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Responses of field grown fennel (*Foeniculum vulgare* Mill.) to different mycorrhiza species under varying intensities of drought stress

Sedigheh Gheisari Zardak[a], Mohsen Movahhedi Dehnavi[a], Amin Salehi[a,*], Majid Gholamhoseini[b]

[a] Agronomy and Plant Breeding Department, Yasouj University, Yasouj, Iran
[b] Oil Seed Crops Department, Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

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ABSTRACT

The influence of colonization of fennel (*Foeniculum vulgare* Mill.) roots by two different species of arbuscular mycorrhizal (AM) fungi (*Glomus mosseae* and *Glomus intraradices*) and different irrigation treatments (well-watered, moderate water deficit stress, severe water deficit stress and very severe water deficit stress) were examined on growth, osmotic adjustment and qualitative and quantitative yield during two consecutive growing seasons. The experiments were conducted at Yasouj University, Yasouj, Iran located in semi-arid region of Iran. The results indicated that irrespective of mycorrhizal species and water deficit stress intensity, inoculated seeds produced taller plants, more dry matter and seed yield as well as seed essential oil content compared with non-inoculated plants. In both years of the experiment and in AM free plots, irrigation treatments showed a significant effect on leaf P concentration so that P concentration decreased with increasing water deficit stress intensity. Interestingly, mycorrhizal inoculation increased P accumulation and soluble sugars in fennel leaves compared with control plants. The data presented in this study suggest that different AM fungi species even within the same genus have distinct effects on fennel growth and yield. Overall, the results showed that *G. mosseae* was more efficient under water deficit stress. The application of AM fungi could be critical in cultivation of fennel under arid and semi-arid conditions, where water is the most important factor in determining plant growth and yield.

1. Introduction

Conventional farming practices negatively affect soil and water quality worldwide. In recent decades, agricultural goods production caused changes in environment functions resulting in unsuitable resource management. It has been reported that irrigated arid and semi-arid areas, where intensive agricultural is practiced, tend to experience problems of water resource deficiency and environmental pollution by excessive irrigation and chemical fertilizer applications (Iqbal et al., 2012; Magesan et al., 2002). Therefore, recent research has focused on new and practical management options for reducing water and nutrient loss, improving soil quality and increasing crop yield (Edmeades, 2003).

Organic and low input cropping systems are the most important objectives of sustainable agriculture, so application of biofertilizers to reduce chemical fertilizers use is a big step towards sustainability. Biofertilizer application improves physical, chemical and biological properties of soil and increases soil fertility without destructive effects on the environment. A promising strategy to achieve sustainability in agriculture is application of useful microorganisms, such as arbuscular mycorrhizal (AM) fungi, which have an important role in water and nutrient supply for plants in organic and sustainable agriculture systems (Ishizuka, 1992). AM fungi belong to a group of microorganisms that live in a symbiotic relationship with most of the crops and medicinal plants (Wu and Xia, 2006). These fungi form essential components of sustainable soil-plant systems and improve crop growth and productivity (Goussous and Mohammad, 2009).

Water shortage is being intensified by population growth, climate change and bio-fuel crop expansion, leading to concerns about global food security and environmental sustainability (Qiu et al., 2012). Since agriculture is the largest water consuming sector of the economy, it should be considered as an important part of solution (Jing et al., 2012). On the other hand, water is one of the major limiting factors affecting plant growth and development, especially in arid and semi-arid regions, where plants are often exposed to periods of water shortage. Drought stress is an important cause of crop losses worldwide, reducing average yield by more than 50%

* Corresponding author.
E-mail address: aminsalehi@yu.ac.ir (A. Salehi).

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(Wang et al., 2003). Fortunately, AM fungi can mitigate adverse effects of drought stress in plants. AM fungi are able to produce a very extensive network of hyphae in the soil when in symbiosis with host plants. During the formation of arbuscular mycorrhizae, fungal hyphae enter the epidermal, exodermal and cortical cell layers of the roots, reaching the inner cortex, where the functional units, the arbuscules, develop (Gholamhoseini et al., 2013). The fungi also form hyphae outside of the plants, extending the root–soil interface to facilitate nutrients and water uptake (Kistner and Parniske, 2002). AM fungi enable host plants to establish itself and grow more efficiently, even under biotic and abiotic stress conditions (Porcel and Ruiz-Lozano, 2004), through a series of complex communications between the host and the fungus (Harrier, 2001). There are numerous reports of fungal symbionts conferring host plant tolerance to various stresses, including drought, heat, salt, metals, and even diseases (Waller et al., 2005; Márquez et al., 2007; Rodriguez et al., 2008). Therefore, the symbiotic interactions of plants with mycorrhizal fungi are agriculturally and ecologically important (Indrasumunar, 2007). In addition, species-specific interactions between host plant and fungal pathogens highlight the importance of screening of different associations to maximize the benefits of the symbiosis (Miller et al., 1987). Considering the physiological differences within species and even within geographic isolates (Bethlenfalvay et al., 1989), the biodiversity of AM is great. There is little information about the physiological specialization and functioning of AM. In fact, AM fungi species differ in their tolerance and in their ability to adapt to environmental conditions (Sylvia and Williams, 1992). The variation within AM species and also different symbiotic strategies occurring in response to water deficit stress, as well as the compatibility with different environmental conditions, suggest that it may be likely to choose the most effective species.

Moreover, mycorrhizal colonization has been shown to increase water deficit tolerance in many crops such as corn (Subramanian et al., 2006), wheat (Bryla and Duniway, 1997), soybean (Bethlenfalvay et al., 1988), onion (Azcón et al., 1996) and lettuce (Tobar et al., 1994; Azcón et al., 1996). However, the majority of these experiments have been conducted under controlled conditions like growth chambers or greenhouses. In addition, there is little information on the use of different mycorrhizal fungi species under field conditions to improve medicinal plant yield and quality, especially in semi-arid regions.

Although medicinal plants have been used since Biblical times, the interest in essential oils increased during the past decades. Today, the traditional crops are not the only plants used in agricultural systems, medicinal plants whose essential oils are valued for their characteristic aromatic or therapeutic attributes. Increase in demand of pharmaceutical factories for primary materials and conservation of natural genetic resources, enhances the importance of the research and production of medicinal plants. Fennel (*Foeniculum vulgare* Mill.), as an important medicinal plant, is a member of the Apiaceae family. It is an herbaceous perennial plant originated from Mediterranean regions (Nourimand et al., 2012). Fennel is an aromatic and flavorful plant with culinary and medicinal use. Fennel seeds are anise like in aroma and are used in baking, meat and fish dishes, ice cream, alcoholic beverages and herb mixtures (Diaaz-Maroto et al., 2005). The fennel bulb, leaves and seeds are extensively used in many of the culinary traditions across the world. The major components of fennel seed essential oil are *trans*-anethole, fenchone, estragol (methyl chavicol), and α-phellandrene. The concentration of the compounds depends on phenological stage and origin of the plant (Diaaz-Maroto et al., 2006; Omidbeigy, 2000).

Fennel is broadly used around the world as mouth fresheners, toothpastes, desserts, antacids and in various culinary applications. Although, more attention has been paid to this plant in the world

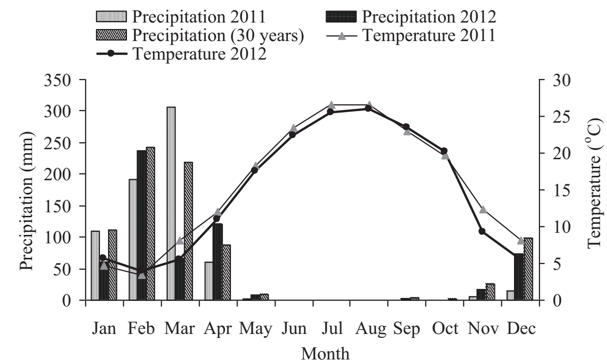

Fig. 1. Monthly temperature and precipitation during the growing seasons in 2011 and 2012.

and its cultivation area has been increased four times during past two decades, there is still little information on the use of different species of mycorrhizal fungi under field conditions to improve fennel yield and quality in semi-arid regions. This study investigates two different species of mycorrhizal fungi, in terms of their efficiency under four different irrigation regimes, by quantifying fennel growth, yield, and essential oil percentages and yield.

2. Material and methods

2.1. Experiment location and climatic characteristics

Field experiments were conducted at the Faculty of Agriculture, Yasouj university, Iran (30° 38′ N and, 51° 32′ E, altitude 1832 m), during the 2011 and 2012 growing seasons. The region is characterized as semi-arid, with mean annual precipitation of 86 mm, which mostly falls during the autumn and winter months. The annual mean temperature was recorded 15 °C. The average precipitation and temperature in 2011 and 2012 were similar to the long-term meteorological data trend (Fig. 1). The field was kept fallow during the previous year to reduce the endogenous mycorrhizal fungi population and eliminate their propagules, and decomposition crop residue remained from the previous year.

2.2. Soil sampling and land preparation

Prior to the beginning of the experiment, a composite soil sample was collected at the depths of 0–30 cm, and the air-dried, crushed and tested for various physical and chemical properties. The soil type was found to be a clay loam with 0.19% total N, 348 ppm available K, 15 ppm available P, EC = 0.8 ds m^{-1}, pH = 7.5, water content at field capacity (FC) = 35% volumetric moisture and water content in permanent wilting point (PWP) = 15% volumetric moisture. In addition, the soil was evaluated in terms of biological factors for this purpose 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. Since the number of extracted propagules from the soil was extremely low (2–3 kg^{-1}), no attempt was made to fumigate the soil before applying the treatments.

Experimental plots were prepared after plowing and disk-harrowing. The plots were 5 m long and consisted of six rows, 50 cm apart. There were 2.5 m gaps between the blocks, and 1.5 m alley was established between the plots to prevent lateral water movement and other interferences. 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 also in