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ORIGINAL PAPERS

EFFECTS OF DIFFERENT TYPES OF MYCORRHIZA ON THE DEVELOPMENT AND THE ELEMENTAL CONTENT OF LUPIN (*LUPINUS ALBUS* L.)

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Abstract

The effects of different types of arbuscular mycorrhizal (AM) fungi on the root inoculation and plant elemental content of lupin (*Lupinus albus* L.) were investigated in the present study. The growth and development of lupin were examined to determine the species of AM fungi that can help to grow lupin with a high protein content and economic value. In this study, which was carried out as a pot experiment under controlled greenhouse conditions, first the pots were inoculated with *Glomus geosporum, Glomus mosseae, Glomus caledonium, Glomus etunicatium* mycorrhizal spores and then lupin (*Lupinus albus)* seeds were sown. The plants were watered with pure water during the experiment. The trial was terminated after a 60-day plant gowing period. In the study, inoculation occurred at lupin roots at rates varying between 13.3 and 30.0%. However, there was no statistically significant difference among the types of arbuscular mycorrhizal fungi applied to the plant in the inoculation rate. The examination of the effect of the application of different AM (*Glomus geosporum, Glomus mosseae, Glomus caledonium, Glomus etunicatium*) spore on the plant development showed that AM inoculation did not have an effect on the lupin development. The effect of AM inoculation on the plant's nutrient content revealed no significant difference in the content of crude protein, P and K, while demonstrating a significant increase in the sulphur and magnesium content versus the control. The plant content of crude protein varied between 185.6 and 226.5 (g kg^{-1}), phosphorus – 0.61-0.74 (g kg^{-1}) and potassium – 9.6-11.1 (g kg^{-1}). The concentrations of Zn, Cu, B and Mo in lupin did not show statistically significant modifications caused by the inoculation of different types of AM. However, the plant Mn content showed a decrease due to AM inoculation, whereas a significant increase was observed in the Na content after AM inoculation. AM fungi were observed in plant roots after the inoculation with any of the four different types of mycorrhiza. But no positive effects of mycorrhizal inoculation were not observed on crude protein and the uptake of plant nutrients.

Keywords: arbuscular mycorrhizal, lupin, inoculation, nutrient.

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INTRODUCTION

It is common knowledge that plant nutrients are taken up through the plant roots, but it is also worth remembering that arbuscular mycorrhizal fungi (AM) contribute to this process, as mycorrhiza increase water and mineral uptake by enlarging the root surface of the host plant (Marschner 1998). It has been demonstrated that as many as 80% of the known plant species are in a natural symbiotic interaction with AM (Smith et al. 2011).

Members of the genus *Lupinus* are resistant to colonization by AM fungi (Trinick 1977) and can inhibit mycorrhizal formation in adjacent roots of other plants (Morley, Mosse 1976). *Lupinus* roots are known to contain flavonoids and alkaloids with antifungal properties (Lane et al. 1987), which may have an adverse influence on AM fungi if contained in their exudates. The first reports that lupins might be non-mycorrhizal were those of Schlicht (1889) and Jones (1924). Recent studies have verified the popular assertion that Lupinus spp. are colonized by mycorrhizal fungi like other legumes. Trinick (1977) reported that *Lupinus angustifolius* L., *L. cosentinii* Guss. and *L. luteus* L. in Australia were poorly colonized at <10% of root length. The mechanisms which determine the non-host nature of Lupinus species in AM symbiosis are very poorly known. Hyphal branching in the vicinity of host roots is a host recognition response of AM fungi (Akiyama, Hayashi 2006, Giovannetti et al. 1993, Giovannetti et al. 1994). The responses of various species and races of *Lupinus* to inoculation with various *Glomus* spp. are reported.

As mentioned above, some researchers reported lupin to be a non-host plant, whereas others observed dense AM colonization depending on a lupin species, and different degrees of AM colonization were demonstrated for different lupin species and types of AM inoculate. Our aim has been to investigate the inoculation of plant roots by different types of AM fungi in lupin, a popular crop in our region owing to its high protein content and economic value.

MATERIAL AND METHODS

Soil and preparation

The acidic soil used in this experiment was taken from a depth of 0-30 cm in the village called Desdigin, in Doganhisar-Konya. The soils in Desdigin are specially suited for lupin cultivation. The soil chosen for the experiment had a moderately acid pH, low in salts as evidenced by EC, with a medium share of lime and a small amount of organic matter. The soil contained sufficient amounts of Cu, Fe and B but inadequate quantities of Zn and Mn (Table 1).

Table 1

The soil analysis results

Type of analysis	Value
pH (1:2.5, soil: water)	6.35
EC (1:5, soil: water) (μS cm^{-1})	43.00
$CaCO_3$ (%)	6.20
Organic matter (%)	1.24
P (mg kg^{-1})	30.96
K(mg kg^{-1})	40.30
Ca(mg kg^{-1})	780.80
Mg (mg kg^{-1})	141.10
Na (mg kg^{-1})	51.10
B (mg kg^{-1})	1.27
Cu (mg kg^{-1})	0.83
Fe (mg kg^{-1})	7.00
Zn (mg kg^{-1})	0.45
Mn (mg kg^{-1})	13.30

In the experiment, the soil was dried, passed through a 4 mm sieve and autoclaved at 121°C twice at one-hour intervals. The sterilized soil was filled into pots of 5 kg. The experiment was conducted according to a randomized plots experimental design with three replications. After weighing batches of the soil and filling the pots, the pots were first inoculated with *Glomus geosporum, Glomus mosseae, Glomus caledonium* or *Glomus etunicatium* mycorrhizal spores at a depth of 5 cm from the surface and with 500 spores per pot. Afterwards, 8 lupin (*Lupinus albus)* seeds were sown into each pot on 11 May 2011. The plants were watered with pure water during the experiment. Four weeks after the seed planting, inoculation was performed. The experiment was terminated after a 60-day plant growing period.

Staining of mycorrhizal roots

Root cleaning and staining were done according to the method described by Koske, Gemma (1989). The percentage of root colonization was calculated by the gridline intersect method or, if the amount of roots was low, by the slide method (Giovanetti, Mosse 1980). The AM colonisation percentage was calculated as the number of segments infected out of 100 segments that were examined under a light microscope (Krüss MBL-2000) at 40X magnification (Giovanetti, Mosse 1980).

Determination of the pant mineral content

Ground plant shoot material at constant weight was incinerated in a closed-system microwave oven using a mixture of HNO_3 and H_2O_2. Then, the

elements were determined using an ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) (Varian-Vista Model, axiel) multi-element analysis and the mineral content of the samples was calculated in mg kg^{-1} and %. The lupin seed crude protein content was determined through the Kjeldahl method by using a Kjeldahl micro-device (BAYRAKLI 1986). The crude protein content of lupin seeds were calculated by multiplying the seed nitrogen content, determined analytically, by the coefficient 6.25 (BREMNER 1965).

Statistical analysis

The data obtained from the above determinations were statistically analysed using Minitab and Mstat-C software.

RESULTS AND DISCUSSION

In the experiment, both hyphae and vesicule-arbuscular formations were observed in plant roots following the inoculation with any of the four different types of mycorrhiza. The root inoculation rate in lupin varied between 13.3-30.0%; the lowest inoculation rate was observed for the *G. mosseae* species of mycorrhiza, while the highest one was observed for *G. etunicatum*. There was no statistically significant difference among the different types of AM in terms of the inoculation results. It was found that the AM inoculation rate in lupin, which is known to be a non-mycorrhizal plant, varied depending on the type of AM. The root inoculation rate (30%) for the *G. etunicatum* species was higher than for the other three types of AM fungi.

The variance analysis conducted to investigate the effect of different AM applications on the plant development showed that AM fungi did not have a significant effect on the plant height, shoot weight and root weight. The highest plant height was observed in the plants inoculated with *G. mosseae* and the highest shoot weight was achieved by the plants inoculated with *G. etunicatium* (Table 2).

The observations of the effect of AM inoculation on the lupin's nutrient content showed that there was no significant difference in crude protein, P and K, whereas a significant increase was observed in the S, Ca and Mg content compared to the control ($P < 0.05$ and $P < 0.01$) – Table 3. The content of crude protein varied between 185.9 and 226.5 g kg^{-1}, P – 0.61-0.74 g kg^{-1} and K – 9.6-11.1 g kg^{-1}.

The plant sulphur content showed a significant increase after AM applications compared to the S content in the control plants ($P < 0.05$). The sulphur content was 2.0 g kg^{-1} in the control and 2.8 g kg^{-1} in *G. caledonium* inoculated plants. The plant Ca content also showed a significant increase compared to control ($P < 0.01$). It was 8.2 g kg^{-1} in the control but 13.6 g kg^{-1} after *G. caledonium* inoculation. Similarly, the Mg content also showed a si-

Table 2

The effect of AM inoculation on plant morphological traits

Mycorrhiza applications	Plant height (cm)	Shoot weight (g)	Shoot dry weight (g)	Root weight (g)	Mycorrhiza infection rates in plant roots (%)
G. geosporum	34.25±3.98	33.96±2.06	5.90±0.43	13.08±0.51	16.7±20.8
G. mosseae	38.13±3.75	43.56±5.82	7.21±1.19	9.63±0.94	13.3±5.8
G.caledonium	35.80±1.25	41.28±4.34	6.80±0.83	10.83±2.32	20.0±10.0
G. etunicatum	37.40±0.92	45.07±8.08	7.40±0.79	10.45±4.65	30.0±10.0
Nonmycorrhiza	36.07±2.23	44.86±7.19	7.19±1.33	11.67±0.97	0.0±0.0
LSD value ($P < 0.01$, $P < 0.05$)	ns	ns	ns	ns	ns

gnificant increase compared to control ($P < 0.01$). It was 2.0 g kg^{-1} in the control and 2.4 g kg^{-1} in *G. geosporium* inoculated plants.

The inoculation of lupin roots with different AM species did not cause a statistically significant difference in the plant content of Zn, Cu, B and Mo. Zn decreased slightly in AM-inoculated plants compared to non-inoculated ones. The zinc content was found at 18.30 mg kg^{-1} in AM non-inoculated plants, 14.54 mg kg^{-1} in *G. caledonium* inoculated plants. The plant Mn content showed a decrease after AM inoculation. It reached 4771.4 mg kg^{-1} in the control, 2438.7 mg kg^{-1} in *G. geosporium* inoculated plants and 3030.2 mg kg^{-1} in *G. caledonium* inoculated ones ($P < 0.01$).

The plant Na content showed a significant increase caused by AM inoculation ($P < 0.05$). The Na content, which was 6.07 g kg^{-1} in control plants, increased to 8.05 g kg^{-1} because of *G. caledonium* inoculation (Table 3).

In the present study, the mycorrhizal infection rates of *Lupinus albus* were found to be low. Based on a study on the AM infection of Lupinus species, Trinick (1977) reported that no vesicles were sighted under poor light conditions, a few vesicles were observed with the improved light conditions in the glasshouse, and the highest frequency of vesicles was observed in the field-collected material, which received the most light. The effects of lupin plants on spore germination and growth of germinative hyphae of *G. mosseae* were studied and an attempt was made to induce mycorrhizal infection in lupin plants grown under low phosphorus conditions (Avio et al. 1990). Con-sidering the phosphorus content of the soil used in the present study, the high available phosphorus content of the soil (30.96 mg kg^{-1}) can explain the low AM infection rate.

The overall results of the current study implicate that AM inoculation did not have a significant effect on the growth and development of the plants and their crude protein, P, K, Zn, Cu, B and Mo uptake. The mycorrhizal

Table 3

The effect of AM inoculation on the content of elements and crude protein in lupin

Mycorrhiza applications	Crude protein (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	S (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
G. geosporum	185.9±14.07	0.61±0.04	9.6±1.38	2.7±0.39 a	11.9±0.73 ab	2.4±0.04 a
G. mosseae	199.0±13.60	0.72±0.13	10.4±1.49	2.3±0.26 b	9.5±0.87 c	2.1±0.05 b
G.caledonium	217.6±8.76	0.61±0.05	11.1±1.14	2.8±0.18 a	13.6±1.48 a	2.3±0.13 a
G. etunicatium	226.5±20.93	0.68±0.09	10.9±0.63	2.3±0.13 b	9.9±0.61 bc	2.0±0.14 b
Nonmycorrhiza	200.3±22.35	0.74±0.04	10.3±0.36	2.0±0.23 b	8.2±0.89 c	2.0±0.15 b
LSD value	ns	ns	ns	0.4448 ($P < 0.05$)	2.343 ($P < 0.01$)	0.1934 ($P < 0.05$)
Mycorrhiza applications	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Mo (mg kg^{-1})	Na (g kg^{-1})
G. geosporum	17.59±6.16	2438.7±575 b	4.58±2.88	34.36±2.25	1.33±0.27	7.04±1.19 ab
G. mosseae	16.89±3.39	3565.3±262 ab	2.80±0.40	39.55±1.51	1.20±0.20	7.04±0.24 ab
G.caledonium	14.54±0.50	3030.2±496 b	2.46±0.42	37.18±3.17	1.28±0.12	8.05±0.48 a
G. etunicatium	15.92±0.43	4632.8±733 a	2.83±0.45	46.08±7.24	1.16±0.03	7.08±0.28 ab
Nonmycorrhiza	18.30±0.86	4771.4±82 a	3.10±0.42	44.46±6.44	1.09±0.11	6.07±0.53 b
LSD value	ns	1262 ($P < 0.01$)	ns	ns	ns	1.129 ($P < 0.05$)

inoculation did not increase the lupin's crude protein content, which might have happened because the type of rhizobium most suitable for lupins was not inoculated to the plant. Besides, the generally low rate of AM inoculation might have influenced this result. Also, mycorrhizal fungi may affect the plant nitrogen content through their effect on the sugar supply for nitrogen fixation by rhizobia and bradyrhizobia in nodules (Hayman, 1982, Koch et al. 1997). Ciesiolka et al. (2007) reported that the protein content varied between 25.2 and 35.1% in lupin seeds and an analogous percentage detected by Yorgancilar and Bilgicli (2014) was 33%.

Similarly, the plant K content did not undergo significant modifications in response to AM inoculation. But some authors have demonstrated conclusively that plant colonization with AM fungi enhanced the K uptake from soil and improved the K status of colonized plants (Sylvia et al. 1993, Subramanian, Charest 1997). Ready and Waugh (1981) reported that K content varied between 239 and 510 μmol g^{-1} DW in different sections of the plant colonized with *Lupinus albus* whereas an analogous value varied between 281 and 583 μmol g^{-1} DW in *Lupinus angustifolius*.

The plant P content did not increase after AM applications. In fact, the P content in the soil was high and there was no significant difference in the concentrations of phosphorus between AM inoculated and non-inoculated

plants. Although mycorrhiza are known to help plants uptake phosphorus, they did not show this effect on lupin. The P uptake of AM non-inoculated plants and the plants inoculated with the different types of AM were found to be similar. This can be explained by referring to a study by HELMKE et al. (2000), which shows that lupin develops an effective mechanism for taking the phosphorus from soil organic P compounds and mineralizing the taken element. This concept is supported by the observation that plants can utilize organic phosphorus supplied as synthetic organophosphorus compounds (sodium glycerophosphate, sodium salt of inositol hexaphosphate) in the presence of phosphatases, especially in sand and solution culture (TARAFDAR, MARSCHNER 1994, HE 1998). Phosphorus deficient conditions stimulate the lupin roots to develop proteoid root structures that excrete large quantities of citrate ion and phosphatase and phytase (HE 1998). White lupin develops proteoid roots that secrete large quantities of citrate or citric acid as an adaptive response to phosphorus deficiency.

In our study, the Zn content varied between 14.54 and 18.30 mg kg^{-1}, the Cu content ranged between 2.46 and 4.58 mg kg^{-1} and the B content was between 34.36 and 46.08 mg kg^{-1}. In a study conducted by YORGANCILAR et al. (2009), the content of Zn, Cu and B in sweet lupin seeds varied between 48.12-6.40 and 29.31 mg kg^{-1}, respectively, and the rate of seed nutrient content was found to be higher compared to that of the inner nutrient content.

The plant Mn content showed a decrease in AM inoculated plants, especially in plants inoculated with *G. geosporium* and *G. caledonium*. Lupins have been reported to accumulate Mn from several types of soil (GLADSTONES 1962, GLADSTONES, DROVER 1962, CHAMBERLAIN, SEARLE 1963), some of which were found to be slightly acidic, so that Mn and Fe oxides were most likely to be solubilized in the rhizosphere. In a study conducted by READY and WAUGH (1981) on Mn accumulation in *L. angustifolius* and *L. albus*, Mn accumulation is explained as follows: reduction of iron oxides (and manganese) in the rhizosphere of *L. albus* is supported by the observation of a blue-grey zone about 10 mm diameter around the roots that fades shortly after breaking the soil open. This colour was not visible around *L. angustifolius* roots. The presence of such a zone, together with manganese accumulation, iron enrichment and failure to accumulate aluminium in lupins growing in this soil, suggests that a reduction of insoluble oxides rather than a decrease of pH enhances the availability of manganese for accumulation by lupins. In this study, the decrease in Mn content through AM inoculation can be explained by the low acidity of the soil, the low level of manganese and the high level of iron in the soil.

The plant Na content showed a significant increase after AM inoculation and the highest Na uptake was observed in the treatment with *G. caledonium* inoculation compared to non-mycorrhizal plants. HOCKING and PATE (1978) determined that leaflets were the major site of accumulation of calcium, iron and manganese; the stem and petioles accumulated substantial amounts of potassium, phosphorus, magnesium, copper and sodium. Similar-

ly, Reay (1987) also reported that Na was enriched in the stems whereas Ca and Mn were redistributed only in *L. albus*.

Finally, both hyphae and vesicule-arbuscule formations were observed in plant roots as the result of the inoculation of four different types of mycorrhiza. The AM inoculation treatments did not have a significant effect on the growth and development of the plants and their crude protein, P, K, Zn, Cu, B and Mo uptake.

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