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SHORT NOTE

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The mycorrhizal status of the woody Mediterranean shrub *Myrtus communis* L.

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Abstract *Myrtus communis* L. (myrtle), a typical Mediterranean plant species belonging to the family Myrtaceae, was shown to form arbuscular mycorrhizal symbioses in nature. Many different spore types were isolated from its rhizosphere and grown in pot cultures; six of them were identified as *Glomus* species. In the laboratory, the myrtle root system was colonized by indigenous endophytes as well as by an Italian isolate of *Glomus intraradices*. In greenhouse experiments, mycorrhizal inoculation reduced transplant stress in 60-day-old myrtle seedlings; their growth was renewed immediately after transplanting, whereas non-mycorrhizal plants stopped development. Significantly larger growth responses were obtained using indigenous fungi than the Italian isolate of *Glomus intraradices*.

Key words Arbuscular mycorrhizas · *Myrtus communis* L. · *Glomus intraradices* · Indigenous endophytes · Transplant stress

Introduction

Mycorrhizas constitute the absorbing root system of approximately 90% of land plants (Harley and Smith 1983). Different mycorrhizal types have evolved to prominence in diverse land plant ecosystems; all known types of association have been reported to occur in Mediterranean biomes (Read 1991). Nevertheless, there is little information about the mycorrhizal status of typical Mediterranean plant species (Lansac et al. 1995; Requena et al. 1996).

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Common myrtle (*Myrtus communis* L.) is an aromatic evergreen shrub and a component of Mediterranean macchia, which is important in degraded *Quercus ilex* forests. Beside being a garden plant, it can also be used for revegetation and reclamation of coastal zones. However, the nursery production of myrtle is difficult because of the transplant stress of young seedlings. Myrtle transplant stress might be reduced by mycorrhizal inoculation, which was shown to improve acclimation of micropropagated fruit rootstocks, vines (Fortuna et al. 1992; Lovato et al. 1992; Sbrana et al. 1994) and woody legumes (Salamanca et al. 1992).

In the present work, we investigated firstly the mycorrhizal status of native myrtle plants in an undisturbed ecosystem within the Brioni National Park, Croatia. Experiments were then performed to evaluate the potential of indigenous endophytes as an inoculum source for mycorrhizal infection of myrtle plants. The growth responses and survival of transplanted myrtle seedlings inoculated with native vesicular-arbuscular mycorrhizal (VAM) fungi or an Italian isolate of the widely used species *Glomus intraradices* Schenck and Smith were compared.

Materials and methods

Sampling area and sample collection

Myrtle was sampled in northwest Veliki Brijun, the central and largest island of the Brioni archipelago (approximately 44° 55' N, 13° 43' E), at an undisturbed site of about 8 ha from which animals were excluded. In addition to myrtle shrubs, up to 3 m tall, other important plant species typical of Mediterranean macchia and supporting different types of mycorrhizas were present: *Quercus ilex* L. (ectomycorrhizal), *Pistacia lentiscus* L. (VAM), *Viburnum tinus* L. (VAM), *Phillyrea latifolia* L. (VAM), *Cistus monspeliensis* L. (ectomycorrhizal), *Arbutus unedo* L. (arbutoid), *Erica arborea* L. (ericoid). Replicate samples (15) of myrtle roots and rhizospheric soil were collected. Only young plants were sampled, to ensure that the samples were part of a myrtle root system.

Plant material

Myrtle fruits were collected in October 1993 and maintained at 4 °C for 4 months to overcome seed dormancy. Seeds were removed from the fruits, washed in water, scarified with 96% H_2SO_4 for 15 min and rinsed for 24 h in distilled water. Single seeds were germinated in 20-ml plastic pots containing a 2:1 v/v mixture of autoclaved peat and sand. After 60 days, seedlings were transplanted into 1-l pots with a 2:1 v/v mixture of autoclaved native soil and sand. The available P concentration of the substratum was 22 ppm (Olsen). All plants were kept under greenhouse conditions.

Fungal material

Indigenous endophytes were recovered from each rhizosphere soil sample (100 g) by wet-sieving and decanting. Roots, spores and sporocarps retained on sieves of pore size 400, 250 and 100 μm were flushed into Petri dishes for retrieval, examination and species identification. Aliquots of this sieved material were used as native inocula, 10 g per pot.

The other inoculum (also 10 g per pot) consisted of spores, external mycelium and root fragments obtained from pot cultures of *Glomus intraradices* Schenck and Smith (IMA 5), isolated from an Italian serpentine soil (Lioi and Giovannetti 1989) and grown in the collection of the Institute of Agricultural Microbiology, University of Pisa. A filtrate obtained by mixing water with equal amounts of both inocula and filtering through a 50-μm pore sieve was used as a control inoculum.

Experimental design

Three different trials were set up: (A) plants inoculated with the native endophytes, (B) plants inoculated with *G. intraradices* (IMA 5), (C) controls, each with 20 replicate plants. At harvests 3 and 12 months after transplanting, height, fresh and dry weight and leaf area were measured for each plant, and the shoot/root ratio calculated. The mycorrhizal status of native plants was assessed after staining with trypan blue (Phillips and Hayman 1970), and the percentage root colonization was estimated by the gridline intersect method (Giovannetti and Mosse 1980).

The data were analysed by one-way analysis of variance (ANOVA) and means were separated by Tukey's multiple range test.

Results

The root systems of native myrtle showed VAM colonization, with 20–35% infected root length.

The many different spore types found in rhizospheric soil of myrtle were isolated and grown in pot cultures for taxonomical identification. All belonged to the genus *Glomus* and included *G. viscosum*, *G. clarum*, *G. constrictum*, *G. deserticola*, *G. intraradices* and *G. mosseae*. The identification of other isolates probably belonging to undescribed *Glomus* species is still in progress.

Both *G. intraradices* (IMA 5) and native endophytes were able to infect myrtle roots, and no significant differences were detected, after statistical analysis of angular transformed data, between mycorrhizal infection; the means were 24% (IMA 5) and 31% (native endophytes).

In pot trials, mycorrhizal plants grew more than controls, and differences in shoot height were visible as early as 6 weeks after inoculation. Both after 3 months and 1 year, non-mycorrhizal plants were the same size as at transplantation, whereas the plants colonized by *G. intraradices* (IMA 5) and by native endophytes showed significant growth (Table 1).

The shoot/root ratios of mycorrhizal plants were higher than the controls 3 months after transplanting, but after 1 year the values decreased to those of the controls (Table 1).

At the end of the experiment, 85% of control plants and 100% of mycorrhizal plants had survived.

Discussion

The results show that myrtle (1) establishes arbuscular mycorrhizal symbioses in nature, (2) can be infected by native endophytes as well as by *G. intraradices* (IMA 5), (3) responds to mycorrhizal inoculation by growth and reduced transplant stress.

Association of the Myrtaceae with ectomycorrhizal symbioses has been shown by several studies of *Eucalyptus* species (Chilvers 1968; Warcup 1975, 1991; Malajczuk et al. 1981; Horan et al. 1988). About 90% of Myrtaceae species reported to form mycorrhizas were shown to possess ectomycorrhizas (Newman and Reddell 1987), and 7% formed arbuscular mycorrhizas. To our knowledge, this is the first report on the mycorrhizal status of *Myrtus communis*, and also confirms the occurrence of arbuscular mycorrhizas within the Myrtaceae (Malajczuk et al. 1981).

All arbuscular mycorrhizal species associated with myrtle roots are *Glomus* species. Interestingly, no *Gigaspora* or *Scutellospora* species were found in Brioni, in contrast to other maritime systems (Koske 1975; Giovannetti and Nicolson 1983; Bergen and Koske

Table 1 Biometrical parameters measured 3 months (*3*) and 1 year (*12*) after mycorrhizal inoculation of *Myrtus communis* L. plants. Means in columns with different letters are significantly different at $P=0.05$ (Tukey's multiple range test)

Treatment	Height (cm)		Leaf area (cm^2)		Dry weight (g)		Shoot/root ratio	
	3	12	3	12	3	12	3	12
Control	1.8a	1.75a	0.35a	0.2a	0.028a	0.018a	1	1.1
Glomus intraradices (IMA 5)	11.6b	26.3b	17.1b	97.7b	0.371a	4.549b	2	0.9
Indigenous endophytes	13.0b	22.2b	18.5b	130.5b	0.456b	6.665b	2.3	0.7

1984; Giovannetti 1984). Indigenous endophytes increased myrtle plant growth more than *G. intraradices* (IMA 5). This supports the suggestion of Dodd and Thomson (1994) that mycorrhizal endophytes should be selected from the soils in which inoculated seedlings are to be transplanted. The selection of efficient isolates of native mycorrhizal fungi should be considered, particularly in programs aimed at the revegetation of degraded natural ecosystems, such as some sites in the Mediterranean area.

The positive growth effect of the mycorrhizal symbiosis found is consistent with previous results (Lovato et al. 1992; Fortuna et al. 1992; Salamanca et al. 1992; Sbrana et al. 1994), and confirms that mycorrhizal inoculation can be used as an effective tool to overcome transplant-induced stress in many plant species. Thus mycorrhizal inoculation at the nursery stage might have a practical application in myrtle nursery production for revegetation of degraded maritime systems.

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