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Effects of arbuscular mycorrhizal inoculation and phosphorus supply on the growth and nutrient uptake of *Kandelia obovata* (Sheue, Liu & Yong) seedlings in autoclaved soil

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ABSTRACT

This study evaluated the interactive effect of arbuscular mycorrhizal fungi (AMF) inoculation and exogenous phosphorus supply on soil phosphotases, plant growth, and nutrient uptake of *Kandelia obovata* (Sheue, Liu & Yong). We aimed to explore the ecophysiological function of AMF in mangrove wetland ecosystems, and to clarify the possible survival mechanism of mangrove species against nutrient deficiency. *K. obovata* seedlings with or without AMF inoculation (mixed mangrove AMF), were cultivated for six months in autoclaved sediment medium which was supplemented with KH_2PO_4 (0, 15, 30, 60, 120 mg kg^{-1}). Then the plant growth, nitrogen and phosphorus content, root vitality, AMF colonization and soil phosphatase activity were analyzed. The inoculated AMF successfully infected *K. obovata* roots, developed intercellular hyphae, arbuscular (Arum-type), and vesicle structures. Arbuscular mycorrhizal fungi colonization ranged from 9.04 to 24.48%, with the highest value observed under 30 and 60 mg kg^{-1} P treatments. Soil P supply, in the form of KH_2PO_4, significantly promoted the height and biomass of *K. obovata*, enhanced root vitality and P uptake, while partially inhibiting soil acid (ACP) and alkaline phosphotase (ALP) activities. Without enhancing plant height, the biomass, root vitality and P uptake were further increased when inoculated with AMF, and the reduction on ACP and ALP activities were alleviated. Phosphorus supply resulted in the decrease of leaf N–P ratio in *K. obovata*, and AMF inoculation strengthened the reduction, thus alleviating P limitation in plant growth. Arbuscular mycorrhizal fungi inoculation and adequate P supply (30 mg kg^{-1} KH_2PO_4) enhanced root vitality, maintained soil ACP and ALP activities, increased plant N and P uptake, and resulted in greater biomass of *K. obovata*. Mutualistic symbiosis with AMF could explain the survival strategies of mangrove plants under a stressed environment (waterlogging and nutrient limitation) from a new perspective.

1. Introduction

Mangroves are the climax formation of hydrohalophytes inhabiting tropical and subtropical estuarine or coastal intertidal zones (Lugo and Snedaker, 1974). As the natural coastal shelterbelts and the major producer of the coastal estuarine ecosystem, mangroves provide precious habitat and food resources for the establishment and stability of coastal biological species diversity, maintaining the ecological balance of the wetland ecosystem. Meanwhile, as open 'interface' ecosystems connecting upland terrestrial and coastal estuarine ecosystems, mangrove wetland ecosystems are fragile eco-sensitive zones. Although mangroves show considerable adaptation to salinity, waterlogging, and nutrient stress, they have degenerated dramatically all over the world mainly due to nutrient elements limitation such as phosphorus deficiency in the habitat. Research showed that the global area of mangrove forests have reduced by 35% in the last 20 years of the 20th century (Valiela et al., 2001). As a result, the protection and reconstruction of mangrove wetland has become a global consensus (Krauss et al., 2008).

Arbuscular mycorrhizal fungi (AMF) are of great ecological significance as they can form mutualistic symbiosis with most terrestrial plant species (Smith and Read, 2008) and a few wetland plant species (Rozema et al., 1986; Tawaraya et al., 2003). While gaining soluble carbohydrates and other growth substances from the host plant, AMF hyphae can absorb water and mineral nutrients from the soil and transport them to the root, acting as

Abbreviations: AMF, arbuscular mycorrhizal fungi; PRC, percentage root colonization; MD, mycorrhizal dependency; HC, hyphae contribution; ACP, acid phosphatase; ALP, alkaline phosphatase.

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a living bridge between soil and plants. Arbuscular mycorrhizal fungi colonization can significantly promote plant P uptake from the soil, so that other functions are often inextricably linked with the improvement of P nutrition status (Cozzolino et al., 2010). The mechanisms involved include extended extraradical hyphae pass through the P depletion zone and expand the absorption area of the host plant root (Li et al., 1991); strengthened P uptake kinetic parameters as P uptake rate of mycorrhizae is six times of the root hair (Sanders and Tinker, 1973); accelerated P transfer rate which is ten times faster in AMF than in the root (Smith et al., 1994); and improved rhizosphere environment in which P solubilization and availability are strengthened. Arbuscular mycorrhizal fungi also can provide other macro- and micro-nutrients to plants such as N, K, Mg, Cu, and Zn usually present in soil in soluble form, especially in low concentrations (Clark and Zeto, 2000; Smith and Read, 2008). In addition, AMF can improve the resistance and survival ability of plant to adverse environment, and thus play a critical role in vegetation recovery and reconstruction process in severely disturbed sites (Bedini et al., 2010; Miller and Jastrow, 1990).

Soil acid phosphotase (ACP) and alkaline phosphotase (ALP) are of particular importance in the enzyme system participated in P absorption, assimilation and metabolism. They can mediate the release of inorganic P (Pi) from organically bound P, and facilitate the transportation of P by AMF to mycorrhizal plant. Former reports have confirmed that AMF could increase soil phosphatase activities (Dodd et al., 1987; Kothari et al., 1990; Mar Vázquez et al., 2000). The AMF hyphae could also excrete organic acids, which changed the rhizosphere pH and dissolved the insoluble phosphate in the soil (Bolan, 1991). Moreover, AMF infection could stimulate plant phosphatase secretion and activities, so that more Pi could be released (Javot et al., 2007).

Mangrove soils in China are mostly silt-clays (Lu et al., 2007) and are poor in P content (Reef et al., 2010). As P is readily adsorbed or co-precipitated with carbonate compounds, growth in mangrove plant communities is limited primarily by low P availability (Koch and Snedaker, 1997). Although the saline and anaerobic conditions of mangrove forests make survival of most microbes that are crucial in nutrient mineralization difficult, AMF have been reported from mangrove ecosystems (Kothamasi et al., 2006; Kumar and Ghose, 2008; Sengupta and Chaudhuri, 2002; Wang et al., 2010). The investigation of AMF resource in mangrove wetland has shown us a new way in mangrove remediation. However, despite the attention given to our knowledge on AMF abundance and diversity in mangrove wetland ecosystems, the ecophysiological roles of the arbuscular mycorrhizal symbiosis are poorly understood. To date there has been little research on the effects of AMF on mangrove nutrition.

Arbuscular mycorrhizal fungi occur as communities in soil and in roots, they are likely to collectively contribute to plant nutrient uptake (Jakobsen et al., 2001). Thus in this experiment, four dominant AMF species isolated from mangrove forests were used as mixed inoculants. Among mangrove plant species, *Kandelia obovata* (Sheue, Liu & Yong) is widely distributed in Southeast Asia, and our former investigation showed that this species has higher AMF root colonization rate in its natural environment than two other mangrove species: *Aegiceras corniculatum* (Linn.) Blanco and *Avicennia marina* (Forsk.) Vierh. (data not shown). It was thus applied as the test material for this study. The major objectives of the present investigation were to examine the effect of exogenous P supply and AMF inoculation on soil phosphotase, plant growth, N and P nutrition of *K. obovata*. After that, we explore the ecophysiological roles of AMF in mangrove plant nutrition, clarify the possible survival mechanism of mangrove species against nutrient deficiency, and discuss the possible use of AMF in mangrove recovery and reconstruction.

2. Materials and methods

2.1. AMF inocula preparation

2.1.1. Sample collection

Soil was collected from Jiulong River mangrove natural reserve, Fujian province, Southeastern China, in May and December 2010. The area (24°24′N, 117°55′E) is characterized by a subtropical maritime monsoon climate, with an annual mean temperature and precipitation of 21.2 °C and 1714.5 mm, respectively. The sampling site mainly consists of a regrowth and mature *K obovata* community, mixed with some other mangrove species (*A. corniculatum* (Linn.) Blanco, *A. marina* (Forsk.) Vierh.). Roots and rhizosphere soils of the subsurface layer (5–30 cm) were collected from representative adult individuals of three mangrove plant species. For the root sample, only the juvenile nutritive roots attached to the plant were collected. The soil that remained adhered to the root after gentle shaking (i.e. the rhizosphere soil) was also collected for AMF identification.

2.1.2. AMF identification and trap culture

The wet sieving and decantation methods (Gerdemann and Nicolson, 1963) were used to isolate spores of AMF from root-associated soil. Then the AMF spores were identified from spore morphology by reference to type descriptions of the species (Schüßler and Walker, 2010). Four AMF species were found to be dominant in mangrove forests, which were: *Funneliformis geosporum* (T. H. Nicolson & Gerd.) (former *Glomus geosporum*), *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) (former *Glomus intraradices*), *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) (former *Glomus claroideum*), and *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) (former *Glomus etunicatum*).

Those four major types of AMF spores were selected and propagated together to prepare the AMF inocula for the pot experiment. Trap culture experiments were conducted using autoclaved (120 °C, 0.2 MPa, 1 h) sand and commercial peat soil (pH = 6.2, OC = 54%) (v:v = 1:1) as the culture medium and *Trifolium repens* L. as the host plant. After the successful trapping process, the mixtures containing AMF spores, mycelium, sandy soil and mycorrhizal root fragments were used as the inocula. Every 100 g of the prepared inocula contained about 400 propagules, and the proportion of those four AMF species was maintained at 6:2:1:1 to mimic the natural environment.

2.2. Pot experiment

2.2.1. Experimental design

The experiment was designed as a two factorial randomized complete block design with the following factors: (a) Inoculation treatments (non-mycorrhizal inocula vs. mangrove AMF inocula); (b) KH_2PO_4 supply (0, 15, 30, 60, 120 mg kg^{-1}). Three pots of each treatment, with a diameter of 20 cm and a depth of 15 cm were used to germinate and grow *K. obovata* seedlings.

2.2.2. Soil preparation and seedlings collection

Soil and mature *K. obovata* seedlings were collected from the same location as above in April and May 2011, respectively. Plant litter and root debris were removed and the soil was then thoroughly mixed for the subsequent experiment. The soil type is sediment and soil texture is silt (75.39 ± 7.62%DW) and clay (23.06 ± 1.91%DW), with pH (soil: water ratio, 1: 5) of 6.63 ± 0.13, salinity of 18.7 ± 1.03‰, and TOC of 1.76 ± 0.85%DW. An autoclave-sterilized procedure (120 °C, 0.2 Mpa, 1 h) was applied in order to eliminate native AMF propagules. Phosphorus was added as an aqueous solution of KH_2PO_4, sequentially, at the range of concentrations previously mentioned. After thorough mixing, the soil was