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Research Paper

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

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A B S T R A C T

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):

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

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