0.02^{c}	3.729±0.16^{c}	0.201±0.01^{cd}	14.8±0.98^{cd}	8.7±0.59^{c}	9.80±0.68^{c}
30	0	4.359±0.24^{cb}	0.268±0.01^{e}	1.355±0.09^{d}	0.088±0.01^{e}	7.5±0.32^{e}	3.1±0.46^{e}	4.82±0.30^{e}
90	50	14.188±1.29^{a}	0.806±0.06^{a}	8.366±0.56^{a}	0.501±0.03^{a}	21.7±1.75^{a}	13.2±0.83^{a}	18.85±1.11^{a}
60	50	11.820±0.74^{a}	0.615±0.03^{b}	6.661±0.27^{b}	0.315±0.02^{bc}	18.6±1.04^{b}	10.3±0.79^{b}	14.46±1.31^{b}
30	50	7.061±0.30^{b}	0.371±0.02^{cd}	2.o32±0.16^{e}	0.143±0.01^{d}	12.6±0.54^{d}	4.9±0.46^{d}	6.59±0.56^{d}
F		11.25	4.25	16.58	5.02	8.96	14.6	17.2
P		0.005*	0.025*	0.001*	0.036*	0.013*	0.003*	0.001*

LSD = Mean indexed by different superscript are significantly different at P≤0.05; * = Significance levels represented by P=0.05.

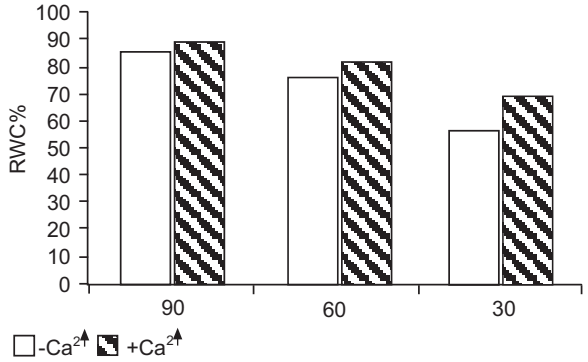

Fig. 1. Changes in the leaf relative water content (RWC%) of 21-d old faba bean in response to drought stress and Ca^{2+} treatment.

It is clearly demonstrated that Ch1.a, Ch1.b contents and Fv/Fm values were significantly decreased with decreasing soil WHC comparing to well-watered conditions (Table 2). The decrease of Chl.a and Chl.b in leaves of faba bean plants under 30% WHC was 69% and 53%, respectively, compared to well-watered plants. This decline was accompanied with a decrease of Fv/Fm from 0.804 in control to 0.668 in 30%WHC-treated plants. On the contrary, the carotenoids content was significantly increased in drought stressed leaves compared to well-watered plants. At severe drought condition, the carrotenoid content was 2-fold of well-watered plants. Irrigation of drought stressed and non-stressed faba plants with 50 mM $CaCl_2$ significantly increased Chl.a. Chl.b and declined caroot. Contents compared to those non Ca^{2+} irrigated plants. The Ch1.a and Chl.b contents in the leaves of severely stressed plants in presence of 50 mM $CaCl_2$ was 1.3-fold and 2.8-fold respectively, compared to those in absence of Ca^{2+}. Whereas, Fv/Fm increased from 0.668 under 30WHC in absence of Ca^{2+} to 0.712 in presence of Ca^{2+}.

Table 2. Changes in photosynthetic pigments content (Chl.a, Chl.b and Carot., mg/g f.m.) of 21-d old faba bean plants in response to drought stress and Ca^{2+} treatment. Each value is the mean of three replicates ±SE

Treatment		Photosynthetic pigments			Fv/Fm
WHC%	Ca^{2+} mM	Chl.a	Chl.b	Carot.	
90	0	32.76±1.93^{b}	10.64±0.71^{b}	5.41±0.49^{c}	0.804^{a}
60	0	24.57±1.21^{c}	8.26±0.59^{c}	9.51±0.63^{a}	0.725^{c}
30	0	10.18±0.85^{e}	4.95±0.31^{d}	10.91±0.68^{a}	0.668^{e}
90	50	43.75±2.41^{a}	12.02±0.53^{a}	4.46±0.37^{c}	0.810^{a}
60	50	40.29±1.61^{a}	10.87±0.78^{a}	5.68±0.41^{c}	0.784^{b}
30	50	13.03±0.77^{d}	12.75±0.45^{a}	7.65±0.48^{b}	0.712^{cd}
F	10.65	5.65	6.55	6.18	
P	0.003*	0.012*	0.009*	0.008*	

LSD = Mean indexed by different superscript are significantly different at P≤0.05; * = Significance levels represented by P≤0.05.

Imposing faba bean plants to drought stress significantly suppressed IAA and GA_3 and increased ABA contents in the shoots and roots (Table3). At 30% WHC the decrease in IAA content in shoots and roots was 82% and 77%, respectively and compared to 90% WHC. Conversely, the increase of ABA content in 30% WHC-treated shoots and roots was 233% and 307%, respectively. On the other hand, irrigation with 50 mM $CaCl_2$ resulted in a significant increase of IAA and GA_3 contents and decrease of ABA content compared to those subjected to drought stress alone. The increase of IAA and GA_3 contents in the shoots of severely drought stressed plants in presence of Ca^{2+} was 63% and 18%, respectively, in comparison to those in absence of Ca^{2+}, while, these values in roots were 97% and 60% respectively. At the same time, the decrease in ABA content in the shoots and roots was 30% and 18%, respectively.

Drought stress significantly declined K, Ca, Mg, P and Fe contents in shoots and roots of faba bean plants. These findings were accompanied with a significant increase of Mn and Zn contents. For instance at 30% WHC, the decrease of K, Ca, Mg, P, Fe in the shoots was 77%, 70%, 71%, 64% and 50%, respectively, compared to well-watered plants. The corresponding values in roots were 73%, 80%,79%,72% and 63%, respectively. It is well seen that application of 50 Mm $CaCl_2$ to drought stressed plants increased the nutrients contents and suppressed Mn and Zn accumulation in comparison to those grown in absence of Ca^{2+} (Table 4).

There was a significant increase of endogenous MDA content in shoots and roots of drought stressed faba bean plants (Table 5). The MDA content in the shoots and roots was 3.9- and 4.8 fold of 30% WHC treated plants compared to 90% WHC, respectively. Application of 50 mM $CaCl_2$ significantly reduced MDA accumulation compared to those grown under the absence of $CaCl_2$. The decline in MDA content in the shoots and roots of 30% WHC treated plants in presence of $CaCl_2$ was 35% and 27%, respectively and compared to those in absence of $CaCl_2$.

Decreasing WHC of soil resulted in a significant increase of SOD and CAT activities in shoots and roots of faba bean plants compared to control (Table 5). The SOD activity in shoots and roots of 30% WHC treated plants was 5.9 and 6.4 fold of 90 WHC treated control, respectively. At same time, the CAT activity was 3.8 and 5.7 fold, respectively. Moreover, application of $CaCl_2$ enhanced SOD and CAT activities in drought stresses plants greater than those in absence of $CaCl_2$. At severe water holding capacity, the SOD and CAT activities of the shoots in presence of 50 mM Ca^{2+} was 1.4 and 1.8 fold respectively, compared to those in absence of Ca^{2+}, while these values in roots were 1.2 and 1.4 fold respectively.

Imposing faba bean plants to drought stress, while in this study, significantly reduced the growth and phenotypic traits, including biomass accumulation, shoot and root lengths, leaf area and leaf RWC. These results in agreement of other recorded observations for several glycophytes such as melon seedlings (Kavas *et al*., 2013), potato (Li *et al*., 2015) and maize (Naeem *et al*., 2017). The suppression of plant growth in response to drought stress might be attributed to induction of ROS generation and oxidative damage of cellular components beside disturbance of plasma membranes structure and

Table 3. Changes ofgrowth hormones content (µg 100 g/f.m.) in shoots and roots of 21 dold faba bean plants in response to drought stress and Ca^{2+} treatment. Each value is the mean of three replicates ±SE

Treatment		Shoot			Root		
WHC%	Ca^{2+}mM	IAA	GA3	ABA	IAA	GA3	ABA
90	0	168.46±11.23^{a}	985.95±65.73^{b}	52.24±4.75^{c}	96.96±5.70^{b}	301.88±21.56^{b}	29.88±2.72^{e}
60	0	45.51±3.25^{d}	625.67±44.69^{d}	85.19±6.55^{b}	55.16±4.24^{d}	219.14±16.86^{c}	61.55±4.10^{c}
30	0	31.10±2.59^{e}	418.81±32.22^{f}	121.85±7.62^{a}	21.97±1.69^{f}	109.78±8.44^{e}	91.86±7.07^{a}
90	50	121.53±9.35^{b}	1076.14±89.68^{a}	53.93±4.90^{c}	127.45±9.10^{a}	388.67±22.86^{a}	24.64±2.05^{c}
60	50	73.34±6.67^{c}	919.49±57.47^{c}	59.90±3.74^{c}	87.66±7.97^{c}	294.16±21.01^{b}	42.91±2.86^{d}
30	50	50.75±3.38^{d}	495.22±38.09^{e}	84.71±6.05^{b}	43.24±2.54^{e}	175.53±12.54^{d}	75.12±6.26^{b}
F		25.6	34.2	28.6	31.2	25.6	18.9
P		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*

LSD = Mean indexed by different superscript are significantly different at P≤0.05; * = Significance levels represented by P≤0.05.

Table 4. Changes of nutrient elements (µg/d.m.) in shoots and roots of 21- d old faba bean plants in response to drought stress and Ca^{2+} treatment. Each value is the mean of three replicates ± SE

WHC%	Ca^{2+}mM	K	Ca	Mg	P	Fe	Mn	Zn
					Shoot			
90	0	3744±340.36^{b}	2192±199.27^{b}	387±25.80^{a}	355±29.58^{a}	287±17.94^{a}	53±3.53^{dc}	71±5.46^{b}
60	0	2185±128.53^{d}	1593±93.71^{c}	210±17.50^{c}	243±18.69^{b}	169±15.36^{c}	74±4.35^{b}	89±5.93^{a}
30	0	877±58.47^{f}	650±40.63^{e}	114±7.60^{ed}	128±8.53^{d}	114±10.36^{ed}	93±5.47^{a}	94±6.27^{a}
90	50	4202±280.13^{a}	3436±214.75^{a}	401±36.45^{a}	387±27.64^{a}	297±24.75^{a}	47±3.13^{ed}	68±5.67^{b}
60	50	2705±193.21^{c}	2665±205.00^{b}	273±16.06^{b}	288±20.57^{b}	226±20.55^{b}	60±4.29^{c}	81±6.75^{a}
30	50	1312±87.47^{e}	909±56.81^{d}	139±11.58^{d}	157±14.27^{c}	126±9.69^{d}	72±4.80^{b}	76±6.33^{a}
F		65.2	42.0	25.6	36.8	24.1	12.3	16.5
P		0.0001*	0.0001*	0.001*	0.001*	0.001*	0.008*	0.005*
WHC%	Ca^{2+}mM				**Root**			
90	0	3533±207.82^{b}	2603±185.93^{c}	285±23.75^{b}	493±32.87^{a}	261±21.2^{a}	37±3.36^{c}	24±2.18^{d}
60	0	2486±191.23^{c}	1482±123.50^{d}	147±11.31^{d}	287±23.92^{cd}	177±16.5^{c}	57±3.35^{b}	106±6.63^{a}
30	0	969±60.56^{f}	518±39.85^{f}	59±3.93^{f}	139±11.58^{ed}	96±8.66^{e}	76±4.75^{a}	124±7.75^{a}
90	50	4012±236.00^{a}	3169±226.36^{a}	453±32.36^{a}	515±30.29^{a}	291±28.3^{a}	33±1.94^{d}	28±1.87^{d}
60	50	2841±202.93^{d}	2347±146.69^{b}	268±15.76^{c}	341±24.36^{b}	201±20.5^{b}	40±3.64^{c}	70±5.00^{cd}
30	50	1415±108.85^{e}	846±70.50^{e}	104±6.12^{e}	203±18.45^{d}	135±14.3^{d}	59±4.92^{b}	89±8.09^{b}
F		52.3	46.2	36.5	45.6	22.3	41.2	30.0
P		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*

LSD = Mean indexed by different superscript are significantly different at P≤0.05; * = Significance levels represented by P≤0.05.

Table 5. Changes of malondialdehyde (µmol/g f.m.) content, superoxide dismutase activity (U/g f.m/min) and catalase activity (µmol H_2O_2/g f.m/min) in shoots and roots of faba bean plants in response to drought stress and Ca^{2+} treatment. Each value is the mean of three replicates ± SE

Treatment		Malondialdehyde		Superoxide dismutase		Catalase	
WHC%	Ca^{2+}mM	Shoot	Root	Shoot	Root	Shoot	Root
90	0	8.46±0.71^{cd}	10.29±0.94^{d}	2.18±0.13^{e}	3.07±0.19^{e}	4.96±0.33^{fe}	4.01±0.36^{fe}
60	0	21.98±1.83^{b}	32.76±2.98^{b}	7.90±0.49^{dc}	10.64±0.82^{d}	8.39±0.65^{d}	5.61±0.51^{ed}
30	0	33.16±3.01^{a}	48.88±3.76^{a}	12.88±0.99^{b}	19.80±1.52^{b}	19.02±1.59^{cb}	22.89±1.43^{b}
90	50	7.91±0.72^{d}	13.57±0.97^{d}	2.85±0.19^{e}	3.38±0.20^{e}	6.56±0.60^{ed}	7.93±0.53^{d}
60	50	10.43±0.80^{c}	19.08±1.59^{c}	10.13±0.92^{bc}	16.99±1.13^{b}	21.54±1.04^{b}	14.57±1.40^{c}
30	50	21.40±1.95^{b}	35.45±2.36^{b}	18.07±1.13^{a}	24.68±1.45^{a}	33.45±2.13^{a}	32.68±3.24^{a}
F		13.5	24.3	32.1	27.6	14.3	19.2
P		0.005*	0.001*	0.001*	0.0001*	0.008*	0.001*

LSD = Mean indexed by different superscript are significantly different at P≤0.05; * = Significance levels represented by P≤0.05.

decline of cell turgor pressure which is reported by Farooq *et al*. (2009). Moreover, the impairment of DNA and mitotic divisions as well as decline of cell elongation and expansion under drought conditions may be resulted in decrease of plant height and leaf area (Hussain *et al*., 2008). In accordance with these views, the results in this study showed that drought stress induced ROS generation and peroxidation of cellular membranes, as indicated by a significant increase of MDA content, causing a marked disturbance of water status and decline of fresh biomass. In addition, ROS may disorder the shoot and root apexices leading to decrease the production and content of growth hormones. The current study showed that, there was a significant decrease of IAA and GA_3 contents in drought stressed faba bean plants and that accompanied with marked decrease of

shoot and root lengths and leaf area. Moreover, the increase of ABA concentration in the shoots (Table 3) and decrease of LA of faba bean under drought conditions may lead to the disturbance of stomatal number and frequency as well as induce the stomatal closure (Anjum *et al*., 2011) resulting in a decrease of trans-pirational rate, CO_2 diffusion and water absorption and plant biomass. Therefore, the reduction of growth traits and impairment of morphological features of faba bean plants. In this study, the degradation of cellular plasma membranes and diminished the growth hormones (IAA, GAs) contents as well as hampered CO_2 diffusion and photosynthetic machinery. Conversely, irrigation of drought stressed plants with 50 mM $CaCl_2$ resulted in a significant increase of biomass content, morphological parameters, leaf RWC and growth hormones content. These observations were associated with a significant decrease of MDA and ABA accumulation compared to those in absence of $CaCl_2$. It has been reported that application of Ca^{2+} can suppress ROS generation (Kaczmarek *et al*., 2017; Ibrahim *et al*., 2016) and decrease of MDA and ABA contents (Mohanta *et al*., 2018; Xu *et al*., 2013). In addition, Naeem *et al*. (2017) suggested that Ca^{2+} induced the accumulation of organic and inorganic osmolytes which resulted in an increase of cellular osmotic potential and RWC in drought stressed maize plants. Tuteja and Mahajan (2007) postulated that Ca^{2+} is required for formation of micro-tubules and improved the mitotic divisions in shoot and root apexices. In agreement with these views, application of $CaCl_2$ significantly increased SOD and CAT activities and that accompanied with a significant decrease of MDA content in drought stressed faba bean plants, comparing to those in absence of $CaCl_2$. Moreover, there was a significant increase of growth hormones (IAA and GAs) as well as nutrient element contents beside leaf RWC, while decrease ABA content. Thus, the ameliorative effect of $CaCl_2$ on the growth traits and morphological features of faba bean plants under drought stress and during this study, induction of enzymatic antioxidant defense mechanism which eliminated the ROS production and improved the integrity of cellular membranes and plant apexices from the oxidative damage. These observations could result in increase of cellular osmotic potential and hormones production, therefore increased organ water content, elongation and expansion. Additionally the decrease of ABA content might induce stomatal opening and gas exchange leading to improve the photosynthesis and growth.

Soil water shortage significantly decreased the Chl.a. Chl.b contents and quantum yield of PS Π (Fv/Fm) values in the leaves of 21-d old faba bean plants. In agreement with these observations, several studies reported that drought stress markedly declined the photosynthetic pigments content and photosynthetic machinery in many plant species including chickpea (Rahbatian *et al*., 2011), maize (Naeem *et al.*, 2017) and tomato plants (Çelik *et al*., 2017). Under drought stress, in this study, there was a significant decrease of nutrient elements (K, Mg, P, Fe) responsible for chlorophylls biosynthesis and that might lead to decline of chlorophylls content. In addition, the increase of MDA content could indicate to the ROS generation and induce of oxidative damage of thylakoid and chloroplast membranes as well as photosynthetic systems associated proteins resulting in a marked reduction of photosynthetic activity and growth. In corporation Kavas *et al*. (2013) proposed that inhibition of chlorophylls synthesis and disturbance of chloroplastic plasma membranes and PSI and PSII aggregated proteins might lead to disorder photosynthetic machinery under drought stress. Moreover, the increase of ABA content in drought stressed faba bean plants might induce stomatal closure (MacRobbie, 2000) and increase CO_2 resistance leading to suppress photosynthesis. Thus, the reduction in growth of broad bean plants under drought stress, in this study, might be related to reduction of photosynthetic machinery *via* stomatal and non-stomatal limitations (Farooq *et al*., 2009). On the contrary, exposure faba bean plants to drought stress resulted in a marked increase of carotenoids content which act as non enzymatic antioxidants. ElSheery and Cao (2008) reported that carrotenoid content was markedly increased under drought stress for scavenging the generated ROS. Whereas, DePascale *et al*. (2009) postulated that the role of carrotenoid is mainly for decline the inhibitory effects of non-radiative energy. Irrigation of drought stressed faba bean plants with 50 mM $CaCl_2$ significantly increased LA, Chl.a, Chl.b, Fv/Fm values and micro elements content compared to those in absence of $CaCl_2$.

These observations were accompanied with a marked decrease of MDA and ABA contents. In agreement with these findings, Xu *et al*. (2013) and Naeem *et al*. (2017) stated that Ca^{2+} application could enhance the photosynthetic machinery in drought stress of *Zoysia japonica* and *Zea maize* respectively. The increase of LA and micro elements content. In this study, might reflect the

increase of photosynthetic area and chlorophylls synthesis. In addition, the decline of MDA content could be related to the enhancement of enzymatic antioxidants (Table 5) and shift off the peroxidation of thylakoid and chloroplastic membranes, while the decrease of ABA content might lead to induce stomatal opening and increase of CO_2 diffusion. Therefore, application of $CaCl_2$ could ameliorate the photosynthetic machinery and diminished the suppressive effects of drought stress, hence increase faba bean growth.

It is well known that plants can develop enzymatic antioxidants defense system to protect themselves under various stress conditions. In this study, the induction of peroxidation of cell membranes and decrease of growth biomarkers in drought stressed faba bean plants, however, was associated with enhancement the activity of SOD and CAT. These observations may indicate the imbalance between ROS generation and their eliminating and scavenging by antioxidant systems, resulting in inhibition of plant growth (Liu *et al*., 2011; Gill and Tutaja, 2010). On the other hand, irrigation with $CaCl_2$ significantly increased the SOD and CAT activities and decreasing of MDA content, revealing the high rate of scavenging and eliminating of the generated ROS and improving the growth. In accordance with these findings, Xu *et al*. (2013) mentioned that the decrease of MDA content in Ca^{2+} pretreated *Zoysia* grass under drought stress might result in an improve the osmotic strength and induce of Fv/Fm value. In addition, Naeem *et al*. (2017) suggested that Ca^{2+} enhanced the activity of SOD and CAT in water stressed maize plants. Kaczmarek *et al*. (2017) and Zhang *et al*. (2017) reported that Ca^{2+} and Ca-binding protein components could induce antioxidant defense mechanisms for scavenging ROS and establishing plasma membranes integrity. Mohanta *et al*. (2018) suggested that Ca^{2+} diminished the production of ROS from PSI and PSΠ complexes *via* induction of SOD activity.

Conclusion

The ameliorative effect of $CaCl_2$ on the growth of faba bean plants under drought stress might be attributed to enhancement the enzymatic antioxidants defense mechanism which improve the cellular and chloroplastic membranes integrity and shoot and root apexices from the inhibitory effect of the oxidative damage by generated ROS. These findings were associated with increase of water and nutrients uptake, growth hormones production and photosynthetic activity, finally improve the growth.

Conflict of Interest. The authors declare that they have no conflict of interest.

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