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Genotype-specific response of *Foeniculum vulgare* grain yield and essential oil composition to proline treatment under different irrigation conditions

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ABSTRACT

Drought stress is a major environmental stress which severely affects growth, yield, as well as quality and quantity of crop products. Proline as a multifunction amino acid has a crucial role in ameliorating of drought stress. In order to study the effect of external proline on the response of fennel to drought stress, three fennel genotypes (namely Urmia, Shiraz, and Kerman) were exposed to two irrigation regimes (drought stress and non-stress control) and two levels of foliar-applied proline (0 and 20 mM) in a pot experiment. Drought led to significant decreases in umbels/plant, plant height, 1000-grains weight, harvest index, above-ground dry mass, grain weight and grain and dry mass WUE, root volume, root fresh and dry mass, ratio of shoot/root, grains and stems essential oil weight/plant and significant increases in root length and grains limonene content. Foliar-applied proline led to notable increases in harvest index and grain essential oil weight in the presence of drought. It also led to increases in umbels/plant, 1000-grains weight, harvest index, above-ground dry mass, grain weight and grain and dry mass WUE and ratio of shoot/root in Urmia and Shiraz genotypes. Anethole, limonene, fenchone, estragole and α-pinene were the main essential oil constituents in both grain and stems. Grain fatty acid and phytosterol compositions were not affected by experimental treatments. It may be concluded that drought stress suppresses fennel growth and grain weight, but foliar-applied proline is potent to mitigate the adverse reaction through increasing growth, grain weight/plant, WUE and grains limonene content in genotypes such as Urmia and Shiraz.

1. Introduction

Fennel (*Foeniculum vulgare* Mill.) is one of the perennial medicinal and aromatic herbs of the Umbelliferae. Its products have applications in diverse pharmacological spectrum and are of considerable importance in particular to food industry (Diao et al., 2014; Moser et al., 2014; Rather et al., 2016). In addition to essential oil, fennel contains lipids and phytosterols that have utilizations in the industry (Barros et al., 2010; Moser et al., 2014; Nguyen et al., 2015). There have been several reports on various biological activities of fennel's essential oil, including hepatoprotective effects, antioxidative, antithrombotic, anti-inflammatory, antidiabetic, antitumor, and acaricidal activities (Badgujar et al., 2014). In addition, the essential oil of fennel seeds has showed significant antifungal as well as antibacterial activities (Salami et al., 2016; Diao et al., 2014; Badgujar et al., 2014).

In the recent decades, essential oils and various plant extracts have received a great deal of attention as sources of bioactive natural products (Figueiredo et al., 2008). Essential oils are volatile secondary metabolites that because of sensory properties and biological activities are extensively used in perfumes, cosmetics, food and beverage flavorings, household cleaning and pharmaceutical industry (Figueiredo et al., 2008; Moser et al., 2014). The ratio of different secondary metabolites varies considerably depending on environment, season, and type of plant material (Figueiredo et al., 2008; Olle and Bender, 2010; Akgul and Bayrak, 1988) as well as within and between taxons (Olle and Bender, 2010; Bettaieb Rebey et al., 2012; Diaz-Maroto et al., 2005). Plants under certain stress conditions often produce higher concentrations of secondary metabolites compared to non-stressed plants (Figueiredo et al., 2008; Olle and Bender, 2010; Bettaieb Rebey et al., 2012; Gholami Zali and Ehsanzadeh, 2018). They also have the different odor, taste, quality and yield potential (Salami et al., 2016).

Drought stress is one the major environmental stresses that imposes profound effects on growth and yield, as well as quantity and quality of metabolites in aromatic plants (Laribi et al., 2009; Bettaieb Rebey et al.,

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2012). Plants exhibit a variety of adaptive mechanisms (ranging from morphological to physiological and biochemical modifications) to cope with the adverse effects of drought stress. Proline accumulation is a common physiological mechanism involved in the adaptation of plants to various stress conditions (Hossain et al., 2014; Hayat et al., 2012). Several reports have indicated that foliar application of proline is capable to enhance stress tolerance and, hence, ameliorate the adverse effects of drought stress (Ali and Ashraf, 2011; Hossain et al., 2014; Gholami Zali and Ehsanzadeh, 2018). Endogenous proline, under stressful conditions, acts as a compatible solute in osmotic adjustment, and a ROS-scavenger in preventing membrane damage and protein denaturation (Hayat et al., 2012; Hossain et al., 2014). However, the enhancement of stress tolerance by foliar application of proline varies with plant species and with type, duration and severity of stress (Ashraf and Foolad, 2007).

Due to the growing occurrence of limited water resources in arid and semi-arid parts of the world, we hypothesize encouraging external application of compatible solutes such as proline and the cultivation of medicinal plant species with low water requirement such as fennel (Askari and Ehsanzadeh, 2015; Gholami Zali and Ehsanzadeh, 2018). It is, however, unfortunate that there is a lacuna of detailed studies on the response of fennel essential oil and its compositions to drought and external proline. Therefore, the aim of this study was to investigate the effects of drought stress and foliar-applied proline on fennel growth and grain weight, as well as its essential oil composition.

2. Materials and methods

2.1. Experiment set up, soil conditions and irrigation regimes

An outdoor pot experiment was conducted at the Research Farm of Isfahan University of Technology (Latitude of 32° 38′ North, Longitude of 51° 39′ East, and an Altitude of 1620 m above sea level), Isfahan, Iran. Two proline concentrations consisting of 0 and 20 mM of pure L-proline (Scharlau, Spain) and two irrigation regimes including irrigation after 35–45% and 75–85% depletion of available soil water (ASW) were applied on three fennel genotypes (Kerman, Shiraz, and Urmia) in a randomized complete block design with four replications. These fennel genotypes were chosen based on the results of our previous field experiment on the response of 11 genotypes to foliar-applied proline and irrigation regimes (Gholami Zali and Ehsanzadeh, 2018). The latter fennel genotypes had been collected from different regions (i.e. north-East, north-west, south-east, and central Iran) in Iran. The present set of three genotypes were chosen for this study on the grounds that genotype Urmia had showed the highest grain yield and essential oil yield in the presence of control irrigation and foliar-applied proline, Shiraz had showed the greatest grain and essential oil yield in the presence of drought and foliar-applied proline, and Kerman had showed notable decreases in grain and essential oil yield in the presence of drought and foliar-applied proline. Healthy seeds (being in botanical terms the indehiscent fruits - mericarps) were washed in distilled water, sterilized in 1% (v/v) sodium hypochlorite solution for approximately 2 min, washed thoroughly again in distilled water and allowed to dry at room temperature (25 °C) for approximately 1 h. The seeds were sown at a depth of one centimeter in germination trays at greenhouse temperature (25 °C). After the seedlings had reached a height of 7–12 cm (45 days after planting), they were transferred to rubber-made containers (25 cm in diameter and 80 cm tall), containing approximately 27 kg of soil on March 15, 2017. Each experimental unit consisted of two containers each containing two plants (i.e. four plants/experimental unit). To reduce compaction and to improve drainage, the field soil was mixed with sand in a proportion of 3:1 (v:v). The sandy loam-textured soil (sand 45.6%, silt 50.4% and clay 4.0%) was characterized by a pH of 7.4, electrical conductivity of 1.1 dS m^{-1}, and phosphorus, potassium, total N and organic matter concentrations of 54 mg/kg, 106 mg/kg, 0.056%, and 1.3%, respectively. From the beginning of the experiment till the establishment of the seedlings, all the containers were irrigated regularly. Irrigation treatments were applied after seedling establishment. Two irrigation regimes including irrigation after 35–45% and 75–85% depletion of available soil water (ASW) were chosen as the control and drought stress levels, respectively. The soil-water potential based on depletion of the available soil water was determined by a soil moisture release curve. The available soil water (ASW) and the volume of irrigation water ($V_{irrigation}$) were calculated based on Eqs. (1) and (2), respectively (Askari and Ehsanzadeh, 2015).

$$ASW = (\theta_{FC} - \theta_{PWP}) \times \rho_b \times V_{pot} \quad (1)$$

$$V_{irrigation} = ASW \times P \quad (2)$$

where θ_{FC} is the gravimetric soil–water content (%) at field capacity, θ_{PWP} is the gravimetric soil–water content (%) at the permanent wilting point, ρ_b is the bulk density (g/cm^3) of the experimental soil, V_{pot} is the volume of the container (m^3), and P is the fraction of ASW (35–45% and 75–85%) that can be depleted from the root zone.

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 four weeks). A 2.3 g/L of the L-proline solution was applied to the plants in each experimental unit (i.e. container) to run-off. Plants of the proline-free experimental units were subjected to the same amount of foliar-applied distilled water.

2.2. Measurement of growth, grain, grain weight, and root components

Plant height, umbels number per individual plant and 1000-grains weight were determined using two plants (i.e. from one of the two containers) at 70–80% physiological maturity. At physiological maturity (approximately 160 days after transfer of plants into the containers) the plants from each container were harvested and air-dried in room temperature (i.e. away from direct sun light) for at least 7 days. Above-ground dry mass and grain weight/plant were determined by threshing and separating the grains from the straw. Harvest index was calculated as the ratio of grain to above-ground dry mass and expressed as percent. Above-ground dry mass and grain weight irrigation water use efficiency were determined by dividing above-ground dry mass (g) and grain weight (g) to total water applied (L) in each irrigation level. Roots were harvested and washed carefully. Root length and root volume, root fresh weight, root dry weight per plant and ratio of above-ground dry weight to root dry weight (shoot/root) were determined using two remaining plants (i.e. from the second container). Root dry weight was determined by drying samples at 72 °C for 72 h.

2.3. Essential oil isolation, compound identification and analysis

Approximately one gram of each harvested sample (grains and stems) was subjected to hydro-distillation in a 250 mL flask containing 80 mL of distilled water for 3 h, using Clevenger's apparatus in three replicates. The essential oil was captured in 1 mL of cyclohexane containing 1 mg of 2-undecanone (before injection was proven that is absent with the investigation material) as an internal standard (IS), and stored in a sealed vial at 4 °C. The essential oils were analyzed by gas chromatography coupled with mass spectrometry (Shimadzu GCMS QP 2120, Shimadzu, Kyoto, Japan). The sample was injected at 270 °C (injector temperature) into a capillary column type ZB-5 Zebron (5% phenyl, 95% dimethylpolysiloxane), Phenomenex, capillary column with a length of 30 m, inner diameter of 0.25 mm, and film thickness of 0.25 μm (Phenomenex USA) using helium as a carrier gas at a flow rate of 1.0 mL/min. The injected volume was 1 μL and the injection mode used was split (split ratio 1:10), the injection port was set up on 270 °C. The oven temperature was raised from 50 to 130 °C at the rate of 4 °C/min, then at the rate of 10 °C/min, to achieve 270 °C for 2 min. Interface temperature was 270 °C; the ion source temperature was 200 °C. The essential oils constituents were identified by their retention indices and