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Changes in root morphology accompanying mycorrhizal alleviation of phosphorus deficiency in micropropagated *Annona cherimola* Mill. plants

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Abstract

The effect of the mycorrhizal inoculation on survival rate, growth, nutrient uptake and root morphology during the acclimatization period and plant establishment of micropropagated juvenile or adult cherimoya plants (*Annona cherimola* Mill.) was determined. Although mycorrhizal colonization did not improve the survival rate of plants, which was already high in non-inoculated plants, it had a positive effect on plant development (shoot length, leaf number, leaf area and fresh and dry weights). Mycorrhizal juvenile plants tripled the macronutrients and increased by four the micronutrient uptake, and mycorrhizal adult plants increased by two phosphorus and all micronutrients, with copper uptake increased five times. Moreover, mycorrhizal colonization changed the root morphology of adult plants increasing three-fold the total number of roots, doubling the number of first-order laterals and increasing second-order laterals by four. Total root length was also increased three-fold, adventitious root length was almost doubled, first-order laterals tripled and second-order roots length increased four-fold. The effect of mycorrhizal colonization seems to be stronger or different in juvenile than in adult plants, suggesting that ontogenic stage is an important factor determining mycorrhizal effect and the plant performance during the acclimatization period.

Keywords: Ex vitro establishment; Fruit tree; Mature; Juvenile; *Glomus*; Survival rate; Ontogenic stage; Acclimatization

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1. Introduction

Cherimoya is a subtropical fruit tree native to South America, well adapted to the subtropical conditions of southern Spain, where it is an economically important crop. Important efforts are being made in this area to improve the production and fruit quality of this species by both traditional and biotechnological methods. Thus, Encina et al. (1994) developed the first micropropagation protocol for juvenile material of cherimoya (cv. Fino de Jete).

Tree clonal propagation using conventional methods, can be achieved using juvenile material in most of the especies, but the ease of propagation tends to diminish as the plant material approaches the adult phase, which is when a reliable evaluation of crop potential can be made. In general, adult material has less morphogenetic capacity than juvenile material, which makes it difficult to root in vivo and in vitro, making necessary biotechnological approaches. These micropropagated plantlets also show slow growth rates or sometimes blocked apical growth just after transplanting, with a low survival percentage (Uosukainen and Vestberg, 1994).

Arbuscular mycorrhizal (AM) fungi are soil inhabitants able to colonize plant roots. AM fungi establish beneficial symbiosis in most higher plants and can improve plant development under environmental conditions of stress. Thus, their inoculation to tissue culture-derived plants can benefit plant growth and survival (Taylor and Harrier, 2003). Inoculation of micropropagated plants during the weaning stage benefits several tropical plant species, such as guava or banana (Estrada-Luna et al., 1998; Declerck et al., 2002) and produces plants with a more efficient root system, as shown by the increased capacity to take up phosphate and other nutrients, and with a increased tolerance to transplant stress, root pathogens or metal contamination (Taylor and Harrier, 2003). Inoculation also produces more uniform plants (Estrada-Luna et al., 2000), renews apical growth (Sbrana et al., 1994), and in some cases AM inoculated plants flower sooner (Wang et al., 1993).

Cherimoya, like many other woody species, shows developed arbuscular mycorrhiza, which enhanced growth and development (Azcón-Aguilar et al., 1994a,b) and increased the root number and root length of adventitious roots, first-order and second-order laterals and the intensity of branching of the first-order laterals in micropropagated cherimoya juvenile plants (Azcón-Aguilar et al., 1996). Micropropagation from cherimoya, cv. Fino de Jete, adult material has recently been achieved (Padilla and Encina, 2004), so we decided to study the effect of mycorrhizal colonization on adult micropropagated plants, especially focusing on the changes induced in the root morphology and nutrient uptake under nutrient deficiency compared to juvenile micropropagated plants.

2. Materials and methods

2.1. Experimental design

The experimental design was a 2×2 factorial study with 20 replications, completely randomized. Treatments included juvenile material, adult material, mycorrhizal plants (M) and non-mycorrhizal plants (N-M).