



## Effect of Native Mycorrhizal Fungi on Morphological and Physiological Traits of Judas Tree (*Cercis siliquastrum*) and Mesquite (*Prosopis cineraria*) Seedlings Under Drought Stress Conditions

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### ABSTRACT

Arbuscular mycorrhizal fungi (AMF) have a mutualistic relationship with a great number of plants. This can offer promising approaches to managing arid ecosystems. In the present study, the effects of native AMF inoculums were evaluated on morphological and physiological traits of *Cercis siliquastrum* and *Prosopis cineraria* seedlings under drought stress conditions. The study was carried out in two independent experiments as a full factorial design with two factors: AM fungal (non- AMF and AMF) and three levels of drought stress (80%, 50%, and 30% of field capacity). The results showed that shoot dry weight and root growth were reduced in response to an increase in drought stress levels on *Prosopis cineraria*. In *C. siliquastrum*, however, the shoot dry weight, root volume and root dry weight increased moderately as a result of AMF but decreased in response to severe drought stress. Native AMF inocula increased proline content by about two-fold, while also increasing root and shoot dry weight and root volume of the inoculated plants of both species. Drought stress increased proline content in both AMF plants and in uninoculated *C. siliquastrum* seedlings. The native AMF colonized the roots of *C. siliquastrum* and *P. cineraria*, by at least 80% and 70%, respectively, which was significantly higher than AMF from soils. Drought stress reduced catalase activity (CAT) in *P. cineraria*, but this was lower in inoculated plants than in uninoculated plants. In response to moderate and severe drought stress, ascorbic peroxidase (APX) activity increased by over 29 and 44%, respectively, compared to well-watered and inoculated *P. cineraria* seedlings. *P. cineraria* seedlings tolerated drought stress by both enzymatic and non-enzymatic ways, while *C. siliquastrum* accumulated osmotic solutes such as proline under drought stress. In conclusion, both species were recommended for xeriscaping purposes, although mesquite proved to be more reliable in adverse conditions.

## Introduction

With regard to biodiversity, some low-maintenance species are defined as such for their low water requirement. They are suitable for landscape design in arid and semi-arid regions as far as xeriscaping is concerned in aspects of worldwide water considerations (Cetin et al., 2018; Sun et al., 2012). From a sustainable perspective, woody legumes are valued as multipurpose species due to their ecological, economic, nutritional, horticultural and ornamental characteristics (Herrera et al., 1993; Johnson, 2016).

Some tree and shrub legumes, including *Cercis* spp. and *Prosopis* spp., are suitable candidates for drought conditions because of their low-to-medium water requirements and low maintenance features (Schuch and Kelly, 2003; Johnson, 2016).

*Cercis* is a genus that consists of 6-10 species which are distributed in warm zones of the Mediterranean, Central and East Asia and North America (More and White, 2003; Liu et al., 2018). *Prosopis* is a commercially important genus of Fabaceae comprising more than 44 species, mostly distributed in arid and semiarid regions of the world (Ross et al., 2014).

Drought stress is one of the most important abiotic stresses worldwide, affecting plant life, biodiversity and agriculture. Various variables in these areas are affected by stress conditions, including morphological characteristics, physiological parameters, biochemical processes, hormonal relationships and molecular networks, cell membrane deterioration and photosynthesis impairment. Furthermore, xylem cavitation and embolism occur in plants that experience drought stress (Vilagrosa et al., 2012; Je et al., 2018). In fact, drought stress reduces photosynthesis by restricting stomatal conductance, ultimately leading to plant growth reduction (Robredo et al., 2010). In such a situation, the overproduction of reactive oxygen species in plants makes a signal for the plant system to control growth and to program cell death (Huang et al., 2017). Osmotic adjustment is important in some plant species as an efficient strategy to cope with environmental stresses, including drought, as reviewed recently by Bahadur et al. (2019). Soluble sugars,  $\alpha$ -tocopherol, carotenoids, glutathione and some amino acids, including proline, are involved in the defense system that acts against oxidative stress (Al-Arjani et al., 2020).

Smith and Read (2008), along with Teste et al. (2020), have suggested that the association of plants with one mycorrhiza at least can benefit

their life cycle and growth in their naturally occurring ecosystems, even as AMF plants are a usual observation in this regard.

The establishment of symbiotic associations in roots (e.g. with arbuscular mycorrhiza) is an efficient and evolutionary developed strategy to increase host plant resistance against environmental stresses (Zhang et al., 2014). Morphological, physiological, hormonal and enzymatic changes are collectively known as plant responses to mycorrhizal inoculation and colonization, mycorrhizal-induced resistance may be obtained through nutrient improvement, metabolite production, hormonal changes, elicitor production and alteration of signaling pathways (Lokhandwala and Hoeksema, 2019; Wu and Zou, 2009).

AMF improves water status by altering root architecture, root hydraulic conductivity and plant aquaporin expression (Khalvati et al., 2005). Meanwhile, plant growth performance, shoot and root biomass, the photosynthesis process and water use efficiency can be potentially improved by AMF application in plant species (Bahadur et al., 2019).

Reactive oxygen species (ROS) are considered as one of the most seriously destructive factors that lead to the oxidization of lipids, DNA and proteins during high-stress conditions so that the balance between ROS production and ROS scavenging would determine ROS accumulation and the oxidative stress response (Mittler et al., 2004). Plants respond to ROS through enzymatic and non-enzymatic strategies (Das and Roychoudhury, 2014).

Antioxidant enzyme activity is a good indicator for evaluating the efficiency of mycorrhizal-induced priming in plant species (Lokhandwala and Hoeksema, 2019).

The protective role of AMF against drought stress-induced ROS accumulation has been demonstrated as AMF caused a considerable increase in the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), ascorbic peroxidase (APX) and proline content in plants, subjected to stress conditions. In particular, this was reported by Marulanda et al., (2007) in *Lavandula spica* inoculated with *Glomus intraradices* and *G. mosseae* under drought stress conditions. Porcel and Ruiz-Lozano (2004) reported similar observations in soybean inoculated with *G. intraradices*, subjected to drought stress, Wu et al. (2007) did the same in *Citrus tangerine* inoculated with *G. mosseae*, *G. geosporum*, *G. versiforme*, *G. etunicatum* and *G. diaphanum* during drought

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stress. Li et al. (2019) reported beneficial effects on *Leymus chinensis* and *Hemarthria altissima* when inoculated with *Glomus* spp., while Zarei and Paymaneh (2013) made such conclusions on *Citrus jambheri* rootstocks inoculated with *G. etunicatum* and *G. intraradices* under salinity stress conditions.

Drought tolerance in trifoliolate orange was improved by enhancing activities and gene expression of antioxidant enzymes such as catalase and superoxide dismutase (He et al., 2019). Accumulation of soluble carbohydrates with osmotic potential has been reported as the main defense mechanism of *Abies alba* (Todea et al., 2020) and *Nerium oleander* (Kumar et al., 2017) under salinity and drought stress.

The origin of AMF and its compatibility with host plants can affect the ability of these fungi under stress conditions (Estrada et al., 2013). Crossay et al. (2019) demonstrated that a mixture of several AMF species could improve plant growth than single AMF species. The adverse effects of stress conditions have been successfully mitigated by AMF being isolated from harsh conditions, indicating adaptation to stress (Estrada et al., 2013) and host plant specificity (Sykorová et al., 2007; Bever et al., 2001).

Considering global warming and drought conditions, xerophytic plant species are preferred for urban and suburban landscapes in areas with inadequate rainfall. Seedling survival and establishment are poor in urban and suburban regions, most possibly because of drought, soil disturbance and/or pollution (Pregitzer et al., 2016; Wang et al., 2018).

In addition, mycorrhizal treatments are practiced to improve plant performance by mitigating transplanting shock during seedling transplanting under stressful conditions, including drought and salt stress, while also benefiting the rehabilitation of degraded lands (Time et al., 2018; Kumar et al., 2017). Furthermore, these soils have lower mycorrhizal fungal species than natural and rural ecosystems. (Bainard et al., 2011). The beneficial effects of AMF have already been reported for ornamental plants used in urban areas (Xie et al., 2020). Pre-inoculation with native mycorrhizal fungi has reportedly improved water content in shoots and general water relations, while also increasing potassium (K) uptake in *Pistacia lentiscus* seedlings after they were planted in degraded soils of southeast Spain (Barea et al., 2011). Mycorrhizal inoculation improved growth and drought tolerance in seedlings of *Acacia seyal* by increasing root length and root count (Abdelmalik et al., 2020).

Arid and semi-arid regions are already experiencing periodic drought stress and,

therefore, drought-tolerant woody species are very important for urban and suburban landscapes in terms of xeriscape purposes. Considering the positive effects of AMF on host plants, this experiment was designed to address the following goals: first, to screen native AMF communities isolated from *C. siliquastrum* and *P. cineraria* plants for their potential to alleviate the deleterious effects of drought stress on seedling growth and antioxidant enzyme activity. Secondly, we aimed to compare native AMF communities with those that inhabit soils in grassless or uncultivated areas. We hypothesized that native AMF communities would be more effective on seedling growth and antioxidant enzyme activity because they are more adapted to each specific host plant.

## Materials and Methods

### *Inoculum production*

To obtain effective native AMF, root and soil samples were collected from the rhizosphere of 20 individual *C. siliquastrum* plants (collected from Kerman city) and also from 10 individual *P. cineraria* plants (collected from Kahnoj and Jiroft cities). The trees ranged in age from 15 to 20 years. One g of root fresh weight was used for staining according to the Koske and Gemma (1989) method in which roots were rinsed in water, cleared in 10 percent potassium hydroxide and stained by 0.05% trypan blue (65 ml lactic acid, 60 ml glycerol, 80 ml water). Mycorrhizal structures were quantified using a light microscope (CH-2 microscope, Olympus, Tokyo, Japan) at 100× magnification. Then, root colonization was assessed by the line-intersect method (McGonigle et al., 1990). The second part of the roots was mixed with the collected soil samples that were used as an inoculum to produce sufficient inocula. Six mycorrhizal communities (i.e. three from *C. siliquastrum* plants and three from *P. cineraria* plants) were selected based on root colonization to produce inoculum by sorghum as a host plant, i.e. compatible with a wide range of AMF species in three replications (Spatafora et al., 2016). A greenhouse experiment was conducted with 18 pots (11 cm height, 13 cm diameter). The pots were filled with autoclaved soil (121°C, 20 min) (1000 g pot<sup>-1</sup>). The harvest was carried out after 90 days. Growth characteristics and root colonization were measured. Then, two AMF communities with the highest root colonization and best growth condition were selected for the next experiments (i.e. one from the *C. siliquastrum* and one from the *P. cineraria* AMF communities). To obtain uniform inoculum, root and rhizosphere soils of three replacement pots were thoroughly mixed to be used in further

experiments. In the inocula, the dominant taxon of arbuscular mycorrhiza was the *genus Glomus* spp.

### Experimental design

The study was carried out in two independent experiments on two plant species (*P. cineraria* and *C. siliquastrum*) in a greenhouse at Shahid Bahonar University. Both experiments were conducted in the greenhouse as factorial with three replications per treatment. Each design had two factors, i) AM fungal (no inoculum or 100 g native AMF inoculum) and ii) water stress with three levels (80%, 50%, and 30% of field capacity) for a total of 36 pots in both experiments. To have seed germination incline towards uniformity, the seeds were collected from a single tree in each species. *P. cineraria* seeds were pretreated with boiling water for 12 h and then kept on a wet paper towel in the refrigerator for one week. *C. siliquastrum* seeds had a thick shell. To break dormancy, seeds were treated with concentrated H<sub>2</sub>SO<sub>4</sub> (97%) for 60 minutes, carefully rinsed in water, and then kept on a wet paper towel in the refrigerator for one month (Olmez et al. 2007). Pots measured 15 cm in height and 17 cm in diameter. They were filled with 1500 g non-sterilized field soil without a history of cultivation from the Shahid Bahonar university campus. The physical and chemical properties of soil in both experiments are documented in Table 1. In both experiments, half of the pots were inoculated with selected native mycorrhizal inoculum. *P. cineraria* was inoculated with a selected inoculum of *P. cineraria*, and *C. siliquastrum* was inoculated with a selected inoculum of *C. siliquastrum*. Half remained uninoculated. The number of spores in the inocula ranged from about 90 to more than 150 spores per gram. To balance the weight of pots, 100 g of sterilized inoculum was autoclaved twice at 121° C for 20 minutes and then was added to uninoculated treatment pots. Both experiments were conducted in a greenhouse from late spring until the end of summer with a temperature between 25-35°C and relative humidity of 45%-65%. Ten pre-germinated seeds were planted in each pot. Two months after planting, three seedlings with uniform sizes and appearance were kept in each pot. During this time, water was supplied daily. The plants were subjected to drought stress after two months of planting. Drought stress levels were imposed by maintaining soil moisture in 80% (control), 50% (moderate stress) and 30% (severe stress) field capacity by daily weighing (Paymaneh et al., 2019).

**Table 1.** Physical and chemical properties of experimental soil.

| Properties                      | values |
|---------------------------------|--------|
| Field capacity(%)               | 25 %   |
| Organic matter(%)               | 0.45 % |
| Available phosphorus(mg kg-1)   | 8.2    |
| pH                              | 7.8    |
| Electrical conductivity(dS m-1) | 1.2    |
| Clay (%)                        | 16.6 % |
| Silt (%)                        | 16.3 % |
| Sand (%)                        | 67.1 % |

To measure field capacity, four pots were weighed and then used for the experiment. They were filled with a specific weight of soil. At first, the soil was saturated with excess water to fill all micropores by water. The surface of the pots was covered by plastic to avoid evaporation, and the soil was allowed to drain freely from the bottom of the pots after about 24h. Moist soil was weighed (FW) and was dried in an oven at 105°C until a constant weight was reached. The dry soil was weighed (DW). Data were used for estimating the amount of moisture held by the soil at field capacity by the following formula (Ogbaga et al., 2014):

$$(FW-DW)/(DW)*100.$$

Plants were harvested four months after the beginning of drought stress treatments. Then, shoots and roots were separated to be weighed. Root volume was measured by the water displacement method (Bruns and Croy, 1985). Root length and plant height were measured by a ruler. Fresh shoots and roots were divided into two parts. One part of fresh shoots was kept at -20 °C to measure enzyme activity. A part of the roots was separated to measure root colonization. Root colonization was assessed by the above procedure. The rest of the roots and shoots were weighed after being dried at 70 °C for 72h.

### Proline, protein and antioxidant enzymes assays

Protein content and enzyme activity were measured in plant leaf extracts. For this purpose, 0.5 g of plant fresh leaves were frozen by liquid nitrogen in a porcelain mortar. Then, they were ground and homogenized in 0.05 M Tris-HCl buffer (pH 7.5). After 10 minutes, the homogenate was centrifuged 10000-× g for 20 minutes. The supernatant was collected to perform assays on protein content and enzyme activity (Zarei et al., 2016).

Protein was assayed by Coomassie brilliant blue G (CBBG) according to Bradford's (1976) method, whereby 100-microliter aliquots of extract and

five ml CBBG solution were thoroughly mixed. After 20 minutes, the absorbance was measured at 529 nm by a spectrophotometer (UV-26, Germany). Protein concentration was assayed by a standard curve of bovine serum albumin (BSA). Catalase activity was estimated based on the decomposition of H<sub>2</sub>O<sub>2</sub> (Chance and Maehly, 1955). The reaction mixture contained 50 mmol potassium phosphate buffer (pH 7), 15 mmol H<sub>2</sub>O<sub>2</sub> and 100-microliter aliquots of plant tissue extract. The change in absorbance was measured at 240 nm by the spectrophotometer.

Ascorbic peroxidase (APX) enzyme was estimated, according to Nakano and Asada's (1981) method. The reaction mixture contained 50 mmol potassium phosphate buffer (pH 7), 0.5 mmol ascorbates, 0.1 mmol H<sub>2</sub>O<sub>2</sub> and 150 microliter aliquots of plant tissue extract. The activity of APX was estimated based on the oxidation of ascorbic acid, while the decrease in absorbance was recorded at 290 nm for two minutes by the spectrophotometer.

Proline content was assayed according to a method by Bates et al. (1973). In this method, 0.1 mg of the fresh, fully developed leaf was homogenized by sulfosalicylic acid (3%, 10 ml). The homogenate was centrifuged 10000-xg for five minutes, and then the supernatant (2 ml) was collected to measure proline content. The extract was allowed to react with ninhydrin acid and then was measured by the spectrophotometric at 520 nm.

### Statistical analyses

All statistical analyses were carried out using SAS 9.1. The data were statistically evaluated using a general linear model (GLM) analysis or by the two-way analysis of variance (ANOVAs) after checking for their conformity with ANOVA assumptions (homoscedasticity). Differences between results arising from treatments on plants were recorded in a pot experiment with *P. cineraria* or *C. siliquastrum* seedlings inoculated or not with native AMF. The plants were subjected to three different levels of drought stress and were tested using Fisher's least significant difference (LSD) ( $P < 0.05$ ).

## Results

### First experiment (*Prosopis cineraria*)

#### Root colonization and growth characters of *Prosopis cineraria*

AMF significantly increased the degree of root colonization in inoculated plants than in uninoculated plants. Native AMF increased root colonization by over 80% in inoculated *P. cineraria*, compared with uninoculated plants. Drought stress could not suppress AMF development in *P. cineraria* plants (Fig. 1f and

Table 2).

Drought stress reduced shoot dry weight and root length in *P. cineraria* plants, while native AMF inoculum enhanced the growth of inoculated plants by increasing shoot and root dry weight and root volume. Also, root volume and root dry weight did not decrease by drought stress but were actually stimulated to cause more root growth in AMF plants (Fig. 1a, b, e and Table 2). The highest shoot dry weight was found in the inoculated plants under well-watered (control) conditions in *P. cineraria* plants. There was a trend of increase in the root length of inoculated plants compared with uninoculated plants under drought stress conditions. The decrease in the root length of uninoculated plants was significantly higher than that of uninoculated plants (Fig. 1d and Table 2).

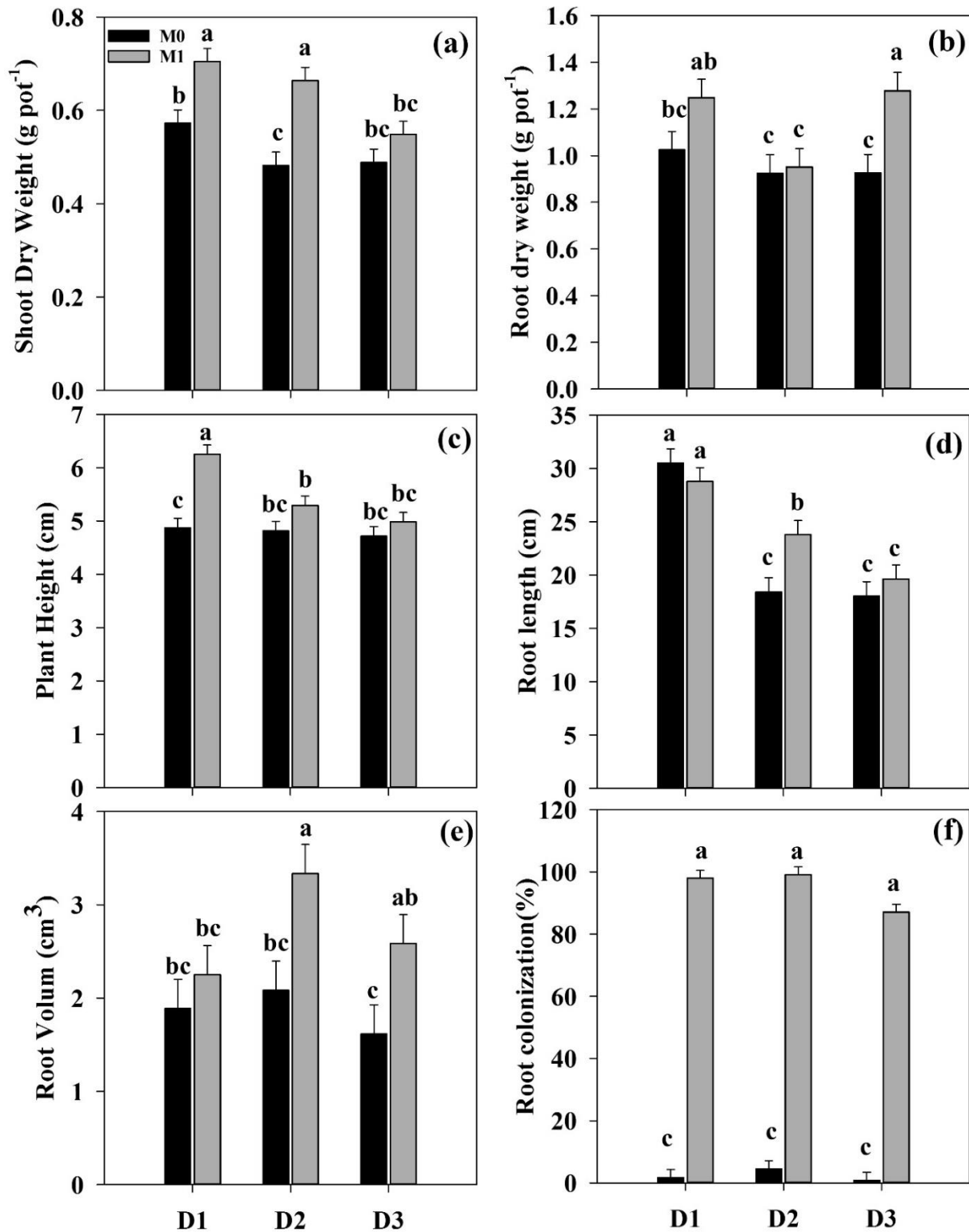
in a pot experiment with *P. cineraria* seedlings. Different letters in each graph indicate significantly different treatments. Means are separated using Fisher's least significant difference (LSD) test at  $P < 0.05$ .

#### *Prosopis cineraria* biochemical characteristics

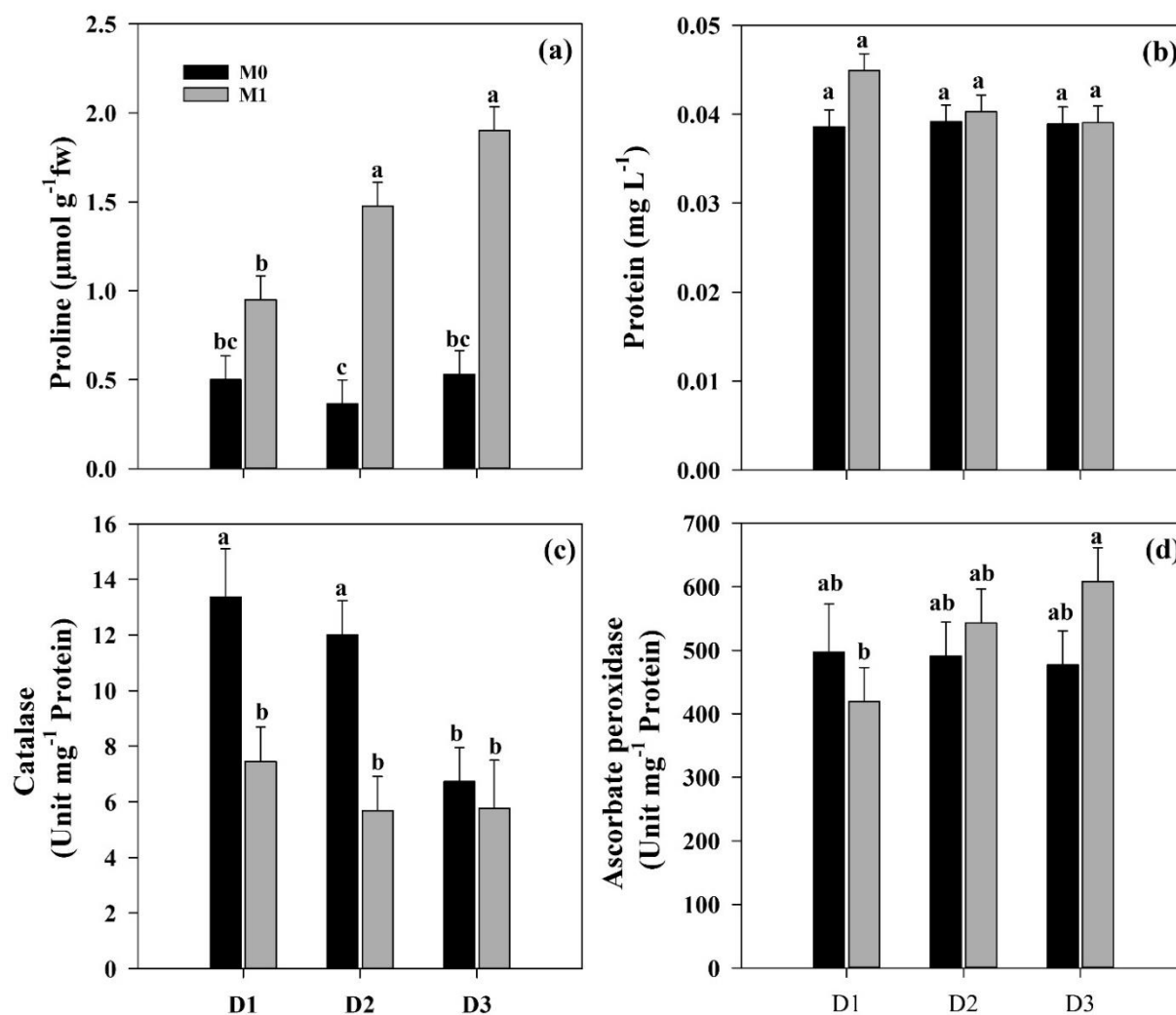
Inoculated *P. cineraria* seedlings with native AMF were able to accumulate higher proline content than uninoculated plants. The proline content was significantly increased by intensified drought stress levels in the inoculated plants, but it was not affected in uninoculated plants. Native AMF increased proline content in well-watered, moderate and severe drought-stressed plants by 100%, 300% and 250%, respectively, compared to non-AMF *P. cineraria* (Fig. 2a and Table 2).

Catalase activity in uninoculated plants was higher than in inoculated *P. cineraria* plants. There was no significant difference between inoculated and uninoculated plants under severe stress conditions. The activity of the catalase enzyme decreased parallel to increasing drought stress levels, although this trend was insignificant in uninoculated plants (Fig. 2c and Table 2).

Protein content was not affected by drought stress and/or AMF in *P. cineraria* plants (Fig. 2b and Table 2). In inoculated plants, the APX activity increased as the drought stress intensified. In response to moderate and severe drought stress, the APX activity increased by 29% and 44%, respectively, compared to well-watered plants. No difference in APX activity occurred, despite increasing levels of drought stress in the uninoculated plants (Fig. 2d and Table 2). In inoculated *P. cineraria*, native AMF increased APX activity in well-watered, moderate and severe drought-stressed plants by 45, 10 and 27%, respectively, compared to uninoculated non-AMF *P. cineraria*.



**Fig. 1.** Effects of selected native arbuscular mycorrhizal fungi (M0 uninoculated with AMF and M1 inoculated with AMF) pre-cultured with sorghum and drought stress (D1, 80% as control, D2, 50% (moderate stress), and D3, 30% (severe stress) field capacity) on shoot dry weight (a), root dry weight (b), plant height (c), root volume (e), root length (d) and root colonization (f)



**Fig. 2.** Effects of the provenance of selected native arbuscular mycorrhizal fungi (M0 uninoculated with AMF and M1 inoculated with AMF) pre-cultured with sorghum and drought stress (D1, 80% as control, D2, 50% (moderate stress), and D3, 30% (severe stress) field capacity) on proline content (a), protein content (b), Catalase activity (c) and Ascorbic peroxidase activity (d) in a pot experiment with *P. cineraria* seedlings. Different letters in each graph indicate significantly different treatments. Means are separated using Fisher's least significant difference (LSD) test at  $P < 0.05$ .

### ***Second experiment (Cercis siliquastrum)*** ***Root colonization and growth characteristics of Cercis siliquastrum***

Drought stress stimulated AMF in the uninoculated Judas tree seedlings, especially in moderate water deficiency, but did not affect native AMF development in the roots of inoculated seedlings. Native AMF increased root colonization by over 70% in inoculated plants, compared to uninoculated ones (Fig. 3f and Table 2).

Different levels of water supply altered the development of *C. siliquastrum* plants. Surprisingly, native AMF suppressed the negative effects of drought stress on the shoot dry weight of *C. siliquastrum* seedlings. Native AMF

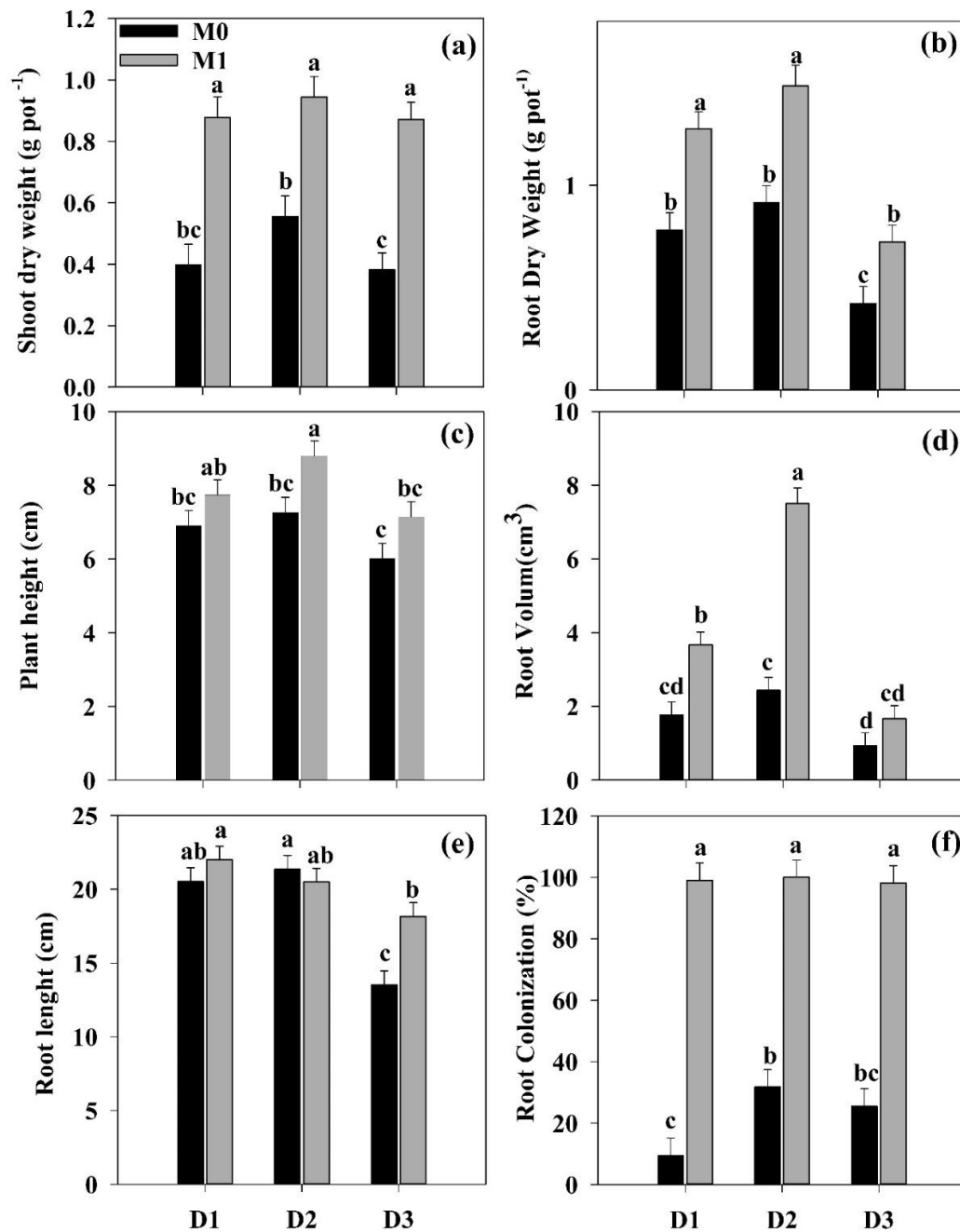
increased shoot dry weight twice as much as the weight observed in non-AMF *C. siliquastrum* with respect to drought stress levels. Root dry weight and root volume increased in response to moderate stress levels and decreased in severe stress levels, compared to well-watered *C. siliquastrum* plants. Root dry weight and root volume were higher in inoculated plants compared to uninoculated plants (Fig. 3a-e and Table 2). Native AMF increased the root volume in well-watered plants, as well as in moderate and severe drought-stressed plants, by more than 100%, 200% and 100%, respectively, compared to non-AMF *C. siliquastrum* (Fig. 3d and Table 2).

Table 2. Results of the general linear model (GLM) analyzes with plant parameters recorded in a pot experiment with *P. cineraria* and *C. siliquastrum* seedlings inoculated or not with native AMF from *P. cineraria* or *C. siliquastrum*, subjected to three different levels of drought stress (n=3).

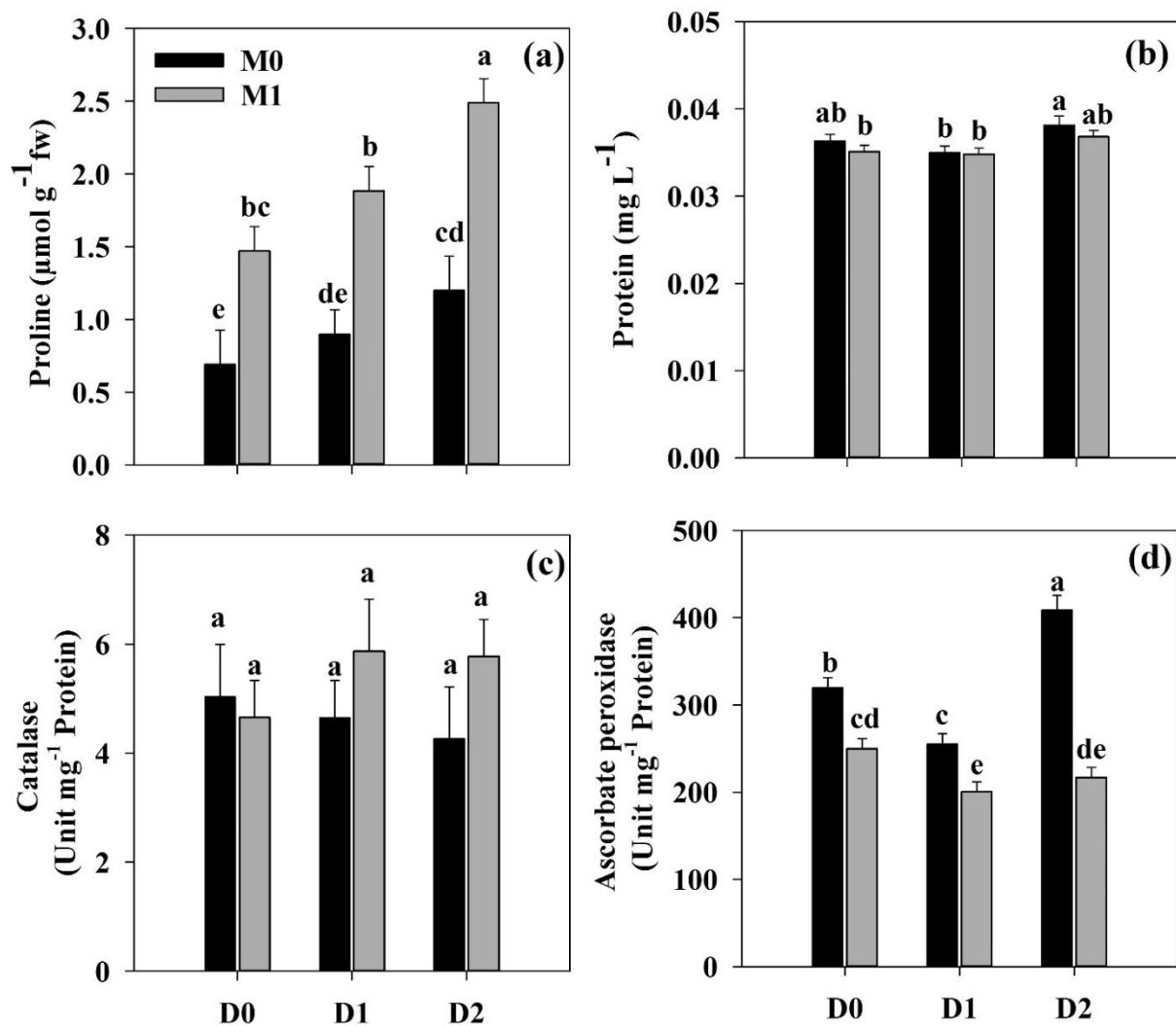
|                               | DF | Root<br>colonization | Shoot dry<br>weight | Root dry<br>weight | Plant<br>height | Root<br>volume | Root<br>length | Proline<br>content | Protein | CAT         | APX           |
|-------------------------------|----|----------------------|---------------------|--------------------|-----------------|----------------|----------------|--------------------|---------|-------------|---------------|
| <b><i>P. cineraria</i></b>    |    |                      |                     |                    |                 |                |                |                    |         |             |               |
| Mycorrhiza (F)                | 1  | 1965.67 ***          | 28.82 ***           | 9.73 **            | 23.71 ***       | 11.45 **       | 2.55 ns        | 78.58<br>***       | 2.67 ns | 28.94*<br>* | 0.78 ns       |
| Drought (D)                   | 2  | 5.17 *               | 8.96 **             | 3.65ns             | 8.55 **         | 2.69 ns        | 37.03 ***      | 6.68 *             | 1.15 ns | 8.82*       | 1.57 ns       |
| F × D                         | 2  | 2.23 ns              | 2.34 ns             | 2.17 ns            | 5.53 *          | 1.07 ns        | 3.61 ns        | 6.18 *             | 1.56 ns | 4.43 ns     | 2.34 ns       |
| <b><i>C. siliquastrum</i></b> |    |                      |                     |                    |                 |                |                |                    |         |             |               |
| Mycorrhiza (F)                | 1  | 279.03 ***           | 153.01 ***          | 49.59 ***          | 12.08 **        | 88.64***       | 5.32 *         | 84.87***           | 2.88 ns | 4.00 ns     | 150.12<br>*** |
| Drought (D)                   | 2  | 2.20 ns              | 4.61 *              | 33.73 ***          | 6.22 *          | 61.34***       | 21.69 ***      | 16.06 **           | 7.61 *  | 0.38 ns     | 33.65 ***     |
| F × D                         | 2  | 2.02 ns              | 0.78 ns             | 1.52 ns            | 0.36 ns         | 22.55 ***      | 4.48 *         | 1.80 ns            | 0.42 ns | 2.26 ns     | 25.38 **      |

DF, degrees of freedom; CAT, Catalase activity; APX, Ascorbic peroxidase activity; ns,  $p \geq 0.05$ ; \*,  $0.01 \leq p < 0.05$ ; \*\*,  $0.001 \leq p < 0.01$ ; \*\*\*,  $p < 0.001$





**Fig. 3.** Effects of the provenance of selected native arbuscular mycorrhizal fungi (M0 uninoculated with AMF and M1 inoculated with AMF) pre-cultured with sorghum and drought stress (D1, 80% as control, D2, 50% (moderate stress), and D3, 30% (severe stress) field capacity) on shoot dry weight (a), root dry weight (b), plant height (c), root volume (d), root length (e) and root colonization (f) in a pot experiment with *C. siliquastrum* seedlings. Different letters in each graph indicate significantly different treatments. Means are separated using Fisher's least significant difference (LSD) test at  $P < 0.05$ .



**Fig. 4.** Effects of the provenance of selected native arbuscular mycorrhizal fungi (M0 uninoculated with AMF and M1 inoculated with AMF) pre-cultured with sorghum and drought stress (D1, 80% as control, D2, 50% (moderate stress), and D3, 30% (severe stress) field capacity) on proline content (a), protein content (b), Catalase activity (c) and Ascorbic peroxidase activity (d) in a pot experiment with *C. siliquastrum* seedlings. Different letters in each graph indicate significantly different effects by the treatments. Mean values are separated using Fisher's least significant difference (LSD) test at  $P < 0.05$ .

### *Cercis siliquastrum* biochemical characteristics

Drought stress significantly increased proline content in both inoculated and uninoculated plants. As a result of native AMF, proline content increased twice as much as the amount observed in uninoculated plants under well-watered and drought stress conditions. Protein content in *C. siliquastrum* plants was not affected by AMF and/or drought stress (Fig. 4a, b and Table 2). Catalase activity increased in inoculated plants

but decreased in uninoculated plants as a result of intense drought stress, although these changes were insignificant (Fig. 4c and Table 2).

APX was lower in the inoculated plants than in uninoculated plants. The activity of APX decreased in response to a moderate intensity of drought stress in both inoculated and uninoculated plants. In severe stress conditions, however, APX activity decreased in inoculated plants but increased significantly (by about 27%) in uninoculated plants (Fig. 4d and Table 2).

## Discussion

Due to its complexity, plant drought tolerance is not fully understood (Wang et al., 2016b). A diverse range of responses is triggered when plant species are exposed to drought stress (Griffin et al., 2004). Plant species, even from the same family or genus, respond differently to stress conditions (Lopez Lauenstein et al., 2013; Fang et al., 2014).

AMF is a promising strategy for plant establishment in arid ecosystems. Many studies have confirmed the higher efficiency and/or compatibility of native AMF, compared to commercial inoculums in host plants (Séry et al., 2016; Emam, 2016). Also, Paymaneh et al. (2019) showed that some commercially-abundant AMF species do not perform efficiently under stress conditions (Badri et al., 2016). AMF community compositions could be changed by stress conditions and the inoculum potential is a key element for the maintenance of AMF in new conditions (Brundrett, 1991).

Our study showed that native AMF could be more useful in colonizing the roots of both species than the AMF of other soils, which is consistent with previous findings by Bahadur et al. (2019). It could be said that native AMF inocula are superior competitors that persist on root niches and adapt to survive in adverse conditions (Hart et al., 2001). Root colonization was not affected by drought stress in inoculated and uninoculated plants of both species. Drought stress increased root colonization of AMF in *C. siliquastrum*. This may be because the plant species is highly dependent on AMF. On the other hand, it may indicate that drought-tolerant plants could support AMF under drought stress conditions. According to reference data, the percentage of colonization was more than 97 percent in non-stress conditions and was more than 86 percent in drought stress conditions (Fig. 1f and 3f and Supplementary Fig. S2 g-i).

*C. siliquastrum* and *P. cineraria* exhibited different root morphologies as presented by root length and root volume. In the case of root length, *P. cineraria* plants had longer roots than *C. siliquastrum* plants (Fig. 1b, d, e and 3b, d, e and Supplementary Fig. S2a-f). Root length revealed that *C. siliquastrum* plants were more susceptible to drought stress than *P. cineraria* plants. In general, *P. cineraria* had a smaller root volume than *C. siliquastrum* plants, regardless of drought stress. Nonetheless, root growth under stress conditions appeared as a drought response mechanism in both plants. On the other hand, root volume was stimulated by moderate drought stress conditions, as suggested in earlier research by Tajabadi Pour et al. (2005).

Horizontal and tap root developments have been

reported when seedlings of *Prosopis juliflora* experienced different soil moistures (Yoda et al., 2012). In the case of *C. siliquastrum*, root and shoot morphology have had an important role under different irrigation regimes as reported by Rahman et al. (2013). Plasticity of the root system and general root distribution have been suggested as strategies against environmental stress on some mesquite species such as *P. glandulosa* (Ansley et al., 2007). *Prosopis* species have developed various mechanisms to cope with harsh environmental stresses, including water deficits (Giordano et al., 2011; Time et al., 2018). Root dry weight was less affected by irrigation regimes in *P. cineraria* than in *C. siliquastrum* (Fig. 1b and 3b, Supplementary Fig. S1g-i and S2a-f)

Native AMF helped both plant species by stimulating root growth under well-watered and stressful conditions, most possibly through water absorption (Khalvati et al., 2005). Similar results were reported by Berta et al. (1990) and Schellenbaum et al. (1991). Native AMF had greater effects on the root volume of *C. siliquastrum*, compared to *P. cineraria*. In moderate drought stress, the root volume of inoculated plants of *C. siliquastrum* and *P. cineraria* increased by 200% and 100%, respectively, compared to non-AMF plants, thereby confirming previous results by Tajabadi Pour et al. (2005).

No significant effects of native AMF were observed on the root length of both species. It might be assumed that root length in both species is more genetically controlled. Root system morphology is related to the strategies of root function under stress conditions (Yoda et al., 2012; Rahman et al., 2013; Berta et al., 1990). To survive in arid ecosystems, xerophytic plants, especially tree species, allocate more biomass to the roots than to shoot biomass (Brunner et al., 2015). The findings of the present study indicated that *P. cineraria* plants tend to stage a greater decrease in their shoot dry weight than in their root dry weight under drought stress.

AMF partially alleviated the negative effects of stress conditions by increasing shoot dry weight and plant height (Supplementary Fig. S2a-c, g-i). Previous studies demonstrated the positive effects of AMF on plants grown under stress conditions by increasing the photosynthetic rate, antioxidant enzymatic activity and nutrient uptake of host plants (Alguacil et al., 2006; Paymaneh et al., 2019). Total shoot length, trunk diameter, shoot and root biomass and shoot to root ratio are reportedly some morphological measures that were positively affected by AMF inoculation in *Acacia smallii* and *Fraxinus uhdei* grown on urban soil (Xie et al., 2020).

Moreover, AMF plants mitigate drought stress-induced oxidative stress through the production of antioxidant compounds, ultimately scavenging the ROS and activating the defense system (Bahadur et al., 2019). The increase of antioxidant enzyme activity and the accumulation of proline in plants that are subjected to stress conditions act as primary defense responses to alleviate oxidative stress through osmotic regulation (Wang et al., 2016a; Porcel and Ruiz-Lozano, 2004; Alguacil et al., 2006). Thus, the capacity of antioxidant enzyme activity and proline accumulation usually determine the potential of plant species to bear stressful conditions (Anjum et al., 2017). In our experiment, both plant species accumulated proline to cope with drought stress, particularly in severe stress conditions, corresponding to previous findings by Heerden and Villiers (1996). Meanwhile, native AMF had an effective role in increasing plant resistance, parallel to an increase in proline content in both plants. In particular, uninoculated *C. siliquastrum* used a similar mechanism of tolerance. *C. siliquastrum* appeared more susceptible to stress than *P. cineraria*, while showing higher proline accumulation (Fig. 2a and 4a and Supplementary Fig. S3a-c).

Proline accumulation was an important response to drought stress in *C. siliquastrum* plants. On the other hand, *P. cineraria* inoculation with native AMF dramatically increased proline content. While confirming the effects of severe drought stress on *P. cineraria* and *C. siliquastrum* plants, AMF increased proline content by 3.5 and 2 times, respectively, more than non-AMF plants. Chun et al. (2018) reported that AMF-inoculated plant species responded to drought stress conditions by increasing proline content. Protein measurements followed no specific trend and could not be considered as an adaptive mechanism under drought stress in both species (Fig. 2a,b and 4a, b and Supplementary Fig. S3a-f).

The varying activity of CAT and APX in *P. cineraria* and *C. siliquastrum* plants, as caused by drought stress and/or inoculation with native AMF, is not clear to the authors (Fig. 2c, d and 4c, d and Supplementary Fig. S3g-l). This difference in the behavior of the two enzymes may be due to differences in the tolerance of the two plants species, whereas the effects of mycorrhiza on the two enzymes was completely different. For instance, ascorbate peroxidase was lower in the amount of the *C. siliquastrum* seedlings treated with native AMF than those without this treatment. Drought stress reduced CAT activity in the uninoculated seedlings of *P. cineraria*, meaning that it was higher in *P. cineraria*

inoculated with native AMF. Drought stress increased APX activity in inoculated *P. cineraria* plants by about 44% in response to severe stress, compared to well-watered plants. APX was not significantly affected by drought stress in uninoculated plants, however. AMF enhances the tolerance of plants to drought stress by modifying antioxidant enzyme activity (Li et al., 2019). Huang (2017) demonstrated the ability of roots to promote H<sub>2</sub>O<sub>2</sub> effluxes into the rhizosphere and reported that AMF stimulates the process using aquaporins which can be expressed in extraradical mycelial structures under drought stress conditions (Li et al., 2013), thereby facilitating the diffusion of hydrogen peroxide across membranes (Bienert et al., 2007). The mechanisms mentioned above can be involved in reducing CAT activity. Moreover, a decrease in CAT activity could be compensated by enhanced levels of APX activity in inoculated *P. cineraria* plants. The attenuation of drought-stress-induced effects, mostly detrimental and triggered by AMF in plants, is well documented in the available literature (Marulanda et al., 2007; Zarei et al., 2016). Enzymatic activity may not be the primary response to drought stress in *C. siliquastrum*, although it appears that this species copes with stress conditions through osmotic adjustment. In particular, the accumulation of proline and morphological adaptation assist the plant in drought stress tolerance. While *P. cineraria* responded to stress conditions mostly through enzymatic scavenging-pathways, its antioxidant capacity responds to AMF in a species-specific manner. In general, AMF communities stimulated antioxidant activities to assist plants in tolerance when stress conditions prevailed.

## Conclusion

*Cercis* is a mesic or xeric species, while *Prosopis* is conclusively a xeric one. Some species of *Cercis* and *Prosopis* can be used for urban landscape purposes and xeriscape plantings due to their aesthetic values and their tolerance to drought, high temperature and salinity (Griffin et al., 2004; Schuch and Kelly, 2007; Cetin et al., 2018). Native AMF inocula from *P. cineraria* and *C. siliquastrum* trees successfully colonized seedling roots and were more compatible and effective under stress conditions than the AMF of other soils. It appeared that *P. cineraria* tolerated drought stress by both enzymatic and non-enzymatic ways, whereas *C. siliquastrum* accumulated osmotic solutions such as proline to withstand drought stress. Meanwhile, the underlying physiological and biochemical processes that led to these observations are not fully understood. Various hormones, many biochemical processes

and pathways occur in a complex molecular network and are triggered under stress conditions. For a deep understanding of drought and AMF responses by woody legumes, including those reported in this communication, future research could be carried out on molecular networks and relevant gene expressions in such settings.

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### Conflicts of interest

The authors declare they have no financial interests.

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