

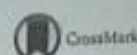


Arbuscular mycorrhiza enhances the production of stevioside and rebaudioside-A in *Stevia rebaudiana* via nutritional and non-nutritional mechanisms

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ABSTRACT

The sweet herb of Paraguay, *Stevia rebaudiana* (Bertoni), is becoming more important worldwide in herbal care for diabetes, as it produces the zero-calorie sweeteners steviol glycosides (SGs)—stevioside and rebaudioside-A. While arbuscular mycorrhizal fungi (AMF) have been shown to enhance production of secondary metabolites in many plant species, their effect on *S. rebaudiana* has not been studied. Moreover, relatively little is known about the mechanisms that may be involved in the increased accumulation of phytochemicals in mycorrhizal plants. Therefore, this study was performed to test the ability of *Rhizophagus fasciculatus* (Thaxt.) C. Walker & A. Schüller to improve the yield of SGs in *S. rebaudiana* and to relate this with some AMF-induced physiological changes in addition to improved phosphorus (P) uptake. The performance of plants inoculated with *R. fasciculatus* was compared with that of non-mycorrhizal plants with similar P concentrations. Mycorrhizal (M) and non-mycorrhizal plants with P-supplementation (NM+P) produced higher concentrations of SGs compared with control plants. However, M plants had more SGs than did NM+P plants. The higher content of SGs in M plants is due to increased concentrations of SGs and to the enhanced biomass of the shoots. The increase in biomass is directly due to the improved uptake of nutrients (N, K, Mg, Cu, Fe, Mn and Zn), and chlorophyll and carbohydrate concentrations in M plants. Higher concentrations of total carbohydrates and jasmonic acid in M plants than in NM+P plants contribute to more biosynthesis of SGs via the methyl erythritol phosphate pathway. This study suggests that AMF-mediated increases in SGs involve both nutritionally and non-nutritionally linked mechanisms.

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1. Introduction

Stevia rebaudiana (Bertoni), a perennial herb of Paraguay, belongs to the Asteraceae family. The plant is becoming increasingly important in the herbal world because it produces a mixture of eight different zero-calorie ent-kaurane glycosides derived from the tetracyclic diterpene steviol (Brandle et al., 1998). Of the eight glycosides, stevioside is the principal sweetening compound, and rebaudioside-A and rebaudioside-C are its main components (Liu and Li, 1995). Because glycosides taste intensely sweet and have

few calories, they have found many uses as sweetening agents and as dietary supplements (Kim et al., 2002).

The increasing demand for herbal care for diabetes calls for intense farming of *S. rebaudiana* to increase the production of its low-calorie sweetening glycosides. Studies reveal that the content of these glycosides, especially stevioside, greatly depends on the total biomass produced, which in turn depends on agricultural practices for cultivation of *Stevia* plants (Geuns, 2003). In pursuit of high production of glycosides, researchers have adopted modern agro-techniques (Das et al., 2008), water management (Fronza and Polengatti, 2003) and fertilizer applications (Mamta et al., 2010). In addition, tissue culture techniques and cultivation of *Stevia* plants in bioreactors have also been tested (Madan et al., 2010). However, in the above techniques, the main shortcomings are the high costs and low in-field practicability. For example, chemical fertilizers constitute a few mineral nutrients and create an imbalance in the whole mineral pattern of the plant body by hindering the uptake of other useful nutrients (Das et al., 2008). Therefore, an

Abbreviations: AMF, arbuscular mycorrhizal fungi; JA, jasmonic acid; M, Mycorrhizal; NM, non-mycorrhizal; NM+P, non-mycorrhizal with P-fertilizers; SGs, steviol glycosides.

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