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Effects of using arbuscular mycorrhizal fungi to alleviate drought stress on the physiological traits and essential oil yield of fennel

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ABSTRACT

This experiment was conducted to evaluate the physiological traits and essential oil yield of fennel affected by mycorrhizal fungal under different irrigation regimes. Field experiments were conducted at the Faculty of Agriculture, Yasouj University, Iran during the 2011 and 2012 years. The experiments were conducted using a randomized complete-block design with a split plot arrangement of treatments through four replications. The first factor included four irrigation regimes as main plot, and the second factor included three mycorrhizal fungus treatments as sub plot. The results indicated that irrespective of the mycorrhizal species and the drought stress intensity, inoculated fennels showed more essential oil yield, leaf and grain nutrient content and osmotic adjustment than did non-inoculated fennels. The positive effect of mycorrhizal symbiosis on leaf nutrient content and osmotic adjustment parameters was higher with high intensity of drought stress than with low intensity of drought stress. Results indicate that different AM fungi species even within the same genus have different effects on medicinal plant response to drought stress. The application of these microorganisms could be critical in the cultivation of medicinal plants under arid and semi-arid conditions, where water is the most important factor in determining plant growth and yield.

1. Introduction

To minimize the adverse effects of conventional agriculture (e.g. polluted water and soil by chemical fertilizers, entering pesticides to the food chain, compaction of the soil by heavy machinery and etc.), different alternative concepts of production have been developed (Gholamhoseini et al., 2013). Currently, low input cropping systems and innovation of resource management are of the most important objectives of sustainable agriculture, so applying of bio-fertilizers and resultantly reduction of inputs application is one step forward to sustainability (Ahmadi-Rad et al., 2016). The connections between fungi and the roots of higher plants are referred to as mycorrhiza (Augé, 2001). Such interactions are mutualistic relationships undertaken by more than 80% of plant species and approximately 6000 species of fungi (Bonfante, 2003; Brundrett, 2002). Arbuscular mycorrhizal (AM) fungi enable the host plant to establish and grow more efficiently under biotic and abiotic stress conditions, including drought (Sun et al., 2017), through a series of complex communications between the host and fungus. Changes in plant hormone levels (Bagheri et al., 2011); enhancement of leaf gas exchange and photosynthetic rate (Birhane et al., 2012); increase water uptake from the soil and transfer to the host plant (Evelin et al., 2009); and enhancement of antioxidant enzyme activities (Baslam and Goicoechea, 2012) are some of the mechanisms have been proposed to explain this protection by AM fungi. There are numerous reports of fungal symbionts (mycobionts) conferring host plant tolerance to various stresses, including herbivory, drought, heat, salt, metals, and disease (Márquez et al., 2007; Rodriguez et al., 2008; Salam et al., 2017). However, most of the relevant experiments were conducted under controlled growth chamber or greenhouse conditions. In addition, there is little information on the use of different species of mycorrhizal fungi under field conditions for improving drought resistance and increasing yield and quality of medicinal plant in semi-arid regions.

Currently, one third of human demands for drugs acquires from plants. Increasing the need of pharmaceutical factories to primary materials and, more importantly, conservation of natural genetic resources emphasis on the research regarding production and processing of medicinal and spice plants. Fennel (*Foeniculum vulgare* L.) is a member of the Apiaceae family. It is an herbaceous perennial plant originated from Mediterranean regions (Nourimand et al., 2012). Fennel essential oil consisted of approximately 70% Anatolia, which is a very important constituent of many pharmaceutical and cosmetic

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products (Omidbeigy, 2000). Attention to this plant in the world has recently been increased and its subculture area in comparison with the past 20 years has been about four times larger.

Fennel like other crops faces water deficit stress due to excessive transpiration caused by high temperature or low water availability, a phenomenon which is considered as one of the most important environmental factors affecting plant growth and productivity, especially in arid and semi-arid regions (Stagnari et al., 2014). Water deficit stress is a major cause of crop losses worldwide, reducing average yields by more than 50% (Kögler and Söffker, 2017). Differences in water availability induce morphological, anatomical and physiological responses such as changes in leaf area and thickness (Guerfel et al., 2009), alterations in gas exchange and assimilate translocation (Morgan et al., 2004), adjustment in water uptake and evapotranspiration (Katerji et al., 2010), antioxidant reactions (Apel and Hirt, 2004), gene expression and enzyme activity (Putpeerawit et al., 2017). According to our literature review, there is no actual information on the effects of different irrigation regimes and mycorrhiza fungi on leaf and grain nutrient content and especially physiological responses of fennel. Because these crucial traits have never been measured in a comprehensive experiment, especially in farm, these experiments were conducted to evaluate the physiological traits of fennel affected by mycorrhizal fungal under different irrigation regimes.

2. Material and methods

2.1. Experiment site

Field experiment was conducted at the Faculty of Agriculture, Yasouj university, Iran (30° 38′ N and, 51° 32′ E, altitude 1832 m), during the 2011 and 2012 years. The average yearly precipitation (over a 30 year period) is 186 mm and the annual mean temperature is 27 °C for the site. The average precipitation and temperature in 2011 and 2012 was similar to the long-term meteorological data trend. The field was kept fallow during the previous year to reduce the endogenous mycorrhizal fungi and eliminate their propagules, and to allow for the decomposition of the root debris from the previous crop. Prior to the beginning of the experiment, a composite soil sample was collected at depths of 0–30 cm, air-dried, crushed and tested for various physical and chemical properties. The experimental soil type was a clay loam with 0.19% total N, 348 ppm available K, 15 ppm available P, EC = 0.8 ds m^{-1} and pH = 7.5. In addition, the soil was evaluated biologically. A wet-sieving technique was used to extract spores, and the most probable number (MPN) test was used to determine the number of propagules (kg^{-1}) in the soil (Giovanetti and Mosse, 1980). Because the number of extracted propagules from the soil was extremely low (2–3 kg^{-1}), based on the wet-sieving technique and the MPN test no attempt was made to fumigate the soil before applying the treatments.

2.2. Field preparation

Plots were prepared after plowing and disk-harrowing. The plots were 5 m long and consisted of six rows, 50 cm apart. The mycorrhizal fungal inoculants consisted of spores and hyphal root fragments from stock cultures of *Glomus mosseae* and *Glomus intraradices*. The dose of inocula was 80 kg ha^{-1}. The *G. mosseae* and G. *intraradices* inocula were selected because of their commercial availability in Iran and in the world. The *G. mosseae* and G. *intraradices* inocula were purchased as pure isolates from the Agri-cultural and Biotechnology Research Institute, Karaj, Iran. Fennel seeds were inoculated with the inoculants, soaked in water for 24–30 h before sowing in order to increase germination performance and then sown during the last week of March in both years. The distance between the plants in the rows was 20 cm; thus, the plant density was approximately 10 plants per m^2. Immediately after sowing, the soil was irrigated. The irrigation cycle of each plot was closed to avoid run off. Plots received uniformly 250 kg ha^{-1} ammonium phosphate at sowing, only in the first year, and 150 kg ha^{-1} urea, at two time points: firs at sowing and second at stem elongation, and repeated in the second year.

2.3. Experimental design and treatments

The experiment was conducted using a randomized complete-block design with a split plot arrangement of treatments through four replications. The first factor included four irrigation regimes (I_1: irrigation was initiated after using 20% of the available water (well-watered), I_2: irrigation was initiated after using 40% of the available water (moderate drought stress), I_3: irrigation was initiated after using 60% of the available water (severe drought stress) and I_4: irrigation was initiated after using 80% of the available water (very severe drought stress)) as main plot, and the second factor included three mycorrhizal fungus treatments (non-inoculation AM fungi, inoculation with *Glomus mosseae* species and inoculation with *Glomus intraradices* species) as sub plot. Weed control, during the vegetative growth period was carried out by hand weeding. Irrigation was performed similarly in all of the plots when 20% of the available water was consumed until umbel formation; different irrigation regimes were performed thereafter. Soil water content (Θv) in experimental plots was monitored using Time-domain reflectometry (TDR, TRIME-FM, England) method. Data were collected every day during the growing seasons. In addition, a polyethylene pipeline and a counter were used to control irrigation water. Deep percolation was calculated using Eq. (1) (Errebhi et al., 1998).

$$\text{Daily deep percolation} = P + I - \Delta SW - ET_C - R \quad (1)$$

Where P is precipitation (mm), I is irrigation water applied (mm), ΔSW is the daily change in soil water content (mm) at the depth of root development (measured by TDR), ET_C is crop evapotranspiration (mm) and R is runoff (mm). Since irrigation cycles in each plot were closed, no runoff was occurred. According to Vazquez et al. (2005) percolation occurs whenever the sum (P + I) is higher than ΔSW + ET_C. It should be noted that irrigation water and rainfall were recorded. The following equation was used to calculate daily crop evapotranspiration.

$$ET_C = ET_0 \times K_C \quad (2)$$

Where ET_0 refers to evapotranspiration calculated by the FAO Penman-Monteith method (Allen et al., 1998), which depends on daily weather conditions at the site, and K_C is the crop coefficient. For each fennel growth stage, the K_C was calculated (Doorenbos and Kassam, 1979). The initial water storage was equivalent to the soil water holding capacity at the depth of 60 cm (prior to seed sowing, when the soil was fully saturated), and following changes in water storage (ΔSW) were estimated daily. In limited irrigation regimes (I_2: moderate drought stress, I_3: severe drought stress and I_4: very severe drought stress) ET_C was adjusted and calculated by the following equation (Allen et al., 1998).

$$ET_{C-adj} = K_S \times K_C \times ET_0 \quad (3)$$

Where KC and ET_0 are the same as in Eq. (2) and K_S is a correction coefficient (with no dimension) for calculating ET_C under water stress conditions. Eq. (4) was used to calculate K_S.

$$\frac{TAW - Dr}{TAW - RAW} \ldots KS = 1 \text{ if } Dr<RAW \quad (4)$$

Where TAW and Dr represent total available water (mm, difference between the water content at FC and PWP), and the amount of water depletion (mm, monitored on a daily basis by TDR) around the roots, respectively. Moreover, RAW stands for readily available water (TAW × MAD, which was defined as an 40, 60 and 80% depletion of available soil water in the limited irrigation regimes.