SHORT NOTE

Analía I. Sannazzaro · Oscar A. Ruiz ·
Edgardo Albertó · Ana B. Menéndez

Presence of different arbuscular mycorrhizal infection patterns in roots of *Lotus glaber* plants growing in the Salado River basin

Abstract Morphological types of arbuscular mycorrhizal (AM) fungi associated with *Lotus glaber* in sodic soils of the Salado River basin were studied. At least eight colonization patterns (IP) of AM fungi in roots of *L. glaber* were observed after 30 plants were analyzed. *Arum*- and *Paris*-type infection were found in the same plant species. This result supports the idea that AM morphology is not solely under plant control, but is also influenced by fungal identity. One infection pattern, presumably corresponding to *Glomus intraradices*, and a second, possibly assignable to *Glomus tenue*, were the most commonly found. Our results reinforce previous suggestions that *G. intraradices* is well adapted to sodic-saline conditions and may play a role in the resistance of *L. glaber* to these soils.

Keywords Arbuscular mycorrhizae · *Lotus* · Infection pattern · *Arum* · *Paris*

Introduction

Lotus glaber is a warm-season, perennial legume from Europe that became naturalized in the flooding pampas of Argentina only a few decades ago. It is the best-adapted species used as forage for cattle production in the Salado River basin (9×10^6 ha). Soils in this region are limed, poorly drained and present a severe phosphorus deficien-

A. I. Sannazzaro · O. A. Ruiz · E. Albertó · A. B. Menéndez
Unidad Biotecnológica 1,
Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús (IIB-INTECH),
7130 Chascomús, Pcia de Buenos Aires, Argentina

A. B. Menéndez (✉)
Departamento de Biodiversidad y Biología Experimental,
Facultad de Ciencias Exactas y Naturales,
Universidad de Buenos Aires,
Piso 4, Pab II, Ciudad Universitaria, 1428 Buenos Aires, Argentina
e-mail: anamen@bg.fcen.uba.ar
Tel.: +54-11-45427396
Fax: +54-11-47872706

cy (2–10 ppm), medium organic matter levels, high alkalinity and salinity, together with periodic exposure to waterlogged conditions, which significantly decrease persistence and yield of common legumes (Mazzanti et al. 1986).

Most vascular plant taxa have symbiotic associations with arbuscular mycorrhizal (AM) fungi, and soils bearing native or cultivated host plants harbor these fungi (Mosse 1973; Harley and Smith 1983). AM fungi have been shown to improve phosphorus nutrition and plant tolerance to abiotic stresses (Ruiz-Lozano et al. 1996; Ruiz-Lozano and Azcon 2000). The taxonomy of AM fungi is based mainly on morphological characteristics of their spores. However, since the number of available taxonomic characters is limited and identification of species that colonize roots in the field is usually difficult, other attempts to classify AM fungi in roots have been made on the basis of their intraradical mycelial morphology (Abbott 1982; Morton 1988; Brundrett et al. 1996; Merryweather and Fitter 1998).

Two morphological types of AM fungi in roots have been described, namely *Arum*- and *Paris*-type (Gallaud 1905; Smith and Smith 1997). The *Arum*-type of colonization is defined by hyphae growing intercellularly in the root cortex and penetrating the cortical cells to produce arbuscules as terminal structures on "trunk hyphae". The *Paris*-type is defined by the absence of intercellular hyphae and the presence of extensive coils of intracellular hyphae within cortical cells, from which arbuscules are formed as intercalary structures and intracellular vesicles.

It was first suggested that AM morphological type is largely dependent on the plant species, and that this could be related to the presence or absence of extensive airspaces in roots (Brundrett and Kendrick 1988). However, Smith and Smith (1997) considered the potential role of the fungal genome in the determination of the morphological type of AM, and some studies have demonstrated differences in morphology of AM in a single plant species when colonized with different fungal species (Abbott 1982; Cavagnaro et al. 2001).

As far as we know, nothing has been reported on colonization patterns of AM fungi in *L. glaber* roots, as affected by saline soil conditions. The aim of the present work was to identify and describe morphological types of AM associations in the roots of *L. glaber* plants growing in the Salado River basin.

Materials and methods

This study was carried out in a 900 ha field located at the Technological Institute of Chascomús (INTECH), Province of Buenos Aires, Argentina (35°35′S, 58°00′W) between March 2001 and April 2002. The field is subjected to periodic waterlogged conditions and the soils, classified as Natracuol (INTA-CIRN 1990), are chemically heterogeneous. In addition to *L. glaber*, vegetation is dominated by plant species that are adapted to saline or flooded soils, namely *Distichlis spicata*, *Paspalum dilatatum*, *Trifolium repens*, *Ambrosia tenuifolia*, *Baccharis* sp., *Cynodon dactylon*, *Cyperus* sp. and *Setaria verticillata*.

L. glaber roots were sampled at five different sites. Soil chemical characteristics were: site 1: phosphorus content (P) 4.2 ppm, electric conductivity (EC) 2.7 mS/cm, pH 8.2, interchangeable sodium percentage (ISP) 36.7%, organic material (OM) 4.9%; site 2: P 5.2 ppm, EC 0.4 mS/cm, pH 6.9, ISP 2.5%, OM 4.5%; site 3: P 5.6 ppm, EC 1.6 mS/cm, pH 8.6, ISP 33.2%, OM 3.1%; site 4: P 6.3 ppm, EC 0.7 mS/cm, pH 8.5, ISP 24.1%, OM 2.3%; site 5: P 5.8 ppm, EC 0.9 mS/cm, pH 8.2, ISP 20.6%, OM 3.1%. At least six samples were randomly taken from each site, air-dried and kept in plastic bags at 4°C until use. The roots were rinsed with tap water, cleared by KOH (10%) for 15 min at 98°C, acidified in HCl (0.2 M) and stained with Trypan Blue for 5 min at 98°C (Phillips and Hayman 1970). Thirty root segments per plant were examined under the microscope. For the characterization of the particular morphological type of AM colonization, roots were examined for the following features as suggested by Merryweather and Fitter (1998): (1) staining intensity (dark/light); (2) auxiliary cells (presence/absence); (3) vesicles (presence/absence); (4) vesicle wall (thick-rigid/thin-plastic); (5) vesicle shape I (globose-lemon/cubic-rectangular/lobed-elongate/romboidal), regarding the

morphology of vesicles; (6) vesicle shape II (similar/diverse) regarding the degree of similarity among vesicles; (7) vesicle vacuole number and size (1–2 large/<5 medium/>5 small); (8) internal hyphae: outline (smooth/irregular) (9) internal hyphal thickness (<1.5 μm/>1.5 μm). In addition, type of arbuscules (*Arum-*/*Paris-*/compound, Smith and Smith 1997), position of vesicles (intracellular/intercellular) and the presence or absence of intraradical spores were recorded.

Results and discussion

All collected root samples were colonized by at least one AM fungus. Eight infection patterns (IP) were observed in roots of *L. glaber* following analysis of 30 plants. Table 1 shows values of morphological characters used to distinguish among different IP. In addition, we detected a number of diverse structures, probably produced by other AM fungi.

Arum-type infection predominated among the roots analyzed, but the *Paris*-type was also present in some roots. The co-occurrence of both colonization types in *L. glaber* supports the finding of Cavagnaro et al. (2001), who concluded that the morphology of AM is not solely under plant control, as traditionally believed, but is also influenced by fungal identity.

The most frequent IPs were IPA and IPB (Fig. 1). The presence of intraradical spores in the first pattern suggests that it could correspond to that of *G. intraradices*. Aliasgharzadeh et al. (2001) found that *G. intraradices* was the dominant AM fungus in saline soils in Iran, and Landwehr et al. (2002) demonstrated its presence in a saline soil in Europe. These findings, along with the present results, suggest that *G. intraradices* is well adapted to saline conditions and may play a role in adaptation of *L. glaber* to the saline environment.

Table 1 Descriptions of the infection pattern (IP) of arbuscular mycorrhizal (AM) fungi in *Lotus glaber*. + present, − absent

| Morphological characters	IP							
	A	B	C	D	E	F	G	H
Staining intensity	Dark	Dark	Light	Dark	Dark	Light	Dark	Dark
Auxiliary cells	N.O.[a]	N.O.	N.O.	N.O.	N.O.	N.O.	N.O.	N.O.
Vesicles	+	+	+	+	N.O.	N.O.	+	+
Vesicle wall	Thick-rigid	Thick-rigid	Thin-plastic	Thick-rigid	N.O.	N.O.	N.O.	N.O.
Vesicle shape I	Globose-lemon	Globose-lemon	Globose-lemon	Globose-lemon	N.O.	N.O.	Lobed-elongate	Cubic-rectangular/lobed-elongated
Vesicle shape II	Similar	Similar	Similar	Similar	N.O.	N.O.	Diverse	Diverse
Vesicle vacuolar nnumber and size	1, Large	0–1	>5 Small	N.D.[b]	N.O.	N.O.	1, Large	1, Large
Position	Intercellular	Intracellular	Intracellular	Intracellular	N.O.	N.O.	Intercellular	Intracellular
Internal hyphae: outline	Irregular	Irregular	Regular	Regular	N.O.	N.O.	Regular	Regular
Internal hyphae: thickness	>1.5 mm	<1.5 mm	>1.5 mm	>1.5 mm	N.O.	N.O.	>1.5 mm	>1.5 mm
Intraradical spores	+	−	−	−	−	−	−	−
Arbuscules	Arum	Paris?	N.O.	Paris	Compound	Paris	N.D.	N.O.
Total frequency of occurrence %	38	31	17	17	3.5	3.5	3.5	3.5

[a] Not observed
[b] Not determined
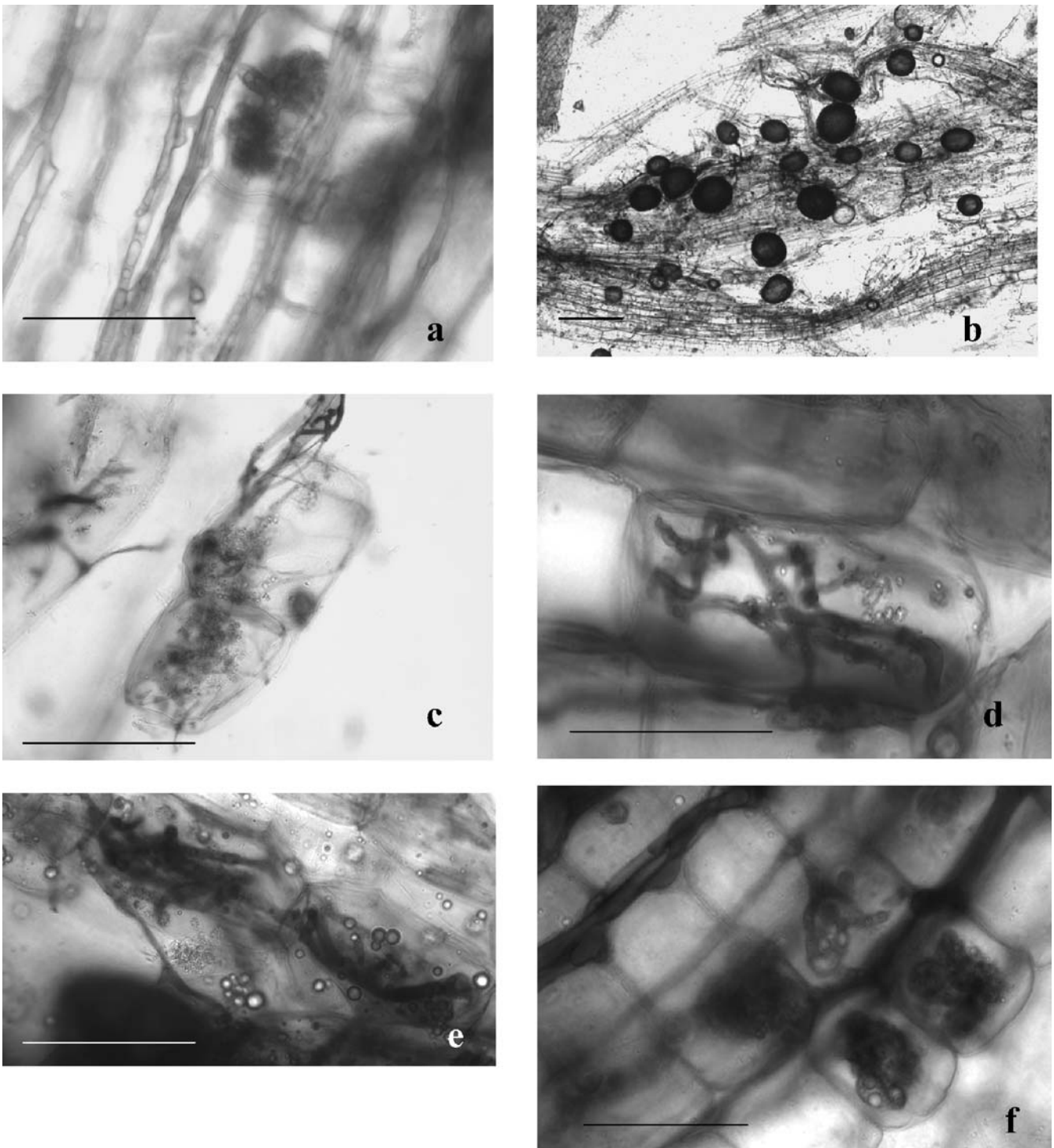
Fig. 1a–f Infection patterns of arbuscular mycorrhizal (AM) fungi found in *Lotus glaber* roots at the Río Salado basin. **a**, **b** *Glomus intraradices* type or infection pattern (IP)A: typical *Arum*-type hyphae and arbuscule (**a**), squashed roots showing intraradical spores (**b**). **c** Fine endophyte or IPB: hyphae and fainting arbuscule. **d**, **e** IPD: intracellular and coiled hyphae resembling *Paris*-type colonization. **f** IPF: intracellular, *Paris*-type arbuscule. *Bars* 100 μm

The second infection pattern most frequently observed (IPB) corresponds to a fine endophyte with hyphal and vesicle diameters and fan-shaped structures identical to those observed by Hall (1977) in AM of *G. tenue* with *Copresma robusta*. The morphology of IPB was also similar to a group of AM fungi with hyphae <1.5 μm in thickness found by Merryweather and Fitter (1998) in *Hyacinthoides* non-scripta but, in that case, the fine

endophyte never bore vesicles and could not be confidently identified as *G. tenue*. The lobed-elongated and cubic-rectangular/lobed-elongated vesicle shapes of IPG and IPH, respectively, suggest that these infection patterns could be assigned to two different *Acaulospora* or *Entrophospora* species, according to the classification of structures of AM fungi in roots of Brundrett et al. (1996).

The absence of IPs assignable to *Gigaspora* or *Scutellospora* genera in this survey is remarkable. In the case of *Gigaspora*, this absence may be explained by inhibition of hyphal growth by NaCl in different species (Juniper and Abbott 1993). Also, *Gigaspora* spp. appear to be more common in acid soils than *Glomus* spp. (Clark 1997) whereas the soils examined tended to be slightly alkaline.

As far as we know, this is the first attempt to characterize the diversity of AM infection patterns of *L. glaber*. Future work should be directed towards isolation and identification of the AM fungal species associated with *L. glaber* and their possible contribution to the tolerance of this host to saline and sodic soils.

References

Abbott LK (1982) Comparative anatomy of vesicular arbuscular mycorrhizas formed on subterranean clover. Aust J Bot 30:485–499

Aliasgharzadeh N, Rastin NS, Towfighi H, Alizadeh A (2001) Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. Mycorrhiza 11:119–122

Brundrett M, Kendrick B (1988) The mycorrhizal status, root anatomy and phenology of plants in a sugar maple forest. Can J Bot 66:1153–1173

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. ACIAR, Canberra, Australia

Cavagnaro TR, Gao L-L, Smith FA, Smith SE (2001) Morphology of arbuscular mycorrhizas is influenced by fungal identity. New Phytol 151:469–475

Clark RB (1997) Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. Plant Soil 192:15–22

Gallaud I (1905) Études sur les mycorhizes endotrophes. Rev Gen Bot 17:5–48

Hall IR (1977) Species and mycorrhizal infection of New Zealand Endogonaceae. Trans Mycol Soc 68:341–456

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London

INTA-CIRN (1990) Atlas de suelos de la República Argentina. Tomo I. SAGyP-PNUD, Argentina, 085/19

Juniper S, Abbott L (1993) Vesicular-arbuscular mycorrhizas and soil salinity. Mycorrhiza 4:45–57

Landwehr M, Hildebrandt U, Wilde P, Nawrath K, Tóth T, Biró B, Bothe H (2002) The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils. Mycorrhiza 12:199–211

Mazzanti A, Darwich NA, Cheppi C, Sarlangue H (1986) Persistencia de pasturas cultivadas en zonas ganaderas de la Pcia. de Buenos Aires. Rev Argent Producción Anim 6 [Suppl 1]:65

Merryweather J, Fitter AH (1998) The arbuscular mycorrhizal fungi of non-scripta—I. Diversity of fungal taxa. New Phytol 138:117–129

Morton JB (1988) Taxonomy of VA mycorrhizal fungi: classification, nomenclature and identification. Mycotaxon 32:267–324

Mosse B (1973) Advances in the study of vesicular-arbuscular mycorrhiza. Annu Rev Phytopathol 11:171–196

Philips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Ruiz-Lozano JM, Azcon R (2000) Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and *Glomus deserticola* under salinity. Mycorrhiza 10:137–143

Ruiz-Lozano JM, Azcon R, Gomez M (1996) Alleviation of salt stress by arbuscular-mycorrhizal *Glomus* species in *Lactuca sativa* plants. Physiol Planta 98:767–772

Smith FA, Smith SE (1997) Structural diversity in (vesicular)-arbuscular mycorrhizal symbiosis. New Phytol 137:373–388