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Changes in Alfalfa (*Medicago sativa* L.) Growth and Biochemical Traits in Response to Silicon Application under Different Irrigation Regime

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ABSTRACT

To decrease adverse effects of water deficit is foliar application with chemical agents such as silicon. However, there is low information on the influence of silicon on alfalfa under drought stress conditions. Thus, the current study was conducted to assessment the effect of different silicon concentrations (0, 1 and 2 mili Molar concentration) on alfalfa growth trend and biochemical traits, which grown under five level of irrigation regimes (100% as control, 85%, 70%, 55% and 40% field capacity; FC) according factorial experiment based on completely randomized design with four replication. The results showed that plant height, dry weight, chlorophyll a and b were reduced in response to water deficit; while water deficit increased chlorophyll a to b ratio as well as activity of superoxide dismutase and catalase in alfalfa crop. Also, water deficit up to 55% FC increased leaf silicon concentration, free proline, total soluble protein and peroxidase; however, severe water stress reduced them. Despite negative impact of water deficit, silicon application increased plant height, dry weight, soluble protein and three antioxidant enzymes as well as leaf silicon concentration. The positive effect of silicon on the most of the measured traits was greater at 2 mM than 1mM concentration, which might be due to higher silicon absorption at higher concentration. Alleviation ability of silicon was greater under severe water deficit compared to no or light water stress conditions. Our results suggested that although water deficit reduced growth and caused some changes in biochemical traits; silicon application, especially at 2mM concentration can be advised to alleviate some of the negative impact of water deficit.

Keywords: *Antioxidant enzyme, Mineral compound, Water deficit.*

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INTRODUCTION

Alfalfa a leguminous plant often suffers from a significant reduction in growth and yield under severe drought stress (Wang and Han, 2007). Alfalfa is an essential hay product which grows in arid and semi-arid regions. Because of its depth and straight roots, this species is able to absorb even depth waters about 5 m depth and more. This advantage saves plant's life in long term drought (Ingram and Bartles, 1996). Plants often encounter unfavorable conditions, which interrupts their growth and productivity. Among the various abiotic stresses, water deficit is the major factor that limits crop productivity worldwide (Tardieu *et al*., 2014). Plants respond and adapt to water deficit by the accumulation of osmolytes and proteins specifically involved in stress tolerance. These molecules are accumulated in plant cells in response to drought stress and act as osmotic balancing agents. In addition of roles in osmotic homeostasis, these organic solutes are free radical scavengers and protect cell structures and membranes against desiccation damages (Krasensky and Jonak, 2012). Synthesis of osmo protectants, osmolytes or compatible solutes including amino acids particularly proline and soluble carbohydrates is one of the mechanisms for adaptation to water deficit (Verbruggen and Hermans, 2008). Water deficit usually causes reduction in crop productivity and inhibits the photosynthesis of plants, which causes changes in chlorophyll contents and components and damages to photosynthetic apparatus (Gong *et al*., 2005, Maghsoudi *et al*., 2015). Water deficit changes the activity of antioxidant enzymes and the accumulation of sugars and proteins in the plant (Zhu *et al*., 2004; Gong *et al*., 2005), resulting in lower plant growth and yield. Plant responses to water deficit have been associated with proline accumulation, which may represent a water loss regulatory mechanism by reducing cell water potential (Ashraf and Foolad, 2007). Proline is a non-protein amino acid that is formed in the leaf tissues of plants exposed to water deficit and, together with sugar, is readily metabolized in leaves after recovery from water stress (Gideon *et al*., 2016). Enhancing generation of reactive oxygen species (ROS) and accumulation of ROS such as superoxide (O_2^-), hydroxyl radicals (.OH) and hydrogen peroxide (H_2O_2) are of the earliest plant responses to water deficit (Ashraf and Foolad, 2007). Such ROS accumulations under water deficit may lead to protein degradation, lipid peroxidation, pigment, and many other deleterious effects (Saed-Moucheshi *et al*., 2014). To protect cells from such deleterious effects, plants increase activities of antioxidant enzymes in the cytosol, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), which are believed to counteract ROS (Foyer and Noctor, 2005). Among mineral compounds, application of silicon (Liang *et al*., 2007) and selenium (Feng *et al*., 2013) attracted much more attention. Several researchers have reported that silicon plays an important role in plant resistance to abiotic stresses such as water deficit (Zhu *et al*., 2004; Gong *et al*., 2005; Hattori *et al*., 2005; Gunes *et al*., 2008, Maghsoudi *et al*., 2015). Silicon (Si) is the second most abundant element existing on earth (Silva *et al.,* 2012). Although it is not considered as an essential element, nevertheless, there is increasing evidence regarding its positive effects on plant growth and development (Karmollachaab *et al.,* 2014). Si acts as a mechanical or physical obstacle in plants and not only acts as cell wall strengthening, but is also actively involved in many physiological processes. Studies have displayed that positive impacts of Si are more prominent under stressful conditions as it can increase plant defense systems against low (Epstein, 1999) and high temperature (Hattori *et al.*, 2005), UV-radiation (Shen *et al.*, 2010), salinity (Biel *et al.*, 2008) and heavy metal toxicity (Shi *et al.*, 2005). Remus-Borel *et al.* (2005) reported mechanism was that Si might decrease the oxidative damage in plants subjected to environmental stresses. Si is also found to enhance water stress tolerance in plants by retaining leaf water potential, leaves erectness, stomatal conductance, structure of xylem vessels under high transpiration rates, and photosynthetic activity (Gong *et al.*, 2003). Si can diminish the electrolyte leakage from plant leaves and therefore raised photosynthetic activity in plants grown under water stress conditions (Epstein, 1999). Gao *et al.* (2006) suggested that Si influences stomata movement and, therefore, affects transpiration rate through stomata. Gong *et al.* (2003) reported greater water use efficiency by application of Si in wheat. Matoh *et al.* (1991) found that application of Si led to formation of a silica cuticle layer on epidermal tissue of leaf, which is responsible for greater leaf water potential under water deficit conditions. Beneficial effects of silicon have demonstrated in some species. As suggested by Hattori *et al*. (2005) silicon increase plant resistance to drought stress, which it may be due to increasing in capacity of the plant in absorbing water from the soil. On the other hand this role can be associated with an increase in the action of antioxidant enzymes, a reduction in the oxidative damage of functional molecules and membranes, and maintenance of many physiological as well as photosynthetic processes, under drought stress conditions (Carlos *et al*., 2009). Asgharipour and Mosapour (2016) reported silicon application increased concentration of proline, soluble sugars in seed, leaf area index, seed yield, essential oil percentage, concentration of sodium and potassium in fennel seed by 24, 13, 20, 17, 31, 41, 24 and 9%, respectively, over those of the control (non-application of Si). The greatest essential oil percentage (2.17%) was achieved in fennel plant by sprayed with 7.5 mM of Si under mild water deficit. These results suggested that Si spraying improved growth and physiological indices hence could increase the ability of plants to resistance water stress. Gong *et al*. (2005) suggested that the improvement of silicon on drought tolerance of wheat plants was associated with the increase of antioxidant defense abilities, therefore alleviating oxidative damage of cellular functional molecules induced by over produced reactive oxygen species (ROS) under drought and maintaining many physiological processes of stressed plants. Silicon application decreased the transpiration rate and electrolyte leakage from leaves (De Souza *et al*., 2015); also enhanced dry matter accumulation and stomatal conductance (Hattori *et al*., 2005), thus preventing the structural and functional deterioration of cell membrane of plant under drought stress conditions (Carlos *et al*., 2009). Role of silicon in reduction of adverse impact of drought stress documented in cucumber [*Cucumis sativus* L. (Zhu *et al*., 2004)], wheat [*Triticum aestivum* L. (Gong *et al*., 2005, Maghsoudi *et al*., 2015)], sorghum [*Sorghum bicolor* L. (Hattori, *et al*., 2005)], barley [*Hordeum vulgare* L. (Gunes *et al*., 2007a)], Chickpea [*Cicer arietinum* L. (Gunes *et al*., 2007b)], Sunflower [*Helianthus annuus* L. (Gunes *et al*., 2008)] and potato [*Solanum tuberosum* L. (Carlos *et al*., 2009)]. The purpose of this study was to investigate the effects of Si application on agro-physiological parameters such as plant height, dry weight, leaf silicon concentration, chlorophyll content, antioxidant enzymes (peroxidase, super oxide dismutase and catalase), amount of osmotic regulator (proline and total soluble protein) of alfalfa under different irrigation regimes.

MATERIALS AND METHODS

Field and treatments information

This research was conducted to evaluate the changes in alfalfa growth trend and biochemical traits in response to silicon application under water deficit conditions at controlled glass house Conditions College of agriculture, Shiraz University, Shiraz, Iran during 2015. The treatments were consisted of three level of silicon concentrations (0, 1 and 2 mili Molar concentration) and five irrigation regimes: 100 (as control), 85, 70, 55 and 40% of field capacity (FC); which arranged in a factorial experiment based on completely randomized design (CRD) with four replications. The irrigation regimes were applied based on F.C. percentage of soil (100%, 85%, 70%, 55% and 40%). Field capacity was determined using pressure plate method (22.5% by weight). Throughout growing season, the needed water for each pot was determined by regular weighing and was added to each pot. The irrigation regimes were imposed after complete establishment, however, before that; pots were irrigated as 100% FC. Silicon was applied slightly after the beginning of 3-4 leaf stage as foliar application using a precision sprayer (AH-15N, Daegu, Korea; 0.3 MPa pressure) with the rate of 500 $l.ha^{-1}$. The pots not receiving silicon was similarly sprayed with equivalent volume of distilled water.

Lab management

Alfalfa plants were grown in 5 liter plastic pots filled with field soil, washed sand and humus by 2:1:1 weight ratio. The physical and chemical properties of the soil are shown in Table 1. Before sowing, the seeds were sterilized using ethanol 70% and mercuric chloride 0.1% for 5 minutes. The uniform and viable scarified seeds of alfalfa (cultivar Bami) were sown at 2-3 cm depth on October, 10. Seedling emerged 4-5 days after planting (DAP). Each pot was considered as an experimental unit. The soil properties were mentioned in table 1.

Table 1. Physical and chemical properties of soil used in the experiment

N (%)	O.C (%)	P (mg kg^{-1})	K (mg kg^{-1})	EC ($ds.m^{-1}$)	pH	F.C. (%)	Texture
0.065	0.83	18.9	232.1	1.02	7.05	22.5	sandy clay

N: Nitrogen, OC: organic carbon, P: Phosphorus, K: Potassium, EC, Electrical conductivity, pH: Acidity, F.C.: Field capacity.

Traits measure

At the 70 after plantings, four plants in each pot were selected randomly and were dried in a forced-air oven at 70°C for 48 h, to determine dry weight. The plant height was measured from the soil surface to the highest free-standing point of the crown. The top fully expanded leaves of three randomly selected plants in each pot were individually harvested, immediately frozen in liquid nitrogen, freeze-dried, and stored at -80 °C for biochemical measurements. The content of free proline was determined using a spectrophotometer according to the acid-ninhydrin method (Bates *et al*., 1973) with minor modifications. Total soluble protein was measured according to Bradford (1976) using bovine serum albumin as a standard. The activity of superoxide dismutase (SOD) was measured according to the method described by Dhindsa *et al.* (1980). The peroxidase (POD) and catalase (CAT) activity were assayed according to Polle *et al.* (1994) and (Aebi 1984) methods, respectively. Leaf chlorophyll *a* and *b* contents were extracted and measured according to Lichtenthaler and Wellburn, (1983) method. Leaf silicon concentration was assayed according to Elliott and Snyder (1991).

Statistical analysis

Statistical analysis (ANOVA) was applied with using the by the SAS software (Ver. 9.1). The differences between treatment means were assessed by least significant difference (LSD) test at 1% probability level. Also correlation coefficients were calculated by Minitab software (Ver. 16).

RESULT AND DISCUSSION

Plant height (PH)

Plant height was significantly affected by different level of irrigation regime and silicon concentration at 1% and 5% probability level, respectively, but interaction effect of treatments was not significant (Table 2). Mean comparison result indicated that in different irrigation regime the maximum plant height (18 cm) was observed in 100% FC and the lowest one (5.66 cm) was found in 40% FC treatment, also between different concentrations of silicon maximum plant height (14.20 cm) was obtained for 2 mM concentration and minimum of that (11.80 cm) was for control (non-silicon application) treatment. Application of silicon especially for 2 mM concentration significantly increased plant height compare to control (20.33%) (Table 3). Water deficit leads to the perturbation at all or some of physiological and biochemical processes and consequently reduces plant growth and yield (Gideon *et al.*, 2016). In this research, plant height was reduced at 40% FC treatment (68%) than to control. It is proven that Si through modification of plant water relation stimulates cell division and cell elongation (Na and Jiashu, 2001) boosts plant immune system (Liang *et al.*, 2003) and enhances plant growth. The sharp decline in seed yield under water stress could be attributed to the negative impact of water deficit on growth and yield attributes, because stomatal closure and reduced turgidity in water deficit conditions led to reduce growth and yield.

Plant dry weight (PDW)

Result of analysis of variance showed effect of different level of irrigation regime and silicon concentration on plant dry weight was significant at 1% probability level, but interaction effect of treatments was not significant (Table 2). According mean comparison result the maximum and the minimum amount of plant dry weight between different levels of irrigation regime belonged to 100% FC (1.25 gr) and 40% FC (0.40 gr), in other word dry weight decreased 32% by increase water deficit. Also maximum plant dry weight (1.03 gr) was obtained for 2 mM concentration and minimum of that (0.66 gr) was for control treatment (Table 3). Plant dry weight was increased in both silicon treatments, so silicon application at 1 and 2 mM led to 48% and 56% increase in PDW compared to control. Another researcher suggested that silicon could increase drought tolerance of plants (Hattori *et al.*, 2005; Gong *et al.*, 2008; De Souza *et al.*, 2015; Maghsoudi *et al.*, 2015). Aranjuelo *et al.* (2001) reported that with the increase in water deficit stress, alfalfa dry forage yield decreased due to the decrease in photosynthesis which resulted from the decrease in leaf area and reduction of RUBISCO enzyme activity. In plant leaves the most of silicon remains in the apoplasm mainly in the outer walls of the epidermal cells on both surfaces, so it will decrease the water loss by stomatal transpiration of leaves (Gong *et al*., 2008; Maghsoudi *et al*., 2015). Shu and Liu (2001) also found that stress decreased crop dry materials, water content and transpiration rate, which were all increased by addition of silicon, and with the increase of silicon concentration, the improvement was better.

Table 2. Analysis of variance of the effect of different level of irrigation regime and silicon concentration on measured traits

S.O.V	df	PH	PDW	LSiC	Chl. *a*	Chl. *b*	Chl. *a/b*
Different level of irrigation regime (I.R)	4	119.80**	1.25**	0.106**	331.45**	21.49**	1.78**
Different concentration of Silicon (Si)	2	63.40*	1.60**	0.005*	42.12^{ns}	5.95^{ns}	0.47^{ns}
I.R*Si	8	4.40^{ns}	0.03^{ns}	0.002^{ns}	7.59^{ns}	1.35^{ns}	0.37^{ns}
Error	45	3.38	0.01	0.001	42.39	3.32	0.12
CV (%)	-	13.93	11.78	9.12	13.17	12.19	8.81

PH: plant height; **PDW**: Plant dry weight; **LSiC**: Leaf silicon concentration; **Chl. *a***: Chlorophyll *a* concentration; **Chl. *b***: Chlorophyll *b* concentration; **Chl. *a/b***: chlorophyll *a* to chlorophyll *b* ratio.
ns, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

Continue Table 2.

S.O.V	df	FP	TSP	POD	SOD	CAT
Different level of irrigation regime (I.R)	4	8.41**	13.49**	44.71**	8.98**	13.29**
Different concentration of Silicon (Si)	2	3.17*	4.18*	31.06**	5.42*	18.54**
I.R*Si	8	0.57^{ns}	0.82^{ns}	20.73*	1.719^{ns}	19.42**
Error	45	1.02	0.80	4.77	1.33	1.67
CV (%)	-	11.98	9.87	13.39	7.74	11.55

FP: Free proline content; **TSP**: Total protein solute; **POD**: Peroxidase activity; **SOD**: Superoxide dismutase activity; **CAT**: Catalase activity.
ns, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

Leaf silicon concentration (LSiC)

Assessment result of analysis of variance indicated effect of different level of irrigation regime and silicon concentration on LSiC was significant at 1% and 5% probability level, respectively, but interaction effect of treatments was not significant (Table 2). Evaluation mean comparison result of different level of irrigation regime revealed the maximum LSiC (4.49 mg.gr^{-1}) was noted for 55% FC and minimum of that (2.31 mg.gr^{-1}) belonged to 100% FC treatment. Also among different concentrations of silicon the highest value of LSiC was belonged to the 2 mM concentration (4.01 mg.gr^{-1}) and the lowest one was found in control treatment as 3.02 mg.gr^{-1}. Water deficit up to 55% FC increased LSiC; while, it was reduced in 40% FC treatment. Although so silicon application at 1 and 2 mM silicon concentration than to control led to increase LSiC, 17.88 and 32.78% respectively (Table 3). Asgharipour and Mosapour (2016) confirmed that result. Maghsoudi *et al.* (2015) indicated that accumulated silicon in plants has a function of regulating water loss. Some researchers also believed that accumulation of silicon could led to the formation of a double cuticle silicon layer, which through the reduction of transpiration, leads to a decrease in water requirement (Gao *et al.*, 2004; De Souza *et al.*, 2015). In general, plants accumulating silicon would be able to form the same amount of dry matter with less water consumption.

Table 3. Mean comparison effect of different level of irrigation regime and silicon concentration on measured traits

Treatment	PH (cm)	PDW (gr)	LSiC ($mg.gr^{-1}$)	Chl. *a* ($mg.gr^{-1}$)	Chl. *b* ($mg.gr^{-1}$)	Chl. *a/b*	FP ($\mu mol.gr^{-1}$)	TSP ($mg.gr^{-1}$)	SOD ($U.mg.protein^{-1}$)
Irrigation regime									
100% FC	18.00^{*a}	1.25^{a}	2.31^{c}	64.63a	25.30a	2.59c	5.73^{c}	5.88^{c}	11.90^{b}
85 % FC	16.33^{ab}	1.23^{ab}	3.41^{b}	62.60a	21.63b	3.03bc	8.10^{b}	10.56^{a}	11.96^{b}
70 % FC	15.00^{b}	0.93^{b}	4.3^{ab}	48.62b	12.83c	3.85b	10.11^{a}	10.60^{a}	16.30^{a}
55 % FC	11.00^{c}	0.63^{c}	4.49^{a}	35.55c	9.00d	4.08b	9.97^{ab}	9.40^{ab}	16.70^{a}
40 % FC	5.66^{d}	0.40^{d}	3.14^{bc}	33.66c	6.00e	6.13a	8.26^{b}	8.86^{b}	17.70^{a}
Silicon concentration									
Control	11.80^{b}	0.66^{b}	3.02^{b}	-	-	-	8.03^{b}	8.08^{b}	12.90^{b}
1 mM	13.60^{ab}	0.98^{ab}	3.56^{ab}	-	-	-	8.35^{ab}	9.31^{ab}	16.12^{a}
2 mM	14.20^{a}	1.03^{a}	4.01^{a}	-	-	-	8.93^{a}	9.80^{a}	15.72^{a}

PH: Plant height, **PDW**: Plant dry weight, **LSiC**: Leaf silicon concentration, **Chl. *a***: *C*hlorophyll *a* concentration; **Chl. *b***: Chlorophyll *b* concentration; **Chl. *a/b***: chlorophyll *a* to chlorophyll *b* ratio, **FP**: Free proline; **TSP**: Total soluble protein, **SOD**: Superoxide dismutase.
*Means in each column for each treatment with similar letter(s) are not significantly different via LSD (0.01).

Chlorophyll *a* (Chl. *a*) and Chlorophyll *b* (Chl. *b*)

Result of analysis of variance showed effect of different level of irrigation regime on Chlorophyll a and b traits was significant at 1% probability level, but effect of different concentration of silicon and interaction effect of treatments was not significant (Table 2). Chlorophyll *a* concentration showed 24%, 44% and 48% reductions under 70%, 55% and 40% FC treatments, respectively. Also these reductions were 49%, 65% and 77% for Chl. *b* concentration, respectively. In other words both of Chl. *a* and Chl. *b* were decreased by water deficit, however, Chl *b* was more damaged (Table 3). Another researchers such as Hajiboland *et al.* (2015) reported same result.

Chlorophyll *a* to chlorophyll *b* ratio (Chl. *a/b*)

Result of analysis of variance revealed effect of different level of irrigation regime on Chl. *a/b* was significant at 1% probability level, but effect of different concentration of silicon and interaction effect of treatments was not significant (Table 2). Thus water deficit increased Chl. *a/b* ratio, so that these increasing were 16%, 48%, 57% and 136% in 85%, 70%%, 55 and 40% FC treatments, respectively (Table 3). Our findings are agreement with the results Nyachiro *et al*. (2001), who described a significant decrease of chlorophyll *a* and *b* caused by water deficit in wheat cultivars. A decrease in chlorophyll concentration with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Liang *et al*., 2003).

Free proline (FP)

Free proline content was significantly affected by different level of irrigation regime and silicon concentration at 1% and 5% probability level, respectively, but interaction effect of treatments was not significant (Table 2). Mean comparison result showed in different level of irrigation regime the maximum free proline (10.11 $\mu mol.gr^{-1}$) was observed in 70% FC and the lowest one (5.73 $\mu mol.gr^{-1}$) was found in 50% FC treatment, also among different concentration of silicon maximum free proline (8.93 $\mu mol.gr^{-1}$) was obtained for 2 mM concentration and minimum of that (8.03 $\mu mol.gr^{-1}$) was for control treatment (Table 3). Proline accumulation in response to water deficit has been widely reported and may play a role in stress adaptation within the cell. Proline accumulation has a positive relationship with increased resistance to water deficit (Saneoka *et al.*, 2004). In this experiment also water deficit increased concentration of proline. There were similar reports about the impact of water deficit on barley (Gunes *et al*., 2007a), chickpea (Gunes *et al*., 2007b) and pepper plants (Gideon *et al*., 2016). In this experiment, Si through increasing proline concentration increased osmotic adjustment during the water stress. There are similar reports regarding increasing in proline content following Si application in barley (Haddad and Moshiri, 2008) and borage (Gagoonani *et al*., 2011).

Total soluble protein (TSP)

The effect of different level of irrigation regime and silicon concentration on total soluble protein was significant at 1% and 5% probability level, respectively, but interaction effect of treatments was not significant (Table 2). Water deficit increased FP and TSP up to 55% FC, and more level of water deficit decreased them. Increasing in FP due to 85%, 70%, 55% and 40% FC treatments were 41%, 76%, 73% and 44%, respectively. These increasing were 79%, 80%, 59% and 50% for TSP, respectively. In the present study the results obtained with higher soluble protein content are in agreement with the findings of Pirasteh-Anosheh *et al.* (2012), who also reported a high protein content in drought stressed wheat plants. Silicon application increased TSP by 13% and 17% at 1 and 2 mM concentrations, respectively (Table 3). Silicon application led to increasing in protein content. In maize plant, water stress affects protein metabolism and nucleic acids and through this plantlet's growth decreases (Safarnejad, 2008). Zhu *et al*. (2004) and Gong *et al*. (2005) observed higher protein concentrations in cucumber and wheat plants that received silicon than in plants that did not receive Silicon when exposed to saline and water stress, respectively.

Peroxidase (POD), Super oxide dismutase (SOD) and Catalase (CAT)

According to result of analysis of variance different level of irrigation regime and silicon concentration had significant effect on activity of antioxidant enzymes, including: POD, SOD and CAT. Furthermore, interaction effect of treatments on activity of POD and CAT was significant (Table 2). Water deficit at 70% and 55% FC increased POD activity; however, 40% FC treatment reduced it. Environmental stresses increase the formation of reactive oxygen species (ROS) that oxidizes photosynthetic pigments, membrane lipids, proteins and nucleic acids. Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater tolerance to oxidative damage (Saed-Moucheshi *et al*., 2014). In stressed barley, it was found that addition of silicon increased the SOD activity in the leaves and SOD, POD, CAT activities in the roots (Liang *et al*., 2003). In all water deficit treatments, silicon application increased activity of POD. This positive effect was more in 2 than to 1 mM silicon concentration only at non stress conditions, and there was no significant difference between these two concentrations in other water regimes (Fig. 1).

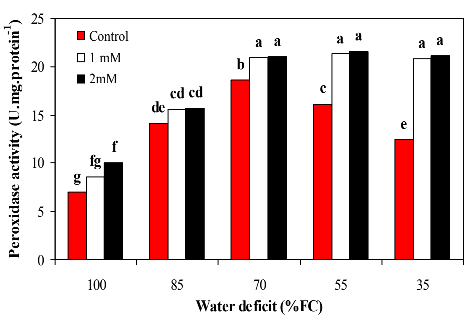

Fig. 1. Interaction effect of different level of silicon and different irrigation regime on peroxidase activity under via LSD test at 1% probability level.

On the other hand, activity of SOD was kept unchanged up to 75% FC and 75% FC and more water stress enhanced SOD activity. Silicon application increased SOD activity at both 1 and 2mM concentrations, and these two concentrations showed no significant difference (Table 3). Water deficit at 85% FC and more water stress increased CAT activity. In no and light stress, silicon application had no significant effect on activity of CAT; however, in more water deficit (i.e. 70, 55 and 40% FC) silicon had positive effect on CAT activity. In such conditions, CAT activity was more in 2 than to 1mM silicon concentration (Fig. 2).

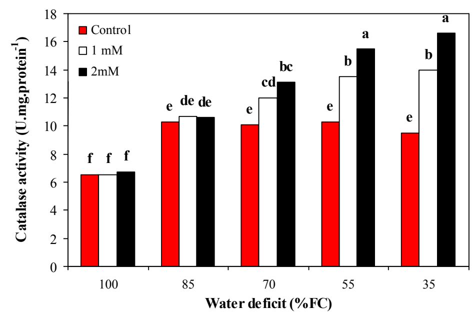

Fig. 2. Interaction effect of different level of silicon and different irrigation regime on catalase activity under via LSD test at 1% probability level.

In the present research, compared with the non-silicon treatment, application of silicon could increase the antioxidant defense abilities. It was suggested that higher activities of antioxidant enzymes are induced by addition of silicon might protect plant tissues from membrane oxidative damage under salt stress (Liang *et al*., 2003). It has been reported that silicon application could alleviated some of negative effects of stress through enhanced antioxidant enzymes activity in the plants of wheat (Maghsoudi et al., 2015), barley (Liang *et al*., 2003), grasses (De Souza *et al*., 2015), cucumber (Zhu *et al*., 2004) and wheat (Gong *et al*., 2008). Abedi and Pakniyat (2010) by study responses of antioxidant enzyme activities under drought stress in rapeseed cultivars observed a significant increase was observed in SOD and POD activities under 30% FC (110% and 148%, respectively) when compared to the control (100 % FC).

Correlation between plant dry weight and other traits

As shown in this research, PDW was significantly associated with plant height (0.791**), biochemical attributes including: chlorophyll *a* (0.847**) and *b* (0.826**) and their ratio (-0.889**), free proline (-0.551**) and three antioxidant enzymes (Peroxidase: -0.502*, Superoxide dismutase: -0.516* and Catalase (-0.841**) (Table 4).

Table 4. Correlation coefficients between plant dry weight and other traits

Traits	Plant height	Leaf silicon concentration	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a*/*b* ratio
plant dry weight	0.791**	-0.091^{ns}	0.847**	0.826**	-0.889**
Traits	**Free proline**	**Soluble protein**	**Peroxidase**	**Superoxide dismutase**	**Catalase**
plant dry weight	-0.551**	0.101 ^{ns}	-0.502*	-0.516*	-0.841**

- ns, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

CONCLUSION

The results showed that water deficit reduced growth and chlorophylls content and enhanced chlorophyll a to b ratio. Activity of antioxidant enzymes in alfalfa plants were also increased by water deficit. On the other hand, silicon concentration, free proline and total soluble protein were increased upon water deficit conditions up to 55% F.C; however, severe water deficit reduced these variables. Despite negative impact of water deficit, silicon application increased growth and improved biochemical traits, especially at 2 mM compared to 1 mM concentration, due to higher silicon absorption. In general, it was concluded that silicon application, especially at 2 mM could ameliorate some negative impacts of salt stress in alfalfa.

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