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Original Paper

Effect of mycorrhization and water stress on morphological parameters, concentration of phenolic compounds and antioxidant capacity of methanolic extract of *Lupinus albus*

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Abstract

Secondary metabolites and mycorrhizal symbiosis play important roles in the adaptation of plants to environmental conditions, but mycorrhizal symbiosis favours also accumulation of secondary metabolites. Our objective was to study effect of interaction between mycorrhization and water deficiency on morphological parameters, phenolic compound concentration in *Lupinus albus* and to perform an evaluation of the antioxidant potential of methanolic extract of this plant. Plants of *L. albus* were subjected to three levels of water defficiency (80, 50 and 30% of field capacity). Half of the plants in each treatment were inoculated with arbuscular mycorrhizal fungi. Total concentrations of polyphenols, flavonoids, and condensed tannins were measured, and antioxidant potential of methanolic extracts from leaves and roots of white lupin plants was evaluated. In general, mycorrhizal plants of *L. albus* had better growth indices in all watering regimes in comparison to non-mycorrhizal plants. The concentrations of phenolic compounds were maximal in the leaf extracts from *L. albus* plants inoculated with arbuscular mycorrhizal fungi grown under 30% of field capacity water regime, and in root extract of non-inoculated plants grown under 30% of field capacity. Methanolic extracts of all plants showed good antioxidant activity, with higher values in mycorrhizal drought-stressed plants.

Key words: antioxidants, *Lupinus albus*, morphological parameters, mycorrhization, secondary metabolites, water stress.
Abbreviations: AMF, arbuscular mycorrizal fungi; CE, catechin equivalent; DM, dry matter; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FC, field capacity; GAE, gallic acid equivalent; QE, quercetin equivalent; ROS, reactive oxygen species.

Introduction

Lupinus albus L. is a legume plant; its seeds have been used as food and feed from ancient times due to their nutritional and therapeutic characteristics (Kinder, Knecht 2011). The green plant itself has been used extensively in some countries as forage and as an organic material for soil enrichment, or in crop rotation, because plant is capable of forming a symbiosis with rhizobacteria to fix atmospheric nitrogen (Yoneyama, Natsume 2010). *L. albus* is rich in bioactive molecules such as polyphenols, flavonoids and tannins, which have antioxidant properties (Petterson 2016; Karamać et al. 2018). Indeed, plants have evolved both enzymatic and non-enzymatic defense systems for scavenging and detoxifying reactive oxygen species (ROS), resulting in relatively high antioxidant defense capacity (Regnault et al. 2008).

Water shortage or drought stress is one of the most widespread environmental factors that negatively affects both crop growth and yield (Lisar et al. 2012). It has been extensively reported that drought stress results in inhibition of photosynthesis, increased oxidative stress and changes in metabolism of plants (Farooq et al. 2009; Sohrabi et al. 2012; Fahad et al., 2017; Michaletti et al. 2018; Kapoor et al. 2020; Parkash, Singh 2020). However, some antioxidant compounds such as ascorbic acid, rutin, quercetin, flavonoids and tannins, produced in plants play a significant role in scavenging ROS (Ashraf et al. 2018).

Arbuscular mycorrhizal fungi (AMF) are known to stimulate growth performance and to increase tolerance

to unfavourable conditions such as drought (Bahadur et al. 2019). Mycorrhization can improve mineral nutrition of the host plant and forms an extended network of hyphae, considerably improving mineral nutrient absorption capacity of roots (Teotia et al. 2017). Moreover, symbiosis with AMF protects plants from unfavourable environmental conditions by inducing the enzymatic antioxidant system (Hashem et al. 2016) and stimulating accumulation of secondary metabolites (Walter, Strack 2011). Higher concentration of polyphenols, flavonoids and tannins has been observed in foliar and root parts of mycorrhizal plants under drought stress (Jugran et al. 2015). Mycorrhizal plants counteract water deficit-induced oxidative stress by enhancing capacity of antioxidant compound production and improved the enzymatic antioxidative activitiy (Sohrabi et al. 2012).

The objective of the present study was to assess the effect of mycorrhization on growth and phenolic compound levels of *L. albus* plants grown at different levels of water availability, and to evaluate antioxidant potential of methanolic extract of this plant.

Materials and methods

Plant material and experimental conditions

Seeds of white lupin, *Lupinus albus* L., were provided by the National Institute of Agronomic Research (INRA) of Sidi Bel Abbes (Algeria).

The experiment was carried out in a semi-controlled greenhouse at the Institut de Technologie Moyenne de l'Agriculture (ITMA) of Tiaret (Algeria). The plants were maintained in the greenhouse at conditions of 16/8 h light/dark cycle, 252 lux light intensity, 28 °C and 60% relative humidity.

Native strains of mycorrhizae were used. These strains were trapped in leek (*Allium porrum* L.) roots. Leek plants were grown in non-sterile soil from the Tiaret area to trap spores of arbuscular mycorrhizal fungi. To ensure that trapping was successful and that leek roots were infected by the mycorrhizal fungi, arbuscular mycorrhizal structures were observed at the root level following the protocol of Kormanik and McGraw (1982) and Vierheilig et al. (1998).

L. albus seeds were disinfected as described by (Redon et al. 2009). The seeds were immersed in 30% H_2O_2 for 5 min and then rinsed five times with distilled water. Seeds were then soaked in distilled water containing three drops of Tween 80 for 3 min and then rinsed several times with distilled water. Disinfected seeds were placed on moistened blotting paper at 20 °C. The germination rate of a seed batch, calculated as the number of germinated seeds by the number of tested seeds, was 98%.

Sterilized seeds were immediately placed in trays containing potting soil sterilized at 120 °C for 3 h in a ventilated oven. They were irrigated daily with demineralized water. After one week, *L. albus* seedlings were planted individually in plastic pots (22 cm in height and 15 cm in diameter) filled with 6.5 kg of substrate [mixture of phosphorus-deficient calcareous soil and sand, 2:1 (v/v); Table 1].

Roots of leek plants infected with the mycorrhizal fungus were washed, cut into small pieces, mixed with sterilized substrate and used as an inoculum. The latter represented 10% of the total weight of substrate used in cultivation (650 g). The inoculum was placed at the base of the roots of seedlings transplanted according to the protocol of McGonigles et al. (1990). *L. albus* was used in two regimes (in the presence or absence of mycorrhizal inoculum) with three levels of water efficiency (80, 50 and 30% of field capacity, FC). Uninoculated plants cultivated under 80% of FC were considered as the control plants.

Ten days after sowing, different water regimes were applied to the plants at a rate of 30, 50 and 80% of the field capacity until the end of the experiment. Soil field capacity was estimated by the following equation:

$$FC = [(W2 - W1] / W2) \times 100,$$

where FC is field capacity, W1 is dry weight of the substrate, W2 is substrate weight at saturation.

The experimental design was a total randomization with three replicates. Each replicate consisted of five plants in individual pots from the same treatment. The pots of AMF-inoculated plants were separated from the pots of AMF-uninoculated plants by a 10 m space to avoid fungal contamination. Five plants from each treatment in each replicate were used to give a total of 15 plants for the different measurements.

Morphological parameters

The length of roots was determined using a graduated tape measure. Root volume was measured by immersing the root system in a graduated cylinder filled with water, according to Archimedes' thrust principle: the volume of an immersed body is equal to the volume of the displaced liquid (difference in level).

Biomass of aerial parts and roots, both fresh and dry, were determined using a precision balance. Fresh weight

Table 1. Results of chemical analysis of the substrate (soil + sand) used in the experiment. Results are averages of 15 replicates. The methods of analysis as well as the expression of the results were performed according to Mathieu, Pieltain (2003)

Parameter (unit)	Value
pH	8.12
Organic matter (%)	2.73
Total C (%)	22.73
Total N (%)	0.45
Potassium (K_2O, mg kg^{-1})	18.70
Available P (P_2O_5, mg kg^{-1})	11.52

was determined directly after plant removal from substrate. Dry weight was obtained after keeping plants in open air in the shade until a constant weight was obtained.

Phytochemical analysis

All leaves and roots of the white lupin plants from each treatment were recovered individually, homogenized, left to dry in the open air in shade until a constant weight was obtained and then used for extract preparation and determination of phenolic compounds. In order to preserve bioactive compounds, the leaves and the roots of *L. albus* were dried naturally and kept at ambient temperature.

The extraction of bioactive molecules was carried out according to the maceration method described by Dif et al. (2015) and by Liezhou et al. (2019). Each 0.2 g sample of dried leaves or roots of *L. albus* was macerated in a mortar containing 10 mL 80% methanol. After vortex homogenization, the mixture was filtered on filter paper No. 1 and preserved at –20 °C until use as recommended by Dif et al. (2015).

Polyphenols were determined with the Folin-Ciocalteu reagent, which in alkaline medium are reduced to tungsten-molybdenum oxide, giving a blue color in the presence of polyphenols (Bouyahya et al. 2017). A sample (125 µL) of extract was added to 125 µL Ciocalteu-Folin reagent (1:10, v/v). The solutions were mixed and incubated for 3 min at room temperature. After incubation, 1250 µL 20% Na_2CO_3 solution was added. The mixture was then adjusted with 1 mL distilled water and incubated for 90 min in the dark at room temperature. The absorbance was measured at 760 nm (Rover, Brown 2013).

The quantification of flavonoids was carried out using the colorimetric method with $AlCl_3$ and NaOH. $AlCl_3$ forms a yellow complex with flavonoids and NaOH forms a pink complex that absorbs in visible light at 510 nm according to the method described by Hebi, Eddouks (2015). 250 µL extract and 1 mL distilled water were added to 75 µL 5% $NaNO_2$ solution. After 6 min incubation at room temperature, 150 µL of a freshly prepared aluminum chloride solution (AlCl3, 10%) was added to the previous mixture. After 5 min rest, still at room temperature, 500 µL 1 N NaOH was added. The resulting homogeneous mixture was adjusted to 2500 µL with distilled water (Hebi, Eddouks 2015).

Condensed tannins were determined by the vanillin method in acidic medium. This method is based on the ability of vanillin to react with the condensed tannin units in the presence of acid to produce a color complex measured at 550 nm (Palacios et al. 2021). A volume of 50 µL of each extract was added to 1500 µL 4% vanillin/methanol solution and then mixed vigorously. Next, a volume of 750 µL of concentrated HCl was added. The resulting mixture was allowed to react at room temperature for 20 min. Absorbance was measured at 550 nm against a blank (Ali-Rachedi et al. 2018).

Determination of antioxidant activity

In the antioxidant test, the antioxidants reduce the purple-colored 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) to a yellow compound (Brand-Williams et al. 1995; Ali-Rachedi et al. 2018). An extract sample (1 mL) from leaves or roots of L. albus was added to 1 mL DPPH solution. DPPH solution was prepared by solubilizing 0.004 mg of this product in 100 mL methanol. The mixture was left in the dark for 30 min, and then the solutions were read with an UV spectrophotometer at 517 nm against a negative control containing only DPPH solution alone, which was prepared under the same conditions, and its absorbance was recorded. The positive control was represented by standard antioxidant ascorbic acid (Sanchez-Morzeno 2002; Pyrzynska, Pękal 2013). The results of anti-free radical activity were expressed as a percentage inhibition (I %) estimated according to the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A0 - A1) / A0] \times 100,$$

where A0 is the absorbance of the blank and A1 is the absorbance of the sample.

Data analysis

Analysis of variance was performed using SPSS V.25.0 statistical software. The results were presented as the mean with its standard deviation. The determination of significance was performed using the ANOVA test. Differences were considered significant at $P \leq 0.05$.

Results

Morphological parameters

L. albus plants inoculated with AMF, regardless of the level of water stress, had higher values for morphological parameters (average of stem height, fresh and dry weight of aerial parts and roots, root length, root volumes) than non-mycorrhizal plants (Table 2). The highest value for stem height of plants was recorded in plants cultivated at 80% FC (both mycorrhizal and non-mycorrhizal) and it decreased with severity of water shortage.

The weight of fresh aerial biomass was higher in inoculated plants gown at 80% FC. It was lower in non-inoculated plants as well as when drought was higher (Table 2). Simultaneously, the weight of dry aerial biomass was higher in mycorrhizal plants grown at 50% FC and this parameter did not significantly differ in inoculated plants grown at 80 and 30% FC and in uninoculated plants grown at 50% FC. The parameter was lower in uninoculated plants at 30% FC.

The longest roots were from *L. albus* plants cultivated at 30% FC (Table 2). The inoculated plants grown at 30% FC had roots 28.6% longer than these for non-inoculated plants at the same water regime. The shortest roots were found in plants grown at 80% FC. These plants also showed the highest root volume and fresh root biomass. The mean values of the latter two parameters were lower in the case

Table 2. Means of morphological parameters of *Lupinus albus*. Values represent the mean of three replicates ± standard deviation (each replication is the mean of values of five plants). Identical letters represent homogeneous groups according to a Tukey test when the trait was significantly different at the level of $P < 0.05$

Parameter	FC (%)	Aerial parts		Roots	
		Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
Length (cm)	80	17.05 ± 0.67B	24.77 ± 3.40b	13.00 ± 1.73A	8.25 ± 2.35a
	50	9.27 ± 1.59A	14.50 ± 2.29a	12.17 ± 2.22A	14.39 ± 2.08b
	30	8.46 ± 1.78A	11.26 ± 1.03a	14.17 ± 1.03A	18.26 ± 2.15b
Volume (mL)	80	-	-	22.67 ± 2.31B	22.67 ± 3.06b
	50	-	-	14.67 ± 2.31A	19.33 ± 1.16ab
	30	-	-	12.09 ± 2.25A	16.66 ± 2.25a
Fresh biomass (g)	80	4.393 ± 0.014B	6.507 ± 1.756a	1.072± 0.094C	1.603 ± 0.179b
	50	4.594 ± 0.04B	5.443 ± 1.264a	0.512 ± 0.056B	0.666 ± 0.110a
	30	1.219 ± 0.069A	3.648 ± 0.02a	0.252 ± 0.032A	0.402 ± 0.022a
Dry biomass (g)	80	0.585 ± 0.025B	0.857 ± 0.022a	0.103 ± 0.004A	0.084 ± 0.040a
	50	0.807 ± 0.028C	0.932 ± 0.022a	0.119 ± 0.025A	0.114 ± 0.020a
	30	0.386 ± 0.007A	0.854 ± 0.018a	0.087 ± 0.003A	0.085 ± 0.007a

of severe water shortage in the absence of mycorrhizal inoculum, as the lowest values were recorded in non-mycorrhizal plants grown at 30% FC.

The dry root biomass of lupin plants ranged from 0.084 to 0.119 g (Table 2). This parameter appeared to be relatively insensitive to the presence or absence of mycorrhizal inoculum and the severity of the water shortage.

Phytochemical analysis

The total polyphenol assay showed that the methanolic extract of leaves of *L. albus* contained highest polyphenol concentration compared to root extract (Table 3). Leaves of AMF-inoculated plants grown under 30% FC had the highest total polyphenol concentration (Table 3), which was 93.8% higher than in control plants extract (uninoculated plants grown under 80% FC). Leaf extracts from mucorrhizal *L. albus* plants cultivated under 80% FC had the lowest total polyphenol concentration, which was by 50.3% lower than in extract from control plants. An increase by 26.5% in polyphenol concentration was evident in the methanolic leaf extracts of non-mycorrhizal *L. albus* plants grrown at FC of 50%, compared to the leaf extracts of control plants (Table 3).

The highest level of polyphenols was recorded in root extracts from uninoculated plants cultivated at 30% FC (59.8% higher than control) and root extracts from inoculated plants grown at 50% FC (30.6% higher than the control). However, the lowest level of polyphenols occurred in root extracts of mycorrhizal plants grown at FC 30 and 80%, with respective values being 48.0% and 44.2% lower than in control plants (Table 3).

Extracts from leaves of inoculated plants grown at 30%

Table 3. Concentration of total polyphenols, flavonoids, condensed tannins in methanolic extracts from leaves and roots of *Lupinus albus* and their percentages of inhibition of free radicals (antioxidant activity). Values represent the mean of three replicates ± standard deviation (each replication is the mean of values of five plants). Identical letters represent homogeneous groups according to a Tukey test when the trait was significantly different at the level of $P < 0.05$

Parameter (unit)	FC (%)	Leaves		Roots	
		Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
Polyphenols (mg GAE g^{-1} DM)	80	4.81 ± 2.92A	2.39 ± 0.43a	2.45 ± 0.68A	1.37 ± 0.32a
	50	6.08 ± 0.88A	5.36 ± 1.35b	2.62 ± 0.25A	3.20 ± 1.28b
	30	4.37 ± 2.66A	9.31 ± 0.53c	3.92 ± 0.79B	1.28 ± 0.29a
Flavonoids (mg QE g^{-1} DM)	80	1.91 ± 1.51A	1.27 ± 0.29a	1.06 ± 0.12A	0.24 ± 0.26a
	50	2.63 ± 1.01A	1.57 ± 0.17a	1.20 ± 0.11A	2.54 ± 1.07b
	30	1.21 ±0.33A	6.75 ± 0.79b	1.82 ± 0.29B	1.45 ± 0.10ab
Tannins (mg CE g^{-1} DM)	80	0.55 ± 0.36A	0.32 ± 0.04a	0.66 ± 0.04B	0.57 ± 0.07b
	50	0.29 ± 0.03A	0.28 ± 0.05a	0.38 ± 0.03A	0.38 ± 0.01a
	30	0.30 ± 0.04A	0.33 ± 0.09a	0.42 ± 0.01A	0.36 ± 0.01a
Antioxidant activity (%)	80	71.26 ± 4.48AB	82.54 ± 2.13a	95.63 ± 0.68C	86.30 ± 1.49a
	50	85.84 ± 2.243B	73.97 ± 14.53a	90.82 ± 1.50B	92.58 ± 1.23a
	30	75.27 ± 5.29A	82.18 ± 1.33a	76.35 ± 2.56A	90.14 ± 4.92a

FC had 253.9% higher flavonoid concentration than in extract from control plants (Table 3). Leaf extracts from non-mycorrhizal plants cultivated at 50% FC had 37.9% higher flavonoid concentration than from control plants. However, inoculated plants grown at 50 and 80% FC, and inoculated plants grown at 30% of FC had leaf extracts with 17.9, 33.4 and 36.5% lower flavonoid concentration than control plants, respectively.

Extracts from roots of inoculated plants grown at 50% FC had the highest concentration of flavonoids (40.5% higher flavonoid concentration, compared to control plants; Table 3). The lowest flavonoid concentration was found in root extracts of mycorrhyzal plants cultivated at 80% FC (77.6% lower flavonoid concentration than for control plants). Inoculated drought-stressed plants (30% FC) and uninoculated drought-stressed plants (30 and 50% FC) had also higher flavonoid concentration than control plants (37.0, 72.4 and 22.8% more, respectively).

Methanolic extract of leaves and roots of control plants (80% FC) had the highest values of condensed tannins (Table 3). Methanolic extracts of roots had higher concentration of tannins than leaf extracts. Leaf extract of mycorrhizal plants had more tannins than that of non-mycorrhizal plants. However, at the root level, extracts of non-inoculated plants had higher tannin concentration.

Leaf extracts of inoculated and uninoculated plants grown at 50% FC had the lowest concentration of tannins (Table 3). They contained 49.0 and 47.1% lower tannin concentration than control plants, respectively. Root extracts of inoculated plants growing at 30% FC had the lowest tannin concentration (45.3% lower than that of control plants).

Antioxydant activity

The The antioxidant activity of leaf and root extracts was estimated by the percentage inhibition of free radicals. All tested methanolic extracts of L. albus showed relatively high antioxidant activity (Table 3). However, this parameter was relatively insensitive to mycorrhizal status of plants and conditions of water availability. Antioxidant activity significantly decreased with diminishing of water supply only in root extracts of non-mycorrhizal plants. When drought-stressed (30% FC) plants were compared, mycorrhization resulted in significantly higher antioxidant activity in both leaf and roots extracts in comparison to that in non-mycorrhizal plants.

Discussion

Growth and morphological characteristics of *Lupinus albus* plants were significantly affected by water shortage and by plant mycorrhizal status. Drought stress at the levels of 50 and 30% of FC reduced plant size (by 15.0% and 53.1%), weight of aerial parts (by 17.0 and 72.3%) and root biomass (by 37.9 and 76.4%), but increased roots length (by 7.7 and 38.5%) of both inoculated and uninoculated plants, respectively (Table 2). It has been already shown that water deficit clearly affects morphological characteristics of plants in the absence of mycorrhizal association by reducing growth of vegetative parts (Mahmoudi et al. 2017).

Water shortage significantly suppresses cell growth due to the low turgor pressure (Shao et al. 2008) resulting in low cell metabolism resulting in inhibition of both cell division and elongation (Singh et al. 2018). Reduction in plant height is usually associated with a decline in cell enlargement and induction of leaf senescence (Bhatt, Srinivasa Rao 2005). Under severe drought stress, cell elongation of plants is inhibited by interruption of water flow from xylem to the surrounding elongating cells (Esmaeilpour et al. 2015). Drought stress inhibits dry matter production largely through its inhibitory effects on leaf expansion and leaf development (Anjum et al. 2011). In contrast, larger length of the root system provides plant access to a greater volume of soil for water acquisition (Sánchez-Blanco et al. 2014). This elongation of the main root leads to a better exploration of deep horizons and consequently a better absorption of water when its presence is limited in the superficial layers of the soil, which explains why roots of drought-stressed *L. albus* plants were longer than these of the plants grown at optimum water supply.

Mycorrhizal symbiosis improved morphological traits of drought-stressed *L. albus* plants. In the presence of mycorrhizal inoculation, roots were longer, with larger volume and with more fresh mass as compared to those of non-inoculated plants grown under water shortage conditions (Table 2). Mycorrhizal inoculation also promoted development of aerial parts, evident as an increase in plant height, fresh and dry mass compared to non-inoculated plants subjected to drought stress conditions. Similar to the results of the present study, inoculation of *Acacia seyal* by *Glomus aggregatum* stimulated the development of fresh biomass of the aerial and root parts in the situation of stress (Manga et al. 2018). Also, arbuscular mycorrhization had a positive impact on improvement of tolerance to water stress in Phaseolus vulgaris, resulting in an increase in dry root and aerial biomass in stressed inoculated plants compared to non-inoculated stressed plants (Ganjeali et al. 2017). Other studies also reported that plants inoculated with AMF produced more dry weight than control plants (Thakur, Shinde 2020).

It has been already demonstrated that mycorrhization improves plant performance in conditions of drought stress. Several studies showed that inoculation with AMF increased plant height, biomass and dry weight in *Solanum lycopersicum* (Ruiz-Lozano et al. 2015; Chitarra et al. 2016); increased plant height, biomass, shoot and root dry matter, root length, shoot length in *Lycopersicon esculentum* (Subramanian et al. 2006; Padmavathi et al. 2016); increased biomass, water content and reduced antioxidant compounds in *Lavandula spica* (Marulanda

et al. 2007); increased fresh and dry weight in *Allium cepa* (Nelsen, Safir 1982); and increased shoot and root weight and flavonoid concentration in *Pistacia vera* (Abbaspour et al. 2012). Water uptake per unit root length in mycorrhizal plants was twice as high as in control plants (Boutasknit et al. 2020; Gurdeep Singh et al. 2021). In addition, root volume increases in inoculated plants subjected to different water stress conditions, because mycorrhizal association is beneficial for production of new roots, their proliferation (increased root volume), their elongation (increase in length) and their maintenance under the effect of water deficit (Zou et al. 2017; Wu, Zou, 2017; He et al. 2019).

Besides increase in plant growth, mycorrhizal inoculation increased concentration of phytochemical constituents (total phenolics, tannins, and flavonoids; Table 3). Total polyphenol and flavonoid concentration increased, but tannin concentration decreased in leaves of non-inoculated plants grown at 50% FC and in roots of non-inoculated plants cultivated at 30% FC, in comparison to control plants grown at 80% FC. In leaves of mycorrhizal plants, concentration of total polyphenols and flavonoids increased with increasing severity of water shortage. However, the highest levels of polyphenols and flavonoids in roots of inoculated plants were obtained in the 50% CF plants. It has been reported that under the effect of water deficit, the phenolic concentration of the aerial and root parts significantly increased in stressed and mycorrhized plants, compared to non-mycorrhized plants (Benjelloun et al. 2014; Koné et al. (2019). In this context, flavonoids play a role in plant-arbuscular mycorrhizal fungi interactions and their concentration was maximal in response to water deficit (De Matos et al. 2014). Flavonoids have the potential to help plants tolerate, resist and escape water stress (Shah, Smith 2020). It was found that there is a positive correlation between root flavonoids, water supply and AMF colonization (Pei et al. 2020).

Concerning mycorrhizal inoculation of *L. albus* in the present study, concentration of phenolic compounds increased significantly in leaf and root extracts, as compared to non-inoculated plants under water shortage conditions. The most likely mechanism involved in increasing concentration of certain phytochemicals is the improved nutrition that AMF can provide to plants (Crişan et al. (2018). High production of phenolic compounds may be related to an increased activity of enzymes such as chalcone synthase and chalcone isomerase, which are involved in the synthesis of flavonoids, and phenylalanine ammonia lyase, responsible for catalyzing the deamination of phenylalanine, which is an important regulating stage in the formation of phenolic compounds, and which may have its activity increased by environmental factors and by biotic factors, such as colonization by fungi (Pedone-Bonfim et al. 2015).

Water deficit also induces endogenous oxidative stress with the formation of ROS and free radicals, which are very harmful to cellular constituents (Bouchemal et al. 2018). As a response to increased ROS, plants have developed protective mechanisms, involving both induction of the enzymatic antioxidative system and accumulation of non-enzymatic antioxidants. This mechanism may be associated with plant resistance to water deficit (Cruz De Carvalho 2008; Gill, Tuteja 2010).

In the present study, capacity of extracts for free radical removal in leaves of *L. albus* mycorrhizal plants grown at 30% of FC and in leaves of non-mycorrhized plants grown at 50% of FC were higher than in control plants (Table 3). Root extracts of mycorrhized *L. albus* subjected to 30 and 50% of FC had higher antioxidant activity as compared to the non-inoculated plants subjected to the same water shortage conditions. Similarly, a number of studies have confirming that antioxidant levels increased in plants under water stress and in the presence of mycorrhizal colonization (Abreu, Mazzafera 2005; Mohamed, Latif 2017; Ghassemi et al. 2019). In particular, concentration of ROS in leaves and roots of mycorrhizal stressed plants was significantly lower as compared to non-mycorrhizal plants (Babita et al. 2018). Moreover, mycorrhizal colonization decrease the level of ROS in the aerial and root parts of the host plant through the enhancement of antioxidant activities in mycorrhizal plants (Wu et al. 2014; Chen et al. 2020). Increase in antioxidants resulted in a lower accumulation of ROS than in non-mycorrhizal plants, indicating lower oxidative damage in symbiotic plants.

Conclusions

The obtained results indicate clearly that arbuscular mycorrhizal fungi had the potential to improve plant growth and development, and increase antioxidant activity and phenolic compound concentration in leaf and root methanolic extracts of *L. albus*, thereby reducing the negative impact of water stress. Our study results can provide perspective means to increase supply of bioactive molecules.

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