Arbuscular Mycorrhizal Fungi

Subjects: Plant Sciences

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Low arbuscular-mycorrhizal (AM) sporulation in arid field soils limits our knowledge of indigenous species when diversity studies are based only on spore morphology. Our aim was to use different approaches (i.e., spore morphological approach and PCR–SSCP (single-strand-conformation-polymorphism) analysis) after trap plant multiplication strategies to improve the knowledge of the current richness of glomalean AM fungi (Glomerales; Glomeromycota) from the Argentine Puna. Indigenous propagules from two pristine sites at 3870 and 3370 m of elevation were multiplied using different host plants; propagation periods (2–6 months), and subculture cycles (1; 2; or 3) from 5 to 13 months. The propagule multiplication experiment allowed the detection of different glomoid taxa of Funneliformis spp. and Rhizoglomus spp., which were considered cryptic species since they had never been found in Puna soils before. On the other hand; almost all the generalist species previously described were recovered from cultures; except for Glomus ambisporum. Both plant host selection and culture times were critical for Glomerales multiplication. The SSCP analysis complemented the morphological approach and showed a high variability of Glomus at each site; revealing the presence of Funneliformis mosseae. This study demonstrates that AMF trap culture (TC) is a useful strategy for improving the analysis of AM fungal diversity/richness in the Argentinean highlands.

Keywords: Glomerales ; trap plant multiplication strategy ; biodiversity ; highlands ; single strand conformation polymorphism (SSCP)

1. Introduction

Arbuscular mycorrhizal fungi (AMF) favour plant growth by playing an important role in the exchange of nutrients and metabolites. Diversity studies of AMF in this ancient symbiotic relationship are carried out through both classical taxonomy and molecular techniques provide a better understanding of their different functions and roles in ecosystem functioning [1].

AMF diversity has been traditionally assessed by the morphological identification of fungal spores [2]. Nevertheless this methodology poses some difficulties: (i) spore production is highly dependent on AMF physiology and the environment (edaphic and climatic); (ii) some fungi are non-sporulating; and (iii) spores could be parasitized or degraded in the soil [3]. Despite its detractors, AMF trap culture (TC) has been successfully implemented to circumvent these problems and to multiply soil AMF propagules under controlled conditions to increase the chances of species detection [3][4] and possibly serve as a source of an accurately identified AMF inoculum. Besides the morphological characterisation of AMF spores, a plethora of molecular techniques has been usefully developed in the last decades that allow the assessment of both AM fungal diversity from different ecosystems (i.e., forest, grassland, rangelands, and agroecosystems) [5] and the genetic diversity of specific AM fungal taxa (i.e., different karyotypes produced by the genus *Funneliformis* (= *Glomus*) and the wide interisolate genetic diversity of model AMF such as *Rhizophagus irregularis*) [6][7]. Next-generation sequencing (NGS) allows for better characterization of AMF communities in different ecosystems. However, its cost and the computational capacity needed to process and understand the huge amount of information generated limit the extensive adoption of these techniques. On the other hand, fingerprinting analysis based on DNA banding patterns has been broadly used for decades to unveil the genetic variability of soil microbial communities (i.e., denaturing and temperature gradient gel electrophoresis (DGGE and TGGE, respectively), single-strand conformation polymorphism (SSCP), length heterogeneity-PCR (LH-PCR), terminal-restriction fragment length polymorphism (T-RFLP)). At present, these techniques are still valid due to their high genotypic resolution, low cost and high versatility, reliability and reproducibility. Among these techniques, SSCP has been widely used for both assessing AMF diversity in peculiar environments, such as arid gypsum sites [8], and detecting shifts in soil fungal community structure related to different land uses and agricultural management [9][10]. Thus, the use of SSCP could be considered a first approach before deciding to perform NGS.

Despite the growing interest in knowing and safeguarding the biological diversity associated with extreme environments, some biodiversity hotspots such as the Argentine Puna are being increasingly threatened and not extensively investigated. The Puna is a harsh South American biogeographical region characterised by highlands with a desert

climate and unique properties, such as a wide daily temperature range, high solar radiation, and particular flora and fauna [11]. AMF diversity has been scarcely studied in this environment [12]. Different AMF indigenous species from field soils at elevations higher than 3320 m have been identified by means of direct spore isolation and their morphological characterisation [12] without previous propagule multiplication. A low number of AMF spores directly retrieved from field samples and an inverse relationship between AMF spore diversity and height above sea level were found in Puna [12].

This work aims to evaluate the impact of soil propagule multiplication on the diversity and richness of the indigenous glomoid AMF species detectable in two soils of the Puna by comparing the results with those previously obtained, without multiplication, on field soils of the same sites: Abra del Cóndor (AC) and Iturbe (It). We hypothesised that different strategies for soil AMF propagule detection (direct propagule isolation vs TC multiplication) and characterisation (morphological and molecular SSCP approaches) could improve knowledge about the AMF community composition in this extreme environment.

2. Development and Findings

In this study, different strategies of multiplication of arbuscular mycorrhizal fungi (AMF) propagule indigenous to two pristine soils of the Argentine Puna (elevation over 3000 m a.s.l.) were evaluated. The diversity and richness of species with an emphasis on glomoid AMF were analysed, using both a morphological approach as well as a simple and low-cost molecular tool. Our results were compared with previous field AMF spore diversity reports at these sites, which confirmed the effectiveness of TC in detecting cryptic AMF species never before described for the Puna. Seven cryptic AMF species were identified by spore morphology (*Funneliformis* sp., *F. geosporus*, *F. monosporus*, *Rhizoglomus microaggregatum*, *Septoglomus constrictum,* and *Sclerocystis* sp.) and by sequencing (*F. mosseae*). The generalist AMF identified included *Funneliformis* sp., *F. geosporus*, *F. mosseae*, *R. microaggregatum*, *R. aggregatus*, *Sclerocystis sinuosa,* and *Septoglomus constrictum*, while some rare species included *F. monosporus* and *Sclerocystis* sp. The combination of trap plant species used was crucial to detecting cryptic glomoid species. The SSCP in combination with the morphological approach, allowed to characterise the diversity of the indigenous glomoid AMF multiplied in TC from the studied sites. The results of this study seem to indicate that the TC approach played an important role in multiplying AMF propagules, and that increasing the number of multiplication cycles did not negatively impact the composition and richness of the glomoid AMF. Overall, we emphasized the importance of combining morphological and molecular strategies in diversity studies and the maintenance of AMF diversity through the variation of host plants and the application of long multiplication cycles for propagules multiplication.

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