Micronutrient accumulation in mycorrhizal citrus under different phosphorus regimes

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ABSTRACT. The objective of this research was to quantify micronutrient accumulation in citrus that received different levels of P fertilizer, with or without the inoculation of the arbuscular-mycorrhizal (AM) fungus *Glomus intraradices*. *Citrus reshni* (Tangerina Cleópatra) (Rutaceae) was grown under with four P regimes (0, 50, 150 e 250 mg kg^{-1} soil), and with five replicates per treatment. There was a significant accumulation of all micronutrients in citrus roots. This enhanced accumulation of micronutrients may affect vital physiological processes in citrus and also yield and disease resistance.

Key words: arbuscular mycorrhizal fungi, citrus rootstock, phosphorus, copper, iron, manganese, zinc.

RESUMO. Acúmulo de micronutrientes em porta-enxerto cítrico micorrizado em diferentes doses de fósforo. Com o objetivo de se estudar a influência de doses altas de P e fungo micorrízico arbuscular, na partição de nutrientes absorvidos por porta-enxerto de citros, instalou-se experimento com quatro níveis de P (0, 50, 150 e 250 mg kg^{-1} de solo), sem e com fungo micorrízico arbuscular (*Glomus intraradices*). Determinaram-se os micronutrientes (Cu, Fe, Mn e Zn) nas raízes, caules e folhas de Tangerineira "Cleópatra", *Citrus reshni* (Rutaceae). Os resultados sugerem o acúmulo de Cu, Fe, Mn e Zn nas raízes de plantas micorrizadas nas doses mais altas de P, o que pode afetar processos fisiológicos vitais e impactar a produção de mudas e interferir na resistência a doenças em citros.

Palavras-chave: fungo micorrízico arbuscular, porta-enxerto de citros, fósforo, cobre, ferro, manganês, zinco

Introduction

In general, citrus requires P fertilization and colonization by arbuscular-mycorrhizal (AM) fungi for satisfactory growth and development. This plant-fungus interaction affects the host plant nutrition, mainly due to the larger leaf blade area, higher photosyntate demand (and thus lower stomatal resistance), transpiration rates, water uptake rates per unit root lenght and the rates of mass flow to the root surface in relation to nonmycorrhizal plants. Mycorrhizal plants may also reduce Al^{+3} and Mn^{+2} toxicity and change the pH of the rhizoplane and rizosphere. However, the intensity of this response depends on edafoclimatic conditions and on the symbiont species (plant and fungus). Under controlled conditions the extraradical mycelium of AM fungi may supply 80-3.133% of P, 25-250% of N, 10-1.011% of K, 70% of Ca, 25-67% of Zn and 60% of Cu (Cardoso, 1986; Chu and Silva, 1991; Marschner and Dell, 1994). Uptake of other micronutrients, as B and Fe via arbuscular mycorrhizal hyphae is not well established (Marschner and Dell, 1996). The uptake of Mn is most commonly reduced when plants are mycorrhizal (Cardoso, 1994; Marschner, 1997; Smith and Read, 1997; Gomes, 1997). This effect has been attributed to a lower Mn^{+4} reducing potential in the rhizosphere of mycorrhizal plants, probably because of lower populations of manganese reducers (Kothari *et al*., 1991a). A number of different mechanisms may be involved in the interactions between mycorrhizal colonization and accumulation of heavy metals, including tissue dilution of the toxic element due to interactions with P nutrition, sequestration of toxic metal in the fungus followed by the development of tolerance in the fungus (Smith and Read, 1997), and production of chelating agents. In Endogone species it has been observed that high incorporation occurs into metal-precipitated fraction and it has been argued that this fraction may also have a storage function (similar to glycogen) (Bevege *et al*., 1975).

The micronutrient content of mycorrhizal plants in relation to P levels has been reported as depending on the species or genera of the plant and the AM fungus (Gomes, 1997). Under high P levels, micronutrient uptake by the host can be reduced and the antagonic effect between P and Zn can not be observed (Marschner and Dell, 1994; Smith and Read, 1997; Gomes, 1997). By varying the phosphorus supply in the hyphal compartment the redox ratio of P/Cu transport in the hyphae could have varied significantly, indicating that hyphal uptake and/or transport of both mineral nutrients are separately regulated (Li *et al*., 1991). In relation to the roots the micronutrient content has been rarely investigated. Therefore one of the objectives of this work was to evaluate the micronutrient accumulation in mycorrhizal citrus under different phosphorus regimes.

Material and methods

Seeds of tangerina Cleópatra (*Citrus reshni* Hort. Ex Tanaka, cv Cleópatra) (Rutaceae) were germinated in autoclaved sand and transplantated (three months old) to pots (5 L) into an autoclaved, limed mixture of washed sand and soil (3:1) substrate. The citrus plants received different levels of P (0, 50, 150 and 250 mg kg^{-1}) and one half of the plants were inoculated with the AM fungus *Glomus intraradices,* while the others remained non-inoculated. The experiment was conducted under greenhouse conditions, with a completely randomized factorial design and four replicates per treatment. Nitrogen (80 mg kg^{-1}; NH_4NO_3), K (50 mg kg^{-1}; KCl), Mg (45 mg kg^{-1}; $MgSO_4.7H_2O$), and S (46 mg kg^{-1}) were added along with micronutrients (B, Cu, Fe, Mo and Zn) according to Hoagland and Arnon (1950), 1 mL per pot. After 6 months, plants were harvested, the leaves, stems and roots were separated and dried (60°C/72 h). The micronutrient (Cu, Fe, Mn and Zn) levels were measured in each plant part by atomic absorption spectrophotometry following acid digestion (Malavolta *et al*., 1989).

Results and discussion

Significantly more Fe (a 10-fold increase) and Mn (a 2-fold increase) was detected in AM roots than in non-AM roots, whereas levels of Fe and Mn in citrus leaves or stems did not differ significantly between +AM and -AM plants (Figures 1 C-D and E-F). At the highest level of P fertilization (250 mg . kg^{-1} soil), there was a 40% decrease in Fe and Mn found in the AM roots, when compared to the level of 150 mg P kg^{-1} soil. Increases in P fertilization did not affect the amount of either Mn or Fe in the leaves and stems of all plants.

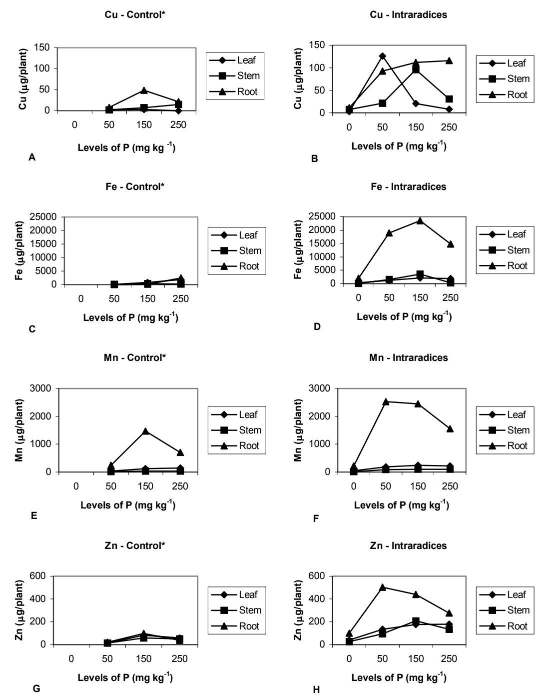

Figure 1. Cu, Fé, Mn and Zn content of Citrus rootstocks (Tangerina Cleópatra) with and without AM fungi in relation to P levels (Average of 4 replicates). *Plants died or insufficient material for analyses at lowest P level

Significantly greater accumulation of Zn and Cu was found in all AM plant organs compared to non-AM plants. Glomus-colonized citrus leaves and stems contained 4- to 8- fold more Zn than non-colonized plants (Figure 1 A-B and G-H). AM citrus micorrhizae contained 4- to 20-fold more Zn than did non-inoculated roots. Phosphorus fertilization caused an increase in total plant Zn between 0 and 150 mg kg^{-1}, but there was a relative decline in plant Zn above 150 mg kg^{-1}. Leaves and stems of mycorrhizal citrus accumulated between 50 and 500% more Cu than non-colonized plants. Colonized roots of citrus acquired from 2 to 10 times more Cu than did the roots of non-AM plants.

The greater accumulation of Fe and Mn in AM citrus roots indicates that the reduction potential in the mycorrhizosphere is significantly greater than that which exists in the rhizosphere of non-inoculated plants. Such an effect may explain why mycorrhizal plants display less negative interactions between P and either Fe or Mn. Both of these cations have very low solubilities with phosphate, resulting in precipitation, which limits P availability in the soil and plant. The accumulation of Mn in mycorrhizal roots was also observed in soybean. In that study, mycorrhizal plants accumulated most of the Mn in their roots, with lower shoot concentrations (Navarro and Cardoso, 1992; Cardoso, 1986), which confirms the protective effect of mycorrhiza against toxic levels of Mn in the soil. Kothari *et al*. (1991b) reported that arbuscular mycorrhiza affect qualitatively the microbial populations in the rizosphere, inhibiting the growth of Fe- and Mn-reducers and consequently decreasing the availability of Mn.

Increased levels of Zn and Cu in Glomus-colonized citrus indicated that the absorption and assimilation of these relatively immobile micronutrients was significantly enhanced in root system that supported mycorrhizal colonization. The increased soil volume that is available due to ramification by mycorrhizal hyphae leads to a greater and complete mining of the soil. The enhanced accumulation of these micronutrients affects vital physiological processes in citrus, which impact yield and disease resistance.

Conclusion

Higher micronutrient (Cu, Fe, Zn and Mn) content was found in AM roots compared with non-AM roots. Only for Cu and Zn, the contents in stems and leaves were significantly greater in mycorrhizal plants, when compared to the non-mycorrhizal ones. The increase of P levels resulted in a decrease of Mn and Zn root contents between 50 and 250 mg P kg^{-1} soil, while the same effect occured only above 150 mg kg^{-1} for Fe. The root content of Cu increased with the increase of the P level.The negative effect of P for micronutrient content was observed only for Cu in stems and leaves of AM-plants where Cu contents only showed decreases at levels higher than 50 mg P kg^{-1} soil, whereas in stems, Cu contents peaked at 150 mg P kg^{-1} soil, and presented very low values at all the other P levels. The results suggest accumulation of Cu, Fe, Mn and Zn in roots of AM-plants. At the highest P level (250 mg kg^{-1}), the micronutrient content in each AM-plant part showed the following tendency: Cu (Roots>> Stems> Leaves), Fe (Roots>> Leaves> Stems), Mn (Roots>> Leaves> Stems), Zn (Roots> Leaves> Stems).

Acknowledgements

This work was supported by grants from FAPESP (Tematic Project number 93/3553-5)

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Received on August 22, 2001.
Accepted on June 12, 2002.