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Effects of low magnesium and an arbuscular mycorrhizal fungus on the growth, magnesium distribution and photosynthesis of two citrus cultivars

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ABSTRACT

The effects of the arbuscular mycorrhizal (AM) fungus, *Glomus versiforme*, on plant growth, magnesium (Mg) concentration and distribution, and photosynthesis in the 'Newhall' (*Citrus sinensis* Osbeck cv. Newhall) navel orange and 'Ponkan' (*Citrus reticulate* Blanco cv. Ponkan) tangerine grafted on the rootstock trifoliate orange (*Poncirus trifoliata* L. Raf.) were studied in potted cultures under Mg-poor and Mg-rich conditions. The low soil Mg inhibited the 'Newhall' seedlings growth more than that of 'Ponkan'. This was accompanied by greater decreases of Mg concentrations and CO_2 assimilation rate in the basal leaves, and lower soluble sugar concentrations in the roots of 'Newhall'. AM inoculation improved the growth, the Mg concentrations in various plant parts, and the photosynthesis of the two cultivars, especially the 'Newhall' seedlings under the Mg-poor condition. These results suggest that *G. versiforme* has the potential to enhance growth and Mg distribution in 'Newhall' seedlings grown in low Mg soil in a greenhouse.

1. Introduction

Magnesium (Mg) is an essential macronutrient required for the normal growth of higher plants. Mg deficiency is a widespread problem in agriculture that affects the structure and integrity of chloroplasts, photosynthesis, sugar accumulation and other metabolic activities (Hermans and Verbruggen, 2005; Shen et al., 2011a, 2011b; Yang et al., 2012). In China, distinct chlorotic symptoms of Mg deficiency are frequently present on mature leaves of 'Newhall' (*Citrus sinensis* Osbeck *cv.* Newhall) navel oranges, which are mainly planted in major citrus areas, affecting fruit yield and nutritional quality (Zhang et al., 2010; Li et al., 2001). Under the same field conditions, other citrus cultivars, such as 'Ponkan' (*Citrus reticulate* Blanco *cv.* Ponkan) tangerines, rarely display the chlorotic symptoms of Mg deficiency (Li et al., 2001). The mechanisms underlying such variances are not well understood.

Arbuscular mycorrhizal (AM) symbiosis offers protection against abiotic and biotic stresses as a consequence of the host's nutritional improvement (Marschner and Dell, 1994; Watanarojanaporn et al., 2011). Improvements in the nutrition of plants colonized by AM fungi can be attributed to the uptake of nutrients via the mycorrhizal pathway, and to the indirect effects brought about by morphological and physiological changes to the roots (Cavagnaro, 2008). AM fungi may also influence nutrient availability via their effects on soil physicochemical properties (Li and Christie, 2001), nutrient cycling (Jackson et al., 2008) and microbial communities (Cavagnaro et al., 2007). 'Newhall' navel oranges and 'Ponkan' tangerines are important citrus cultivars that are generally grafted on trifoliate orange (*Poncirus trifoliata* L. Raf.), which is used as the rootstock in most citrus producing areas in China. However, *P. trifoliata* has short or even rare root hairs in field systems, and is thus dependent on AM fungi for optimal growth (Wu and Xia, 2006; Wang et al., 2012). Previous studies showed that high Mg levels generally decreased the mycorrhizal development and sporulation of *Glomus* sp. colonizing sweet potato and onion (Jarstfer et al., 1998). Additionally, spores of *Glomus* and *Acaulospora* tend to be found in high Mg soils, while those of *Scutellospora* and *Gigaspora* usually occur in low Mg soils (Schenck and Sequeira, 1987). Among three different AM fungi, *Glomus versiforme* was the most beneficial to the growth of trifoliate oranges in low Mg soil (unpublished data). These findings indicated that Mg excess or deficiency could affect AM symbiosis. However, under Mg-poor conditions, the effects of the AM fungus *G. versiforme* on mycorrhizal colonization, growth and Mg distribution in 'Newhall' and 'Ponkan' scions grafted on the trifoliate orange remain unclear.

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There is insufficient information on the differential responses of various citrus genotypes to AM fungal colonization when exposed to a Mg deficiency, and very few reports on citrus scion-rootstock combinations under Mg-poor conditions. Thus, the purpose of this study is to evaluate the effects of mycorrhization by *G. versiforme* on 'Newhall' and 'Ponkan' grafted on trifoliate orange under Mg deficient conditions. Comparisons in plant growth, photosynthesis, and Mg concentration and distribution were investigated.

2. Materials and methods

2.1. Plant culture and experimental procedure

'Newhall' (*C. sinensis* Osbeck *cv.* Newhall) navel orange and 'Ponkan' (*C. reticulate* Blanco *cv.* Ponkan) tangerine grafted on 2-year-old trifoliate orange (*Poncirus trifoliate* L. Raf.) seedlings with uniform rootstock stem diameter (1.3–1.4 cm) were selected, the buds of two scion varieties did not sprout before our experiment, and the initial shoots (about 4–5 cm above the grafting unit) of the rootstock seedlings were removed. All the virus-free materials (rootstock seedlings and buds of scion) used in the present experiment were harvested from the Fine Varieties Breeding Center of Citrus at Zigui county, Hubei province, China.

According to Sheng et al. (2009), the major part of the root system of the seedlings were severely pruned to maintain highly similar root systems before the treatments and to induce new root development. A small number of the roots may have been infected with native AM fungal species, which should not have differed significant among these plants because they had so few roots. Subsequently, all the seedlings were washed with deionized water to remove surface contaminations, followed by transplantation to black pots (one plant per pot) containing 3.0 kg of autoclaved experiment mixture (0.11 MPa, 121 °C, 2 h) of clay loam, quartz sand and vermiculite (3:1:1, v/v/v): pH value 6.85, organic matter 13.8 g kg^{-1}, total nitrogen (N) 0.24 g kg^{-1}, available potassium (K) 17.18 mg kg^{-1}, available phosphorous (P) 10.5 mg kg^{-1}, available calcium (Ca) 356.18 mg kg^{-1}, available magnesium (Mg) 11.25 mg kg^{-1}, available zinc (Zn) 0.32 mg kg^{-1}, available copper (Cu) 0.112 mg kg^{-1}.

The mycorrhizal inoculum, provided by the Institute of Plant Nutrition and Resources in the Beijing Academy of Agriculture and Forestry Sciences, consisted of spores, soil, hyphae and infected jowar root fragments from a stock culture of *G. versiforme* (No. BGC HUN02B). The inoculated dosage was 50 g of inoculum per pot containing ~600 spores. The inocula were placed 5 cm below roots at transplantation time and non-AM treatment also received the same weight of autoclaved mixture.

According to the standards for classifying the soil nutrient status of citrus orchards (Zhuang, 1994; Tang et al., 2013), the experimental soil mixture had extremely low levels of available Mg, Zn, K, Cu, P and Ca. To maintain the nutrient supply, the plants were initially irrigated with distilled water every 2–3 days, until the sprouting shoots of the scions were about 10 cm in length. Then, the plants were supplied with 1/2 Hoagland's No.2 nutrient solution, in which the micronutrients were supplied at full strength, and macronutrients at half strength, except Mg, which was supplied at two concentrations (0 and 24 mg L^{-1}) every 15 days. According to Yang et al. (2012), treatments with 0 mg L^{-1} and 24 mg L^{-1} Mg represented Mg-poor (MP) and Mg-rich (MR) conditions, respectively. The Mg treatments were created by withholding the supply of $MgSO_4$ and replacing it with equivalent moles of Na_2SO_4 to maintain the supply of sulfur and the osmoticum of the nutrient solution. The modified Mg-free full strength nutrient solution contained 4 mmol L^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, 6 mmol L^{-1} KNO_3, 1 mmol L^{-1} $NH_4H_2PO_4$, 46 μmol L^{-1} H_3BO_3, 9 μmol L^{-1} $MnCl_2{\cdot}4H_2O$, 0.3 μmol L^{-1} $CuSO_4{\cdot}5H_2O$, 0.1 μmol L^{-1} H_2MoO_4 and 50 μmol L^{-1} Fe-EDTA.

Each pot was placed in a greenhouse for 6 months with natural daylight from April to September, the average day/night temperature was 25/20 °C. The site is located at 31°20′ N latitude, 119°21′ E longitude, at a 10 m above sea level.

2.2. Plant growth and Mg analyses

At harvest time, plant height was determined by a measuring tape. A fraction of fresh roots were carefully washed and cut into 1 cm root pieces to fix in formalin-acetic acid-alcohol solutions, these roots were cleared with 10% (w/v) KOH and stained with 0.05% (w/v) trypan blue in lactophenol for mycorrhizal colonization determination (Phillps and Hayman, 1970). The AM colonization percentage was calculated as follows: AM colonization percentage (%) = 100 × root length infected/root length observed.

When samples were collected, leaf, scion stem, rootstock stem and root, were separately sampled. For the leaves, basal leaves (spring-flush) and upper leaves (summer and autumn-flush) were collected based on different phases of shoot growth. The plant material was then dried at 75 °C for 48 h and the dry weights (DW) measured, ground to fine powder and wet digested in HNO_3–$HClO_4$ (4:1, v/v) before Mg analysis by inductively coupled plasma optical emission spectrometry (ICP-OES, Pekin Elmer Optimal 2100 DV).

2.3. Chlorophyll, soluble sugar and gas exchange measurements

Leaf chlorophyll and root soluble sugar concentrations were extracted and measured according to Wang (2006). Leaf gas exchange parameters including net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were determined using a CI-340 portable photosynthesis system (USA) at ambient CO_2 concentration under a controlled light intensity of 1000 μmol m^{-2} s^{-1} between 10:00 and 11:00 on a clean day.

2.4. Experimental design and statistical analysis

The experiment was a completely randomized block design with two cultivars under two Mg conditions that were either inoculated with the AM *G. versiforme* or remained non-inoculated. Three replications (two plants in each) were designated for each treatment making a total of 48 pots.

The data were subjected to an analysis of variance (ANOVA) using the Statistical Analysis System software (SAS Institute Inc., 1996), and the differences were compared by the least significant differences test at a 5% level.

3. Results and analysis

3.1. Plant growth, leaf chlorophyll and root soluble sugar

Low Mg decreased plant height, dry weight per plant, and the leaf chlorophyll $a+b$ concentrations of both cultivars. Low Mg decreased the mycorrhizal colonization of 'Ponkan' and the soluble sugar content in the roots of 'Newhall', but did not significantly affect mycorrhizal colonization of 'Newhall' or the soluble sugar content in the roots of 'Ponkan'. Compared with the Mg-rich condition, dry weights per plant of 'Newhall' and 'Ponkan' under the Mg-poor condition were reduced by 21.04% and 13.51%, respectively. The mycorrhizal colonization level was higher in 'Newhall' seedlings than in 'Ponkan' seedlings under the Mg-poor condition (Fig. 1). However, AM inoculation significantly enhanced the dry weight per plant of both cultivars, soluble sugar content in