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The arbuscular mycorrhizal fungus *Diversispora spurca* ameliorates effects of waterlogging on growth, root system architecture and antioxidant enzyme activities of citrus seedlings

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ABSTRACT

Citrus plants are often exposed to heavy rain and subsequent periods of soil waterlogging which severely restrict tree growth. We assessed the effect of one arbuscular mycorrhizal fungus species (*Diversispora spurca*) on growth, root system architecture (RSA), and antioxidant enzyme activities of young citrus (*Citrus junos*) seedlings. Waterlogging for 37 d significantly restricted mycorrhizal colonization but increased the number of entry points and vesicles. Compared with non-mycorrhizal controls, mycorrhizal seedlings had significantly greater plant height, fresh mass, total root and taproot lengths, projected and surface root areas, root volume, and numbers of lst, 2nd and 3rd order lateral roots regardless of waterlogging treatment. *D. spurca* significantly increased root catalase (CAT) activity in non-stressed seedlings and increased root soluble protein concentration and leaf CAT activity in waterlogged seedlings, thereby inducing lower oxidative damage. These results suggest that *D. spurca* ameliorates effects of waterlogging on growth, RSA and antioxidant enzyme activities.

Introduction

Soil waterlogging is a major abiotic stress affecting growth, development and survival of plants in natural, agricultural and horticultural systems (Parent *et al.* 2008). Waterlogging mainly causes hypoxic conditions within the soil, which affects water and nutrient absorption, sugar mobilization, photosynthesis and reactive oxygen species metabolism (Ahmed *et al.* 2002; Grassini *et al.* 2007; Sairam *et al.* 2009a).

Arbuscular mycorrhizal fungi (AMF), a monophyletic group in the phylum Glomeromycota, can establish symbiotic associations with 70–90 % of land plant species (Wang & Qiu 2006). Mycorrhizal symbionts receive about 20 % of the host's photosynthates in citrus plants grown in high-P soil and in exchange, facilitate nutrient and water uptake by their hosts (Peng *et al.* 1993). AMF can increase the host plant's ability to cope with several abiotic stresses, including salinity, drought, high temperature and heavy metals (Miransari 2010;

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Wu & Zou 2011). Some studies also showed that AMF enhance the tolerance of the host plant to waterlogged soil (Miller & Sharitz 2000; Neto *et al.* 2006; Fougnies *et al.* 2007). Under rooting-zone waterlogging stress, mycorrhizal *Panicum hemitomon* and *Leersia hexandra* plants exhibited improved P nutrition compared to non-mycorrhizal plants (Miller & Sharitz 2000). *Aster tripolium* plants colonized by *Glomus* species (mainly *Glomus geosporum*) showed better tolerance to waterlogging, mediated through improved osmotic adjustment and N nutrition (Neto *et al.* 2006). In *Pterocarpus officinalis* seedlings, mycorrhizal inoculation improved plant growth and P acquisition, thus resulting in better tolerance to waterlogging (Fougnies *et al.* 2007).

Citrus plants are highly dependent on arbuscular mycorrhizas (Wu *et al.* 2011) and are also flooding-sensitive (Hossain *et al.* 2009). The Jianghan Plain, China, located in the middle region of Yangtze River valley, is an important region of citrus cultivation in the South of China. The Jianghan Plain is an alluvial plain, with groundwater level relatively higher than other regions (Yu 1992); prone to periodic waterlogging. Heavy rain and subsequent periods of soil waterlogging often occur in the citrus orchards. Such conditions seriously inhibit tree growth as well as fruit development, and frequently lead to untimely decline in orchard productivity (Hossain *et al.* 2009). Matsumura *et al.* (2008) demonstrated that a mycorrhizal network formed by inoculation with *Gigaspora margarita* allowed trifoliate orange (*Poncirus trifoliata*) to acquire oxygen from the root aerenchyma of bahiagrass (*Paspalum notatum*) under waterlogged conditions. However, limited efforts have been made in the past to expand our understanding of the citrus plant responses to waterlogged conditions through mycorrhizal inoculation.

The arbuscular mycorrhizal fungus, *Diversispora spurca*, exhibits higher tolerance to salt stress in terms of significant responses to root system architecture (RSA) and growth of citrus plants (Zou & Wu 2011). However, it is not clear whether *D. spurca* alleviates waterlogging stress of citrus. The present work assessed the ability of this species to alleviate waterlogging stress in citrus (*Citrus junos*) seedlings through effects on antioxidant enzymes, RSA and growth.

Materials and methods

Experimental design

The experiment was a completely random 2 × 2 factorial design, which consisted of inoculation with AMF (with or without *D. spurca*) and water treatment (waterlogging or no waterlogging). Each of the four treatments was replicated three times for a total of 12 pots.

Fungal inoculum

Spores of *D. spurca* (No.: BGC SD03A) were isolated from the rhizosphere of *Lycopersicon esculentum* in Shouguang, Shandong, and propagated on white clover (*Trifolium repens*). The clover grew on an autoclaved substratum of soil and river sand (1:1, v/v) in a controlled environment chamber (PQX, Life Apparatus, Ningbo Life Science and Technology Ltd., China) with a 16:8 photoperiod (light intensity = 603 μmolm^{-2} s^{-1}) at 25 °C/19 °C day/night temperature and 80 % relative humidity. After 13 weeks, the shoots were removed and the infected root systems (73.15 %) and growth substratum were collected as the mycorrhizal inoculum. The growth substrata contained ~12 spores g^{-1}, according to wet sieving and decanting (Gerdemann & Nicolson 1963). Meanwhile, fungal inoculum included spores, growth substratum, intraradical hyphae and infected root segments of *T. repens* (Klironomos & Hart 2002).

Plant culture

Six-leaf citrus (*Citrus junos*) seedlings approx. 9 cm in height were transplanted into plastic pots (17.5 cm upper diameter × 13 cm height × 11 cm bottom diameter). All plants had been grown in sterilized soil and had not been colonized by AMF. These pots contained 2.8 kg of autoclaved soil (Xanthi-udic ferralsol: pH = 6.2, organic matter = 9.4 g kg^{-1}, and Olsen-P = 16.2 mg kg^{-1}) obtained from a citrus orchard of Yangtze University. AMF-treated seedlings received 40 g of fungal inoculum, which was mixed into the rhizosphere of the citrus seedlings at planting. The non-AMF citrus seedlings received 40 g of autoclaved mycorrhizal inoculum together with a 2 ml aliquot of a filtrate of mycorrhizal inoculum to provide a common background microbial community for AMF and non-AMF seedlings. All mycorrhizal and non-mycorrhizal seedlings grew in a plastic greenhouse (Jingzhou, China), where the light intensity ranged from 721 to 967 μmolm^{-2} s^{-1} with 25/19 °C (day/night) and 70–95 % relative humidity.

Waterlogging stress

Waterlogging stress began 116 d after the mycorrhizal inoculation. Waterlogging was imposed for 37 d by placing the treated pot into a larger plastic bucket (28 cm upper mouth diameter × 18 cm height × 20 cm bottom mouth diameter) filled with tap water to 2 cm above the pot. The non-waterlogged pots were well-watered (~25 % soil water content) throughout the experiment.

Measurement of variables

Plant height and stem diameter were recorded after harvest of the seedlings. The shoots and roots were washed free from soil under a stream of tap water and fresh weights of shoots and roots were recorded.

The root systems were scanned with an Epson Flatbed Scanner, Epson Perfection V700 Photo Dual Lens System (J221A, Indonesia) and analyzed using WinRHIZO software (Regent Instruments Inc., Quebec, Canada). The traits of RSA, including mean diameter, length, projected area, surface area and volume were automatically obtained. The taproot length was measured using a vernier calliper, and numbers of first, second and third order lateral roots were counted. The 1-cm fresh root segments were cleared with 10 % KOH and stained with 0.05 % trypan blue (Phillips & Hayman 1970). The root colonization by AMF was calculated by the method previously described by Wu *et al.* (2008).

The 0.2 g of fresh leaves and roots were homogenized in 5 ml of 50 mM phosphate buffer (pH 7.8) at 4 °C in an ice-