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Water use efficiency of three mycorrhizal Lamiaceae species (*Lavandula officinalis, Rosmarinus officinalis* and *Thymus vulgaris*)

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ABSTRACT

As a mechanism of plants to adapt to soil water deficits, promotion of water use efficiency has been the focus of strategies to increase crop tolerance to drought. Probable improvement of arbuscular mycorrhizal fungi (*Funnelliformis mosseae* or *Rhizophagus irregularis*) for the essential oil and biological yield of watered and rainfed host plants (lavender, rosemary and thyme) are the main aim of this study. Physiological responses of the host plants to inoculation with two species of fungi were evaluated under different irrigation regimes (irrigation at 75 and 50% of field capacity, and rainfed). A factorial (two factors) experiment was conducted for two years (2015–2016) based on a randomized complete block design with three replications at Urmia University. Inoculation with these root symbionts increased the colonization of the plants as compared to non-inoculated plants. The seedlings inoculated with fungi and subjected to water stress had more successful colonization. Stress reduced biological yield of inoculated and non- inoculated plants. Drought-induced reduction of biomass was significantly compensated for by mycorrhizal fungi. The highest essential oil percentage was obtained in rainfed condition. Since water use efficiency is affected by economic performance and volume of used water in different years, the results were variable. Increasing irrigation intervals can help the plants to adapt to water stress and prevent significant reduction in water use efficiency. However, in general, this study showed that inoculation with fungi is effective in alleviating adverse effects of water stress.

1. Introduction

The Lamiaceae family (formerly called Labiatae) with 236 genera and more than 7000 species is best known as herbs with sharp fragrance (Berry, 2013). In the industrialized world, herbs support the natural function of body systems and help as natural remedies (Comar and Kirby, 2005; Horne, 1996). Lavender (*Lavandula officinalis* syn. *L. angustifolia,* Lamiaceae family), a small fragrant shrub native to the Mediterranean mountains, the Arabian Peninsula, Russia and Africa, is cultivated in Southern Europe, the United Kingdom, the United States, and Australia (Basch et al., 2004). This plant has gained popularity with a variety of therapeutic and cosmetic uses (Denner, 2009). Lavender essential oil is used in a wide range of therapeutic settings and aromatherapy after suffering severe burns (Glenn, 2007). Rosemary (*Rosmarinus officinalis* L., Lamiaceae family), native to the Mediterranean regions, is cultivated worldwide in Algeria, Spain, France, Portugal, Russia, China, former Yugoslavia, Tunisia, Morocco, Italy and USA. The biggest producing countries of rosemary essential oil are Spain, Tunisia, Morocco and France (Charles, 2013). Rosemary has been shown to have strong antioxidant (Jiang et al., 2011), antifungal and antibacterial properties (Bozin et al., 2007). Thyme (*Thymus vulgaris* L., Lamiaceae family), a culinary and medicinal herb, is native to Southern Europe, although it now grows more widely both in the wild and in cultivation. It is cultivated in many countries worldwide (Russia, Poland, Switzerland, USA, Spain, France, Italy, Morocco and South Africa) with a great range of climatic conditions (Özgen, 2016). Thyme species are one of the widely used herbal medicinal plants in the treatment of renal diseases, hypertension, inflammation, infections, pain, and for washing skin and mouth (Abebe et al., 2003; Parvev and Yadav, 2010).

Nowadays, maximizing yield per unit of used water requires a better understanding of crop response to various levels of water stress (Singh Rao et al., 2016) and irrigation water qualities (Ali-Shtayeha et al., 2018; Shtull-Trauring et al., 2016). In water-limited conditions, yield should be determined by drought resistance, and/or water use efficiency (WUE) (Blum, 2005). The effect of drought on growth and development of medicinal and aromatic plants has been studied. Previous studies indicated the significant effect of irrigation on yield-determining morphological and physiological characteristics of plants. In addition, irrigation levels affected yields (drug, seed and essential oil)

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and some major constituents of the essential oils (Llorens-Molina and Vacas, 2017; An and Liang, 2013; Mathobo et al., 2017; Rioba et al., 2015; Sánchez-Blanco et al., 2004).

In order to increase food production without pressure on the ecosystem in water-limited regions, it is important to increase the water use efficiency in agriculture (Gliñski et al., 2011). This has brought into focus the urgent need for planned action to manage water resources effectively (Brockerhoff, 2000). An alternative strategy to enhance drought tolerance of plants is arbuscular mycorrhizal fungi (AMF) symbiosis. Mycorrhizal plants can enhance water balance in irrigated and drought conditions (Auge, 2001) by improving shoot and root growth, biomass and the antioxidant system (Li et al., 2015). In drought condition, improved uptake and transport of water and nutrients, especially immobile mineral nutrients (e.g. phosphorous) resulting in the plant tissues hydration and growth promotion of mycorrhizal plants (Auge, 2001). The non-nutritional mechanisms by which AMF increase plants' tolerance to drought include, hormonal changes, increased plants' photosynthetic rate, accumulation of compatible osmolites (Al-Karaki, 2013; Birhane et al., 2012), disease resistance, plant chemical defense, soil aggregation, and allelochemical transport and protection (Delavaux et al., 2017).

This effect depends on the plant-AMF interaction and the bioactive compound (Tarraf et al., 2015). Yield assessments under water-limiting conditions, and reduction of damage by AMF symbiosis, shows that it is a sustainable solution for crop production in dry climates (Gholamhoseini et al., 2013). There are reports on AMF-plant relations of Lamiaceae, including *Thymus granatensis* (Navarro-Fernandez and Aroca, 2011), *Thymus polytrichus* (Whitfield et al., 2004), *Lavandula multifida* (Bakkali Yakhlef et al., 2011), *Lavandula stoechas*, *Lavandula dentate, Lavandula multifida* (Ouahmane et al., 2006), and *Rosmarinus officinalis* (Camprubi et al., 2013; Varela-Cervero et al., 2016).

The aim of the present study was to (i) examine the impact of *Funnelliformis mosseae* or *Rhizophagus irregularis* symbiosis on the yield (biological and essential oil) and water use efficiency of lavender, rosemary and thyme in different water stress levels; (ii) compare the effect of two species of fungi on these mentioned plants.

2. Materials and methods

2.1. Site description and climatic characteristics

Two-year seedlings of *L. officinalis* cv. Silver, *R. officinalis* cv. Boule and *T. vulgaris* cv. Varico3, from Lamiaceae family were supplied by the Research Institute of Forests and Rangelands. For each plant species, a 2-year (2015–2016) factorial experiment was conducted based on randomized complete block design (RCBD) with three replications at Agricultural Research Farm of Urmia University (latitude 37°33′09″ N, 45°05′53″ E and 1362 m above sea level), Iran. This region enjoys a semi-arid climate. Total monthly rainfall, average monthly air temperature and relative humidity for the two growing seasons are presented in Fig. 1.

2.2. Field preparation and treatments application

The three levels of irrigation including irrigation at 75% field capacity (well-watered, 75FC), 50% field capacity (moderate stress, 50FC) and rainfed (severe stress) were applied after the last rainfall in both years as the first factor. Two mycorrhizal fungi species, namely *Funnelliformis mosseae* syn. *Glomus mosseae* and/or *Rhizophagus irregularis* syn. *Glomus intraradices* and a non-AMF inoculated treatment, were considered as the second factor.

The seedlings of lavender, rosemary and thyme were transplanted on the 22nd of April 2015 in nine rows (50 cm inter and 45 cm intra row spaces). The mycorrhizal inocula (initially isolated from the endemic AMF community of a maize farm), a mixture of sterile sand, mycorrhizal hyphae and spores (20 spores/g inoculum) and colonized root fragments, were provided by Dr. Y. Rezaee Danesh (Department of Plant Protection, Urmia University). The biological fertilizers (AMF inocula) were incorporated into the soil at depth of 10–30 cm. Inoculum was placed in the holes (20 g per hole) through the plant seedlings roots and lightly covered with soil from the hole on the day of planting (only the first year). For non-AMF inoculated plants, seedlings were transplanted with no inoculation. Inoculum potentials were 3400 and 3360 propagules per plant for "*F. mosseae*" and "*R. irregularis*", respectively. The main soil physicochemical properties are presented in Table 1. The plots were set at adequate distances (250 cm) from each other with the purpose of triggering no interference. Weeds were controlled manually during the experiment.

2.3. Irrigation water calculation

The irrigation water supply at each plot was measured with a water counter. Irrigation water needed prior to irrigation (VN) is the amount of water needed during irrigation to replenish the soil moisture deficit, thereby, taking the soil back to the field capacity. VN was calculated using Eq. (1) (Benami and Ofen, 1984):

$$VN = [(FC - WP) \times BD \times D \times (1 - ASM) \times A]/100 \qquad (1)$$

Where, VN is the irrigation water needed before irrigation (m^3), FC is field capacity (%), WP is the wilting point (%), BD is bulk density (g/cm^3), D is the root zone depth (m), ASM is the available soil moisture before irrigation (a fraction) and A is the area of the field (m^2) determined with a pressure plate; water holding content at Field Capacity (−0.33 kPa) was 28.6% and at the Permanent Wilting Point (–1500 kPa), it was found to be 14.5%. Based on the above method, the amounts of the used irrigation water were 2187, 1500 and 0 m^3/ha (for the first year), and 2625, 2250 and 0 m^3/ha (for the second year) for irrigation at 75FC, 50FC and rainfed condition (without irrigation), respectively.

Three of the Lamiaceae species (lavender, rosemary and thyme) were harvested on the 20th of September of the two years.

2.4. Measurements

2.4.1. Colonization

The percentage of plant root colonization was determined in 10 plants per experimental unit. To this end, fresh roots were cleared in 10% potassium hydroxide (KOH) for 10 min at 90 °C, and then stained in 0.05% lactic acid-glycerol-Trypan Blue (Phillips and Hayman, 1970). Root colonization was assessed using the gridline intersect method of Liu and Luo (1994).

2.4.2. Biological yield

At plant maturity, the yield was measured from 3 m^2 of each experimental plot. Shoot dry biomass was equal to the sum of aerial vegetative plant parts and was expressed in kg/ha. Flowers, leaves and shoots were dried at ambient temperature (mean 22 ± 1 °C for 96 h).

2.4.3. Essential oil

Floral foliage was obtained from 3 m^2 of each experimental plot in the flowering stages (in thyme mixed of two-stage flowering), air dried and weighed to extract the essential oil. Shade drying was done under conventional open air conditions for 96 h. Dried materials (with 14% moisture content) from each of these treatments were hydro distilled for 3 h using a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate and preserved in a sealed vial at 4 °C until further analysis (the sodium sulfate absorbs any water and clumps together) (Clevenger, 1928). The essential oil content was measured as percentage of weight. Then, oil yield was determined using Eq. (2):

$$\text{Yield of essential oil} = \text{Yield of dried aerial parts} \times \text{Essential oil percentage.} \qquad (2)$$