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Research Paper

Exogenous proline improves osmoregulation, physiological functions, essential oil, and seed yield of fennel

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ABSTRACT

Proline is an amino acid with pivotal role in plant responses to environmental constraints. Effects of its foliar application (20 mM) on physiological functions of 12 fennel (*Foeniculum vulgare* Mill.) genotypes at the presence of different moisture conditions (non-stress control and drought stress) was studied in a 2-year field study. Drought led to notable increases in mean leaf polyphenol, proline and total soluble carbohydrates and essential oil concentrations, despite decreases in carotenoids and chlorophyll concentrations, leaf water potential, relative water content, plant above-ground dry mass and water use efficiency. Exogenous proline resulted in significant increases in mean carotenoids, polyphenol, chlorophyll, proline, total soluble carbohydrates and essential oil concentrations and relative water content, but it caused a decrease in leaf water potential. Proline amendment positively affected plant water relations, due mostly to enhancement in osmoregulation, as certain genotypes with greater mean leaf proline concentration and relative water content appeared to produce greater above-ground dry mass, when exposed to the external proline. Ameliorative effects of exogenous proline tended to be greater in drought-stressed plants, as it led to the enhancement of chlorophyll concentration and relative water content of fennel in drought conditions. Genotypes Urmia and Yazd were found to be able to withstand better against drought and benefit more from external proline. Our findings suggest that while indigenous proline is the most sensitive osmoticum in fennel's response to drought, its external amendment may bring about improvements in water relations and osmoregulatory measure in this medicinal plant.

1. Introduction

Drought is known as a prominent environmental constraint that imposes serious limitations to crop productivity world-wide (Mirjahanmardi and Ehsanzadeh, 2016). More or less 45% of the world cultivated area is faced with frequent and continuous drought and this poses a menacing threat to the food security of at least 38% of the world population that reside in these drought-prone areas. Both saline and water-limited conditions affect plant growth and physiological functions primarily through causing an osmotic stress and, hence, decreasing chemical activity of water and losing of cell turgor (LiXin et al., 2009). Plants may take advantage of the synthesis and accumulation of organic osmolytes to combat osmotic stress (Yoshiba et al., 1997). The accumulation of osmolytes brings about increasing in osmotic adjustment and, thereby, overcoming the negative consequences of drought on plant growth and dry mass and seed production through the maintaining of adequate water absorption (LiXin et al., 2009; dos Santos et al., 2013). Not only varietal differences exist in the degree of accumulation of stress-associated indigenous osmolytes, but such differences have also been postulated in relation to mitigative effects of exogenously applied osmolytes (LiXin et al., 2009). Diverse organic osmoticums are potent to play mitigative roles, but proline is the preferred substance in a wide range of plant species (Hare and Cress, 1997). Proline is an amino acid that its accumulation in plant cells is a function of the interplay and balance between biosynthetic and degenerative processes (Yoshiba et al., 1997). Proline accumulation occurs in a wide range of biota (Lehmann et al., 2010) and in response to an array of stresses (Verbruggen and Hermans, 2008) and, hence, it is an organic compound that effectively takes part in plant stress tolerance. However, not all plants are capable to produce sufficient amount of this amino acid to warrant averting negative effects of environmental stresses. Thus, external application of this amino acid has been proposed to partial relief of the plants from the stress. Enhancement of plant metabolism and resistance to abiotic and biotic stresses as a result of amino acid applications has been attributed, in part, to the involvement of these compounds in nitrogen uptake and nitrate metabolism (Cerdán et al., 2013). Besides, osmoregulating and ROS-scavenging roles of these osmolytes have, also, been emphasized in

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some plant species (Ali and Ashraf, 2011; Moustakas et al., 2011).

Fennel is a perennial medicinal plant that its products have proven useful in the treatment of a variety of complaints including diabetes, chronic coughs and kidney stones (Askari and Ehsanzadeh, 2015). Furthermore, fennel's widespread uses in folk medicine have been ascribed to the antioxidative potential of its products (Barros et al., 2009). Fennel is mainly grown in arid and semi-arid regions, including Iran, and it might be a suitable medicinal crop for drought-prone environments. Improvement of physiological and photosynthetic responses of different plant species to environmental stresses, in general, and the projected water scarcity and associated osmotic stress in the face of global warming, in particular, has been the focus of study and debate in recent decades (Moustakas et al., 2011). However, the stress-relieving potential of osmolytes in medicinal plants have not been dealt with sufficiently. The premier aim of this work was, thus, to assess whether external application of proline as foliar spray is effective in altering some physiological responses of fennel to unfavorable water status.

2. Materials and methods

2.1. Experiment set up, soil conditions and irrigation regimes

This 2-year field experiment was carried out at the Lavark Research Farm of Isfahan University of Technology, located in Najaf Abad (32°32′N, 51°23′E, 1630 m above mean sea level, 14.5 °C mean annual temperature, and 140 mm mean long-term annual precipitation), Iran in 2015 and 2016. Two proline concentrations consisting of 0 and 20 mM of L-proline ($C_5H_9NO_2$, Molar mass 115.13 g/mol, Scharlau, Spain) and two irrigation regimes including irrigation after 35–45% and 75–85% depletion of available soil water (ASW) were applied on 12 fennel genotypes. The fennel genotypes that had been collected from different regions in Iran, were 'Ardabil', 'Avicenna', 'Birjand', 'Bushehr', 'Isfahan', 'Hamadan', 'Kashan', 'Kerman', 'Mashhad', 'Shiraz', 'Urmia', and 'Yazd'. A 3-replicate split factorial randomized complete block design was conducted, in which main plots consisted of the two irrigation regimes, subplots consisted of the 12 fennel genotypes and two foliar proline application levels. The external proline was applied on the foliage at two steps, 10-days apart, when the plants had been subjected to irrigation regimes for six weeks (i.e. BBCH-scale stage 51) (Meier, 2003).

The experimental field, seed preparation, sowing, irrigation and soil conditions have been described in a previous publication (Askari and Ehsanzadeh, 2015). Each sub-plot consisted of five rows that were 2 m long and 0.5 m apart. Spacing between plants in the same row was 0.2 m. The soil (Fine Loam Typical Haplargid) N, P, K, Zn and Fe contents were detected to be 740, 25.0, 225.0, 3.8 and 9.4 mg/kg, respectively. According to the chemical analysis of the soil, P and K macro elements were sufficient and only a urea fertilizer (i.e. 46% of N) was given at a 120 kg ha^{-1} basis to the soil at mid-April 2015 and late April 2016, i.e. before commencing irrigation treatments. The plants were watered twice at late winter and early spring 2015 and three times at late winter and early spring 2016, and then when the plants were approximately at BBCH-scale stage 31 in 2015 and BBCH-scale stage 35 in 2016 watering regimes were applied and continued to approximately 75% physiological maturity (BBCH-scale stage 85), i.e. mid-September 2015 and late-September 2016.

Total ASW, i.e. amount of the soil water in the root zone between field capacity and the permanent wilting point, was calculated based on Eq. (1).

$$ASW = (W_{FC} - W_{WP}) \times Bd \times V \quad (1)$$

Where W_{FC} is the gravimetric soil-water content (%) at field capacity, W_{WP} the gravimetric soil-water content (%) at the permanent wilting point, Bd the bulk density of the soil (g/cm^3) and V is the volume of soil layer in the root zone (m^3). Readily available soil water (RAW), i.e. the fraction of ASW that a plant can readily extract from the root zone without suffering drought stress, was calculated according to Eq. (2) (Allen et al., 1998).

$$RAW = \rho \times ASW \quad (2)$$

The ρ factor varies for different plants from 0.3 for shallow-rooted crops at high rates of plant evapotranspiration, ET_c (> 8 mm/day) to 0.7 for deep rooted crops at low rates of ET_c (< 3 mm/day) (Allen et al., 1998). The factor ρ was used to estimate the required time of irrigation to prevent water stress. The value of ρ was considered to be 0.4 for fennel (Askari and Ehsanzadeh, 2015). The two levels of irrigation were scheduled based on the maximum allowable depletion (MAD) percentage of ASW (Kramer and Boyer, 1995) and were applied when 35–45% and 75–85% of the ASW were depleted from the root zone, respectively. A soil moisture release curve was developed and used for determination of depletion of the available soil water based on the soil water potential. Eq. (3) was used for determining the volume of irrigation water (V_{irrig}) necessary to increase the water content in the root zone depth to field capacity.

$$V_{irrig} = \frac{ASW \times f}{E_a} \quad (3)$$

In this equation f is the fraction of ASW (35–45% and75–85%) that can be depleted from the root zone, and E_a is the irrigation efficiency (%). Irrigation efficiency was assumed to be 70% throughout the growing season (Tafteh and Sepaskhah, 2012). The irrigation water was applied with a pipe and the volume was measured with a flow meter. Number of irrigations and total volume of water applied over the course of growing season in 2015 for control plots were 17 and 0.861 m^3/m^2 and for drought-stressed plots were 7 and 0.717 m^3/m^2, respectively. Number of irrigations and total volume of water applied over the course of growing season in 2016 for control plots were 16 and 0.810 m^3/m^2 and for drought-stressed plots were 6 and 0.608 m^3/m^2, respectively.

2.2. Measurement of leaf water relations, proline, photosynthetic pigments and polyphenols

At 50–70% flowering stage (BBCH-scale stage 64) leaf water potential, relative water content, proline and chlorophyll concentrations of three plants per experimental unit were measured in both years. The mid-day water potential was determined using a portable pressure chamber instrument (*PMS Model 600*, USA). On a sunny day, second fully developed upper leaves were excised at the petioles close to leaf collars. The chamber was pressurized with compressed air until the tissue water was returned to the open end of the petiole and could be seen in the cut surface. Then the measured balance pressure was explained as the water potential and expressed as Mega Pascals (MPa). A mean of three measurements was reported for each plot.

Relative water content was measured on leaf sections obtained from the second fully developed upper leaves. They were quickly sealed within plastic bags and fresh weights were determined immediately after excision. After placing them in distilled water in test tubes for 4 h at room temperature (nearly 22 °C) and under the low light environment of the laboratory, turgid masses were estimated. Leaf dry masses were measured after drying the leaf samples in oven for 48 h at 72 °C. Finally, relative water content was calculated by Eq. (4) (Smart and Bingham, 1974):

$$RCW\,(\%) = \left(\frac{Fresh Weight - Dry Weight}{Turgid Weight - Dry Weight}\right) \times 100$$

Free proline content in the leaves was measured using the method of Bates et al. (1973). 200 mg of fresh mature leaves were crushed in 10 mL of 3% aqueous sulphosalycylic acid and the extract was filtered using Whatman filter paper. Two mL of the extract was added into the test tube containing 2 mL of ninhydrin reagent and 2 mL of glacial

acetic acid. The reaction mixture was heated in a boiling water bath at 100 °C for 1 h. After cooling the mixture on ice, 4 mL of toluene was added and thoroughly mixed. Finally, the toluene phase was separated and its absorbance measured at 520 nm using a spectrophotometer (*Hitachi U1800, Japan*) against toluene blank.

The concentration of chlorophyll and carotenoids in fresh leaves was measured by the spectrophotometer using the method of Lichtenthaler and Buschmann, 2001. 500 mg of fresh leaf tissue was crushed using mortar and pestle containing 10 mL of acetone (80%). The light absorption of leaf extract solution was recorded and pigment concentrations were measured and calculated according to the details given in Askari and Ehsanzadeh (2015).

For polyphenol measurement one gram of fresh leaf sample was obtained from mature healthy fennel leaves, powdered in a grinder, mixed by a magnetic stirrer and extracted in 100 mL of ethanol 95% at 22 °C for 1 h. The extract was filtered through Whatman filter paper to remove plant tissue particles. Then polyphenol content of the extract was determined using Folin-Ciocalteu reagent according to Singh et al. (2002). The procedure consists mixing the extracted sample with 1.0 mL of 10-fold-diluted Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution and letting to stand at room temperature for at least 1 h. Polyphenol concentration was assessed using the spectrophotometer by reading the absorbencies at 765 nm and expressed as mg galik/g FW.

2.3. Measurement of plant dry mass, seed yield, seed essential oil content and water use efficiency

At 70–80% physiological maturity (BBCH-scale stage 95) the plants from the central 2 m^2 portion in each plot were harvested, air dried for 7 days and seed weight/plant was determined for 5 plants per plot. Above ground dry mass was determined by drying a subsample at 72 °C for 72 h and expressed as g/m^2. A 20 g sample of seeds of each plot was grind to a powder and hydrodistillation of essential oil at 200 mL of deionized water was done according to Clevenger (1928) by the Clevenger's apparatus (Borosil, India). Distillation process continued for 4 h at 100 °C. The essential oil phase was separated, dried over anhydrous sodium sulfate, and kept in a dark glass bottle at 4 °C. Irrigation water use efficiency was determined by dividing above-ground dry mass (g/m^2) to total water applied (L/m^2) in each irrigation level.

2.4. Statistical analysis

A combined analysis of variances over the data obtained from the 2-year study was done using the general linear model (GLM) in SAS software (SAS, 1999). Least significant difference (LSD, $P \leq 0.05$) test was employed to separate the means, where F-test was found statistically significant at $P \leq 0.05$.

3. Results

Irrigation, exogenous proline and genotype led to statistically significant effects on leaf carotenoids, polyphenol, chlorophyll, proline, total soluble carbohydrates, and seed essential oil concentrations, leaf water potential, relative water content, and seed yield,. Plant above-ground dry mass was significantly affected by irrigation and genotype and water use efficiency was significantly affected by genotype (Table 1). Proline × genotype interaction effect was found statistically significant for leaf polyphenol, proline and seed essential oil concentrations, leaf water potential, relative water content, plant above-ground dry mass and water use efficiency. Irrigation × proline interaction effect was found significant for leaf carotenoids, polyphenol, chlorophyll and proline concentrations, leaf water potential and relative water content. Irrigation × genotype interaction effect was found statistically significant only for leaf polyphenol and proline concentrations. In contrary to increases in mean leaf polyphenol, proline, total soluble carbohydrates and seed essential oil concentrations, drought led to significant decreases in mean leaf carotenoids and chlorophyll concentrations, water potential, relative water content, plant above-ground dry mass, and seed yield (Table 2). Foliar-applied proline appeared to negate, at least in part, the adverse effect of drought on the above-mentioned physiological attributes, as it led to notable increases in mean leaf carotenoids and chlorophyll concentrations, seed yield, and relative water content, although it was not found effective on mean plant above-ground dry mass and water use efficiency and it resulted in a decrease in leaf water potential. Statistically significant genotypic differences were evident in all examined traits. Though, genotypes Urmia, Yazd and Kashan (albeit in a lesser degree) tended to out-number the remaining genotypes in terms of leaf chlorophyll concentration and osmoregulatory and water relation attributes, i.e. either mean proline concentration, total soluble carbohydrates concentration or relative water content. Furthermore, genotype Urmia outperformed most of the remaining genotypes in terms of plant above-ground dry mass, seed essential oil concentration and water use efficiency and Yazd was found to be among the higher-yielding (i.e. seed yield) and more water-use efficient genotypes.

External proline led to contrasting effects on non-stressed and drought-stressed plants, in that it resulted in significant increases in leaf chlorophyll concentration and relative water content at the presence of drought, despite lack of such effects at the absence of drought (Table 3). Furthermore, it led to significant increases in leaf polyphenol and proline concentrations of non-stressed plants, despite a decrease in leaf polyphenol concentration and lack of such effects on proline concentration of the stressed plants. Application of proline depressed leaf water potential of fennel plants, irrespective of soil water condition, albeit the depression was a bit greater in the stressed plants, leading to the statistically significant interaction. Despite differential, though significant, decreases in leaf water potential and increases in leaf relative water content of all fennel genotypes, proline treatment affected leaf polyphenol, proline and seed essential oil concentrations, plant above-ground dry mass and water use efficiency in a genotype-specific manner (Table 4). Apart from decreases in leaf proline and seed essential oil concentrations in a pair of the genotypes and leaf polyphenol concentration, plant above-ground dry mass and water use efficiency in three of the genotypes, application of exogenous proline resulted in either significant increases in these attributes or lack of significant effect in the remaining genotypes. Genotypes Urmia and Yazd appeared to be more responsive to external proline, at least because they out-numbered the other genotypes in terms of leaf polyphenol and proline concentrations, relative water content, above-ground dry mass and (in a lesser extent) water use efficiency. Drought led to significant increases in leaf proline concentration of all fennel genotypes, albeit the extent of the increase varied with genotype (Table 5). Genotypes Kashan, Yazd and Urmia were more responsive to drought in this respect, as they indicated the greatest concentrations of proline among the examined genotypes. Drought led to significant increases in leaf polyphenol concentration of a majority of genotypes, though some genotypes did not indicate significant modifications. The greatest concentration of leaf polyphenol was found in the drought-stressed plants of genotype Kashan. Drought led to significant decreases in seed yield of all fennel genotypes, albeit the extent of the decrease differed among genotypes. While genotype Yazd was the highest-yielding at the absence of drought, genotype Kashan out-yielded the remaining genotypes under drought condition.

4. Discussion

Even though leaf proline of drought-stricken fennel genotypes was found to be at modest concentrations (Table 2), it appeared to play decisive roles in coping with drought in this medicinal plant. Being regulated by diverse factors such as water status, diurnal light variability and intensity, nitrogen availability, cell, tissue and organ type and

Table 1
Analysis of variance (mean squares) for different traits of twelve fennel genotypes (G) evaluated at two levels of irrigation (I) and two levels of exogenous proline (P) in 3 replicates (R) in 2 years (Y).

	DF	Caroten	Polyph	Chl	Proline	LWP	TSC	RWC	SDM	SeedY	EO	WUE
Y	1	0.022**	0.644^{ns}	2.48**	32.5*	0.89^{ns}	0.012^{ns}	1369**	1957808^{ns}	257^{ns}	7.33*	15.2*
Y(R)	4	0.0008	1.71	0.082	1.92	0.085	0.066	51.3	791669	70.1	0.775	1.53
I	1	0.108**	70.1**	5.17**	546**	34.5**	20.3**	10320**	25590514**	16463**	23.1**	4.77^{ns}
Y × I	1	0.0001^{ns}	2.95^{ns}	0.002^{ns}	27.5^{ns}	0.013^{ns}	0.791^{ns}	13.3^{ns}	65031^{ns}	60.4^{ns}	4.14*	0.522^{ns}
R × I(Y)	4	0.0008	1.30	0.012	10.0	0.298	0.378	52.6	476330	33.3	0.336	0.751
P	1	0.014**	9.82**	0.534**	152**	4.78**	4.62**	1387**	98076^{ns}	601**	4.24**	0.208^{ns}
G	11	0.003**	5.20**	0.195**	23.6**	0.359**	1.83**	140**	861223**	201**	2.16**	1.70**
P × G	11	0.0008^{ns}	7.30**	0.030^{ns}	15.4**	0.010*	0.115^{ns}	98.7**	527213**	97*	0.846**	0.966**
I × P	1	0.012**	88.3**	0.573**	97.0**	0.247*	0.047^{ns}	946**	12097^{ns}	12.4^{ns}	0.151^{ns}	0.003^{ns}
I × G	11	0.001^{ns}	4.92**	0.050^{ns}	8.53**	0.086^{ns}	0.311^{ns}	57.0^{ns}	208083^{ns}	78.4*	0.327^{ns}	0.499^{ns}
I × P × G	11	0.0004^{ns}	5.64**	0.025^{ns}	13.8**	0.077^{ns}	0.874**	52.1^{ns}	372549*	55.5^{ns}	0.858**	0.721*
Y × P	1	0.001^{ns}	0.012^{ns}	0.029^{ns}	4.69^{ns}	0.027^{ns}	0.456^{ns}	74.0 ^{ns}	216027^{ns}	222*	0.065^{ns}	0.477^{ns}
Y × G	11	0.001^{ns}	1.76^{ns}	0.061^{ns}	17.8**	0.218**	0.471*	98.1**	288396^{ns}	21.8^{ns}	0.241^{ns}	0.517^{ns}
Y × I × P	1	0.003^{ns}	1.48^{ns}	0.360**	0.188^{ns}	0.070^{ns}	0.002^{ns}	4.5^{ns}	41820^{ns}	151^{ns}	8.36**	0.132^{ns}
Y × I × G	11	0.0009^{ns}	1.94^{ns}	0.047^{ns}	12.0**	0.099^{ns}	0.226^{ns}	45.4^{ns}	303308^{ns}	22.6^{ns}	0.344^{ns}	0.518^{ns}
Y × G × P	11	0.0008^{ns}	1.02^{ns}	0.042^{ns}	8.93**	0.031^{ns}	0.376^{ns}	18.9^{ns}	258722^{ns}	90.8*	0.398^{ns}	0.519^{ns}
Y × I × G × P	11	0.0008^{ns}	0.923^{ns}	0.037^{ns}	14.2**	5.42^{ns}	0.560*	64.2^{ns}	356290*	48.0^{ns}	0.530*	0.705*
Error	184	0.001	1.37	0.036	3.08	0.053	0.244	35.3	171928	42.4	0.263	0.263

DF, degrees of freedom; Caroten, carotenoids; Polyph, polyphenol; Chl, chlorophyll; LWP, leaf water potential; TSC, total soluble carbohydrates; RWC, relative water content; SDM, above-ground dry mass; SeedY, seed yield per plant; OE, seed essential oil percent; WUE, irrigation water use efficiency for above-ground dry mass. Ns, non significant.

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

Table 2
Mean comparison for different traits of twelve fennel genotypes evaluated at two levels of irrigation and two levels of exogenous proline in 2 years.

Traits	Carotenoids (mg/g FW)	Polyphenols (mg galik/g FW)	Chlorophyll (mg/g FW)	Proline (μmol/g FW)	Water Potential (-MPa)	Relative water content (%)	Soluble carbohydrates (mg/g FW)	Dry mass (g/m^2)	Seed yield (g/plant)	Essentail oil (%)	Water-use efficiency (g/L)
Experimental factor											
Irrigation											
Control	0.18^{a}	4.99^{b}	1.1^{a}	4.28^{b}	1.93^{b}	78.6^{a}	2.82^{b}	1928^{a}	23.3^{a}	2.98^{b}	2.31^{a}
Drought	0.14^{b}	5.98^{a}	0.80^{b}	7.04^{a}	2.62^{a}	66.7^{b}	3.35^{a}	1331^{b}	8.18^{b}	3.54^{a}	2.05^{a}
$LSD_{(0.05)}$	0.009	0.37	0.03	1.03	0.18	2.4	0.20	226	1.88	0.19	0.28
Proline											
0	0.15^{b}	5.30^{b}	0.89^{b}	4.93^{b}	2.15^{b}	70.4^{b}	2.96^{b}	1611^{a}	14.3^{b}	3.14^{b}	2.15^{a}
20 mM	0.17^{a}	5.67^{a}	0.98^{a}	6.39^{a}	2.41^{a}	74.8^{a}	3.21^{a}	1648^{a}	17.2^{a}	3.38^{a}	2.21^{a}
$LSD_{(0.05)}$	0.007	0.27	0.04	0.40	0.05	1.38	0.11	96.4	1.51	0.12	0.14
Genotype											
Ardabil	0.169^{abcd}	5.58^{bc}	0.973^{abc}	4.49^{c}	2.39^{ab}	73.0$^{a–d}$	2.93^{bc}	1135^{d}	18.0^{ab}	2.07^{de}	1.48^{d}
Avicenna	0.159^{cde}	5.43^{bc}	0.978^{ab}	4.99^{bc}	2.16^{e}	75.1^{ab}	2.94^{bc}	1888^{a}	14.6^{bc}	2.21^{cde}	2.55^{a}
Birjand	0.180^{a}	5.92^{ab}	1.066^{a}	4.96^{bc}	2.30^{abcd}	71.4$^{c–f}$	3.18^{b}	1646^{bc}	16.8^{ab}	3.34^{cd}	2.19^{bc}
Bushehr	0.156^{cde}	5.03^{cd}	0.849^{de}	5.23^{bc}	2.26^{cde}	75.2^{ab}	2.97^{bc}	1754^{ab}	14.9^{bc}	3.12^{de}	2.34^{ab}
Hamadan	0.161^{bcde}	4.72^{d}	0.979^{ab}	5.14^{bc}	2.01^{f}	68.4^{f}	2.69^{c}	1531^{bc}	8.82^{d}	2.92^{e}	2.05^{bc}
Isfahan	0.156^{cde}	5.30^{bcd}	0.908^{bcd}	5.70^{b}	2.30^{bcd}	72.2$^{b–e}$	2.97^{bc}	1664^{abc}	16.2^{ab}	3.10^{de}	2.23^{abc}
Kashan	0.160^{bcde}	6.55^{a}	0.997^{ab}	6.80^{a}	2.33^{abcd}	73.3$^{a–d}$	3.58^{a}	1596^{bc}	18.3^{ab}	3.23^{ab}	2.18^{bc}
Kerman	0.172^{abc}	5.33^{bcd}	1.004^{ab}	5.37^{bc}	2.24^{de}	74.2^{abc}	2.99^{b}	1735^{ab}	12.2^{cd}	3.05^{e}	2.34^{ab}
Mashhad	0.152d^{e}	5.62^{bc}	0.820d^{e}	5.38^{bc}	2.16^{e}	70.6^{def}	3.07^{b}	1482^{c}	17.2^{ab}	3.43^{bc}	1.99^{c}
Shiraz	0.143^{e}	5.13^{cd}	0.777^{e}	5.06^{bc}	2.39^{abc}	67.9^{ef}	2.97^{bc}	1482^{c}	16.2^{ab}	2.96^{e}	2.34^{ab}
Urmia	0.154^{cde}	5.64^{bc}	0.864^{cde}	7.45^{a}	2.43^{a}	73.6^{a-d}	3.67^{a}	1723^{ab}	16.1^{ab}	3.90^{a}	2.31^{abc}
Yazd	0.178^{ab}	5.64^{bc}	1.023^{a}	7.41^{a}	2.39^{abc}	75.8^{a}	3.10^{b}	1667^{abc}	19.5^{a}	3.34^{cd}	2.21^{abc}
$LSD_{(0.05)}$	0.018	0.67	0.109	1.00	0.13	3.38	0.28	236	3.71	0.291	0.335

$LSD_{(0.05)}$, least significant differences at $P \leq 0.05$. In each column and within each experimental factor means with at least one similar letter are not significantly different according to the $LSD_{(0.05)}$.

plant growth regulators, homeostasis of endogenous proline is tightly controlled as a result of an interplay of its biosynthesis, degradation and internal transport (Lehmann et al., 2010). The greater level (64%) of proline concentration (Tables 2, 3 and 5) in drought-stressed fennel plants (i.e. compared to non-stressed plants), thus, may have been resulted from an enhanced biosynthesis, depressed degradation or altered inter- and intra-cellular transport. Even though functioning of proline and soluble carbohydrates in signaling, ROS-scavenging and thus protecting chloroplast structure (i.e. from oxidative damage) is not ruled out (Moustakas et al., 2011), from our data one may propose that the latter osmolytes acted also as osmoticums and, thus, helped fennel plants to undergo osmotic adjustment. The above proposition was put forth as increases in leaf proline and soluble carbohydrates concentrations seem to have led to increases in relative water content and, contrarily, decreases in leaf water potential of stressed fennel plants (Tables 2 and 3). Osmoregulation is critical to the preservation of growth under drought and salinity stress conditions. Besides inorganic ions, synthesis and accumulation of osmolytes are key to the latter process (Ben Ahmed et al., 2011). Proline biosynthesis and accumulation in the stressed tissues is potent to be upregulated by one or even two orders of magnitude (Verbruggen and Hermans, 2008), but upregulations in the range of several-fold are more common in the plant cells and organs

Table 3
Mean comparisons for interaction effects of irrigation × proline on different traits of twelve fennel genotypes.

Traits	Carotenoids (mg/g FW)		Polyphenols (mg galik/g FW)		Chlorophyll (mg/g FW)		Proline (μmol/g FW)		Water potential (−MPa)		Relative water content (%)	
Exogenous Proline	0	20 mM	0	20 mM	0	20 mM	0	20 mM	0	20 mM	0	20 mM
Irrigation												
Control	0.181^{a}	0.181^{a}	4.26^{c}	5.73^{b}	1.07^{a}	1.06^{a}	2.98^{c}	5.60^{b}	1.84^{d}	2.03^{c}	78.2^{a}	79.0^{a}
Drought	0.129^{b}	0.126^{b}	6.35^{a}	5.61^{b}	0.71^{c}	0.89^{b}	6.89^{a}	7.18^{a}	2.47^{b}	2.79^{a}	62.7^{c}	70.7^{b}
$LSD_{(0.05)}$	0.010		0.39		0.06		0.58		0.08		2.0	

$LSD_{(0.05)}$, least significant differences at $P \leq 0.05$. In each trait means with at least one similar letter are not significantly different according to the $LSD_{(0.05)}$.

(Ben Ahmed et al., 2011).

Even though proline is best known for its role in osmoregulation, it may, potentially, act as a singlet oxygen quencher in the plant cells (Alia et al., 2001). While we have presented evidence for an osmoregulatory role of proline, we cannot discount other possible roles of this amino acid, i.e. it serves more than one function in fennel. This osmoprotectant plays adaptive roles through either acting as a carbon and nitrogen storage, scavenging ROS, stabilizing the structure of proteins, buffering cytosolic pH, or signaling stress (Hare and Cress, 1997). We measured four different non-enzymatic compounds which are potent to display ROS-scavenging roles, i.e. leaf carotenoids, polyphenols, soluble carbohydrates and proline. While concentration of leaf carotenoids indicated a 22% decrease, those of polyphenols, soluble carbohydrates and proline were increased by 20%, 19% and 64%, respectively, in drought-stricken fennel plants. The notably greater increase in proline concentration, i.e. relative to the other organic osmoprotectants (Table 2), may be attributed to the somewhat unique feature of proline metabolism, i.e. its extreme sensitivity to adverse environmental conditions (Hare and Cress, 1997). This amino acid is the terminal product of a rather short metabolic pathway. Proline accumulation, i.e. due to environmental stresses, therefore, will not affect a great number of metabolic reactions involved in intermediary metabolism, in comparison to accumulation of certain multi-functional substances, e.g. glutamate. As has been argued by Hare and Cress (1997), proline biosynthetic pathway is associated with a high rate of consumption of reductants (e.g. NADPH) and its degradation (oxidation) is capable of yielding 30 ATP equivalents. Thus, its accumulation could serve as an excellent means of storing energy and/or a resource of value either in the acclimation to or relieving from stress. Considering the available documents proving the importance of proline in tackling drought stress in other species, for example Gadallah (1995) findings on cotton and Ben Ahmed et al. (2011) report on olives, and evidence gathered in the present study, this multi-functional endogenous amino acid seems to be a key player in fennel response to drought.

An association between drought-induced modifications in plant water status (reflected in an attribute such as relative water content) and photosynthetic pigments (e.g. chlorophyll concentration) of fennel is supported by similar associations reported by other works. Examples are decreases in relative water content and leaf chlorophyll content of drought-stricken soybean plants (Mutava et al., 2015) and water-stressed fennel (Mirjahanmardi and Ehsanzadeh, 2016). As has been emphasized in these reports and might be true with fennel plants of the present study, prolonged and severe drought often lead to impairment of chlorophyll synthesis and/or extravagation of chlorophyll degradation. The substantial drought-associated decrease in above-ground dry mass of fennel genotypes might be related to probable depressions in several components of photosynthetic apparatus. In addition to degradation of chlorophyll, drought is known to suppress photosynthetic electron transfer and CO_2 fixation and assimilation and increase photorespiration of C_3 plants (Massacci et al., 2008).

An array of plant metabolic compounds are among the polyphenols, many of which known to play ROS-scavenging roles. Polyphenols are known to increase in different plant species and in response to an array of stressor factors, for instance date palm exposed to Cd (Zouari et al., 2016) and grapevine stricken by drought (Griesser et al., 2015). Albeit, increases in polyphenols are expected to be more notable when plants are exposed to prolonged drought and, presumably, an induced oxidative stress. Polyphenols may donate electrons and react with free radicles and, hence, act as reductants and terminate free radical chain reaction (Singh et al., 2002). Therefore, increase in polyphenols concentration of drought-stressed fennel plants is suggestive of adoption of a ROS-relieving response by the stressed plants.

Increase in seed essential oil concentration of drought-stricken fennel plants may be justified in part by a dilution effect brought about by decreases in plant tissue and organ sizes (e.g. smaller seed sizes in stressed plants). A similar conclusion has also been drawn by Simon et al. (1992) where they have put forth the possibility of increase in density of oil glands due to the reduction in leaf area of basil plants. Moreover, enhancement of biosynthesis of plant secondary metabolites is known to be an adaptive species-specific measure, taken across plant kingdom and in response to a wide range of environmental constraints, including drought (Németh-Zámbori et al., 2016; Llorens-Molina and Vacas, 2017). Similar to our results, some researchers reported that water stress increased essential oil content of *Foeniculum vulgare* (Mohamed and Abdu, 2004; Mirjahanmardi and Ehsanzadeh, 2016) and *Thymus daenensis* (Bahreininejad et al., 2013; Ghasemi Pirbalouti et al., 2014).

Response of plant water use efficiency to water deficit is known to be dependent on plant species, plant growth stage at which water is withheld and the severity and duration of drought (Maroco et al., 2000). However, severe drought often results in a depression in water use efficiency (Song et al., 2010). We did not attempt measuring photosynthetic gas exchange in the fennel plants, but drought-induced stomatal closure is expected to lower plant transpiration and hence to increase water use efficiency. Nonetheless, stomatal limitation to CO_2 diffusion to the chloroplasts of stressed plants may bring about a photooxidative stress to the photosynthetic apparatus (Fini et al., 2013) and a consequent decrease in plant dry mass. Therefore, it could be inferred that a type of non-stomatal impairment of the photosynthetic machinery concomitant to the stomatal limitation have, perhaps, resulted in a lower water use efficiency in the drought-stressed fennel plants at the present experiment.

Since osmolytes production is one of the few conserved (i.e. among different planta) basic plant physiological responses to water deficit (Ashraf and Foolad, 2007), their exogenous application has been a focus of attention of certain workers whose aim is provoking plant osmoprotecting and osmoregulating measures. Since stress-induced proline normally accumulates in the cytosol and thus contributes to osmotic adjustment and maintains cytosolic water volume (Ashraf and Foolad, 2007), its external application is sought as a means to handle, at least in part, the imminent water scarcity episodes facing a vast area of agricultural lands. Correction of plant water relations, as has been the case with leaf relative water content and water potential in the examined fennel genotypes, due to the external application of proline has been

Table 4
Mean comparisons for interaction effects of genotype × proline on different traits of twelve fennel genotypes.

Traits	Polyphenols (mg galik/g FW)		Proline (μmol/g FW)		Water Potential (−MPa)		Relative water content (%)		Dry mass (g/m^2)		Seed yield (g/plant)		Essential oil (%)		Water-use efficiency (g/L)	
Exogenous Proline	0	20 mM	0	20 mM	0	20 mM	0	20 mM	0	20 mM	0	20 mM	0	20 mM	0	20 mM
Genotype																
Ardabil	5.83^{b-g}	5.32^{e-h}	4.70^{e-h}	4.28^{ghi}	2.27^{d-g}	2.51^{ab}	67.3^{f}	78.7^{ab}	1056^{j}	1213^{ij}	11.6^{g-j}	24.4^{a}	2.69^{ij}	3.46^{cde}	1.37^{j}	1.58^{ij}
Avicenna	4.35^{ij}	6.51^{abc}	3.11^{i}	6.87^{b}	1.92^{i}	2.39^{a-d}	74.2^{a-e}	76.0^{abc}	1972^{a}	1805^{a-d}	14.2^{d-i}	15.0^{d-h}	3.20^{efg}	3.22^{efg}	2.60^{a}	2.49^{abc}
Birjand	5.24^{e-i}	6.60^{ab}	4.59^{fgh}	5.34^{c-g}	2.09^{ghi}	2.42^{a-d}	67.4^{f}	75.4^{a-d}	1427^{f-i}	1866^{abc}	13.2^{f-j}	20.4^{abc}	3.47^{cde}	3.22^{efg}	1.90^{f-i}	2.48^{abc}
Bushehr	5.04^{fgj}	5.02^{f-i}	4.73^{e-h}	5.73^{b-f}	1.91^{e-h}	2.11^{gh}	75.8^{a}	74.7^{a-d}	1739^{a-f}	1770^{a-e}	15.3^{b-h}	14.5^{d-i}	3.12^{e-h}	3.11^{e-h}	2.31^{a-f}	2.36^{a-d}
Hamadan	5.65^{c-h}	3.78^{j}	3.76^{hi}	6.51^{bc}	2.13^{fgh}	2.47^{a-c}	68.1^{f}	68.8^{f}	1399^{ghi}	1663^{a-h}	8.17^{j}	9.46^{ij}	2.76^{hij}	3.10^{e-i}	1.90^{f-i}	2.20^{a-h}
Isfahan	5.52^{d-h}	5.07^{f-i}	6.32^{bcd}	5.09^{b-h}	2.19^{e-h}	2.47^{a-c}	68.0^{f}	76.3^{abc}	1883^{abc}	1445^{e-i}	15.2^{c-h}	17.2^{b-f}	2.93^{f-j}	3.27^{d-g}	2.53^{ab}	1.93^{e-i}
Kashan	6.12^{a-e}	6.98^{a}	5.07^{d-h}	8.53^{a}	2.13^{fgh}	2.35^{b-e}	70.8^{c-f}	75.8^{abc}	1472^{d-i}	1721^{a-g}	18.9^{bcd}	17.6^{b-f}	3.43^{cde}	4.02^{ab}	1.96^{d-i}	2.39^{abc}
Kerman	4.90^{ghi}	5.76^{b-g}	5.53^{b-g}	5.20^{c-g}	2.07^{hi}	2.54^{a}	74.0^{a-d}	74.3^{a-e}	1903^{ab}	1566^{c-h}	11.2^{hij}	13.2^{f-j}	2.87^{g-j}	3.22^{efg}	2.58^{a}	2.09^{c-h}
Mashhad	5.52^{d-h}	5.72^{b-g}	4.75^{e-h}	6.02^{b-e}	2.09^{ghi}	2.24^{d-h}	67.4^{f}	73.8^{cde}	1588^{b-h}	1375^{hij}	17.6^{b-f}	16.8^{b-g}	3.19^{efg}	3.69^{bc}	2.14^{b-h}	1.84^{hi}
Shiraz	4.71^{hij}	5.54^{d-h}	4.42^{f-i}	5.69^{b-g}	2.35^{b-e}	2.42^{a-d}	68.8^{f}	69.0^{f}	1778^{a-e}	1699^{a-h}	13.6^{e-i}	18.7^{b-e}	2.63^{j}	3.29^{c-f}	2.38^{abc}	2.29^{a-g}
Urmia	5.85^{b-f}	5.41^{e-h}	6.17^{bcd}	8.72^{a}	2.30^{c-f}	2.57^{a}	69.7^{ef}	77.6^{abc}	1703^{a-h}	1744^{a-f}	13.8^{d-i}	18.3^{b-f}	4.13^{a}	3.66^{bcd}	2.32^{a-e}	2.32^{a-e}
Yazd	4.90^{ghi}	6.37^{a-d}	6.10^{b-e}	8.72^{a}	2.35^{b-e}	2.42^{a-d}	74.0^{b-e}	77.7^{abc}	1419^{f-i}	1915^{ab}	18.5^{b-e}	20.5^{ab}	3.30^{c-f}	3.38^{cde}	1.88^{ghi}	2.55^{ab}
LSD$_{(0.05)}$	0.94		1.41		1.87		4.8		334		5.3		0.41		0.41	

LSD$_{(0.05)}$, least significant differences at $P \leq 0.05$. In each trait means with at least one similar letter are not significantly different according to the LSD$_{(0.05)}$.

Table 5
Mean comparisons for interaction effects of genotype × irrigation on different traits of fennel genotypes.

Traits	Polyphenols (mg galik/g FW)		Proline (μmol/g FW)		Seed yield (g/plant)	
Irrigation	Control	Drought	Control	Drought	Control	Drought
Genotype						
Ardabil	4.83^{fg}	6.32^{a-d}	2.69^{l}	6.29^{d-h}	27.8^{ab}	8.24^{ghi}
Avicenna	4.35^{gh}	6.51^{ab}	4.03^{jkl}	5.94^{fgh}	20.0^{de}	9.21^{ghi}
Birjand	5.45^{c-f}	6.39^{abc}	3.23^{kl}	6.70^{d-g}	25.1^{a-d}	8.54^{ghi}
Bushehr	5.02^{efg}	5.03^{efg}	4.83^{hij}	5.62^{ghi}	21.6^{cde}	8.17^{ghi}
Hamadan	4.28^{gh}	5.16^{efg}	3.80^{jkl}	6.47^{d-g}	13.1f^{g}	4.54^{i}
Isfahan	5.51^{c-f}	5.08^{efg}	4.15^{jk}	7.25^{c-f}	24.6^{a-d}	7.78^{hi}
Kashan	5.87^{b-e}	7.23^{a}	4.36^{ijk}	9.23^{a}	25.7^{abc}	10.8^{gh}
Kerman	4.77^{fg}	5.88^{b-e}	4.36^{ijk}	7.37^{b-e}	18.1^{ef}	6.35^{hi}
Mashhad	5.31^{ef}	5.93^{b-e}	3.17^{kl}	7.60^{bcd}	26.9^{ab}	7.40^{hi}
Shiraz	3.70^{h}	6.55^{ab}	4.26^{ijk}	5.85^{fgh}	23.2^{b-e}	9.09^{ghi}
Urmia	5.39^{def}	5.88^{b-e}	6.42^{d-g}	8.48^{abc}	23.9^{bcd}	8.25^{ghi}
Yazd	5.46^{c-f}	5.81^{b-e}	6.14^{e-h}	8.68^{ab}	29.4^{a}	9.68^{ghi}
LSD$_{(0.05)}$	0.94		1.41		5.25	

LSD$_{(0.05)}$, least significant differences at $P \leq 0.05$. In each trait means with at least one similar letter are not significantly different according to the LSD$_{(0.05)}$.

observed in diverse species, e.g. cotton (Gadallah, 1995) and grapevine (Griesser et al., 2015). Furthermore, improvement in concentration of photosynthetic pigments in proline-treated fennel plants was not far from our expectation, because endogenous proline is assumed to be synthesized in chloroplast in stress conditions (Lehmann et al., 2010) and known to be effective in protection of chloroplast structure by dissipating the excess energy of PS II and scavenging of drought-induced ROS (Moustakas et al., 2011). Moreover, the greater concentrations of thylakoid-based chloroplastic pigments, i.e. chlorophylls and carotenoids, found in the proline-amended fennel plants reinforce the notion that proline brings about protection against destabilization of cellular membrane structures (Gadallah, 1995; Ben Ahmed et al., 2011). It is, at present, not clear to which extent exogenously-applied proline contributes to photosynthetic functions in fennel, as we did not attempt measuring CO_2 assimilation and light reactions in the examined fennel genotypes. Nonetheless, given the data of previous workers (e.g. Ben Ahmed et al., 2011 report on olive) and notable increase in the concentrations of photosynthetic pigments of drought-stressed fennel plants of present study (Table 3), accelerating photosynthetic rate and a consequent increasing in seed yield of proline-amended fennel plants is imaginable. From the partial proportionality among changes in polyphenols, carotenoids, proline and soluble carbohydrates concentrations and seed and dry mass of fennel genotypes, we are confident that an interplay between these defensive compounds is key to conferring increased tolerance to drought stress and maintaining seed and dry mass in proline-amended plants of this medicinal species. As proline has been found potent to enhance the antioxidative enzymes activities and non-enzyme antioxidative compounds (including carotenoids and phenolics) concentrations in response to diverse stresses, including Cd-treated date palm plants (Zouari et al., 2016) and drought-stressed maize (Ali et al., 2013), alleviation of stress-induced oxidative damages to fennel by exogenous proline is not ruled out. The increment of antioxidant molecules (e.g. carotenoids and polyphenols concentrations) by exogenous proline application in association with increases in chlorophyll concentration of fennel leaves is a further confirmation for the protective role played by this amino acid in alleviation of drought stress. In fact, increases in polyphenol and carotenoids concentrations in proline-treated fennel plants (Table 2) is a clear evidence for the proline-driven protective roles and/or measures in the present medicinal plant. Although data around fennel is scarce, enhancement of accumulation of proline and soluble carbohydrates due to foliar application of proline has been documented in the other species including *Arabidopsis thaliana* model plant (Moustakas et al., 2011) and olive tree

plant (Ben Ahmed et al., 2011). Moreover, proline-induced enhancement in polyphenol concentration in the fennel leaf tissue is in line with the previous reports wherein phenolic compounds have been accumulated in response to drought in diverse plant species such as maize (Ali et al., 2013) and grapevine (Griesser et al., 2015). The fact that exogenous proline led to notable decreases in leaf water potential of all genotypes (Table 4) is suggestive of osmoregulatory role of this amino acid. In addition, the observation that proline-treated plants of a majority of the examined fennel genotypes produced a greater seed yield and above-ground dry mass (Table 4) provides further convincing evidence in support of a cause-and-effect relationship between proline application, protective functions enhancement and stress-relieving in this medicinal plant species.

As has been proposed by some reports (LiXin et al., 2009), those plant species or genotypes that are not much efficient in terms of endogenous biosynthesis/accumulation of osmolytes (and thus are perhaps sensitive to drought) are more responsive to exogenously-applied osmolytes, when grown at the presence of drought. Our data support, at least in part, the above notion, where some of the examined fennel genotypes (e.g. Hamadan, Avicenna and Kashan) with a low capability of proline synthesis and/or accumulation (Table 2) were found to be more responsive to proline spray. The proline-exposed plants of latter fennel genotypes indicated 70–120% increases in their proline concentration, compared to the proline-deprived plants. Besides, lack of a substantial impact of exogenous proline on some growth attributes of well-watered fennel plants is not surprising, given the established possibility of lack of efficacy (and perhaps detrimental effects due to over-supply) of exogenous proline on non-stressed plants (Verbruggen and Hermans, 2008). A lack of responsiveness of certain attributes in well-watered plants to exogenous osmolytes (as evidenced by chlorophyll concentration in present study) (Table 3) doesn't seem to be limited to fennel response to the external proline, as such behavior has been documented for maize in relation to exogenous glycine-betaine (LiXin et al., 2009). Scientific data tend to suggest that ameliorative effects of exogenous application of proline are widespread among plant species. However, the extent of this effectiveness seems to be dependent on a number of factors. The effectiveness (i.e. of exogenous application of osmolytes such as proline) may vary with plant species, physiological state and concentration, stage and number of applications (Ashraf and Foolad, 2007). Thus, the somewhat moderate effectiveness of exogenous proline on different physiological, growth and yield attributes of fennel was not surprising.

Some controversy, among the literature, surrounds the type of response from non-stressed plants to external proline. Question has arisen as to whether an application of proline bear adverse consequences for non-stressed plants. Although some reports have confirmed positive consequences for proline application on plants, regardless of being stressed or non-stressed, others have challenged the merits of external proline application, on the account that it may harm non-stressed plants. Sperdouli and Moustakas (2015), for example, have reported that application of proline on non-stressed *Arabidopsis thaliana* plants elicits a malfunctioning in photosynthetic electron transfer. The toxicity stems from the involvement of this amino acid in buffering redox potential in cytosol and chloroplasts. In fact, since proline synthesis produces NAD^+ and its degradation generates NADPH, a cycle of proline synthesis and degradation is key to the maintenance of a balanced NAD^+/NADPH ratio in photosynthetic machinery. Exogenously-supplied proline leads to the production of NADPH and a decreased $NADP^+$/NADPH ratio. Accumulation of NADPH in stroma of chloroplast may result in the blockage of electron flow and over-reduction of the PQ pool, due to depletion of electron acceptors, i.e. NAD^+. According to this proposition, a decreased NAD^+/NADPH ratio may bring about a photosynthetic redox imbalance in the non-stressed plants, if exposed to external proline. An interesting finding of the present study is that proline amendment led to positive effects on many traits of fennel plants, irrespective of soil and plant water status (Tables 2, 3 and 4), albeit the extent of these effects varied with genotype. This finding suggests that in contrary to some reports, external proline benefits non-stressed fennel plants. Since external proline was applied in a greater dose on the fennel plants of the present study (i.e. 20 mM), as compared to a rather low dose (i.e. 10 mM proline application on *A. thaliana*) in the report of Sperdouli and Moustakas (2015), we conclude that species-specificity (and may be genotype-specificity, at least in fennel) must not be underestimated in examining plant responses to foliar proline applications.

5. Conclusions

From the data gathered in the present study it could be surmised that fennel is a somewhat drought-tolerant medicinal plant species. This study provides evidence that fennel is a plant species that is responsive to external application of proline. Even though a prolonged drought is potent to adversely affect the fennel physiological functions, but exogenous application of proline is capable of partial counteracting the harmful effects of drought on these attributes. From the presented data, proline amendment appeared potent to relief, in part, fennel plants from harm of drought through enhancing photosynthetic, antioxidative and water relations attributes. Although the necessity of further examinations of probable differences in the type and extent of fennel responses with developmental stage and number of applications cannot be ignored, presented results depicted a moderate response of this medicinal plant to external proline.

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