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Influence of arbuscular mycorrhizal fungi and water regime on the development of endemic *Thymus* species in dolomitic soils

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ABSTRACT

Dolomitic soils characteristically contain high levels of magnesium and calcium, and have a low water holding capacity. Such constraints enhance plant speciation and the emergence of endemic species. *Thymus granatensis* is an example of an endemic species from Southern Spain (Mediterranean climate) and a bioindicator of dolomitic soils that can be found in the Sierra de Baza Natural Park on pure dolomite (PD) soils (those containing more than 90% dolomite). Acknowledging the beneficial role of arbuscular mycorrhizal (AM) symbiosis against abiotic stress factors, this research was established to ascertain the importance of the AM community in supporting the growth of *T. granatensis* in PD soils. To study this, another *Thymus* species i.e., *T. mastichina*, was chosen for comparison. *T. mastichina* does not grow naturally in PD soils, but it is able to grow in a wide range of soil types including those having lower dolomite content. The results showed that: (i) AM establishment increases the biomass production of both species; (ii) the AM fungal community from PD soil might be fundamental for the growth of *T. granatensis* in the PD soil, particularly under drought conditions; (iii) the inoculation with AM fungi from the PD soil lowered the shoot Mg and Ca concentrations of both *Thymus* species growing in PD soil. Accordingly, AM fungi management could be used as a biotechnological tool to address the restoration and conservation of Mediterranean dolomitic habitats.

1. Introduction

Dolomitic soils contain high levels of magnesium and calcium which can be detrimental to growth and development of non-adapted plant species (Mota et al., 2008). Among other eco-physiological constraints, supra-optimal concentrations of Ca and Mg are known to interfere with cellular homeostasis, stomatal function and photosynthetic performance (Shaul, 2002; Broadley et al., 2003). In addition, the "pure dolomite" (PD) soils (those containing more than 90% dolomite) are usually poorly developed and have a low water holding capacity with sandy texture and low organic matter content (Fernández-Gálvez et al., 2009). Due to these adverse growth conditions only adapted plants, most of them endemic species, can thrive in the PD soils (Baskin and Baskin, 2000; Allison and Stevens, 2001; Dixon and Tood, 2001; Siebert and Siebert, 2005; Mota et al., 2008). In general, dolomite-adapted plants have a reduced shoot size, small leaves with whitish hairs to protect them from sunlight, and a strong root system to help the plants withstand the low stability of the soil (Allison and Stevens, 2001; Mota et al., 2008).

A representative area of pure dolomitic soils occurs in the Baetic Range in southern Spain, which harbours a "thyme-shrub" community with a representative presence of endemic species such as *Thymus granatensis*, *Pterocephalus spathulatus*, *Jurinea pinnata* and *Centaurea boissieri* (Blanca and Morales, 1991; Lorite et al., 2001; Mota et al., 2008). In addition to constraints imposed by their dolomitic character, these soils and plant communities are affected by Mediterranean climate conditions, typically defined by the coincidence of high temperatures and scarce rainfall in a long, dry and hot summer (Quézel, 1985). This provokes a high atmospheric evaporative demand which generates a progressive reduction of the vegetation cover coupled with concomitant soil erosion of semiarid Mediterranean ecosystems (Quézel, 1985; Oyonarte et al., 2008). To enhance conservation of endemic and endangered species and to promote restoration at risk areas, management of native adapted species has been proposed to minimize the negative environmental impact, while keeping the ecosystem equilibrium (Tamás, 2003; Mota et al., 2008).

Among the different eco-physiological factors that help with the development of endangered plant species and restoration of degraded areas, the impact of arbuscular mycorrhizal (AM) fungi has been investigated (Requena et al., 2001; Jeffries et al., 2003; Renkel et al., 2004; Zubek et al., 2009). A positive effect of AM fungi is expected in dolomitic soils because of the well-known enhancement of plant nutrient uptake capacity, root growth and plant

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response against abiotic stresses by AM symbiosis (Ruiz-Lozano, 2003; Barea et al., 2005; Turnau et al., 2006; van der Heijden et al., 2006; Smith et al., 2009). Additionally, AM fungi improve soil structure and promote plant diversity and succession (Barea et al., 2005; Rillig and Mummey, 2006; van der Heijden et al., 2006; Bedini et al., 2009).

There are several reports of the role of ectomycorrhizal symbiosis with species belonging to the family Pinaceae in dolomitic soils (Bücking et al., 2002; Baier et al., 2006). Similarly, the role of AM fungi in the remediation of dolomitic soils contaminated with heavy metals has been studied (Turnau et al., 2006; Zubek et al., 2009). However, the impact of the AM symbiosis on the functioning of plant communities growing in pure dolomite soils has received less attention. In this context, a series of experiments were designed to investigate the importance of the AM fungi community in supporting the growth of *T. granatensis*, an endemic species bioindicator of dolomitic soil from the Baetic Range in Southern Spain (Blanca and Morales, 1991; Lorite et al., 2001; Mota et al., 2008). For this goal, another *Thymus* species i.e., *T. mastichina*, was chosen for comparison. *T. mastichina* is distributed throughout the Iberian Peninsula and grows in neighbouring areas to the pure dolomite target site. It is a component of a rosemary grove community which also includes *Rosmarinus officinalis*, *Thymus zigys*, *Genista cinerea* and *Lavandula latifolia* (Blanca and Morales, 1991; Blanco-Salas et al., 2009; Fernández-Gálvez et al., 2009).

The two *Thymus* species and their naturally associated AM fungal populations appear as an interesting model system to: (i) examine the impact of AM symbiosis on the growth of the target *Thymus* species in dolomitic soils, (ii) to study their "degree of dolomitophily" (Mota et al., 2008), and (iii) to assess their degree of tolerance to drought conditions.

2. Materials and methods

2.1. Target dolomitic area, soil and plant analysis

The study area is situated in the "Sierra de Baza" Natural Park, which belongs to the Baetic Range (Southern Spain, located in Granada Province between 37°20′N and 2°48′W). The Park has a surface area of 53,649 ha and is topographically abrupt with altitudes ranging from 900 to 2200 m.a.s.l. The climate is Mediterranean-type, with an annual rainfall of about 450 mm. Winters are cold with a mean temperature of 5 °C, with frequent snowfall while summers are very dry and warm with a mean temperature of 23 °C (Blanca and Morales, 1991; Fernández-Gálvez et al., 2009).

The target dolomitic thyme-shrub community occupies 632 ha and was declared as a "Reserve Territory" with a special level of conservation and protection, to prevent the use of this soil type for industrial purposes (http://www.sierradebaza.org/siebaza_legis_decreto101.htm).

This research involved two test soils: the "pure dolomite soil" (PD soil) where the thyme-shrub community grows and the "lower dolomite content soil" (LD soil) where the rosemary grove is established. Non-sieved soil samples were collected from root-associated areas (0–15 cm in depth) of thyme plants randomly distributed in either PD or LD soils. Samples from the same plot were packed in sacs and mixed homogeneously. After transportation, several subsamples were air-dried and sieved at 2 mm before determination of physical, chemical and mineralogical properties. The mineralogical characterization was carried out by X-ray diffraction (PANalitical X'Pert Pro Multi Purpose X-ray Diffractometer, Netherlands). The particle-size distribution (texture) was determined by the pipette method (Robinson, 1922) and pH measurements were made in a 1:2.5 soil–water solution using a glass electrode. Organic carbon content was measured by the potassium dichromate oxidation method (Yeomans and Bremner, 1989). Soil N content was measured by the C/N elemental analyzer and the available P was analyzed according to the method of Olsen and Sommers (1982). Other nutrients like K, Mg and Ca were measured after acid digestion, by means of ICP-OES Intrepid II XDL with Thermo Electro Corporation procedure. The Mg and Ca concentrations in field-grown target plant species were measured in leaves, stems and roots to determine possible differences in accumulation patterns.

To assess the mycorrhizal infectivity of the soils, the most probable number of AM fungal infective propagules (AM spores and mycelium as unit with the capacity to colonize plants root) was determined in the rhizosphere soil from the field-grown target plants (Sieverding, 1991).

2.2. Pot experiment

2.2.1. Experimental design

This experiment consisted of a randomised complete block design with three factors: (i) soil type, (ii) AM fungal treatment and (iii) water regime, to investigate their effects on the growth of two target plant species (*T. granatensis* and *T. mastichina*). There were two soil types (PD and LD), three AM fungal treatments (non-AM fungi, AM fungi from PD soil and AM fungi from LD soil) and two water regimes (well watered and drought stress). Each of the three factor combinations was replicated five times for a total of 60 pots per plant species.

2.2.2. Preparation of soils

Non-sieved soil samples collected from the same plot (PD or LD) were mixed homogeneously. These samples were heated at 90 °C for 1 h (3 consecutive days). The soils were maintaining at room temperature after each heating to allow germination of the remaining spores (those that were not killed after heating) and form vegetative cells which are more vulnerable to destroy in successive heat shocks. Then, the soils were left to stabilize for 15 days before distributing into the corresponding pots of 500 ml capacity. Each pot received 10 ml of a soil suspension filtrate to restore a general microbial population free of AM fungal propagules. The soil filtrates were obtained by suspending 100 g of each experimental soil in 1 l of sterile water. The suspension was stirred and then left 24 h for decanting. After that, the suspension was filtered twice (Whatman Grade 1 filter paper).

2.2.3. AM fungal inoculum

Three AM fungal treatments were considered: non-AM fungi, AM fungi from PD soil (PD-F) and AM fungi from LD soil (LD-F).

Non-AM fungi treatments consisted of the prepared soil plus a filtrate free of AM fungal propagules, as described in Section 2.2.2. To obtain the AM fungal component from either PD or LD soils, the classical "wet sieving and decanting" technique (Brundrett et al., 1996) was used. Briefly, 310 g of the corresponding 2 mm sieved soil (which is an equivalent volume of 500 ml of each soil with the original texture) was suspended in 2 l of water. Then, the suspension was strongly stirred for sample homogenization and left for soil decantation, and the supernatant was poured out through coupled sieves of 700 μm, 500 μm, 250 μm and 50 μm. This procedure was carried out twice with the same soil sample. Finally, the material from all sieves except that of 700 μm was added to each pot in the planting hole, according to the corresponding treatment.

2.2.4. Water management

The irrigation variable consisted of two water regimes: well watered (WW) and drought stress (DS). The available water for the plants of each soil type was determined on sieved samples by

Table 1
Characteristics of the two experimental soils: pure dolomite (PD) and lower percentage of dolomite (LD).

Parameter	PD soil	LD soil
Dolomite (%)	99	82
Quartz (%)	1	8.5
Muscovite (%)	–	6
Chlorite (%)	–	1.5
Albite (%)	–	1.5
Other phyllosilicates (%)	–	0.5
Organic carbon (%)	1.6	3.6
Texture	Sandy-loam	Loam
Field capacity (%)	6.5	25.8
Permanent wilting point (%)	3.8	15.1
Available water (%)	2.7	10.7
pH_{water} (1:2.5)	8.2	7.8
N (%)	0.1	0.3
Available P (ppm)	0.5	2.1
K (%)	0.1	1.1
Ca (%)	21.9	10.7
Mg (%)	11.9	6.4

a pressure cell apparatus by means of field capacity (−0.03 MPa) and permanent wilting point (−1.5 MPa) measurements (Richards, 1947). Soil moisture was calculated from the measurement of field capacity for well watered pots and the 65% of field capacity for drought treatments (Table 1). The water content of the soil was maintained by weighing the pots every 2 days and replacing the lost water (pots humidity before watering was approximately 95% and 60% of field capacity in WW and DS respectively).

2.2.5. Plant material and growth conditions

Seeds of *T. granatensis* were collected from the thyme-shrub established on PD soil and those of *T. mastichina* from the rosemary grove established on LD soil. Seeds were germinated on a vermiculite bed in dark conditions at 20 °C and 60% relative humidity. After germination, the seedlings were grown for 3 months in the same substrate in a controlled-environment greenhouse under 16 h light (25 °C) and 8 h dark (20 °C) cycle with 50–60% relative humidity and a photosynthetic photon flux density of 700 μmol m^{-2} s^{-2} for the compensating photophase. After that, the seedlings were transplanted into pots where they received the corresponding treatment and were grown for 7 months under greenhouse conditions as described before.

2.2.6. Plant biomass and nutrient concentration

At the end of the experimental period, plants were harvested and shoot and root dry weights were measured after drying in an oven at 75 °C for 48 h. Shoots were ground and the Mg and Ca concentrations were measured as described before.

2.2.7. Mycorrhizal development: root colonization and spores

For the measurement of the mycorrhizal colonization, randomly sampled roots were cleared with 10% KOH (w/v) and stained with 0.05% Trypan Blue (w/v) in lactic acid (modified from Phillips and Hayman, 1970). Stained root pieces were examined under a compound microscope (Nikon, Japan) and the extent of AM colonization was estimated by the methods described by Trouvelot et al. (1986).

Non-AMF treatments were checked and no mycorrhizal colonization was found.

Spores were isolated from the soil by wet sieving and sucrose density gradient centrifugation (Brundrett et al., 1996) mounted in polyvinyl alcohol–lactic acid–glycerol (PVLG), as described by Koske and Tessier (1983) and examined under a compound microscope. The spores were identified by morphological approaches by following current species descriptions and identification manuals (Morton http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm).

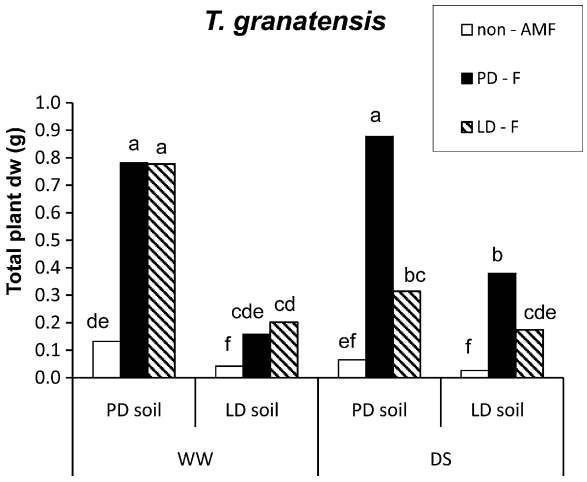

Fig. 1. Total plant dry weight (dw) of *T. granatensis* growing in either pure dolomite (PD) or lower percentage of dolomite (LD) soils, under three AM fungi treatments, non-AM fungi (non-AMF), AM fungi from PD soil (PD-F) and AM fungi from LD soil (LD-F), affected by two water regime, well watered (WW) and drought stress (DS). Mean values ($n = 5$) with a common letter are not significantly different by LSD test ($P < 0.05$).

2.3. Statistical analysis

Three-way ANOVA were performed to assess the effects of soil origin, AM fungi treatment, water regime and their interactions, on *Thymus* spp. biomass and shoot Mg and Ca concentrations. Data normality and homogeneity of variance were verified by means of Kolmogorov–Smirnov and Levene's tests respectively. Generally, biomass production in *T. mastichina* is greater than in *T. granatensis* in natural conditions (see plant species description in http://www.floraiberica.es/floraiberica/texto/pdfs/12_140_21_Thymus.pdf) and in this experiment (see Figs. 1 and 2). Accordingly, the impact of the different treatments on the dependent variables was analyzed separately for each target plant species.

Partial Eta squared (PES) is a parameter used in some ecological studies (Griffith et al., 2001; Daniels et al., 2008) to calculate the magnitude of the effect attributable to each factor and/or interaction on dependent variables and therefore it was used in this study. Thus, PES is a number between 0 and 1 inclusive, with higher scores representing more desirable, larger effect size (Norusis, 1990). Dif-

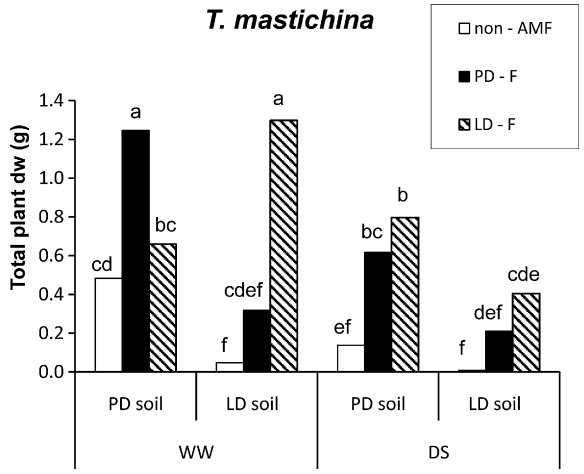

Fig. 2. Total plant dry weight (dw) of *T. mastichina*. Mean values ($n = 5$) with a common letter are not significantly different by LSD test ($P < 0.05$). For other abbreviations, see Fig. 1.

ferences among treatments were tested with Fisherĭs Protected least significant difference (LSD) ($P < 0.05$). Bivariate correlation analysis was carried out to test the covariance among dependent variables by means of the Pearson coefficient. The software used for the statistical analysis was SPSS 15.0 (2006).

3. Results

3.1. Soil characteristics

Physical and chemical properties of the target dolomitic soils (PD and LD) are recorded in Table 1. Mineralogical studies showed that the PD soil had 99% of dolomite and 1% of quartz while LD soil had 82% of dolomite, 8.5% quartz and a proportion of different siliceous minerals (muscovite, chlorite, albite and others) which were not present in PD soil. Both soils have a basic pH. The texture of PD soil was sandy-loam while LD soil was loam. Organic carbon and available water content were lower in PD than in LD soil. The levels of Mg and Ca were higher in PD than in LD soil while N, K and available P concentrations were higher in LD than in PD soil.

The values of the most probable number of AM fungal infective propagules were 60 and 74 per 100 g of 2 mm sieved for PD and LD soil, respectively. These data were not statistically different, therefore, both soils can be considered to have a similar potential for AM root colonization.

3.2. Plant growth in the pot experiment

The growth of *T. granatensis* was significantly affected by the factors "AM fungi" and "soil" but not by the factor "water regime" (Table 2). There was a remarkable influence of AM inoculation on biomass production so that the development of plants with non-AMF treatment was negligible (Fig. 1 and Table 2, AM fungi, PES = 0.769). The PD soil supported the strongest effect on the growth of *T. granatensis* (Fig. 1 and Table 2, soil, PES = 0.597). Under WW conditions, *T. granatensis* responded similarly to AM inoculation irrespectively of the fungal origin. However, under DS regime, fungi from PD soil were more effective than those from LD soil at maintaining the growth of *T. granatensis* plants (Fig. 1 and Table 2, AM fungi × water regimen, PES = 0.254).

All three factors had a significant effect on *T. mastichina* biomass production (Table 2, PES parameter, $P < 0.005$). Inoculation of *T. mastichina* plants was non-effective when the test soil and the AM fungi community had a different origin (Fig. 2 and Table 2, soil × AM fungi, PES = 0.418) except in the case of plants growing in PD soil under DS conditions. In contrast, the highest biomass production of *T. mastichina* was found under WW conditions when soil and AM fungal had the same origin (Fig. 2 and Table 2, soil × AM fungi × water regime, PSE = 0.449).

Table 2
Influence of the three factors tested (AM fungi, soil and water regime) on biomass production of *T. granatensis* and *T. mastichina* plants.

Factors and interactions	d.f.	*T. granatensis* PES	*T. mastichina* PES
Soil	1	0.597**	0.349**
AM fungi	2	0.769**	0.658**
Water regime	1	0.044	0.409**
Soil × AM fungi	2	0.100	0.418**
Soil × water regime	1	0.137**	0.008
AM fungi × water regime	2	0.254**	0.050
Soil × AM fungi × water regime	2	0.034	0.449**
R^2		0.848**	0.835**

Effect size is given by partial Eta squared (PES). d.f., degrees of freedom.
** Significant factors $P < 0.005$.

Table 3
Mg and Ca concentrations in *T. granatensis* (*T. gr*) and *T. mastichina* (*T. m*) plants growing in the field from either PD or LD soils, respectively.

Field-grown plants	Mg conc. (%)		Ca conc. (%)	
	T. gr	*T. m*	*T. gr*	*T. m*
Leaf	0.56 a	0.44 b	2.18 a	1.63 b
Stem	0.69 a	0.25 b	1.58 b	0.65 c
Root	0.56 a	1.14 a	1.44 b	2.52 a

For each column, mean values (three replicates) with a common letter are not significantly different by LSD test ($P < 0.05$).

3.3. Plant Mg and Ca concentrations

Field-grown plants of *T. granatensis* accumulated Mg equally in all tissues, while Ca concentrations were higher in leaves (Table 3). However, *T. mastichina* plants growing in LD soil accumulated more Mg and Ca in their roots. These data are in accordance with those found in the pot experiment (Table 4). There was not enough root and shoot biomass in non-AMF treatments for nutrient analysis.

Results from the pot experiment show that inoculation with AM fungi appears as a significant factor affecting shoot Mg concentrations (Table 5, $P < 0.05$) for both *Thymus* species, although this is not a significant dependent variable for *T. granatensis* (Table 5, PES = 0.329, $P > 0.05$). Regardless of water regime, inoculation with PD-F caused a lower concentration of Mg in shoots of both *Thymus* plants growing in PD soil in comparison to plants inoculated with AM fungi from the LD soil (Table 4). It is noteworthy that plants growing under WW conditions on PD soil with LD-F inoculation had the highest Mg concentration in their shoots.

The "AM fungi" factor was the most influential independent variable affecting the Ca accumulation in the shoots of *T. granatensis* but not *T. mastichina* (Table 5, PES = 0.147). However, the "soil" factor was the most influential independent variable affecting the accumulation of Ca in the shoots of *T. mastichina*, with higher Ca shoot levels in plants growing in LD than in PD soil (Table 4 and 5, PES = 0.450).

3.4. AM colonization and spores

Mycorrhizal colonization in both *Thymus* species ranged between 7 and 29% (Table 6). The percentage of arbuscules under DS conditions was significantly higher in plants growing in their own natural soil and inoculated with the native AM fungi from their corresponding soils (Table 6). A total of 9 AM fungal morphotypes of spores were distinguished as associated to any of the test *Thymus* species. Six of them belonged to the genus *Glomus* and the others to *Diversispora*, *Scutellospora* and *Pacispora* (Table 7). The most common species found were *G. claroideum*, *G. etunicatum* and *G. macrocarpum* which were present in all of the treatments (Table 8, species 1, 3 and 5 respectively).

3.5. Correlation analysis

There was a significant positive correlation between plant biomass and AM colonization level (Table 8, $P < 0.005$) and a negative correlation between plant biomass and shoot Ca concentration for both *Thymus* species (Table 8, $P < 0.05$ in *T. granatensis* and $P < 0.005$ in *T. mastichina*). Plant biomass was negatively correlated with shoot Mg concentration ($P < 0.005$) in *T. mastichina* but not in *T. granatensis*. Furthermore, there was a high positive correlation between shoot Mg and Ca concentrations in both *Thymus* species (Table 8).

Table 4
Shoot Mg and Ca concentrations in *T. granatensis* (*T. gr*) and *T. mastichina* (*T. m*). For other abbreviations, see Fig. 1.

Water regime	Soil	AM fungal origin	Shoot Mg conc. (%)		Shoot Ca conc. (%)	
			T. gr	*T. m*	*T. gr*	*T. m*
WW	PD	PD-F	0.53 b	0.30 c	1.15 bc	0.63 c
		LD-F	0.98 a	0.58 a	1.72 ab	0.95 ab
	LD	PD-F	0.59 ab	0.40 bc	1.19 abc	1.02 ab
		LD-F	0.58 ab	0.39 bc	0.96 c	1.03 ab
DS	PD	PD-F	0.48 b	0.35 bc	0.99 c	0.76 bc
		LD-F	0.70 ab	0.40 bc	1.66 a	0.93 ab
	LD	PD-F	0.52 b	0.43 abc	1.39 abc	1.12 a
		LD-F	0.56 ab	0.50 ab	1.57 ab	1.12 a

For each column, mean values (five replicates) with a common letter are not significantly different by LSD test ($P < 0.05$).

Table 5
Influence of the three factors tested (AM fungi, soil and water regime) on shoot Mg and Ca concentrations.

Factors and interactions	d.f.	Shoot Mg conc. (%)		Shoot Ca conc. (%)	
		T. gr PES	*T. m* PES	*T. gr* PES	*T. m* PES
Soil	1	0.046	0.026	0.008	0.450**
AM fungi	1	0.156*	0.291*	0.147*	0.153
Water regime	1	0.034	0.000	0.094	0.068
Soil × AM fungi	1	0.125	0.158	0.172*	0.151
Soil × water regime	1	0.001	0.167	0.110	0.006
AM fungi × water regime	1	0.000	0.057	0.084	0.018
Soil × AM fungi × water regime	1	0.004	0.203*	0.002	0.015
R^2		0.329	0.538*	0.417*	0.582*

Effect size is given by partial Eta squared (PES). (Tg) *T. granatensis*, (*T. m*) *T. mastichina*. d.f., degrees of freedom.
* Significant factors $P < 0.05$
** Significant factors $P < 0.005$.

Table 6
Mycorrhizal colonization (% M) and arbuscules richness (%A). For other abbreviations, see Fig. 1.

Water regime	Soil	AM fungal origin	*T. granatensis*		*T. mastichina*	
			% M	% A	% M	% A
WW	PD	PD-F	26.8 a	1.7 b	27.3 a	11.8 ab
		LD-F	25.9 a	18.7 ab	17.7 ab	30.0 ab
	LD	PD-F	13.8 bc	7.8 ab	8.6 b	20.6 ab
		LD-F	19.4 ab	6.9 ab	22.4 a	9.7 ab
DS	PD	PD-F	17.0 abc	26.3 a	10.2 b	2.9 b
		LD-F	7.2 c	14.8 ab	10.4 b	0.4 b
	LD	PD-F	10.0 bc	6.2 ab	10.5 b	8.4 ab
		LD-F	7.0 c	2.5 b	29.4 a	35.9 a

For each column, mean values (five replicates) with a common letter are not significantly different by LSD test ($P < 0.05$).

Table 7
Diversity of spore morphotypes per treatment in *T. granatensis* (T. gr) and *T. mastichina* (T. m). For other abbreviations, see Fig. 1.

Plant sp.	Water regime	soil	AM fungi	1	2	3	4	5	6	7	8	9
T. gr	WW	PD	PD-F	+	+	+	+	+	+	–	–	+
			LD-F	+	+	+	+	+	+	–	–	–
		LD	PD-F	+	–	+	–	+	+	+	–	–
			LD-F	+	–	+	–	+	–	+	–	–
	DS	PD	PD-F	+	–	+	+	+	–	–	–	+
			LD-F	+	–	+	–	+	–	–	+	–
		LD	PD-F	+	+	+	–	+	+	–	–	–
			LD-F	+	+	+	–	+	+	+	+	–
T. m	WW	PD	PD-F	+	–	+	+	+	+	+	+	–
			LD-F	+	–	+	–	+	–	–	–	–
		LD	PD-F	+	+	+	–	+	+	–	–	–
			LD-F	+	–	+	–	+	–	–	–	-
	DS	PD	PD-F	+	–	+	–	+	+	–	+	+
			LD-F	+	–	+	–	+	–	–	–	+
		LD	PD-F	+	–	+	–	+	–	–	–	–
			LD-F	+	–	+	–	+	–	+	+	–

(1) *G. claroideum*, (2) *G. constrictum*, (3) *G. etunicatum*, (4) *G. intrarradices*, (5) *G. macrocarpum*, (6) *G. mosseae*, (7) Diversispora sp., (8) *Pacispora dominikii*, (9) *Scutellospora calospora*.

Table 8
Correlation among biomass, mycorrhizal colonization (%M) and shoot Mg and Ca concentrations in the pot experiment.

Plant species	Variables	Biomass	% M	Mg conc.
T. granatensis	% M	0.659**		
	Mg conc.	−0.262	−0.045	
	Ca conc.	−0.366*	−0.305	0.764**
T. mastichina	% M	0.682**		
	Mg conc.	−0.487**	−0.123	
	Ca conc.	−0.528**	−0.344	0.709**

* Significant factors $P < 0.05$ tested by the Pearson correlation coefficient.
** Significant factors $P < 0.005$ tested by the Pearson correlation coefficient.

4. Discussion

4.1. Soil characteristics

The analysis of the target dolomitic soils confirmed that the PD soil was a "pure dolomitic soil" i.e., those containing more than 90% dolomite (Allison and Stevens, 2001; Dixon and Tood, 2001; Mota et al., 2008). The loam texture and the higher percentage of organic carbon in the LD soil could explain the higher water availability for plants in this soil with respect to PD soil.

4.2. Plant growth

By means of the PES parameter it was possible to demonstrate that AM fungi inoculation was the most impacting factor for *T. granatensis* biomass production compared with the other two independent variables tested (soil and water regime). The use of this parameter also supports that *T. granatensis* can be considered as a "dolomite bioindicator" (Blanca and Morales, 1991; Lorite et al., 2001; Mota et al., 2008) due to the impact of the soil factor on its growth. This species is also adapted to drought conditions as shown by its ability to produce the same biomass regardless of the water regime. *T. granatensis* plants have in fact a variety of anatomic characteristics to avoid high evapotranspiration, such as a reduced shoot and small leaves (Mota et al., 2008). In addition, this research showed that native AM fungal community (PD-F) was the most influential treatment measured to alleviate drought stress in *T. granatensis.*

The three factors studied and their interactions, significantly affected the growth of *T. mastichina*, with the "soil" factor being the least important. This agrees with some reports describing the versatility of *T. mastichina* for growing in a wide range of soil types including siliceous, gypseous, loams and limestone soils (Blanca and Morales, 1991; Blanco-Salas et al., 2009). The "AM fungi" factor was the most influential ones among those tested. The highest effect on plant growth was produced under WW conditions when the soil and the AM fungal community had the same origin. Moreover, *T. mastichina* seems to have higher water requirements for growth and development than *T. granatensis*.

4.3. Plant Mg and Ca concentrations

T. granatensis plants accumulated high concentrations of Mg in their shoots (0.48–0.98%) in comparison with the average levels (0.15–0.35%) described for other species (Shaul, 2002). Conversely, *T. mastichina* plants accumulated Mg and Ca in their roots (root tissues and associated microorganisms) as compared with the shoots.

The "AM fungi" factor had a considerable influence on shoot Mg and Ca concentrations. This suggests that AM symbiosis could be involved in their acquisition and/or shoot translocation in these plants. Particularly, the inoculation with AM fungi from the PD soil lowered the concentration of Mg and Ca in the shoots of both *Thymus* plants growing in PD soil, with respect to plants inoculated with AM fungi from the LD soil. This agrees with previous reports concerning ectomycorrhizal fungi which seem to have a function as a partial barrier in Mg and Ca uptake by the plants in dolomitic soils (Bücking et al., 2002; Baier et al., 2006).

4.4. AM fungal colonization and spores

There was a positive relationship between AM colonization and plant growth in the pot experiment. The percentage of arbuscules in DS conditions was higher when plants, soil and AM fungi had the same origin. This suggests a certain preference for AM fungi by plants under water stress. However this was not reflected in a plant growth response. This apparent discrepancy could be caused by the cost-benefit balance for the plant and AM fungi in the mutualistic AM symbiosis depending on the environmental conditions (Smith et al., 2009).

Nine AM fungal species were detected by morphological spore identification. Three of these species (*G. claroideum*, *G. etunicatum* and *G. macrocarpum*) were present in all treatments. These species seem to be generalists, independent of plant species, soil types and growth conditions (Stutz et al., 2000; Blaszkowski et al., 2003). Moreover, a new AM fungal species has been isolated from the target pure dolomitic area (Palenzuela et al., 2008), but this fungus was not found in the present study. This species, *Otospora bareai* belongs to family Diversisporaceae (Palenzuela et al., 2008) and seems to be a specific ecotype because it has only been found in the "Reserve territory" at the Sierra de Baza Natural Park, associated with *T. granatensis* and *P. spathulatus*.

4.5. Correlation analysis

Pearson correlation coefficient demonstrated that the biomass of both *Thymus* spp. was positively correlated with the mycorrhizal colonization levels. This supports that AM symbiosis is crucial for growth increase of both plant species. Moreover, there was a positive correlation between shoot Ca and Mg concentrations in both *Thymus* spp. which could be related with the shoot Ca to Mg concentration ratio (Dixon and Tood, 2001). For the target plant species, this proportion ranged between 2 and 3 (Table 4). These are low values due to the elevated Mg concentrations found for both plant species.

T. granatensis biomass production was independent of shoot Mg levels. However, plants with greater shoot Ca concentrations grew less, even though they were able to maintain Ca:Mg ratios around 2. These results suggest that *T. granatensis* may have an additional adaptation mechanism for the accumulation of high shoot levels of Mg as a strategy for its establishment in pure dolomitic soils. Similarly, plants adapted to serpentine soils (with high levels of Mg and water restrictions), also accumulate high Mg and Ca concentration in their leaves. In this serpentine soil type, some studies have reported that chelation of Mg and Ca by soluble carboxylates such as malate, citrate and isocitrate located in the vacuole may be involved in the protective mechanisms to support supra-optimal Mg and Ca concentrations in plant tissues (Brady et al., 2005). The formation of these metal–ligand complexes could be tested in dolomitic plants. The negative correlation found between *T. mastichina* biomass production and Mg and Ca levels suggest a lower tolerance of shoot Mg and Ca variability in comparison with *T. granatensis.*

5. Conclusions

AM symbiosis was critical for improving the growth of *T. granatensis* and *T. mastichina* in dolomitic soils with supra-optimal Mg and Ca concentrations and water restrictions. Particularly AM fungal community from the PD soil could play a key role for *T. granatensis* plants under drought conditions. Moreover, the results

suggest that AM fungi from PD soil might have a function in the control of Ca and Mg acquisition and/or shoot translocation by the two target plant species in PD soil. *T. granatensis* plants showed a great tolerance to high Mg concentrations in their shoots and lower water requirements than *T. mastichina*. For these reasons, this study supports that *T. granatensis* is more adapted to dolomitic soils than *T. mastichina* and therefore has a higher degree of dolomitophily.

This research supports that native AM fungal species could be used as a tool for propagation of both *Thymus* species, a basis for restoration strategies and conservation "*in situ*" and "*ex situ*" of these plant species and their natural habitats.

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