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Manganese modulates the responses of nitrogen-supplied and *Rhizobium*-nodulated *Phaseolus vulgaris* L. to inoculation with arbuscular mycorrhizal fungi

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ABSTRACT

In Venezuela, low yields of black bean crops are attributed, in part, to the low manganese (Mn) and phosphorus (P) contents in the Quartzipsamment soils where this crop is usually sown. To test this hypothesis, black bean plants were grown in sterilized sand to simulate soil physical properties, were fertilized with increasing Mn concentrations (0.1–20 μM) and inoculated with a commercial mixture of *Rhizobium leguminosarum* bv *phaseoli* strains 127K44, 127K89, 127K105 (+Rh), in combination with arbuscular mycorrhizal fungi *Scutellospora heterogama* and *Entrophospora colombiana* (+AMF). Non-inoculated plants fertilized with 6 mM NO_3 and 2 mM P served as controls. Plants were harvested at 18, 25, 33, and 40 days after emergence. At all harvests, the greatest growth and highest P and iron (Fe) leaf concentrations occurred in control plants grown in 5 μM Mn. The growth of +AMF plants was promoted at 0.1 μM Mn and inhibited at higher than 1 μM Mn. Whereas, concentrations of 5–10 μM Mn enhanced the growth and the Mn concentrations in leaves of +Rh plants 40 days after emergence. The tripartite symbiosis (+Rh+AMF) decreased growth, nodulation and leaf ureide and chlorophyll concentrations in plants grown in less than 20 μM Mn, imputed to severe ultrastructural alterations in the leaf and nodule tissues. Only +Rh+AMF plants grown in 20 μM Mn were effectively nodulated, AMF colonized and reached the flowering stage, although with diminished growth and low chlorophyll concentrations. Results confirm the high Mn requirement of +Rh plants for growth and nodulation and question the implementation of the tripartite symbiosis to improve yields in early flowering black bean varieties planted in soils deficient in Mn and P.

1. Introduction

Black beans (*Phaseolus vulgaris* L.) constitute one of the main sources of protein for the low-income population. Yields of this crop are, however, very low in Venezuela attributed, in part, to the low organic matter, phosphorus (P) and manganese (Mn) contents in the Quartzipsamment soils (Blue et al., 1981; Frazao et al., 2010; Izaguirre-Mayoral et al., 1992; López et al., 1981; Woodard et al., 2007) where black beans are usually sown.

Black beans can overcome soil nitrogen (N) deficiencies via the establishment of an efficient symbiosis with *Rhizobium* (Blanco et al., 2009). However, deficiency of P is known to reduced growth of N-fertilized black beans (Boutraa, 2009), and it is expected to hinder the growth and nodulation of the *Rhizobium* (+Rh) plants as shown for other +Rh legume species (Araújo et al., 2008; Hernández et al., 2009; Izaguirre-Mayoral et al., 2002; Rotaru and Sinclair, 2009; Sharma et al., 2009; Zaman-Allah et al., 2007). To ameliorate the soil P deficiencies, plants can establish a tripartite symbiosis with arbuscular mycorrhizal fungi (AMF) to promote the uptake of P as well of other elements directly involved in the symbiotic process (Chalk et al., 2006; Izaguirre-Mayoral et al., 2000; Javaid, 2009; Mortiner et al., 2008; Talaat and Abdallah, 2008). The tripartite symbiosis with *Rhizobium* and AMF (+Rh+AMF) has, however, a calculated cost of up to 20% of photosynthates and can become a metabolic burden to the host plant under nutrient deficiencies (Lynch and Ho, 2005). Concomitantly, deficiency of Mn is also known to affect nodulation, symbiotic efficiency, growth, nutrient uptake, N-metabolism, ultrastructure of cell organelles and the catabolism of ureides exported from nodules to leaves of +Rh legumes (Fageria et al., 2008; Heckman et al., 1993; Izaguirre-Mayoral and Olivares,

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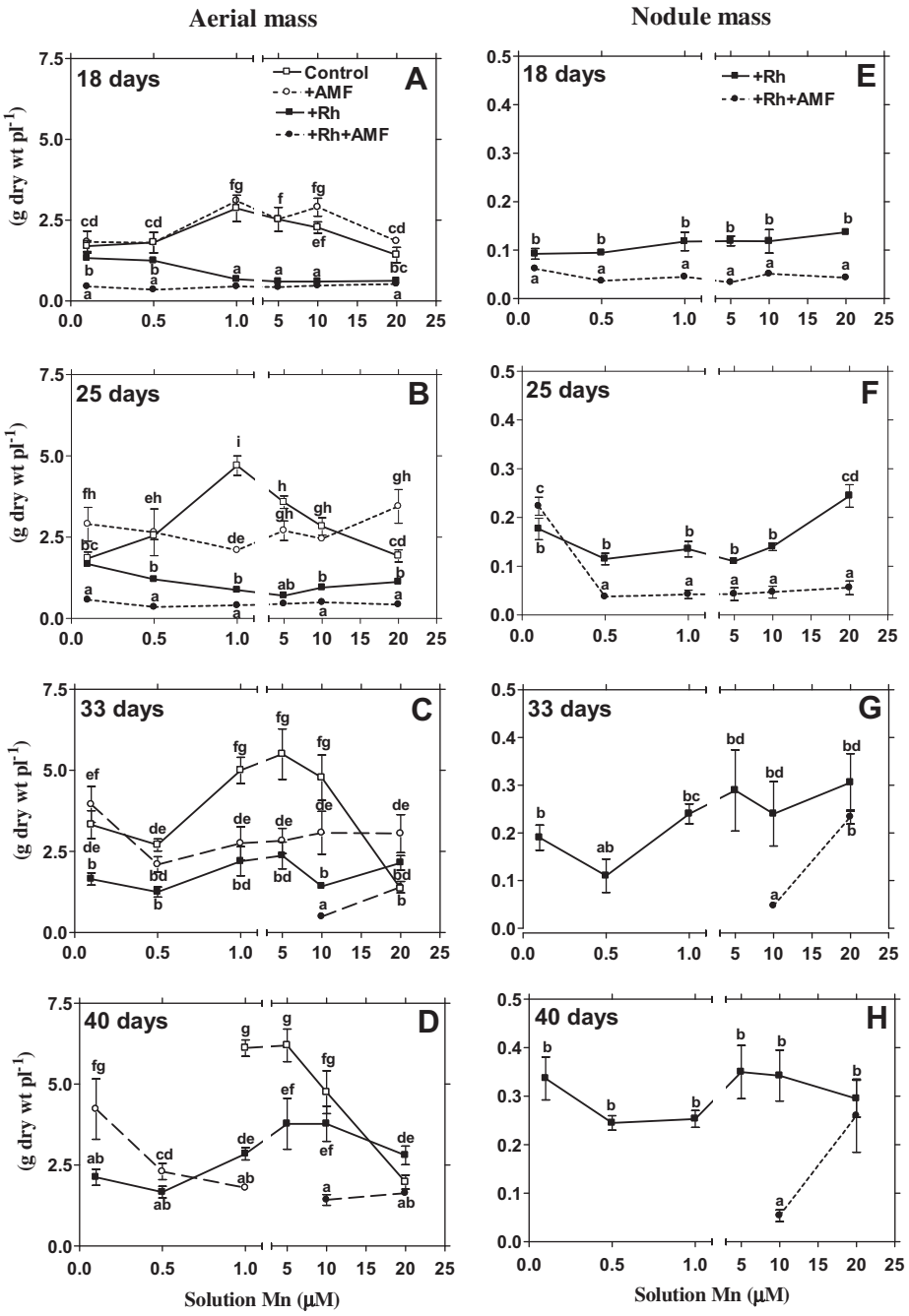

Fig. 1. Aerial (A, B, C and D) and nodule mass (E, F, G and H) of 18, 25, 33 and 40 days old AMF-inoculated (+AMF), *Rhizobium*-inoculated (+Rh) and *Rhizobium*–AMF-inoculated (+Rh+AMF) plants grown in increasing solution Mn concentrations. Non-inoculated plants served as controls. Means and standard errors followed by the same letter(s) are not significantly different (LSD, $p < 0.05$).

2006; Izaguirre-Mayoral and Sinclair, 2009). Mycorrhizal plants generally show reduced uptake of Mn (Clark, 2002).

The aim of this investigation was to test the hypothesis that deficiencies of Mn and P underlies the poor performance of black beans in the field (Nleya et al., 2009). For this purpose, plants inoculated with *Rhizobium* in combination with a mixture of AMF *Scutellospora heterogama* and *Entrophospora colombiana* were grown in sand to simulate soil physical properties and were fertilized with increasing concentrations of Mn in the nutrient solution. The responses of plants to the different treatment combinations were analyzed in terms of growth and nodulation. Concentrations of ureides and P were also determined as indicators of the effectiveness of the +Rh and +AMF symbiosis, respectively. In addition, the nutritional status of the plants subjected to the different inoculation treatments was analyzed by measuring the chlorophyll, Mn and iron (Fe) concentrations in the leaves.

2. Materials and methods

Seeds of black bean (*P. vulgaris* L. var La Palma), an early flowering variety, were surfaced-sterilized with ethanol (70% v/v, 5 min), rinsed six times with sterile distilled water and sown in sterilized Leonard jars (one seed per jar) containing 0.9 kg of sand and 0.8 L of a nutrient solution in the upper and lower