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Co-occurrence of three fungal root symbionts in *Gaultheria poeppigii* DC in Central Argentina

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Abstract The roots of *Gaultheria poeppigii* (Ericaceae) were examined for fungal symbiont colonization. Typical structures of ericoid mycorrhizas (hyphae and intracellular coil hyphae complexes), dark septate fungal endophytes (hyphae and sclerotia), and arbuscular mycorrhizas (hyphae, coils, vesicles and arbuscules) were found in the roots of all the individuals examined. The evolutionarily derived position of *Gaultheria* within the Ericales may suggest that *G. poeppigii* recently acquired the ability to form arbuscular mycorrhizas rather than having retained it from ancestral lines.

Keywords Ericaceae · Ericoid mycorrhizas · Dark septate fungal endophytes · Arbuscular mycorrhizas · Colonization

Introduction

Gaultheria poeppigii DC is a South American species of the Ericaceae that is distributed in southern Chile and central and southern Argentina (Sleumer 1985), showing a disjunct distribution. *G. poeppigii* is the only member of the Ericaceae in the mountains of central Argentina. This shrubby species grows at 1,500–2,500 m elevation, in sheltered crevices of granite outcrops, especially in cool and humid south- and south-east-facing slopes. These outcrops are distributed as patches surrounded by grassland communities above the timberline border (Funes and Cabido 1995).

The members of the Ericaceae often show association with ericoid mycorrhizal (ERM) fungi, and some of them have arbutoid mycorrhizas (Smith and Read 1997). Another type of fungal symbiont, commonly but not exclusively reported for roots of Ericaceae and many other plant families, are the so-called dark septate fungal endophytes (DSE) (Jumpponen and Trappe 1998). Ecto-

mycorrhizal (ECM) fungi are also found in association with ericaceous plants, albeit less frequently and mostly restricted to trees (Largent et al. 1980). Although arbuscular mycorrhizal (AM) fungi are not generally thought to occur in ericaceous plants (Harley and Harley 1987; Treu et al. 1996; and others), there are some reports of this type of symbiosis (Koske et al. 1990).

The aim of the present study was to determine the type of mycorrhizal symbioses in *G. poeppigii*.

Materials and methods

Study area

The study site is Pampa de Achala, located in the Córdoba mountains, central Argentina (29°30′–33°30′S, 63°40′–65°20′W), between 1,700 and 2,200 m elevation. The mean annual temperature is 8°C, and frosts are likely to occur at anytime during the year. Annual precipitation is 750–970 mm, most of which falls during spring and summer (Cabido and Acosta 1986). Soils have a pH ranging from 4.6 to 6.6 with high organic matter content that may greatly accumulate in the outcrop crevices (Cabido et al. 1987). The plant communities in these microsites are dominated, together with *G. poeppigii*, by shrubs such as *Stevia achalensis* (Asteraceae), *Baccharis tucumanensis* (Asteraceae), *Berberis hieronymi* (Berberidaceae), *Satureja odora* (Lamiaceae), *Polylepis australis* (Rosaceae), *Solanum incisum* (Solanaceae), and ferns, mostly *Blechnum penna-marina* (Blechnaceae). Further descriptions of these communities are found in Cabido and Acosta (1985, 1986).

Sampling methods

Entire root systems of *G. poeppigii* were collected from three sites (two of them separated by a distance of 5 km and the third approximately 60 km away from the other two) of Pampa de Achala during the growing season, that is when mycorrhizal fungi are supposed to be more active (November 1999, February, October and November 2000). Three samples (a total of 12 individuals) were taken each time.

Clearing and staining methods

Within 48 h of collection, the roots were separated from adhering rhizosphere soil and washed. All the lateral fine roots (<2 mm, no obvious tannins) of each seedling were cleared in 20% potassium hydroxide solution, acidified with 10% hydrochloric acid and stained with 0.05% aniline blue solution according to the method

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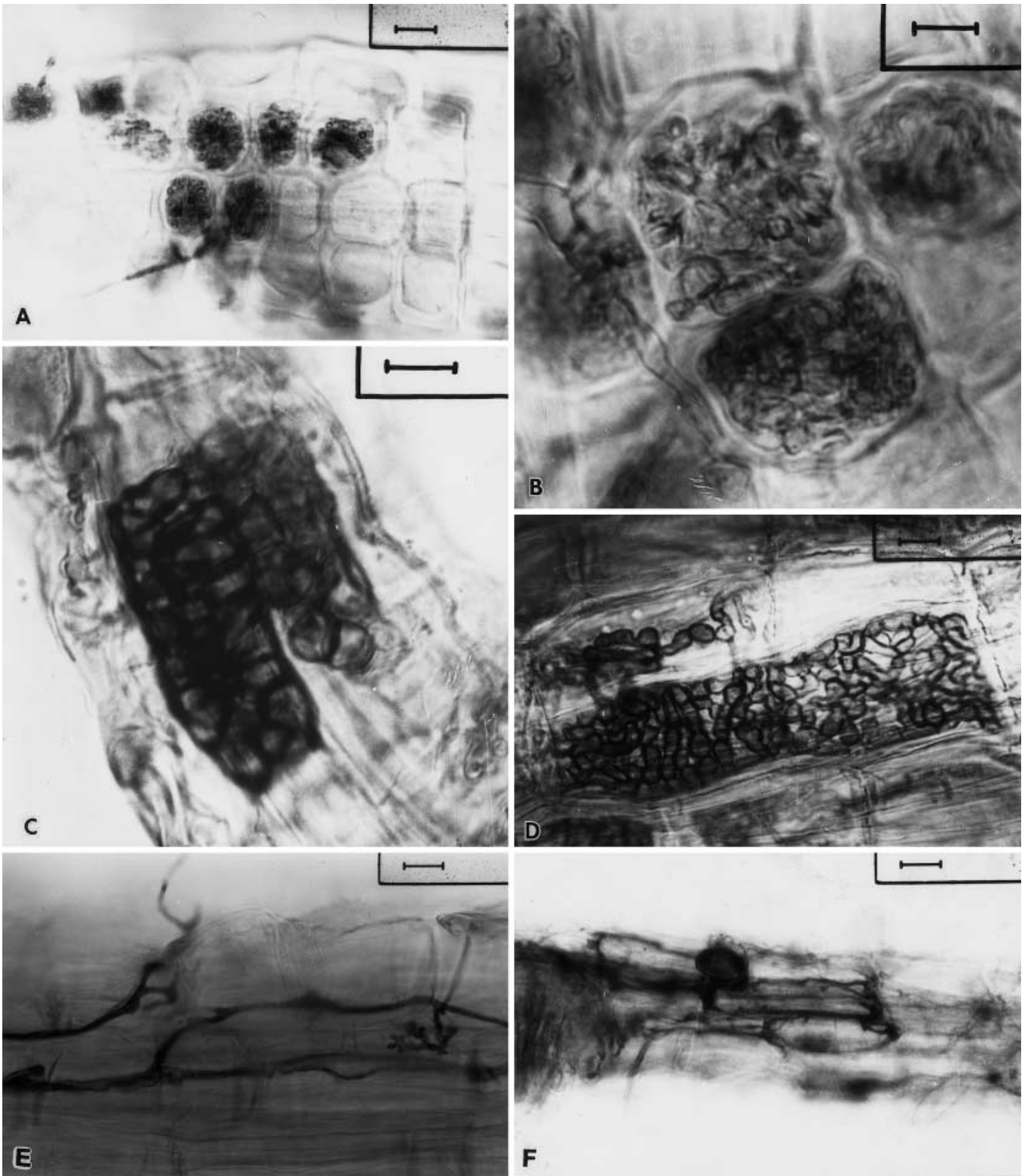


Fig. 1 *Gaultheria poeppigii* roots showing **A, B** typical ericoid hyphal complexes filling each root cell, **C** sclerotia of the *Rhizoctonia* type, **D** sclerotia of the *Phialocephala* type, **E** arbuscular mycorrhizal hyphae and a developing arbuscule, **F** arbuscular mycorrhizal hyphae and intracellular vesicle. Bars represent: **A** 12.5 μm ; **B** 6.3 μm ; **C, D** 10 μm ; **E** 12.5 μm ; **F** 25 μm

of Grace and Stribley (1991). Roots that remained dark after clearing were bleached in hydrogen peroxide prior to staining (Koske and Gemma 1989).

Spore extraction

The rhizosphere soil from the February 2000 samples was examined and glomalean spores were recovered by wet sieving and centrifugal flotation (Daniels and Skipper 1982).

Mycorrhizal structures in roots and the spores were mounted in Polyvinyl-alcohol (Omar et al. 1979) and observed with a normal light microscope (Kyowa), using the standard 10-40-100 objectives. They were photographed using an Axiophot Zeiss microscope with differential interference contrast objectives.

Plant and spores voucher specimens were deposited in the Herbarium of the Museo Botánico de la Facultad de Ciencias Exactas, Físicas y Naturales de la Universidad Nacional de Córdoba.

Results

All *G. poeppigii* individuals' roots sampled in all 4 months of the growing season showed three types of fungal symbionts: ERM, DSE, and AM. The ericoid fungi were present in the terminal portion of the finer roots (generally ranging from 60 to 120 μm diameter). These lateral roots (typical ericaceous "hair roots") consisted of a small stele surrounded by endodermal and cortical layers, four to seven cells thick. The endophytes presented hyaline to brownish hyphae (depending on the age), with septa and thickened walls at maturity, 2–4.5 μm diameter, spread over the root surface. The hyphae penetrate the cortical cells filling each cell with a well-stained dense coil hyphal complex (Fig. 1A, B). At maturity, this complex appears granular, brownish and poorly stained, but remains clearly distinguishable. The main function of these coils is presumed to be nutrient exchange (Smith and Read 1997).

DSE appeared outside and inside roots broader than 70 μm in diameter. The internal hyphae were extracellular but they penetrated the cell to form two distinct sclerotia morphologically similar to the *Rhizoctonia* DC type (Fig. 1C) and the *Phialocephala* Medlar. type (Fig. 1D) described by Haselwandter and Read (1980), Read and Haselwandter (1981), Stoyke and Currah (1990), and Jumpponen and Trappe (1998).

AM fungi were present in wider roots, 80–250 μm diameter, and showed some of the characteristic features of this type of association: irregular hyphae without septa, entry points or appressoria, coils, vesicles and some scattered arbuscules. The vesicles were regular (Fig. 1F) and irregularly shaped, mainly intracellular but some develop extracellularly, suggesting that this type of association may involve more than one species. The observed arbuscules developed laterally (Fig. 1E). Glomalean spores of three unidentified species belonging to the genera *Glomus*, *Acaulospora* and *Scutelospora* were found in the adjacent rhizosphere soil.

Discussion

G. poeppigii has been consistently found associated with ERM fungi, dark septate endophytes and AM fungi in the Córdoba mountains in central Argentina. The occurrence of ERM–DSE, ERM–AM, and AM–DSE associations in roots of the same ericaceous species has been previously reported by Haselwandter and Read (1980), Koske et al (1990) and Blaschke (1991), respectively. However, there is no previous report of the co-occur-

rence of all three fungal types in the roots of the same species. This is not only the first report of their "unusual" co-occurrence but also the first detailed description of the fungal root symbionts of an ericaceous species in Argentina.

Although both ERM and AM fungi are known to be involved in nitrogen and phosphorus nutrition, ERM fungi are generally mostly associated with nitrogen nutrition by providing access to ammonium ions and by contributing to the utilization of simple organic nitrogen compounds (Read 1983; Leake 1992). Instead, AM fungi are mainly involved in phosphorus nutrition (Allen 1991; Smith and Read 1997), but are also known to have other roles such as limiting the adsorption of toxic heavy metals, protection against pathogens, and improvement of water relations (Newsham et al. 1995). On the other hand, although they have been found associated with plants of many families from the tropics to the Arctic, the function of DSE remains controversial (Jumpponen and Trappe 1998; Jumpponen 2001). It has been suggested that the possible role of these fungal symbionts is related to plant vigour, competitive success (Haselwandter and Read 1980), and protection against pathogens (Deacon 1973; Haselwandter 1987). Treu et al. (1996) proposed that DSE assume the role of AM fungi in arctic-alpine habitats. Nevertheless, more experimental evidence of the ecological significance of this type of association is needed (Smith and Read 1997; Jumpponen and Trappe 1998).

Mutualistic interactions between fungi and plants have arisen independently in different groups of plants at different times during evolution (Pirozynski 1981; Selosse and Le Tacon 1998). It has been proposed that symbiotic fungi (AM) were instrumental in the colonization of land by ancient plants (Pirozynski and Malloch 1975; Simon et al. 1993; Redecker et al. 2000). Trappe (1987) suggested that ancestral plant species were all AM, and in the members of the Ericales this dependence was replaced by ERM. Accordingly, Ericales is one of the very few plant groups in which AM fungi are reportedly mostly absent (Harley and Harley 1987).

Koske et al. (1990) studied the fungal symbionts in the roots of three indigenous species of *Vaccinium* (Ericaceae) in the Hawaiian Islands. After finding ERM and AM fungi, they proposed that the Hawaiian populations of Ericaceae may be a relict group that have ERM but have not yet lost their ability to form AM fungi. Persistence of ancient types in present-day floras, a common phenomenon in islands (Aldridge 1979; Brown and Lomolino 1998), was mentioned as a possible explanation. This explanation of the co-existence of ERM and AM fungi does not apply to my findings because *G. poeppigii* has a continental distribution. Furthermore, within the Ericales, *Gaultheria* is an evolutionarily derived genus (Judd and Kron 1993; Kron et al. 1999). This evidence suggests another hypothesis: in *G. poeppigii* the ability to form AM fungi may have been recently acquired rather than retained from ancestral lines.

Ecological and/or evolutionary processes, interacting to various degrees, may select the type of root symbionts

in vascular plants. As not all endophytes necessarily provide the same benefits, it would be interesting to compare the relative contributions and carbon costs of each type of endophytic fungus present in *G. poeppigii* roots to elucidate the ecological implications of the occurrence of each symbiont.

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