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Exogenously applied proline as a tool to enhance water use efficiency: Case of fennel

Ali Gholami Zali, Parviz Ehsanzadeh*

Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, 84156-83111, Iran

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ABSTRACT

Water is the most limiting factor for grain yield of different crop species in the arid and semi-arid regions. In Iran, eminency of water shortage has added fuel to the craving for finding water-efficient alternative crops or introducing water-saving procedures to the production systems. A 2-year field study with two irrigation regimes (drought versus drought-free control), two levels of proline application (0 and 20 mM), and 11 fennel genotypes was carried out to determine the interactive effects of water supply, proline, and genotype on the essential oil and grain yield, yield components, water use efficiency (WUE) and some physiological traits of fennel. Drought stress led to modifications in the activities of catalase (CAT), ascorbate-peroxidase (APX) and peroxidase (POX), and decreases in chlorophyll (Chl) concentration, umbels/plant, 1000-grains weight, grains/m^2. These drought-induced modifications in latter traits resulted in decreases in grain yield, essential oil yield, harvest index and WUE in all genotypes but the extent of the decreases differed among genotypes. Effect of exogenous proline on different traits was dependent on water condition and genotype, but it led to increases in 1000-grains weight, grain and essential oil yield and WUE of a number of genotypes, irrespective of water condition. The presented data suggests that some fennel genotypes are drought-tolerant and exogenously-applied proline is a procedure capable to decrease the number of irrigations and increase grain, essential oil yield and WUE of this medicinal plant.

1. Introduction

Drought episodes are expected to get heightened in frequency and intensity in the face of global climate change. Water shortage is predicted to pose greater pressure on agricultural production systems than in the past. Indeed, meeting the future demand of food production seems exceedingly challenging, given the fact that the ever-growing demand for urban and industrial water, expansion and perhaps maintaining current quotient of irrigation is questionable (Daryano et al., 2017). Water shortage brings about drought stress and imposes growth depressions and yield penalties to almost all crop plants. Variation exists in the degree and intensity of trouble in plant growth and depression in grain yield, depending on plant species. Plant species native to arid regions or adapted to low water supply are expected to withstand drought-associated troubles and depressions. Considering the urgency of water shortage in arid and semi-arid parts of the world, encouraging cultivation of medicinal plant species with low water requirement seems inevitable. Fennel is a herbaceous medicinal plant grown in arid and semi-arid regions (Ashraf and Akhtar, 2004). Fennel's products have been implicated in remediation of a variety of human diseases, mainly because of their antioxidative and radical scavenging potential (Barros et al., 2009). Since fennel is considered tolerant to various stresses, it might be a suitable medicinal crop for achieving high WUE at least in drought-prone environments. We hypothesize that adoption of semi-arid based medicinal crops such as fennel (*Foeniculum vulgare* Mill.) will improve the sustainability of crop production in the drought-prone regions under changing climate. Our previous studies (e.g. Askari and Ehsanzadeh, 2015) have shown that fennel is potent to be hired as an alternative crop for tackling the water scarcity crisis which isgrowing in the arid and semiarid regions.

Plants often benefit from endogenous organic osmolytes to tackle the osmotic stress through osmotic adjustment and, thereby, maintaining of adequate water absorption (Yoshiba et al., 1997; dos Santos et al., 2013). Among different osmoticums the amino acid proline is the main substance in a wide range of biota (Lehmann et al., 2010) and plant species (Hare and Cress, 1997) and in response to an array of stresses (Verbruggen and Hermans, 2008). Exogenous proline may be applied to ameliorate the stress effects, but its effectiveness may vary with plant species (Ashraf and Foolad, 2007). External application of amino acids like proline may improve

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* Corresponding author.
E-mail address: ehsanzadehp@gmail.com (P. Ehsanzadeh).

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Table 1
A synopsis of weather conditions during the growth seasons in 2015 and 2016.

Month	Days	2015				2016			
		Mean		Total		Mean		Total	
		T_{max} (°C)	T_{min}	Pan Evaporation (mm)	Precipitation	T_{max} (°C)	T_{min}	Pan Evaporation (mm)	Precipitation
April	15–31	25.0	10.5	126.7	3.3	24.0	10.7	100.0	20.7
May	1–31	28.7	14.7	243.4	3.4	30.2	15.5	309.7	2.8
June	1–30	36.3	21.1	336.0	0.0	34.1	18.3	374.2	0.0
July	1–31	36.4	20.8	311.4	0.0	38.1	22.6	409.3	0.0
August	1–31	35.5	19.1	318.1	0.0	34.2	18.5	303.2	0.0
September	1–30	30.1	15.6	230.0	0.7	33.3	17.1	285.7	0.0

T_{max}: maximum daily temperature; T_{min}: minimum daily temperature.

nitrogen uptake and metabolism (Cerdán et al., 2013), osmoregulation and scavenging of reactive oxygen species (ROS) (Moustakas et al., 2011) and hence partial relief of the plants from the stress. However, according to some reports (Verbruggen and Hermans, 2008; Sperdouli and Moustakas, 2015), it may injure non-stressed plants. ROS's such as hydrogen peroxide (H_2O_2) have been implicated in plant response to diverse stresses and enzymes such as CAT and POX are thought as the primary antioxidative detoxifiers of stress-induced H_2O_2 in many plant species. Drought stress alters the equilibrium between ROS production and enzymatic defense responses in plant tissue, as evidenced by increase in H_2O_2 concentration with drought prolongation (Lee et al., 2007). Even though a set of antioxidant enzymes comprising of CAT, SOD, APX, POX together to other enzymes of ascorbate-glutathione pathway are supposed to give shape to an effective scavenging system of stress-induced ROS, the response of this defensive system has proven to be contradictory, depending on plant species and type, duration and severity of stress (Cavalcanti et al., 2004).

The objective of this study was to investigate the feasibility of exogenous application of proline as a water management practice to improve grain yield and WUE of fennel under different soil moisture conditions. In order to fulfill this goal, in addition to grain yield components some important physiological attributes were also examined.

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 annual precipitation), Iran in 2015 and 2016. The mean daily maximum and minimum air temperatures, monthly Pan Evaporations and precipitations during the growing seasons in 2015 and 2016 were obtained from the Najafabad Synoptic Weather Station (situated in the 7 km distance from the research farm) and are shown in Table 1. Proline treatment consisted of 0 and 20 mM of L-proline ($C_5H_9NO_2$, Molar mass 115.13 g mol^{-1}, Scharlau, Spain) application, irrigation treatment consisted of irrigation after 35–45% and 75–85% depletion of available soil water (ASW) and 11 fennel genotypes were 'Urmia', 'Hamadan', 'Kerman', 'Shiraz', 'Birjand' 'Yazd', 'Avicenna', 'Kashan', 'Mashhad', 'Bushehr' and 'Isfahan'. Experiment was a 3-replicate split factorial randomized complete block design with main plots consisting of the two irrigation regimes, subplots consisting of the 11 fennel genotypes and two levels of foliar proline application. Proline application was carried out twice at a 10-days interval, at 25–50% flowering (when plants had been subjected to irrigation regimes at least for six weeks). Based on its molar mass, a 2.3 g/L of the L-proline solution was applied to the plants in each experimental plot to run-off. Plants of the proline-free experimental plots were subjected the same amount of foliar-applied distilled water.

Details of the experimental field, sowing, and irrigation 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 was a Fine Loam Typical Haplargid and according to the chemical analysis of the soil, and 120 kg ha^{-1} of a urea fertilizer (i.e. 46% of N) was given to the soil at mid-April 2015 and late-April 2016. When the plants were approximately 25 cm tall in 2015 and 30 cm tall in 2016 watering regimes were applied and continued to approximately 75% physiological maturity, 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).

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

where W_{FC} is the gravimetric soil-water content (%) at field capacity, W_{WP} is the gravimetric soil-water content (%) at the permanent wilting point, Bd is 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).

$$RAW = \rho \times ASW \tag{2}$$

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^{-1}) to 0.7 for deep rooted crops at low rates of ET_c (<3 mm day^{-1}) (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 to determine the 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 to field capacity.

$$V_{irrig} = \frac{ASW \times f}{E_a} \tag{3}$$

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). In fact, Eqs. (4) and