Agricultural Water Management 117 (2013) 106–114

Contents lists available at SciVerse ScienceDirect

Agricultural Water Management

journal homepage: www.elsevier.com/locate/agwat

Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress

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ARTICLE INFO

Article history:
Received 25 October 2012
Accepted 2 November 2012
Available online 12 December 2012

Keywords:
Mycorrhizal inoculated plants
Root colonization
Glomus mosseae
Glomus hoi

ABSTRACT

Drought stress is one of the most important limiting factors for field crops in arid and semi-arid regions. Yield assessments under drought stress conditions, using soil microorganisms to reduce the damage from drought stress, demonstrate that soil microorganisms are sustainable solutions for crop production in such climates. Therefore, we undertook a two year experiment to understand the effects of root colonization by two species of mycorrhizal fungi (*Glomus mosseae* and *Glomus hoi*), under different drought stress conditions, quantifying sunflower growth, nutrient uptake, yield, yield components, oil percentage and irrigation water productivity. The experiment was conducted at Tarbiat Modares University, located in the semi-arid region of Iran, during the 2006 and 2007 growing seasons. Drought stress was induced at two stress levels by irrigating after 60 and 80% water depletion, which were defined as mild and severe drought stress, respectively. Irrigating after 40% water depletion was considered to be normal irrigation (no stress). The results indicated that irrespective of the mycorrhizal species and the drought stress intensity, inoculated plants produced more dry matter, heavier seeds and greater seed and oil yields than did non-inoculated plants. Interestingly, the seed yields of plants inoculated with *G. mosseae* under each irrigation regime were higher than those of plants inoculated with *G. hoi*, or of the non-inoculated plants. Although drought stress reduced the N percentages in the leaves and seeds, mycorrhizal fungi enhanced the N percentages, particularly when the plants were inoculated with *G. mosseae*. In addition, seed P percentages were not affected by mycorrhizae in 2006. In contrast, the highest P percentages in both leaves and seeds were obtained from plants inoculated with *G. mosseae* in 2007. Overall, our results show that *G. mosseae* is more efficient under drought stress, and better supports sunflower plants. The application of these microorganisms could be critical in the cultivation of sunflowers under arid and semi-arid conditions, where water is the most important factor in determining plant growth and yield.

1. Introduction

Water is one of the major limiting factors affecting plant growth, development and yield, especially in arid and semi-arid regions, where plants are often exposed to periods of water shortage (drought stress). Drought is a major cause of crop losses worldwide, reducing average yields by more than 50% (Wang et al., 2003). Among the diverse impacts of drought on plant development in these arid ecosystems, restricted water and nutrient availability are commonly discussed (Agnew and Warren, 1996). Fortunately, arbuscular mycorrhizal symbiosis can protect crops against the detrimental effects of drought stress. The symbiotic interactions of plants with mycorrhizal fungi are agriculturally and ecologically important (Indrasumunar, 2007). Over 90% of plants will engage in arbuscular mycorrhizal symbiosis, which mainly improves the nutrient uptake of phosphorus, and several other (still disputed) nutrients (Bonfante, 2003; Brundrett, 2002). 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. The fungi also form hyphae outside of the plant, extending the root–soil interface to facilitate the uptake of nutrients such as phosphates and water (Kistner and Parniske, 2002). Arbuscular mycorrhizal fungi enable the host plant to establish itself and grow more efficiently, even under conditions of biotic and abiotic stress, including drought (Porcel and Ruiz-Lozano, 2004), through a series of complex communications between the host and the fungus (Harrier, 2001). It has been shown that associations between roots and arbuscular mycorrhizal fungi enhance the amount of P taken up by plant roots under drought conditions (Ruiz-Lozano et al., 1995). As a result of the increased uptake of P, plants inoculated with mycorrhizae frequently produce higher yields than do those without arbuscular mycorrhizal fungi (Smith and Read, 1997). A significant osmotic adjustment based on the organic solutes,

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http://dx.doi.org/10.1016/j.agwat.2012.11.007

including soluble sugar, proline, etc., and inorganic ions, including potassium, calcium and magnesium, in the leaves of mycorrhizal plants have been reported (Wu and Xia, 2006). Also, improved P uptake due to the presence of arbuscular mycorrhizal fungi during periods of water shortage has been postulated as a primary mechanism for the enhanced drought tolerance of the host plants (Bethlenfalvay et al., 1988). However, other researchers consider that the drought tolerance of the host plants is independent of the P uptake stimulated by the arbuscular mycorrhizal fungi (Augé et al., 1994). Mycorrhizal colonization of the roots has been shown to increase drought tolerance in 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). There are also numerous reports of fungal symbionts (mycobionts) 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). However, most of the relevant experiments were conducted under controlled growth chamber or greenhouse conditions.

Sunflower (*Helianthus annuus* L.) is known as one of the most useful sources of edible oil in the world. The plant has a short vegetation period, is relatively drought tolerant and has shown potential for reducing the existing gap between the production and the consumption of edible oil because it contains 40–50% oil and 17–20% protein (Wang et al., 1997). Like other cultivated crops in arid and semi-arid climates, sunflower is subjected to drought stress. Yet, there is little information on the use of different species of mycorrhizal fungi under field conditions for improving sunflower yield and quality in semi-arid regions. This study investigates two different species of mycorrhizal fungi, investigating their efficiency under two different conditions of drought stress, by quantifying sunflower growth, nutrient uptake, yield, yield components, oil percentages and irrigation water productivity.

2. Materials and methods

2.1. Study site and climatic characteristics

Field experiments were conducted at the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran (35°44′ N, 51°10′ E, altitude 1352 m), during the summers of 2006 and 2007. The average yearly precipitation (over a 30 year period), which occurs mostly during the autumn and winter months, is 298 mm for the site. The annual mean temperature is 19 °C. The average temperature in 2006 was similar to the long-term meteorological data trend, while in 2007, the average temperature (17 °C) was lower.

2.2. Soil sampling and analysis

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 and 30–60 cm, air-dried, crushed and tested for various physical and chemical properties. The research field had a sandy loam soil. Details of the soil properties are shown in Table 1. 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. 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.

Table 1
Soil physico-chemical properties.

Properties	Depth (cm)			
	0–30		30–60	
	2006	2007	2006	2007
Organic matter (%)	1.05	1.06	1.2	0.9
pH (water 1:2 ratio)	7.8	7.2	6.5	7.00
Sand (%)	68	62	59	52
Silt (%)	18	22	29	30
Clay (%)	14	16	12	18
EC ($dS\,m^{-1}$)	1.7	1.0	1.2	1.1
N (%)	0. 7	0.55	0.8	0.8
P ($mg\,kg^{-1}$)	7.5	8.9	n.d.[a]	n.d.
K ($mg\,kg^{-1}$)	310	345	n.d.	n.d.
Fe ($mg\,kg^{-1}$)	6.9	7.5	n.d.	n.d.
Zn ($mg\,kg^{-1}$)	1.2	0.7	n.d.	n.d.
$CaCO_3$ (%)	4.5	7.7	n.d.	n.d.
CEC[b] ($cmol_c\,kg^{-1}$)	5.8	6.1	n.d.	n.d.
Field capacity (%)	22	21	n.d.	n.d.
Crop extractable water (%)	10	10	n.d.	n.d.
Available water (%)	12	11	n.d.	n.d.

[a] Not determined.
[b] Cation exchange capacity.

2.3. Field preparation and treatment application

Sunflowers were planted in different sections of the field each year. Plots were prepared after plowing and disk-harrowing. The plots were 5 m long and consisted of eight rows, 50 cm apart. There was a 2 m path between all of the plots, to eliminate the influence of lateral water movement. The mycorrhizal fungal inoculants consisted of spores and hyphal root fragments from stock cultures of *Glomus mosseae* and *Glomus hoi*. The dose of inocula (125 spores per ml of inoculum) was 50 $g\,m^{-2}$. The *G. mosseae* and *G. hoi* inocula were purchased as pure isolates from the Agricultural and Biotechnology Research Institute, Karaj, Iran. These isolates were selected because of their commercial availability in Iran. Early-maturing sunflower seeds (cv. Alestar) were inoculated with the inoculants and sown during the first week of June. The distance between the plants in the rows was 20 cm; thus, the plant density was approximately 100,000 plants ha^{-1}. According to the recommended N requirements and soil N content for sunflowers, 130 $kg\,ha^{-1}$ N was supplied from fertilizer, using urea as its N source, at two time points, before seed sowing and at the flowering stage (R_2 stage, as described by Schneiter and Miller, 1981). According to the results of the soil analysis, no P or K fertilization was required. The experimental design was arranged as completely randomized blocks, with a split-plot arrangement for the treatments, and four replications. To monitor the soil water content (θ_v), the Time Domain Reflectometry (TDR) method was used. TDR probe tubes were inserted into the soil in each experimental plot at a depth of 0–80 cm (at 20 cm intervals). Data for the soil volumetric water content were collected daily during the growing season using a TDR device (TRIME-FM, England). Irrigation was performed whenever 40% of the available water was consumed, until the seedlings were established. The various experimental irrigation regimes were initiated after the third leaves appeared. The three experimental irrigation regimes, irrigation after 40% of the water was depleted (normal irrigation), irrigation after 60% of the water was depleted (mild stress) and irrigation after 80% of the water was depleted (severe stress), were randomized for the main plots, while the three mycorrhizal treatments, *G. mosseae*, *G. hoi* and no inoculation (control), were randomized for the subplots. A polyethylene piping network with a volume counter was used for accurate and uniform irrigation, and the total amount of water consumed during the growing season, under normal irrigation, and the mild and severe stress conditions, was recorded as 6700,