



Original Scientific Paper

Variations in the water potential of stem xylem in Russian olive (*Elaeagnus angustifolia*) seedlings treated with mycorrhizal fungi under drought conditions

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ABSTRACT:

Measures must be taken to reduce the stress caused by water scarcity, which is the greatest obstacle to increasing the success of afforestation in arid areas. Precautions such as site preparation and species change do not ensure sufficient benefits. For this, it is necessary to try alternative methods such as using mycorrhization of seedlings for afforestation. The aim of the present study was to obtain Russian olive (*Elaeagnus angustifolia*) seedlings with high resistance to water stress and ascertain the effects of mycorrhizae on the water potential of water-stressed seedlings. Accordingly, we determined the water potentials of seedlings inoculated with arbuscular mycorrhizal fungi. Reduction in soil water content caused a reduction in the water potential of seedlings in all treatment variants. Mycorrhization reduced stress by increasing the water potential of seedlings in drought conditions, thereby enhancing their resistance to water stress.

Keywords:

mycorrhizae, water potential, water stress, *Elaeagnus angustifolia*

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INTRODUCTION

The most important factor for the success of afforestation in arid areas is the access of plants to water. Due to the low water-retaining capacity of less developed soils, the effects of drought vary on a regional basis. Although selection of different species, site preparation and various planting techniques have been tried to achieve afforestation in drought-affected fields, the desired success could not be accomplished. Apart from these techniques, the use of mycorrhized seedlings, especially in conditions where drought stress occurs, increases the field performance of seedlings, ensures the success of afforestation and reduces the costs (PERRY *et al.* 1987; ALLEN 1991; KOZŁOWSKI *et al.* 1991; DUNABEITIA *et al.* 2004; EDMONDS *et al.* 2005; KALEFETOGLU & EKMEKCI 2005; TOPRAK 2016).

Water stress prevents the growth and development of plants (BAÑON *et al.* 2004) and reduces resistance to

disease and pests (DESPREZ-LOUSTAU *et al.* 2006; BOSTOCK *et al.* 2014). Since mycorrhizae have positive effects on root biomass and architecture (TOPRAK 2020a), it can increase drought resistance of the plant by taking up the water from capillary pores in arid areas. In addition, mycorrhizae can increase soil water content and infiltration due to their effect on soil structure (BERTA *et al.* 2002; KLIRONOMOS 2003; GAMALERO *et al.* 2004; PIOTROWSKI *et al.* 2004). In experimental conditions, the leaf water potential is often higher in mycorrhized plants under drought conditions in comparison with non mycorrhized controls (DUAN *et al.* 1996; ZARIK *et al.* 2016; BUDAK *et al.* 2017; TOPRAK 2020b). However, the effects of mycorrhizae on the nutritional status and growth performance of deciduous seedlings have been studied more often than water relations (HUANTE *et al.* 1993; RIESKE 2001; TOPRAK 2020a).

Russian olive (*Elaeagnus angustifolia* L.) is a drought-resistant species. It is able to grow in a wide

range of climates and soil conditions. It has been shown to have arbuscular mycorrhizae (RIFFLE 1977). It is also an actinohorizal species, participating in a nitrogen-fixing symbiosis (ZITZER & DAWSON 1992).

The aim of the present study was to grow water stress-resistant Russian olive seedlings and determine the effects of mycorrhizae on the water potential of seedlings under water stress.

MATERIALS AND METHODS

Properties of the soil medium. The soil medium used to grow seedlings consisted of soil (70%) + peat (20%) + perlite (10%). The soil in the medium was obtained from Duzce, Turkey. The soil medium was sterilised for 2 h at 120°C in an autoclave. Initial properties of the non-autoclaved soil are presented in Tables 1-3. The soil samples were air-dried, sieved to obtain a < 2 mm-sized fraction and prepared for chemical analysis. Soil texture was de-

termined with the aid of a Bouyoucos hydrometer (GEE & BAUDER 1986). Acidity was determined with a pH meter (a Hanna-HI 221 microprocessor) and a WTW-Inolab (cond level 1) electrical conductivity (EC) meter used for electrical conductivity. The total calcite content was measured with a Scheibler pressure calcimeter (LOEPPERT & SUAREZ 1996). All samples were analysed for their C and N concentrations by means of dry combustion using a LECO Truspec CN-2000 analyser (LECO Corporation, St. Joseph, MI, USA), while P, K, Ca, Mg, Fe, Cu, Zn and Mn concentrations were determined with an ICP-OES instrument (Perkin Elmer Optima 7000 DV). Cation exchange capacities (CEC) were determined with NH₄OAc extracts (SUMNER & MILLER 1996).

Mycorrhizal mixtures. The commercial mycorrhizal mixture (CM) used in the study [RhizoMyx[®] (Novozymes)] contains arbuscular mycorrhizal fungi and some growth regulators (Table 4).

Table 1. Properties of the soil medium used in seedling pots.

Soil texture	OM %	CEC me 100 g ⁻¹	Total lime %	pH	EC μS cm ⁻¹
Sandy clay loam	1.6 ± 0.1	33 ± 1	2.1 ± 0.4	7.4 ± 0.03	140 ± 1

Table 2. Macronutrient concentration of the soil medium used in seedling pots.

C	N	P	K	Ca	Mg
%		mg kg ⁻¹			
1.1 ± 0.1	0.1 ± 0.02	7.2 ± 0.1	78 ± 0.3	6215 ± 94	130 ± 1

Table 3. Micronutrient concentration (mg kg⁻¹) of the soil medium used in seedling pots.

Fe	Cu	Zn	Mn
19.7 ± 0.1	3.2 ± 0.02	0.5 ± 0.1	38 ± 0.3

Table 4. Composition of mycorrhizal mixture [RhizoMyx[®] (Novozymes)].

Arbuscular mycorrhizae	(propagule g ⁻¹)	Inert ingredients	%
<i>Glomus intraradices</i>	25	Humic acids	28.70
<i>Glomus mosseae</i>	24	Cold-water kelp extracts	18.00
<i>Glomus aggregatum</i>	24	Ascorbic acid	12.00
<i>Glomus clarum</i>	1	Amino acids	6.00
<i>Glomus monosporum</i>	1	Myo-inositol	2.50
<i>Glomus deserticola</i>	1	Surfactant	2.50
<i>Glomus brasilianum</i>	1	Thiamine	1.75
<i>Glomus etunicatum</i>	1	Alpha-tocopherol	1.00
<i>Gigaspora margarita</i>	1		

Table 5. Pearson correlation coefficients for xylem water potential and soil moisture of Russian olive seedlings.

	Soil moisture
(CM)	0.81 ****
(IM)	0.85 ****
(Control)	0.87 ****

Significance levels are indicated by **** $P < 0.0001$.

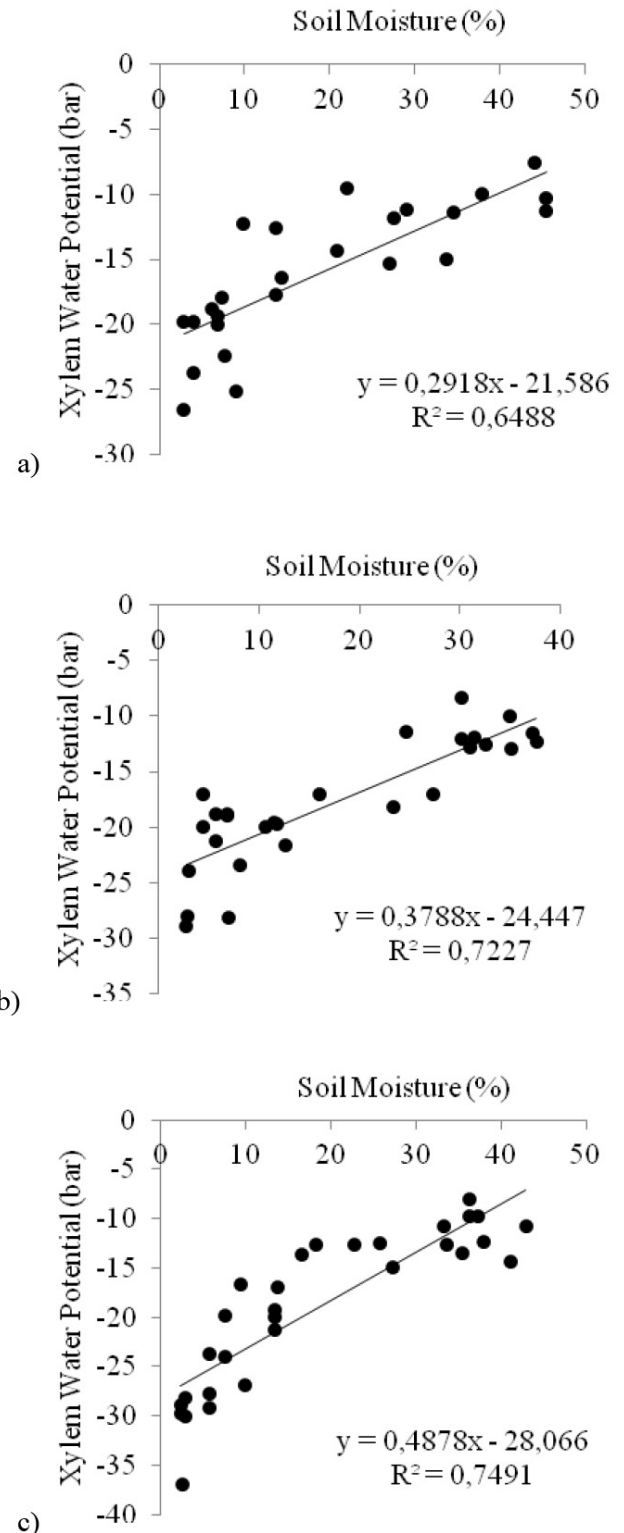
Indigenous mycorrhizal spores were collected from rhizospheres of Russian olive trees in Central Anatolia, Turkey.

Experimental design. A completely randomised design (CRD) was used for the experiment. The experiment was conducted in a greenhouse at the Duzce University in Duzce, Turkey. Russian olive seeds were used for the study.

Seeds subjected to commercial mycorrhizal treatment were dipped in commercial mycorrhizal solutions containing 5 grams of inoculum for 5 minutes and then removed from the solution. The seeds were sown in each of several pots. Two weeks after sowing, solutions of inoculums were prepared by applying 1 g of a cocktail per 100 ml of water and added to the experimental pots. For autochthonous inoculum treatment, 500 indigenous mycorrhizal spores (*Funneliformis*, *Claroideoglomus*) per pot were used and placed 50 mm below the seeds. Spores for this inoculum were collected from rhizospheres of Russian olive trees in Central Anatolia, Turkey. Fifty seedlings were grown per each treatment [commercial mycorrhizae (CM) and indigenous mycorrhizae (IM)]. Another 50 seedlings were grown without any treatment as a control.

To determine arbuscular mycorrhizal colonisation in the roots, root samples were heated in a 10% KOH solution at 90°C for 1 hour. Roots were bleached at room temperature and acidified with 1% HCl. After cleaning, they were stained with 0.05% trypan blue (800 ml glycerine, 800 ml lactic acid, 800 ml distilled water and 1.2 g trypan blue) while being subjected to heating at 90°C for 15 minutes (PHILIPS & HAYMAN 1970; BRUNDRETT *et al.* 1996; UTOBO *et al.* 2011). Segments 1 cm long were used to evaluate the rate of mycorrhizal colonisation following the protocols described by GIOVANNETTI & MOSSE (1980).

Measurement of soil moisture and xylem water potential. Moisture in the pots where seedlings were grown was determined with a moisture meter (Fieldscout TDR 100 Soil Moisture Meter). Seedlings were irrigated at weekly intervals until measurement of the water potential started in the second week of August. Mid-

**Fig. 1.** Relationships between soil moisture and leaf water potential of Russian olive seedlings in CM (a), IM (b) and control (c).

day xylem water potentials (Ψ_p) at the root collar were measured during the gradual decrease in soil moisture every 3 days. To determine the change in Ψ_p caused by the soil moisture drop, water potentials were measured using a pressure chamber (PMS Instruments Company, 1505D-EXP) in the middle of the day, when soil moisture was simultaneously measured in the pots. The water potential of seedlings and soil moisture were measured until the seedlings died.

Statistical analysis. The effects of treatments on Ψ_p were tested by analysis of variance (ANOVA). Tukey's HSD test was performed to compare the means. The relationships between soil moisture and Ψ_p were determined using Pearson's correlation. The results were considered different at a significance level of $\alpha = 0.05$. SAS was used for all statistical analyses (SAS 1996).

RESULTS

The seedling roots that were subjected to both IM and CM treatments developed arbuscular mycorrhiza, while those of the control seedlings were not infected. The colonisation level was much higher in IM-treated seedlings (~70%) than in CM seedlings (~4%) ($P < 0.0001$).

Pearson correlation coefficients between Ψ_p and soil moisture are presented in Table 5. Correlation analysis demonstrated that Ψ_p shows a highly supported positive correlation with soil moisture in the CM, IM and control variants ($P < 0.0001$).

It was determined that there was a positive relationship between soil moisture and Ψ_p of seedlings (R^2 0.65, 0.72, 0.75), and the slope of linear lines was statistically significant ($P < 0.0001$) in the obtained equations in the CM, IM and control variants, respectively. The regression model for estimation of Ψ_p of seedlings depending on soil moisture is given in equations 1, 2 and 3 in the CM, IM and control variants, respectively (Fig. 1).

$$\Psi_p \text{ (bars)} = -21.58603 + 0.29184 \times \text{soil moisture (\%)} \quad (1)$$

$$\Psi_p \text{ (bars)} = -24.44690 + 0.37878 \times \text{soil moisture (\%)} \quad (2)$$

$$\Psi_p \text{ (bars)} = -28.06600 + 0.48776 \times \text{soil moisture (\%)} \quad (3)$$

DISCUSSION

Seedling water potential is an important indicator of the water status in drought conditions (ELSAIED *et al.* 2011). JAFARNIA *et al.* (2018) demonstrated that Persian oak (*Quercus brantii* Lindl) seedlings had a reduced xylem water potential when they were exposed to severe drought stress. TOPRAK (2020a) reported that the water potential of black locust (*Robinia pseudoacacia* L.) seedlings exposed to drought stress decreases with decreasing soil moisture. The reduced water potential of plants

under water stress is a physiological response to enhance drought resistance. A decrease in the water potential of plants due to increased water stress was demonstrated in many studies (GIORIO *et al.* 1999; KIRNAK & DEMIRTAS 2002; TOGNETTI *et al.* 2004; TANG & ZHAO 2006; BOUSSADIA *et al.* 2008; COTROZZI *et al.* 2016, TOPRAK 2020b). As in other studies, loss of soil moisture under progressive drought in the present study was followed by a decrease in Ψ_p of the seedlings. In all treatments, Ψ_p showed a significant decrease under drought stress.

Arbuscular mycorrhizae provide the host plants with more water and some macro- and micro- nutrients, especially P (TOPRAK 2020a, b). Previous studies reported that arbuscular mycorrhizae are able to enhance the drought tolerance of plants (AUGÉ 2001; AUGÉ & MOORE 2005). It was concluded that inoculation with arbuscular mycorrhizal fungi improves the resistance of seedlings to water stress by regulating relationships between the plant and water (AUGÉ 2001; LAMBERS *et al.* 2008; APPLE 2010; RUIZ-LOZANO & AROCA 2010). The water potential of plants was usually higher in arbuscular mycorrhizal plants under conditions of water stress (DUAN *et al.* 1996; AUGÉ 2004; BIRHANE *et al.* 2012; ZARIK *et al.* 2016) because arbuscular mycorrhizae can alter the water relationships of plants (SMITH & READ 2008). In the present study, it was shown that the Ψ_p of mycorrhized seedlings was higher than those of the control seedlings under conditions of about 4% soil moisture (Fig. 1). Arbuscular mycorrhizae improved the drought resistance of Russian olive. Their effect became more visible with an increasing water deficit.

Water stress strongly inhibits seedling growth and has an important role in reducing plantation success (LIVINGSTON & BLACK 1987). Arbuscular mycorrhizal fungi can quickly adapt to soil drought and strongly colonise roots. Russian olive is a nitrogen-fixing species (FISHER & BINKLEY 2000; DECANT 2008). Since it can significantly contribute to the N pool and increase microbial diversity in the rhizosphere, it should be a good candidate to consider for use in afforestation projects to reclaim degraded arid lands (YILDIZ *et al.* 2017).

CONCLUSION

Decrease in soil moisture caused a decrease in the water potential of Russian olive seedlings, but mycorrhized seedlings at lower soil moisture had a higher water potential. Mycorrhizae reduced the water stress of seedlings in drought conditions and increased their resistance to water stress. In addition, Russian olive is known to have nitrogen-fixing root nodules, which allow it to adapt to infertile soils. In order to ensure success of afforestation in arid and semiarid lands, it is necessary to slow down the decrease in the water potential of plants caused by water deficit and promote processes that will enable the water potential to reach higher values at low-

er soil moisture values. The use of mycorrhized Russian olive seedlings can contribute to increasing the success of afforestation in arid and semi-arid areas and it can therefore be recommended to the practitioners.

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REZIME

Botanica
SERBICA

Varijacije u vodnom potencijalu ksilema stabla izdanaka ruske masline (*Elaeagnus angustifolia*) tretiranih mikorizalnim gljivicama u uslovima stresa

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Budući da je stres izazvan nedostatkom vode najveća prepreka uspešnom pošumljavanju u sušnim područjima, potrebno je preduzeti mere kako bi se taj stres smanjio. Priprema lokacije i promena vrsta nisu dali dovoljnu korist, te je potrebno isprobati alternativne metode sa upotrebom mikorize. Cilj ove studije je upotreba klijanaca ruske masline (*Elaeagnus angustifolia*) koji su otporni na nedostatak vode i utvrđivanje efekta mikorize na vodni potencijal klijanaca u uslovima stresa. Zbog toga su određivani vodeni potencijali sadnica inokuliranih arbuskularnim mikoriznim gljivicama. Smanjenje sadržaja vode u tlu uzrokovalo je smanjenje vodnog potencijala sadnica u svim tretmanima. Mikorizacija je smanjila stres povećavajući vodeni potencijal klijanaca u uslovima suše i tako povećala otpornost klijanaca prema stresu.

KLJUČNE REČI: mikoriza; vodni potencijal; vodeni stres; *Elaeagnus angustifolia*

