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Effect of foliar spray of calcium lactate on the growth, yield and biochemical attribute of lettuce (*Lactuca sativa* L.) under water deficit stress

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Key words: anthocyanin, antioxidant enzymes, leaf water status, nutrient uptake.

Abstract: The field experiment was conducted to evaluate the effect of foliar spray of calcium lactate (Ca) on fresh yield and biochemical attribute of lettuce (*Lactuca sativa* L.) under water deficit stress, in a split plot form based on a randomized complete block design with three Irrigation regimes (70, 85 and 100% ETc) and three calcium lactate treatment levels (0, 0.75 and 1.5 g L^{-1}) in three replicates. Results revealed that water deficit stress significantly reduced the growth and yield of plant, leaf relative water contents, excised leaf water retention and N, P and Mg absorption while led to increase anthocyanin, phenol and flavonoids contents, antioxidant activity, peroxidase and catalase activity and water use efficiency. The results of our research indicated that the application of CaL 1.5 g L^{-1} is capable of increasing lettuce yield, under field conditions with 30% less than optimal irrigation. CaL treatment showed a clearly protective effect in stressed plants, enhancing their leaf water status, antioxidant capacity and N and Ca contents in comparison to untreated plants. Therefore, feeding leaves by CaL with increasing antioxidant activity and nutrients content especially N led to increase growth and fresh yield of lettuce under normal irrigation and water deficit conditions.

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1. Introduction

Abiotic stresses such as high temperature, drought, salinity and chemical toxicity, are the most important limiting factors to crop productivity. Drought is undoubtedly one of the most important stresses that have huge impact on growth and productivity of the crops (Fahad *et al.*, 2017; Hussain *et al.*, 2018). Water stress is the most prominent abiotic stress limiting agricultural crop growth and productivity (Gholipoor *et al.*, 2013; Ihsan *et al.*, 2016). Deficit irrigation stress as a consequence of the progressive decrease in water availability has been a hot topic regarding food security during the last two decades (UNESCO, 2012). Growth and development of plants is influenced by reduction in turgor that result in decreased nutrient acquisition from dry soil (Luo *et al.*, 2011). Due to the threat of climate change, there is a need to limit the use of water resources in arid and semi-arid climates. It is therefore important to find new approaches to avoid crop productivity losses in 'limited fresh-water' areas.

Lettuce (*Lactuca sativa* L.), an annual plant of Asteraceae family, is considered as one of the most important salad vegetables as a cool season crop. Lettuce leaves contain vitamins C and E, carotenoids, phenolic acids with anti-free radical activity, and minerals with a lot of fiber, which are an important part of the human diet. Moreover, lettuce contains lactocin and lactucopicrin which improve the quality of sleep (Chakraborti *et al.*, 2002; FAOSTAT, 2016).

Since most of vegetable species are shallow-rooted, they are sensitive to mild water stress. In lettuce production, it is particularly important to preserve optimal growth through a well-scheduled irrigation program, where the harvested part of the plant is the photosynthetic leaf area, (Ahmed *et al.*, 2000; Casanova *et al.*, 2009). Its leaves have high water content and it is sensitive to mild water deficit stress due to its shallow root system (Kizil *et al.*, 2012). Therefore, in lettuce, new strategies will become critical to enhance productivity under deficit irrigation (Malcom *et al.*, 2012).

Foliar application of agro-chemicals has widely been used in agriculture as a rapid, low-cost and effective way for enhancing growth and productivity of many vegetable crops under water deficit stress especially green leafy vegetables like lettuce. Calcium lactate is considered as one of important agro-chemical which can be spray and play important roles in physiological and biochemical processes. Calcium (Ca) is the mineral nutrient most commonly decrease absorption under water deficit condition, so increasing the calcium content in the leafy vegetables could further improve Ca concentrations in plant tissues (Grusak, 2002).

Ca is an essential macronutrient for plant growth and development, and is considered as an important intracellular messenger, mediating responses to hormones, stress signals and a variety of developmental processes. Furthermore, Ca is an important component in the structure of cell walls and cell membranes (Hepler and Winship, 2010). Ca plays a role in the regulation of various mechanisms of plants under environmental conditions such as water stress, heat, cold and salinity. In addition, calcium signaling is required for acquisition of tolerance or resistance to the stress (Cousson, 2009). Positive effect of calcium in improving stress tolerance can be attributed to regulate of water status, antioxidant systems activity, osmolytes accumulation, improving photosynthetic pigment content, and nutritional balances (Kurtyka *et al.*, 2008). Ca plays an important role in oxidative stress signaling, linking H_2O_2 perception and induction of antioxidant genes in plants (Rentel and Knight, 2004). Ca participates in most cellular signaling processes (Sanders *et al.*, 2002) and interacts strongly with reactive oxygen species (Evans *et al.*, 2005).

Since the combined effects of Ca and water deficit stress have hardly been reported, the current study was, therefore, designed to evaluate the influence of foliar application of calcium lactate on the growth, yield and biochemical attribute of lettuce cv. New Red Fire under water deficit stress.

2. Materials and Methods

Experimental design

The field experiment was carried out at the Research farm of the Agriculture faculty, University of Zanjan, Iran, during 2017. The experiment was performed using a split plot based on a randomized complete block design with three Irrigation regimes (70, 85 and 100% ETc) as the main plot and three calcium lactate (CaL) treatment levels (0, 0.75 and 1.5 g L^{-1}) as the sub-plot in three replicates. The soil properties of experimental filed as well as average daily climatic data during the growing seasons was shown in Tables 1 and 2, respectively.

Plant material

Seeds of lettuce (*Lactuca sativa* L.) cv. New Red Fire was obtained from a "Takii seed" company. Lettuce seeds were sown in the nursery on the 2nd of August. Seedlings were transplanted at the 3-4 leaf stage when the seedlings were four weeks old with 25 cm spacing within row and 35 cm spacing between rows that there were about 11.5 plants per square meters (plants m^{-2}).

Table 1 - Soil physical and chemical properties on the site of experimental field

Soil texture	Organic matter (%)	pH	EC (dS m^{-1})	N (%)	Ca (g kg^{-1})	Na (g kg^{-1})	K (g kg^{-1})
Loam clay	0.94	7.4	1.49	0.07	0.12	0.13	0.20

Table 2 - Average daily climatic parameters of Zanjan Synoptic station during the growth seasons (2017) of lettuce

Meteorological parameter	May	June	July	August	September
Rainfall (mm)	0.01	1.11	5.00	0.00	0.02
Average temperature (°C)	22.94	25.71	27.68	24.79	15.73
Minimum temperature (°C)	11.29	16.8	17.61	14.68	7.89
Maximum temperature (°C)	32.47	33.96	36.82	35.12	25.05

Irrigation treatments and calcium lactate applications

After plant establishment, lettuce plants were sprayed with different concentration calcium lactate at 6-7th leaf stage, 10 and 20 days after first spraying for 3 times, during the plant growth. Irrigation treatments were applied one week after the first spraying. All foliar sprayings time were the same and distilled water was used for control treatment. The three irrigation levels were calculated based on actual evapotranspiration (ETc): (1) control, irrigated 100% crop water requirement (I100), (2) deficit irrigation 85% ETc (I85), and (3) deficit irrigation 50% ETc (I50). The Water requirement of the plant for control treatment was estimated using long-term average daily data of meteorological parameters recorded at Zanjan Meteorological Station and following relation.

$$ET_C = ET_0 \times K_C$$

ET_C: Water requirement of lettuce (mm/day), ET_0: Evapotranspiration of grass reference plant (mm/day) and K_c: Vegetable coefficient of lettuce (no unit).

It is necessary to explain that ET_0 values were estimated based on the standard FAO-Penman-Monteith method. Table 2 shows the long-term average of meteorological parameters of Zanjan synoptic station during the period of plant growth which was used to calculate ET0 and ETC values. After calculating the ETC values, the net and gross irrigation water requirements of lettuce were estimated based on cropping intervals, type of irrigation system and irrigation interval and then give the plant at each irrigation time. Based on the calculations, the amount of irrigation water given to the control plants was estimated to be 895.7 $m^3.ha^{-1}$. Water requirement of other treatments was estimated and distributed based on the water requirement of control treatment and water stress (Allen *et al.*, 1998). All necessary management practices such as weeds control were done according to recommended practices during the crop growth.

Measurements

Anthocyanin content. Anthocyanin content in leaf tissue was determined according to the method of Mita *et al.* (2000). Fresh weight of leaves (0.1 g) was homogenized in methanol containing 1% (v/v) HCl and then was filtrated. The filtration was stored at 4°C for 24 hours in dark conditions. The absorbance of filtration was recorded at 550 nm using UV-vis spectrophotometer (Specorp 250 Jena-History) and the anthocyanin was expressed as µmol g^{-1} FW.

Total phenols and flavonoids contents

The fresh leaf tissue (2.0 g) was washed with deionized water, and homogenized in 80% cold methanol (20:80, V/V). The homogenate was centrifuged at 10,000 rpm for 10 min, and the supernatant was collected for the measurement of, total phenolic and flavonoid content. Total phenolics assay was carried out according to the procedure described in the literature (Meda *et al.*, 2005). The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight based on a standard curve using gallic acid as standard. Total flavonoids were determined by the colorimetric method (Kim *et al.*, 2002). Quercetin was used as a reference standard, and the results were expressed as mg quercetin equivalents per 100 g fresh weight of leaf.

Antioxidant activity

As mentioned in the previous paragraph, 2.0 g of leaves were homogenated in methanol and then was centrifuged. The filtration was used to determine free radical scavenging activity using the 2, 2,-diphenyl-2-picryl-hydrazyl (DPPH) method at optical density 517 nm (Sun *et al.*, 2007). Antioxidant activity (%) was calculated using the following equation:

$$\text{Antioxidant activity} = \text{A DPPH} - \text{A sample (517 nm)}/\text{A DPPH} \times 100$$

Catalase (CAT) and peroxidase (POX) enzymes activity

Samples were taken from the fully expanded leaf and transferred to the laboratory in the ice. Leaf sample (0.5 g) was frozen in liquid nitrogen and ground using a porcelain mortar and pestle.

Catalase (CAT) activity was measured by following the decomposition of H_2O_2 at 240 nm with a UV spectrophotometer (Cakmak and Horst, 1991). Samples without H_2O_2 were used as blank. The activity of CAT was calculated by the differences obtained at OD_{240} values at 30 second interval for 2 min after the initial biochemical reaction. Peroxidase (POX) activity was measured using modified method of the Tuna *et al.* (2008) with guaiacol at 470 nm. A change of 0.01 units per minute in absorbance was considered to be equal to one unit POX activity, which was expressed as unit g^{-1} FW min^{-1}.

Leaf water status (RWC, ELWR)

The fresh weight of young leaves (FW) was recorded and then was kept in Petri dishes for 24 hours immersed in distilled water. The turgid weight (TW) was measured after saturation of leaves with water. The leaves were dried at 70˚C to constant weight and then weighted (DW). Leaf relative water contents (RWC) were calculated according to the following formula reported by Hanson and Hitz (1982).

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

For the determination of excised leaf water retention (ELWR), The youngest leaves collected for each treatment were weighed to record fresh weight (FW), kept at room temperature (25˚C) for 6 hours and reweighed (WL). ELWR was calculated using the following formula suggested by Lonbani and Arzani (2011).

$$ELWR= [1-(FW-WL)/FW] \times 100$$

Nutrient contents

The lettuce leaf samples from each treatment were collected at the end of the experiment. For mineral analysis, leaf samples were taken and oven-dried at 70°C until constant weight. Then 0.3 g of the dry samples was taken and digested using a mixture of sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) as described by Allen *et al.* (1974). All the studied elements were assayed in the digest of the concerned plant samples. Total nitrogen was determined using Kjeldahl method as described by Piper (1950). Phosphorus determination was done by complexing it with ammonium molybdate, which on reduction with ascorbic acid gives stable blue colour, the content of P was measured by spectrophotometer at 882 nm according to Watanabe and Olsen (1965). Potassium, calcium and magnesium content were analyzed by flame photometer (Chapman and Pratt, 1961).

Yield and water use efficiency (WUE)

Lettuce plants were weighed after harvest with a digital gravimetric scale. The average weight of single plant was calculated in grams and total yield was estimated in kg/m^2. Also water use efficiency (WUE) was obtained from the ratio of the amount of yield of each treatment to the amount of water consumed by the same treatment in kg m^{-3}.

Statistical analyses of the data

The analysis of variance (two-way ANOVA) and least significant difference (LSD) test ($P \leq 0.05$ and $P \leq 0.01$) used to compare means within each sampling date. The Statistical analysis and standard error calculation were carried out using SAS software (v. 9.1).

3. Results

Anthocyanin content

The data in Table 3 and figure 1, displays the anthocyanin contents of lettuce leaf applied with different concentrations of CaL under water deficit. Mean comparisons of data showed that deficit irrigation led to a significant increase in antioxidant activity compared to control. However, the effect of CaL on the anthocyanin contents was depended to the

Table 3 - Variance analysis (ANOVA) of effect of calcium lactate on physiological characteristics in lettuce under deficit irrigation

S.O.V	df	Mean of squares							
		Anthocyanin	Total phenols	Flavonoids	CAT activity	POX activity	Antioxidant activity	RWC	ELWR
Replication	2	1.003	0.002	0.308	0.057	0.003	16.725	8.614	23.677
Irrigation	2	2.162 **	6.187 **	11.932 **	1.953 **	0.851 **	132.47 **	42.799 *	238.260 **
Error (a)	4	5.648	0.135	0.330	0.009	0.001	23.524	9.036	14.106
Calcium lactate	2	4.292 *	1.057 **	24.265 **	0.702 **	0.052 *	318.416 **	186.691 **	420.124 **
Calcium lactate × irrigation	4	2.770 *	0.475 *	1.149 *	0.075 *	0.030 *	43.004 *	1.302 NS	29.661 *
Error (b)	12	6.388	0.121	0.338	0.023	0.008	9.686	9.323	6.176
Coefficient of Variation (%)	-	11. 18	2. 31	4.19	8.87	16.93	3.73	3.9	3.4

** and * represent significance at the 1 and 5% probability levels, respectively, and NS represents non-significance at p<0.05.

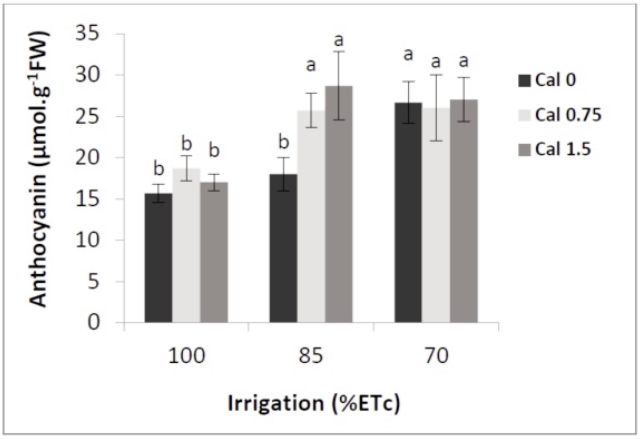

Fig. 1 - Effects of CaL treatments on anthocyanin content of lettuce under deficit irrigation. Values are means with standard errors (n= 3). Data were subjected to two-way ANOVA and different letters mean that values are statistically different P<0.05.

irrigation regime treatment. The highest value of anthocyanin content was obtained from plant treated with 1.5 g L^{-1} CaL under irrigation 85% ETc. According to the results, there was no significant difference between different levels of CaL under irrigation 100% ETc. However, anthocyanin content at 85% ETc was significantly increased by using CaL while under irrigation of 70% ETc with the increase of anthocyanin content did not show any significant difference between different levels of CaL.

Secondary metabolic products, which are intensively biosynthesized under drought, are antioxidants (Do Nascimento and Fett-Neto, 2010). Anthocyanin pigments as one of secondary metabolites and antioxidative systems play many important eco-physiological roles in plants, including roles in stress protection (Winkel-Shirley, 2002). Increased anthocyanin contents are thought to mask chlorophyll and/or act as a filter for preventing high light absorption by leaves and thus minimize photoinhibition (Farrant, 2000). Therefore anthocyanin accumulation in drought-stressed leaves confirms a possible protective role of anthocyanins as sun-screens and reactive oxygen species (ROS) scavengers in stressed plants (Merzlyak and Chivkunova, 2000), that similar results have also been reported by Jazizadeh and Mortezaei Nejad (2016) in chicory.

The obtained results indicated that CaL was effective in preserving and increasing anthocyanin content. These findings were in agreement with Abd-Elhady (2014) findings who also observed that CaL pretreatments proved to be effective for increasing the retention of anthocyanin in frozen strawberry.

Total phenols and flavonoids contents

The exposure to water deficit stress significantly ($P<0.05$) increased total phenols and flavonoids contents (Table 3, Figs. 2A, B). Besides, the results of the present study also showed that foliar application of CaL increased total phenols and flavonoids contents under normal and deficit irrigation, however, the effects of CaL was dependent to the irrigation levels. The maximum value of phenols and flavonoids contents was recorded in plant treated with 0.75 g L^{-1} CaL under irrigation 70% ETc. In all levels of irrigation, application of 0.75 g L^{-1} CaL had the greatest effect on total flavonoid content, although did not show significant difference with CaL 1.5 g L^{-1} under irrigation 70% ETc.

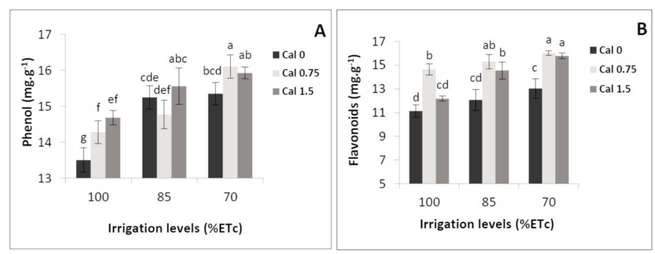

Fig. 2 - Effects of CaL treatments on total phenol and flavonoids contents of lettuce under deficit irrigation. Values are means with standard errors (n= 3). Data were subjected to two-way ANOVA and different letters mean that values are statistically different P<0.05.

Phenolic compounds include many secondary metabolites in plants that display antioxidant properties (Barbagallo *et al.*, 2012). Some of the phenolic compounds, such as phenolic acids or flavonoids, are widely recognized in most of the plant species (Jwa *et al.*, 2006). Phenolic compounds are important because of their contribution to the nutritional quality attributes of fruits and vegetables such as color, astringency, bitterness and flavor (Vinson *et al.*, 2001). The role of phenols as antioxidant is supported by several researches and the recovery methods have a great importance for industrial use (Barbagallo *et al.*, 2012). Environmental stress can cause an increase in the content of phenolic compounds of cell (Weidner *et al.*, 2009).

Aghdam *et al.* (2013) reported that the total phenols and flavonoids contents increased in the cornelian cherry fruit with $CaCl_2$ treated. Their results suggested that $CaCl_2$ treatment may stimulate the accumulation of phenols and flavonoids fruits by activating their biosynthetic pathways. Biosynthesis of phenols such as flavonoids in plants carried out via the shikimate-phenylpropanoid pathways. Ca^{2+} plays a direct role in the biosynthesis of phenols (Castañeda and Perez, 1996). CaL might be a potential molecule for activating phenyl propanoid-flavonoids pathways of fruits by increasing the PAL activity (Jacobo-Velazquez *et al.*, 2011; Aghdam *et al.*, 2013).

Catalase (CAT) and peroxidase (POX) enzymes activity

Significant differences among irrigation treatments were observed for CAT and POX enzyme activity (Table 3, Figs. 3A, 3B). The antioxidant enzyme activates increased with the decrease of irrigation water applied. As the results showed CAT and POX enzymes activity increased with increasing CaL concentration under deficit irrigation, although no significantly differences was observed in normal irrigation. The highest CAT and POX enzymes activity were recorded in plant treated with 1.5 g L^{-1} CaL under irrigation 70% ETc, which had no significant difference with 0.75 g L^{-1}.

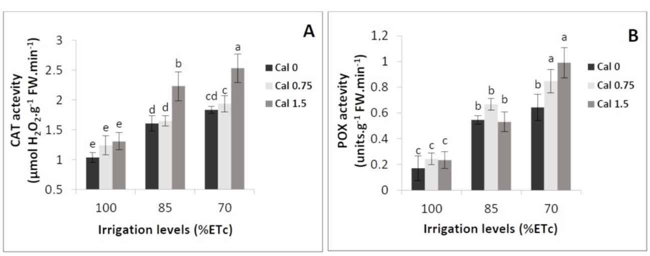

Fig. 3 - Effects of CaL treatments on catalase (CAT) and peroxidase (POX) enzymes activity of lettuce under deficit irrigation. Values are means with standard errors (n= 3). Data were subjected to two-way ANOVA and different letters mean that values are statistically different P<0.05.

The production of ROS (Vurukonda *et al.*, 2016) is another major factor that impairs plant growth under water deficit (Liting *et al.*, 2015). Plants employ a number of mechanisms, at molecular, cellular and physiological levels to persist stress condition (Shinozaki and Yamaguchi-Shinozaki, 2000). The activation of antioxidant enzymes is one of the major types of these mechanisms which enable plants to control ROS (Shahid *et al.*, 2014). CAT, ascorbate peroxidase (APX), superoxide dismutase (SOD) and POX are the key antioxidant enzymes involved in detoxification of superoxide and hydrogen peroxide (Kadkhodaie *et al.*, 2014).

A relationship between antioxidant enzymes activity and water stress or salinity tolerance was confirmed by comparison of a tolerant cultivar with a sensitive cultivar in several plant species, such as tomato (Mittova *et al.*, 2002).

Calcium is known to regulate different metabolisms in plants mediating signaling pathways, which modulate gene expression in response to stress and its adaptation (Upadhyaya *et al.*, 2011). Upadhyaya *et al.* (2011), observed POX activity was increased in the stressed plant as compared to controls, but recovering plants showed POX activity increasing after rehydration, which was enhanced by $CaCl_2$ and reported that $CaCl_2$ treatment resulted in increased non enzymatic antioxidant and enhanced activities of enzymatic antioxidant, including SOD, POX and CAT, and thus reduced ROS accumulation and lipid peroxidation ultimately leading to improved post-drought recovery potential in *Camellia sinensis*. Calcium applied alleviation of drought-induced damage has been clarified in numerous plants e.g. *Zoysia japonica* (Xu *et al.*, 2013), and *Phaseolus vulgaris* (Abou El-Yazied, 2011).

Antioxidant activity (AA)

AA was affected significantly by the irrigation treatments, and water deficit stress increased AA, which no significant difference was observed between irrigation 100 and 85%ETc (Table 3, Fig. 4). In present study, the exogenous application of CaL significantly (P<0.05) increased AA of lettuce under different irrigation regimes compared to control plant. The highest AA (93.03%) was observed in CaL 0.75 g L^{-1} under irrigation 85% ETc, however had no significant difference with CaL 1.5 g L^{-1} treatment under irrigation 70% ETc (Fig. 4).

The antioxidant activity in lettuce arises from phenolic compounds, secondary plant products, such as

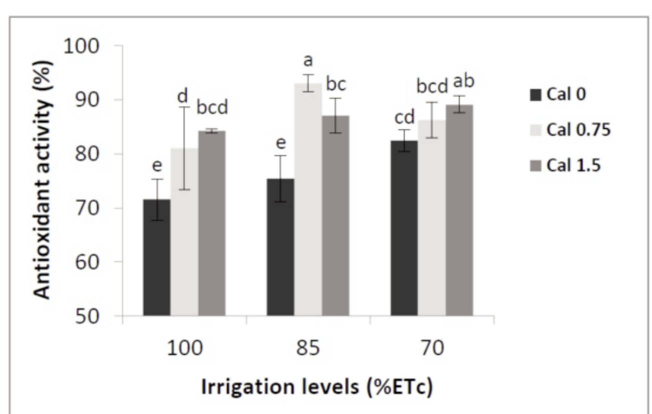

Fig. 4 - Effect of CaL treatments on antioxidant activity of lettuce under deficit irrigation. Values are means with standard errors (n = 3). Data were subjected to two-way ANOVA and different letters mean that values are statistically different P<0.05.

flavonoids and phenols, and also anthocyanin. Also, the antioxidant activity strongly correlated to the presence of efficient oxygen radical scavengers, such as vitamin C and phenolic compounds (Tulipani *et al.*, 2008).

In current study a significant correlation was found between antioxidant activity and anthocyanins, phenols and flavonoids contents; which the anthocyanin, phenolic and flavonoids content and CAT and POX enzymes activity, as well as the total antioxidant activity also increased with increasing water deficit stress and Cal concentration. This finding described that phenolic compounds and anthocyanin, and antioxidant enzyme activity makes an important contribution to the antioxidant capacity in lettuce leaf. Velioglu *et al.* (1998) reported a strong relationship between total phenolic content and antioxidant activity in fresh fruits and vegetables.

Leaf water status (RWC, ELWR)

Based on the findings (Table 3, Figs. 5A, 6), deficit irrigation caused a significant reduction in RWC and ELWR contents. The application of CaL significantly ameliorated relative water content (RWC) and excised leaf water retention (ELWR) contents (Figs. 5B, 6). Mean comparisons of data, displayed that pretreatment with CaL markedly reduced the effects of water deficit stress and also improved ELWR under control irrigation and water deficit stress. The highest value of ELWR content was obtained in plant treated with CaL 1.5 g L^{-1} under irrigation of 85 and 100% ETc.

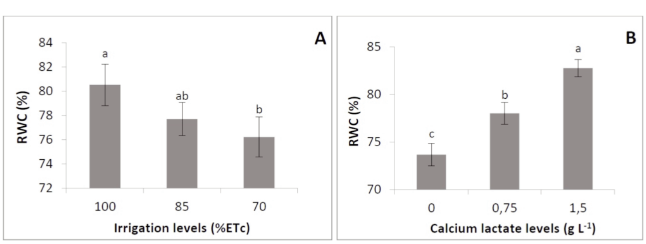

Fig. 5 - Effects of irrigation (A) and CaL (B) treatments on leaf relative water content (RWC) of lettuce. Data were subjected to two-way ANOVA and different letters mean that values are statistically different P<0.05.

RWC and ELWR are among the main physiological criteria that influence plant water relations and have been used for assessing drought tolerance (Xing *et al.*, 2004). Under drought stress, leaf RWC plays an important role in the tolerance of plants to stress by inducing osmotic adjustments due to the accumulation of osmoprotectants (Barnabás *et al.*, 2008; Zhang *et al.*, 2012). The maintenance of a high plant

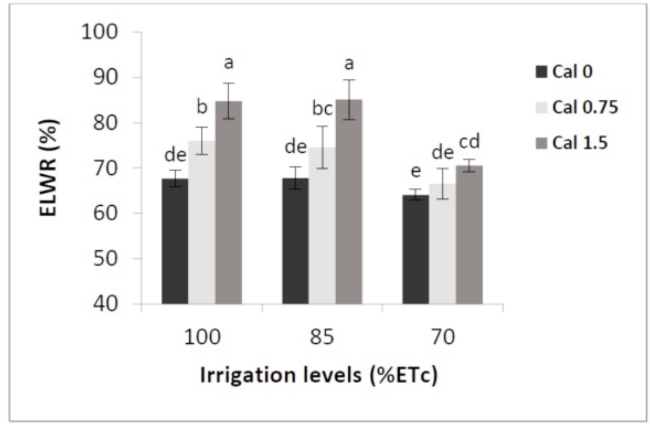

Fig. 6 - Effects of CaL treatment on excised leaf water retention (ELWR) content of lettuce under deficit irrigation. Values are means with standard errors (n= 3). Data were subjected to two-way ANOVA and different letters mean that

water status during stress is a significant defensive mechanism to maintain enough water by minimizing water loss (e.g. caused by stomatal closure, trichomes, reduced leaf area, senescence of older leaves, etc.) and maximizing water uptake (e.g. by increased root growth) (Barnabás *et al.*, 2008). Because of the decrease in leaf area, the accumulation of chlorophyll has increased, but due to high transpiration, the plant loses more water and as a result, the RWC of leaf and consequently photosynthesis decreases (Farooq *et al.*, 2012). Farooqi *et al.* (2000) indicated that RWC of lemongrass leaves decreased in all the cultivars due to drought but after rehydration, RWC gradually increased to pre-stress level, which has also been reported in several crop species such as melon (Mani, 2014). As well as drought stress significantly decreased RWC and ELWR in spring safflower (Balian *et al.*, 2015).

Ruiz-Lozano and Azcon (1997) reported that calcium application significantly increased RWC in lettuce. The results of this research showed that the RWC of leaves increased with calcium application. Increasing relative water content means increasing water holding capacity, which can prevent water loss in leaves in a dry environment (Ma *et al.*, 2005).

Nutrient contents

According to the results (Table 4, Fig. 7), N content in lettuce leaves increased with increasing CaL concentration, indeed the highest value of N was obtained at CaL 1.5 g L^{-1} under irrigation 100 %ETc that had significant difference with deficit irrigation treatments (85 and 70% ETc), whereas in other treatments there were not any significant differences. Mean comparisons of data, showed that deficit irri-

Table 4 - Variance analysis (ANOVA) of effects of calcium lactate on nutrient contents and fresh yield in lettuce under deficit irrigation

Source of variations	df	Mean of Squares						
		N content	P content	K content	Ca content	Mg content	Fresh yield	WUE
Replication	2	0.030	0.008	0.008	0.002	0.004	206838.82	0.380
Irrigation	2	0.208 **	0.111 **	0.153 **	0.621 **	2.157 **	21349043.15 **	3.293 **
Error (a)	4	0.008	0.008	0.003	0.005	0.004	83934.71	0.183
Calcium lactate	2	1.158 **	0.024 NS	0.025 NS	0.291 **	0.095 **	6105376.18 **	10.144 **
Calcium lactate × Irrigation	4	0.116 **	0.006 NS	0.010 NS	0.047 **	0.018 *	413961.66 *	0.317 NS
Error (b)	12	0.012	0.006	0.008	0.004	0.005	101407.46	0.170
Coefficient of Variation (%)	-	1.47	3.48	1.43	2.09	3.4	2.82	2.78

** and * represent significance at the 1 and 5% probability levels, respectively, and NS represents non-significance at p<0.05.

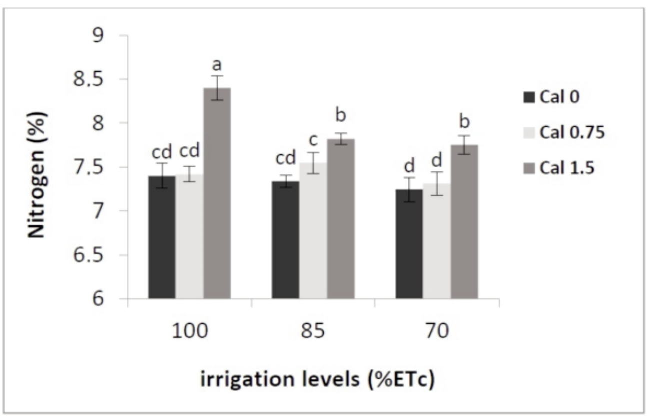

Fig. 7 - Effects of CaL treatments on nitrogen content of lettuce leaves under deficit irrigation. Values are means with standard errors (n= 3). Data were subjected to two-way ANOVA and different letters mean that values are statistically different P<0.05.

gation significantly increased P content in lettuce leaves and decreased K content compared to control irrigation (Table 4, Figs. 8A, 8B).

The Ca content increased with the deficit irrigation treatments, in particular with moderate deficit irrigation (85% ETc). Ca content in lettuce leaves increased in response to higher CaL concentration (Fig. 9A). In fact, the highest value of Ca was

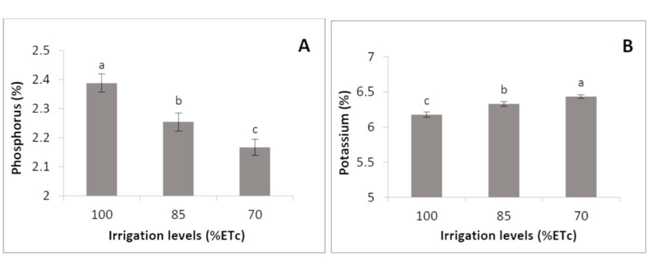

Fig. 8 - Effect of irrigation treatments on phosphorus (A) and potassium (B) contents of lettuce leaves. Values are means with standard errors (n= 3). Data were subjected to two-way ANOVA and different letters mean that values are statistically different P<0.05.

observed in treatments with application of 1.5 g L^{-1} CaL under irrigation 70 and 85% ETc. The overall effect of deficit irrigation on Mg content was negative, with a decrease of 0.79% (Fig. 9B) under deficit irrigation 70% ETc. Mg content in the leaves decreased when the concentration of CaL applied was increased. The lowest value of Mg content was obtained in plant applied with 1.5 g L^{-1} CaL under deficit irrigation 70% ETc.

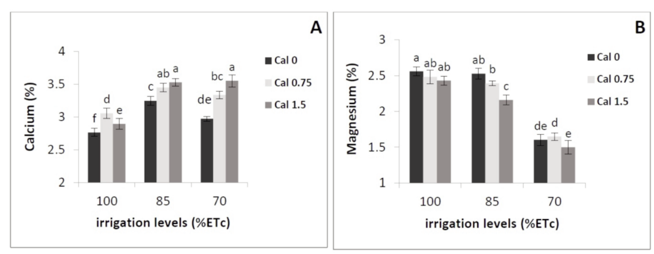

Fig. 9 - Effect of CaL treatments on calcium (A) and magnesium (B) contents of lettuce leaves under deficit irrigation. Values are means with standard errors (n= 3). Data were subjected to two-way ANOVA and different letters mean that values are statistically different P<0.05.

Drought stress and associated reduction in soil moisture can decrease plant nutrient uptake by reducing nutrient supply through mineralization (Sanaullah *et al.*, 2012), and nutrient diffusion in the soil (Chapin III, 1991; Lambers *et al.*, 2008). Drought can depress plant growth by reducing N and P uptake, transport and redistribution (Rouphael *et al.*, 2012). A majority of studies have indicated that plants decrease N and P uptake with a decline in soil moisture (Sardans and Peñuelas, 2012). N uptake was reduced in maize under stress conditions, which indicates that the absorption of nutrients is limited in conditions of water deficit stress, which may be reduced due to reduced transpiration rate, active transfer and membrane permeability (Naeem *et al.*, 2017). Owing to a reduction in stomatal conductance, photosynthesis and transpiration rates also decrease, and CO_2 assimilation rates progressively decline in response to drought (Farooq *et al.*, 2012). Therefore, drought effects on plant may depend on the reduction in N and P uptake relative to the decrease in CO_2 assimilation (He and Dijkstra, 2014).

Based on the current findings, increasing K and Ca contents and decreasing Mg content of lettuce leaves under water deficit stress as compared to well-watered conditions that also reported by Tadayyon *et al.* (2018) in castor plants. Potassium has a positive correlation with the physiological effects of plants, such as water use efficiency, stomatal control, air and underground body biomass, and is likely to play an important role in photosynthesis (Sardans *et al.*, 2012). Increasing K content of leaves with decreasing irrigation rate maybe due to role of this cation in the regulation of osmotic pressure and stomatal control, (Zhao *et al.*, 2000). Nahar and Gretzmacher (2002) reported that with increasing deficit irrigation, Mg concentration in tomato tissues decreased, which is similar to results of the present study.

The same results were reported from other authors, that high concentrations of Ca often result in increased leaf Ca along with a marked reduction in leaf Mg (Nassery *et al.*, 1979; Borghesi *et al.*, 2011). As well as, Naeem *et al.* (2018) revealed that concentration of macronutrients (N, K, Ca) in maize grains was markedly improved by foliar supply of calcium which indicates its synergistic effect on uptake and translocation of these nutrients. Tuna *et al.* (2007) also observed leaf N, K and Ca content increased in tomato plants supplemented with calcium under stress conditions.

In safflower, by decreasing soil moisture K and Mg content decreased. Following this reduction, there was a significant increase in calcium concentration, which is justified by the antagonistic relationship between Ca and Mg (Vafaie *et al.*, 2013). Morard *et al.* (1996) reported an intense antagonistic relationship between Ca and Mg, that Mg transfer to leaves was affected by calcium.

Fresh yield

Lettuce plants grown under control and deficit irrigation conditions exhibited significant differences between CaL treatments in fresh yield (Table 4, Fig. 10). Water deficit stress caused significant reductions in yield. In fact, deficit irrigated plants showed a 6.8 and 15.8% decrease in fresh yield, respectively. As the results showed, with increasing CaL concentrations, lettuce yield significantly increased and reached to highest value (1.37 kg.m^{-2}) at CaL 1.5 g L^{-1} under irrigation 100% ETc (Fig. 10).

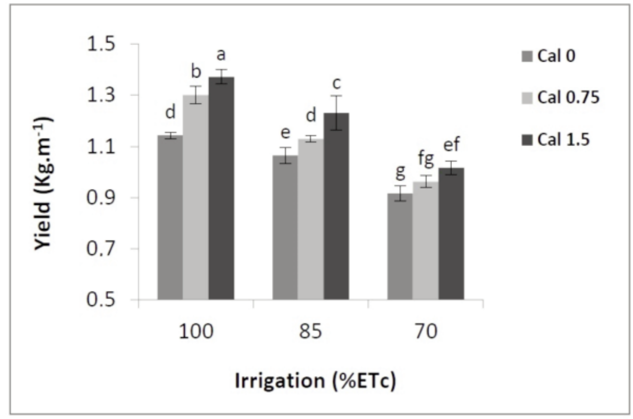

Fig. 10 - Effect of CaL treatments on fresh yield of lettuce under deficit irrigation. Values are means with standard errors (n= 3). Data were subjected to two-way ANOVA and different letters mean that values are statistically different P<0.05.

Lettuce is one of the leaf-edible vegetables that it is extremely sensitive to water deficit stress due to shallow root system (Sabedze and Wahome, 2010). Our results are in agreement with many open-field studies on lettuce (Jiménez-Arias, 2019) and lettuce (Sayyari *et al.*, 2013). Deficit irrigation defined as a practice that applies water below full crop-water requirements, deliberately exposes plants to a certain level of moisture stress. It is well known that drought stress results in dehydration of the cell and osmotic imbalance that impairs numerous metabolic and physiological processes in plants (Mahajan and Tuteja, 2005). Reduction in fresh yield of lettuce with deficit irrigation might be attributed to the suppression of cell division and expansion, and growth due to the low turgor pressure and also closure of stomata leaf and more leaf senescence under drought stress (Sayyari *et al.*, 2013).

Foliar application of CaL enhanced fresh yield of lettuce. Naeem *et al.* (2013) reported that crop productivity and photosynthetic efficiency in *Senna occidentalis* was improved under Ca application. With low calcium availability, a reduction in bean plant height, leaf area and shoot and root growth has been reported (Leal and Prado, 2008). Foliar applied of chelated calcium enhanced the seed yield and related attributes in common bean under water-deficit conditions (Abou El-Yazied, 2011).

Water use efficiency (WUE)

According to the results (Table 4, Figs. 11A, 11B), irrigation and CaL treatments significantly affected WUE, but their interaction showed no significant differences. Water deficit stress significantly increased WUE and the highest WUE was recorded in 70% ETc deficit-irrigated plants that had no significant difference with deficit irrigation 85% ETc (Fig 8A). WUE increased with increasing CaL concentration and the highest WUE (15.8 kg m^{-3}) was obtained at 1.5 g L^{-1} CaL (Fig. 8B).

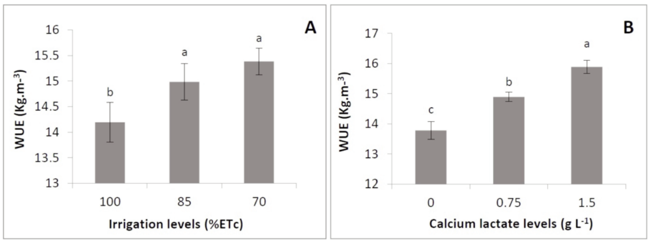

Fig. 10 - Effect of CaL treatments on fresh yield of lettuce under deficit irrigation. Values are means with standard errors (n= 3). Data were subjected to two-way ANOVA and different letters mean that values are statistically different P<0.05.

WUE, the physiological parameter of crop, describes the relationship between plant water use and dry matter production (Cai *et al.*, 2012). Regarding water resource constraints, it is essential to find ways to preserve water and increase water use efficiency in plants (Topcu *et al.*, 2007; Alenazi *et al.*, 2015). The highest WUE value was determined in 70% ETc irrigation. It was calculated that WUE values increased with the decrease in the amount of water. These results are similar to the previous finding of Şimşek *et al.* (2004), who reported that the maximum WUE for watermelon was obtained with low irrigation. With increased WUE, there is a greater biomass production per amount of water transpired, and less water is needed for growth and development (Nemali and van Iersel, 2008).

WUE is strongly related to photosynthetic activity and transpiration efficiency, and can be affected by irrigation (Monneveux *et al.*, 2006). Ca is directly involved in photosynthesis processes, and its deficit reduces the plant's biomass by reducing the efficiency of carboxylation and photosynthesis (Alarcon *et al.*, 1999). The results of the current experiment showed that N content was increased with Ca application, which increasing N content leads to increase dry matter production as well as the WUE. Therefore, Ca maybe increased the WUE by increasing the amount of N and Ca in lettuce plants.

4. Conclusions

The results obtained in this investigation proposed that lettuce is sensitive to water deficit stress during their entire growing period. Hence, it could be concluded that under water deficit, decrease in the relative water content in the leaves is related to the decrease markedly in the fresh yield. Application of CaL showed a clearly protective effect on yield of plants under water deficit stress. The result also revealed that treating the plants with calcium lactate led to increase N and Ca accumulation. CaL appears to promote water deficit tolerance by acting at different levels: leaf water status and antioxidant defenses, without evidence of toxic effects on the soil. Finally, calcium application was determined as an optimum strategy for most desirable traits that decrease stresses effect. However, further studies may be required to determine CaL application rates for optimal response of growth, yield and nutrients uptake.

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