

# Arbuscular mycorrhizal symbiosis as sustainable alternative in the *Stevia rebaudiana* Bertoni production

Cauich-Cauich, Rodrigo Armando<sup>1</sup>; Tun-Suárez, José María<sup>1</sup>; Herrera-Parra, Elizabeth de los Ángeles<sup>2</sup>; Cristóbal-Alejo, Jairo<sup>1</sup>; Lozano-Contreras, Mónica Guadalupe<sup>2\*</sup>

<sup>1</sup> Tecnológico Nacional de México/División de Estudios de Posgrado e Investigación/ Instituto Tecnológico de Conkal, Avenida Tecnológico s/n, Conkal, Yucatán, CP. 97345.

<sup>2</sup> Campo Experimental Mocochoá, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Antigua carretera Mérida-Motul Km. 24.5, Mocochoá, Yucatán, México, C.P. 97454.

\* Correspondence: morbica@gmail.com

## ABSTRACT

**Objective:** *Stevia rebaudiana* Bertoni is a crop that can meet the demand for natural sweeteners; however, this demand requires a sustainable production, as a result of the inclusion of its steviol glycosides or active ingredients in the food and pharmaceutical industries.

**Design/methodology/approach:** Modern agriculture implies the integrated use of valid natural resources, such as the arbuscular mycorrhizal fungi (AMF). These microorganisms establish a symbiotic relationship with, at least, 80% of the plants, to which they provide multiple benefits. They can directly and indirectly improve crop productivity, through nutrient (particularly, phosphorus) translocation of the soil solution.

**Results:** As a sustainable alternative for the production of *S. rebaudiana*, they improve its nutritional state, resulting in a higher biomass production and glycoside concentration —fundamental yield parameters. Additionally, they promote resistance to biotic and abiotic stress factors and improve soil quality.

**Limitations/implications:** It is worth mentioning that this fungi-plant mutualism is approximately 400 million years-old; however, it has only aroused interest during the last few years.

**Findings/conclusions:** Although arbuscular mycorrhizal fungi (AMF) are an exploitable resource, their communities are threatened by biotic factors —such as the interaction with other microorganisms— and abiotic factors —which involve bad agricultural practices.

**Keywords:** mycorrhizae; sustainability, Stevia, biomass, glycosides.

**Citation:** Cauich-Cauich, R. A., Tun-Suárez, J. M., Herrera-Parra, E. de los A., Cristóbal-Alejo, J., & Lozano-Contreras, M. G. (2022). Arbuscular mycorrhizal symbiosis as sustainable alternative in the *Stevia rebaudiana* Bertoni production. *Agro Productividad*. <https://doi.org/10.32854/agrop.v15i1.2093>

**Editor in Chief:** Dr. Jorge Cadena Iñiguez

**Received:** July 20, 2021.

**Accepted:** January 3, 2022.

**Published on-line:** January, 2022.

This work is licensed under a Creative Commons Attribution-Non-Commercial 4.0 International license.



## INTRODUCTION

Crop phytosanitary and nutrient management is usually based on the use of agrochemicals, with the aim of achieving a maximum exploitation of the crop's productivity; this practice requires knowledge about the agricultural needs of the plant species. However, the irrational use of these products creates problems in the agricultural sector, such as pollution, soil infertility, salinity, pest resistance, etc. (Amekawa, 2009). Sustainability is the new development trend for food production. Modern agriculture is adopting biotechnical feasible tools to achieve a sustainable production, through the application of beneficial microorganisms, both for the plant and for the soil. These



Image by Jaromír Novota at Pixabay

microorganisms have similar effects than agrochemicals and can replace them or reduce their use (Jeffries *et al.*, 2003).

Soil microorganisms are an important ingredient; some groups are more studied than others, as a result of the benefits they provide to crops. This review highlights the multiple benefits that arbuscular mycorrhizal fungi (AMF) —considered a feasible alternative for sustainable agriculture— provide to plants.

Mutualistic symbiotic relationships are 400-million-year-old (Bonfante and Genre, 2008). Currently, the AMF establish symbiosis with at least 80% of the plant species (Vierheilig, 2004). However, in an evolutionary context, plants evolved with the help of arbuscular mycorrhizae relationships (Simon *et al.*, 1993), as a result of a most interesting peculiarity of the AMF: they are forced symbionts, *i.e.*, they need a host or phytosymbiont to survive and they require a compatible relationship that provides a mutual benefit (Harrison, 1997).

During the last few years, the successful inoculation of plants of agricultural interest with AMF has been proved to promote growth and enhance the yield of the plant, as a result of an improvement in nutrient absorption.

This positive effect has also been achieved in *Stevia rebaudiana* Bertoni (Giovannini *et al.*, 2020; Tavarini *et al.*, 2018; Mandal *et al.*, 2013). This plant was introduced to Mexico mainly as a consequence of the demand for natural sweeteners, which diabetics can consume; they also replace synthetic sweeteners which cause health problems. Additionally, after its approval as food sweetener in several countries, *Stevia rebaudiana* Bertoni has acquired a high exportation potential. Its intense sweetness and medicinal properties have made it a topic of scientific and commercial interest. The importance of *S. rebaudiana* is also based on the fact that its leaves have a zero-calorie compound that is over 300 times sweeter than sucrose. This compound is made up of steviol glycosides (SG) (stevioside, steviolbioside, rebaudioside-A, B, C, D, and dulcoside-A) (Tavarini *et al.*, 2015), which are biosynthesized in a chain reaction from the pyruvate and the glyceraldehyde 3-phosphate (Brandle and Telmer, 2007; Mandal *et al.*, 2015). Among these glycosides that can be found in the leaves of the plant —which makes the leaves sweet—, stevioside and rebaudioside-A make up the highest proportion (80%).

The leaves of *S. rebaudiana* have a great proportion of its SG content; therefore, its production is mainly focused in the amount of biomass and SG content.

In this context, the main benefit of the AMF is phosphorus translocation, a key element in the SG biosynthesis. Most researches have focused on the improvement of those two important parameters. Mandal *et al.* (2013) evaluated the effect of *Rhizophagus fasciculatus* (Thaxt) on yield and SG concentration. The interaction improved the biomass and the stevioside and rebaudioside-A concentration. Tavarini *et al.* (2018) used *R. irregulare* to modify the growth habit of the plants, obtaining more branches and less height; they also reported an 88% colonization. This confirmed the susceptibility of the plants to symbiosis and AMF colonization. Vafadar *et al.* (2014) also showed that AMF inoculation efficiently increases the dry weight of the leaves of *S. rebaudiana*, using relatively low doses of chemical fertilization.

The positive effects of AMF in agriculture have been widely described; they promise an eco-friendly production. The information about the mechanisms that participate in the multiple benefits that the AMF provide to plants must increase. Most of these benefits have been attributed to the improvement of nutrient uptake by the roots. The objective of this review was to discuss the benefits and mechanisms that participate in the arbuscular mycorrhizal symbiosis for the improvement of the biomass and the metabolite of the *S. rebaudiana* crop, as well as the factors that limit the AMF use.

### ***Stevia rebaudiana* productive potential**

*Stevia* has several phytochemical characteristics which makes it a crop of interest for the food industry—as sweetener, antioxidant, and dietary supplement—and for the pharmaceutical industry—for the treatment of diabetes mellitus, since it does not produce a glycaemic response. The leaves of *S. rebaudiana* can achieve a zero-calorie natural sweetness higher than sucrose (Ramesh and Megeji, 2006); they have a unique composition in the presence of labdane, phenolic acids, sterols, triterpenoid, chlorophyll, organic acids, monosaccharide, and disaccharide (Gardana *et al.*, 2010; Tavarini and Angelini, 2013).

In mid-December 2008, the Food and Drugs Administration (FDA) established that the components of *S. rebaudiana* were safe for human consumption. The same year, the JECFA/FAO (experts on food additives) agreed with the FDA's approval of *S. rebaudiana* for human consumption. In 2010, the JECFA, along with the World Health Organization, awarded the plant the safe food certification.

Since then, it has been considered a crop with high economic potential and, currently, it is considered the second sweetener worldwide. China is the main commercialization point: 50% of the production is sold in its own domestic market. China also provides at least 75% of the world production and its main exportation routes are Japan and the USA (Sarmiento-López *et al.*, 2021). Paraguay is the second producer of *S. rebaudiana* (Mogra and Dashora, 2009). Its market is focused in dry leaves commercialization, mainly supplying the EU, which has a sweetener annual consumption of ten thousand tons. Therefore, the demand of *S. rebaudiana* provides an opportunity for Mexican farmers seeking to generate a profitable crop with exportation potential.

### **Arbuscular mycorrhizae**

Mycorrhizae imply mutualism between the arbuscular mycorrhizal fungi (AMF) and the plant. Its name comes from the Greek terms “mikos” (fungi) and “rhiza” (root). It literary means “fungi root”. Specifically, it belongs to the “endomycorrhizae” type and it is the most abundant group in horticulture. This kind of fungi belong to the Glomeromycota division and are forced symbionts. The AMF have different particular structures such as hyphae, vesicles, and arbuscules. The hypahe are main structures and can be classified as “ineffective”—those that initiate the colonization and establish contact with the roots—, “absorbent”—those that explore the soil and exchange nutrients—, and “fertile”—those in charge of the asexual production of spores (Camarena-Gutiérrez, 2012).

Glomeromycota is made up of nine families (Acaulosporaceae, Archaeosporaceae, Diversisporaceae, Entrophosporaceae, Glomeraceae, Gigasporaceae, Geosiphonaceae,

Pacisporaceae, Paraglomeraceae) and at least 200 species have been described so far (Schübler *et al.*, 2001). They are characterized by the intracellular and intercellular growth of the cortical cells of the root; they show an internal development of arbuscules (dichotomously-divided hyphae, invaginated by the plasma membrane of the cortical cells, and they have short life periods) and vesicles (lipid bodies that are created in the terminal part of the hyphae) (Brundrett, 2004; Rich *et al.*, 2014).

The arbuscular mycorrhizal symbiosis takes place when an AMF spore germinates and branches in all directions, in order to find and establish contact with the roots of the plant. In this first “presymbiosis” stage, the receptive signs of the plant (flavonoids and elicitors) identify and recognize the symbiont (Harrison, 1997). However, the amount of enzymes, catalases, and peroxidases also increases; this process occurs at the same time that the appressorium (initial symbiotic structure) appears and the fungi penetrates the root. The biochemical communication between the two mutualists does not allow the plant to reject or defend itself from the fungi; this communication produces morphogenetic changes that are crucial to initiate the colonization of the roots and to guarantee mutual benefits (Akiyama, 2005).

#### **Advantages of the arbuscular mycorrhizal symbiosis in *Stevia rebaudiana***

The symbiosis benefits for cultivated plants have been reported in survival, morphological, and productive terms. AMF are known to be capable of improving the physiological conditions of plants, even in difficult conditions, such as soil tension caused by heavy metals (Rillig, 2004), soil compaction, salinity, and drought (Miransari *et al.*, 2007). Additionally, an improvement in growth and productivity of the cultivated plants has been reported. The AMF diversity in the soil is very important, because they have a differential effect in the host (Kernaghan, 2005). Although *S. rebaudiana* is highly susceptible to AMF colonization, studies about the effects of mycorrhizal symbiosis on its quantitative and qualitative production are still limited (Giovannini *et al.*, 2020; Mandal *et al.*, 2015).

Most researches focus on the improvement of mineral nutrition: the main benefit that the AMF provides to mycorrhizae plants. However, this benefit also unchains an increase in the chlorophyll concentrations which, on their turn, modify the photosynthetic rate, resulting in a higher physiological productivity (Feng *et al.*, 2002). Although AMF cannot fix atmospheric nitrogen, their interaction with fixing microorganisms can increase the fixation of this nutrient (Rajan *et al.*, 2000).

The inoculation of *S. rebaudiana* with AMF has doubtlessly shown positive results, firstly because it leads to a greater biomass production —the commercial aspect of this crop. In this context, the inoculation of *Glomus intraradices* stimulated height, number of leaves, root length, and biomass production which are related to a higher chlorophyll content (Vafadar *et al.*, 2014; Mandal *et al.*, 2013). Parniske (2008) attributed this response to an improvement in the nutritional state of the plant, as a result of the extensive AMF hyphae network. This network covered a greater soil area, in order to absorb water and nutrients with low availability and mobility.

The AMF symbiosis can modify the quality of *S. rebaudiana*, by improving its growth habit. This situation was particularly observed when 25 mg of phosphorous were applied

as  $P_2O_5$   $kg^{-1}$  of soil, combined with AMF symbiosis (Tavarini *et al.*, 2018), resulting in an increase of the SG yield. Nevertheless, further studies about the optimum phosphorous doses —based on the nutritional requirement of the plant— must be carried out, in order to avoid high concentrations and inhibiting AMF colonization.

On their part, Bidabadi and Masoumian (2008) proved that AMF could mitigate alterations caused by salinity stress, as a result of their positive impact on photosynthesis and the antioxidant defense system.

As essential components of soil biota, AMF also have “non-nutritional” effects: they prevent erosion, as a result of the production of a stable hydrophobic glycoprotein (glomalin), which joins the AMF hyphae, stabilizing soil aggregates and forming their long-term structure (Harrison, 1997). Arbuscular mycorrhizal symbiosis is also important for the phytoremediation of soils that have been polluted by heavy metals; the said metals are immobilized at the root and their translocation is reduced (Pawlowska *et al.*, 2000).

### **Mechanisms of arbuscular mycorrhizal fungi in the production of steviol glycosides**

One of the main mechanisms of symbiosis is its contribution to nutrient translocation (mainly phosphorous) which is essential for the steviol glycoside pathway (Tavarini *et al.*, 2018). Although soils have very low concentrations of phosphorous, it is necessary for this process; additionally, as a result of its minimal mobility, it is not widely or easily available for the root system (Kapoor *et al.*, 2007). Under these conditions, the AMF mycelial network is the most efficient way to increase its capture (Augé, 2004). A low phosphorous level in the soil solution means that there is also a low phospholipid level in the plant membrane, resulting in a greater root exudation that stimulates an AMF colonization (Bonfante *et al.*, 2004).

Phosphorous is absorbed from the soil solution up to the plant as phosphate; the AMF’s extraradical hyphae capture it quicker than diffusion; afterwards, the intraradical hyphae transport it to the cytoplasm. Alternatively, it is accumulated in vacuoles as polyphosphate granules; subsequently, it is sent through the hyphal lumen by cytoplasmic currents towards the arbusculae, where the polyphosphate is hydrolyzed and the phosphorous ion is transferred to the cell (Harrison, 1997).

On its turn, the phosphorous translocation mechanism improves the nitrogen, potassium, magnesium, and zinc assimilation (Jefferies *et al.*, 2003), incorporating an optimal nutritional state that results in the production of biomass and the concentration of steviol glycosides in the leaves.

Steviol glycosides belong to the secondary metabolites that the plant produces. During the last decade, there has been relatively little information about the AMF-induced increases in the secondary metabolite production of *S. rebaudiana*. According to Mandal *et al.* (2013), this situation is the result of the lack of in-depth studies about the effect of inoculating tropical vegetables with AMF; other studies mainly focus in the AMF-driven production of secondary metabolites in medicinal herbs (Zubek *et al.*, 2012). However, despite the growing evidence that AMF favor SG concentration, there is relatively little information about the mechanisms that are potentially involved in this increase.

Clearly, the SG biosynthesis is largely dependent on the carbon's primary metabolism and the energy supplies that the plant can process. In this regard, the AMF-plant symbiosis improves photosynthesis through morphological (*e.g.*, increase in leaf area) and physiological (*e.g.*, regulation of stomatal conductance and chlorophyll concentration) adaptations (Turgeman *et al.*, 2011) —which increases the plants carbohydrate content. SG biosynthesis takes place in the leaves and consists of three stages (Brandle *et al.*, 1998): 1) the 2-C-methyl-D-erythritol-4-phosphate pathway (MEP) (Wanke *et al.*, 2001) which leads to the conversion of pyruvate and glyceraldehyde-3-phosphate into kaurene; 2) the conversion of kaurene into steviol; and 3) the transformation of steviol into stevioside and rebaudioside-A by different glycosyltransferases (Brandle and Telmer, 2007).

A greater carbohydrate concentration increases gene transcription through the MEP pathway (Hsieh and Goodman, 2005); therefore, the AMF-plant symbiosis increases the expression of essential genes for SG biosynthesis (Mandal *et al.*, 2015). In comparison with non-inoculated plants, the transcription levels of 1-deoxy-D-xylulose-5-phosphate synthase (DXS), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), and 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (MDS) increased 1.7-1.8 times respectively in the MEP pathway. In the second stage, the transcription levels of the five genes involved (GGDPS, CPPS, KS, KO, and KAH) increased 1.4-6.5 times when mycorrhization was involved; in the third stage, glycotransferases (UGT) underwent a positive regulation, guaranteeing a greater SG concentration (Guleria *et al.*, 2014).

### **Limitations on the use of arbuscular mycorrhizal fungi**

Biotic and abiotic factors can limit the use of AMF in the field, as well as the diversity and natural structure of the communities in the soil (Kernaghan, 2005). Plant species also determine AMF communities, based on the presymbiosis search compounds oozed by the roots to identify their preferred symbiont (Arnaut *et al.*, 1996).

Despite the scarce evidence, other soil microorganism populations can establish a synergy with the AMF, while others can inhibit spore germination and colonization (Barea *et al.*, 2002). Therefore, rhizosphere-associated microbiota has a positive or negative influence on AMF development (Gange, 2000).

Likewise, the soil's natural conditions —moisture, temperature, structure, and nutrient availability— also control AMF communities, colonization, and actions. Sometimes, these conditions are the consequence of agricultural practices (Jansa *et al.*, 2003).

Soil can contribute to AMF development at one point and hinder it later on; it can directly or indirectly limit the effects of symbiosis on AMF communities or their host plants, respectively (Augé, 2000). Neither moisture deficit (Miller, 2000), nor temperature (Matsubara and Harada, 1996) have an impact on colonization, although they can reduce spore production.

Some agricultural practices —including fertilizer doses application, inadequate crop rotation, tilling, and the use of lime— impact mycorrhizal colonization levels (Entry *et al.*, 2002). Some fertilization levels of phosphorous inhibit AMF efficiency in soy crops (Ezawa and Yoshida, 2002). Although AMF can survive in soils that have been highly polluted by heavy metals, such conditions diminish spore germination and colonization.

Arbuscular mycorrhizal symbiosis is a microbiological tool that can be used as a sustainable fertilization alternative to produce *Stevia rebaudiana*; it provides benefits that influence the improvement of biomass yield and glycoside concentration. The combination of mutual benefit mechanisms (*e.g.*, phosphorous translocation) makes the AMF and *S. rebaudiana* suitable symbionts: the plant species obtains enough phosphorous and other nutrients to improve its productivity and to concentrate its active ingredient (SG), while the AMF obtains the carbon it needs for its development.

## CONCLUSIONS

Market demands require a sustainable production of *S. rebaudiana*; therefore, AMF must be taken into consideration as core elements of the cultivation and must be included in agronomy management plans. Mycorrhizae are a fundamental resource in soil structure and consequently for sustainability maintenance. Biotic or abiotic factors can threaten the communities, which must be incorporated into production plots.

## ACKNOWLEDGMENTS

The authors would like to thank the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) for funding the Technology Research and Transference Project for the Use of Biofertilizers and Organic Manure in Agriculture No. 13451632421. The first author would like to thank the Consejo Nacional de Ciencia y Tecnología (CONACyT) for the postgraduate scholarship he was awarded. The authors would also like to thank the Posgrado en Agricultura Tropical Sustentable, Instituto Tecnológico de Conkal, Tecnológico Nacional de México, for their support for this research.

## REFERENCES

- Augé, R.M. (2000). Stomatal Behavior of Arbuscular Mycorrhizal Plants. *In*: Kapulnik Y., Douds D.D. (eds) Arbuscular Mycorrhizas: Physiology and Function. Springer, Dordrecht. [https://doi.org/10.1007/978-94-017-0776-3\\_10](https://doi.org/10.1007/978-94-017-0776-3_10)
- Augé, R.M. (2004). Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science* 84 (4): 373-381. <https://doi.org/10.4141/S04-002>
- Amekawa, Y. (2009). Reflections on the growing influence of good agricultural practices in the global south. *Journal of Agricultural and Environmental Ethics* 22 (6): 531-557. <https://doi.org/bm4wg4>
- Arnaud, M., Hamel, C., Vimard, B., Caron, M., Fortin, J. A. (1996). Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an *in vitro* system in the absence of host roots. *Mycological research* 100 (3): 328-332. <https://doi.org/cztv3w>
- Akiyama, K. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435: 824–827. <https://doi.org/10.1038/nature03608>
- Barea, J. M., Toro, M., Orozco, M. O., Campos, E., Azcón, R. (2002). The application of isotopic ( $^{32}\text{P}$  and  $^{15}\text{N}$ ) dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and *Rhizobium* to improve the agronomic efficiency of rock phosphate for legume crops. *Nutrient Cycling in Agroecosystems* 63 (1): 35-42. <https://doi.org/bt9tbw>
- Bidabadi, S. S. and Masoumian, M. (2018). Arbuscular mycorrhizal symbiosis improves growth and antioxidative response of *Stevia rebaudiana* (Bert.) under salt stress. *Trends in Horticulture* 1 (3):1-8. <https://doi.org/t77p>
- Bonfante, P., Genre, A., and Bianciotto, V. (2004). The colonisation strategies of arbuscular mycorrhizal fungi: an overview of their cellular interactions with plants and bacteria. *In*: Avance en el conocimiento de la biología de las micorrizas. J.T. Frías-Hernández, V. Olalde-Portugal, R. Ferrera-Cerrato (Eds). Primera Edición. Universidad de Guanajuato. Guanajuato, México, Pp 19-28.
- Bonfante, P. and Genre, A. (2008). Plants and arbuscular mycorrhizal fungi: an evolutionary developmental perspective. *Trends in Plant Science* 13 (9): 492-498. <https://doi.org/cg8hs5>

- Brandle, J. E., Starratt, A. N., Gijzen, M. (1998). *Stevia rebaudiana*: Its agricultural, biological, and chemical properties. *Canadian Journal of plant science* 78 (4): 527-536. <https://doi.org/cn6qdn>
- Brandle, J. E. and Telmer, P. G. (2007). Steviol glycoside biosynthesis. *Phytochemistry* 68(14): 1855-1863. <https://doi.org/d2bt6g>
- Brundrett, M. (2004). Diversity and classification of mycorrhizal associations. *Biological reviews* 79 (3): 473-495. <https://doi.org/bt6pwk>
- Camarena-Gutiérrez, G. (2012). Interacción planta-hongos micorrízicos arbusculares. *Revista Chapingo serie ciencias forestales y del ambiente* 18 (3): 409-421. <https://doi.org/f77d>
- Entry, J. A., Rygielwicz, P. T., Watrud, L. S., Donnelly, P. K. (2002). Influence of adverse soil conditions on the formation and function of arbuscular mycorrhizas. *Advances in Environmental Research* 7 (1): 123-138. <https://doi.org/ds9xtf>
- Ezawa, T., Yamamoto, K., Yoshida, S. (2002). Enhancement of the effectiveness of indigenous arbuscular mycorrhizal fungi by inorganic soil amendments. *Soil Science and Plant Nutrition* 48 (6): 897-900. <https://doi.org/bdcrwz>
- Feng, G., Zhang, F., Li, X., Tian, C., Tang, C., Rengel, Z. (2002). Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza* 12 (4): 185-190. <https://doi.org/bw288z>
- Gange, A. (2000). Arbuscular mycorrhizal fungi, Collembola and plant growth. *Trends in Ecology & Evolution* 15 (9): 369-372. <https://doi.org/fh437d>
- Gardana, C., Scaglianti, M., Simonetti, P. (2010). Evaluation of steviol and its glycosides in *Stevia rebaudiana* leaves and commercial sweetener by ultra-high-performance liquid chromatography mass spectrometry. *Journal of Chromatography A* 1217: 1463-1470. <https://doi.org/bf6f8h>
- Giovannini, L., Palla, M., Agnolucci, M., Avio, L., Sbrana, C., Turrini, A., Giovannetti, M. (2020). Arbuscular mycorrhizal fungi and associated microbiota as plant biostimulants: research strategies for the selection of the best performing inocula. *Agronomy* 10 (1): 106. <https://doi.org/f78b>
- Guleria, P., Masand, S., Yadav, S. K. (2014). Overexpression of SrUGT85C2 from *Stevia* reduced growth and yield of transgenic *Arabidopsis* by influencing plastidial MEP pathway. *Gene* 539 (2): 250-257. <https://doi.org/f5wfr5>
- Harrison, M. J. (1997). The arbuscular mycorrhizal symbiosis. In *Plant-microbe interactions* (pp. 1-34). Springer, Boston, MA. <https://doi.org/dsz2c9>
- Hsieh, M. H. and Goodman, H. M. (2005). The *Arabidopsis* IspH homolog is involved in the plastid nonmevalonate pathway of isoprenoid biosynthesis. *Plant physiology* 138 (2): 641-653. <https://doi.org/cmxcc7>
- Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I. R., Frossard, E. (2003). Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications* 13 (4): 1164-1176. <https://doi.org/fb9fqz>
- Jefferies, P., Gianinazzi, S., Perotto, S., Turnau, K., Barea, J. M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils* 37: 1-16. <https://doi.org/dhjp87>
- Kapoor, R., Chaudhary, V., Bhatnagar, A. K. (2007). Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. *Mycorrhiza* 17: 581-587. <https://doi.org/c72x36>
- Kernaghan, G. (2005). Mycorrhizal diversity: cause and effect?. *Pedobiologia* 49(6): 511-520. <https://doi.org/bj75v9>
- Mandal, S., Evelin, H., Giri, B., Singh, V. P., Kapoor, R. (2013). Arbuscular mycorrhiza enhances the production of stevioside and rebaudioside-A in *Stevia rebaudiana* via nutritional and non-nutritional mechanisms. *Applied Soil Ecology* 72: 187-194. <https://doi.org/f77g>
- Mandal, S., Upadhyay, S., Singh, V. P., Kapoor, R. (2015). Enhanced production of steviol glycosides in mycorrhizal plants: a concerted effect of arbuscular mycorrhizal symbiosis on transcription of biosynthetic genes. *Plant Physiology and Biochemistry* 89: 100-106. <https://doi.org/f67g9v>
- Matsubara, Y. I., and Harada, T. (1996). Effect of constant and diurnally fluctuating temperatures on arbuscular mycorrhizal fungus infection and the growth of infected asparagus (*Asparagus officinalis* L.) seedlings. *Journal of the Japanese Society for Horticultural Science* 65 (3): 565-570. <https://doi.org/bh8cv4>
- Miransari, M., Bahrami, H.A., Rejali, F., Malakouti, M.J., Torabi, H. (2007). Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on corn (*Zea mays* L.) growth. *Soil Biology and Biochemistry* 39: 2014-2026. <https://doi.org/ffs4ww>
- Miller, S. P. (2000). Arbuscular mycorrhizal colonization of semi aquatic grasses along a wide hydrologic gradient. *New Phytologist* 145 (1): 145-155. <https://doi.org/c59vrr>



- Mogra, R. and Dashora, V. (2009). Exploring the use of *Stevia rebaudiana* as a sweetener in comparison with other sweeteners. *Journal of Human Ecology* 25 (2): 117-120. <https://doi.org/f77v>
- Pawlowska, T. E., Chaney, R. L., Chin, M., Charvat, I. (2000). Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal-contaminated landfill. *Applied and Environmental microbiology* 66 (6): 2526-2530. <https://doi.org/cd3xdw>
- Parniske, M. (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* 6: 763-775. <https://doi.org/btrpt2>
- Rajan, S. K., Reddy, B. J. D., Bagyaraj, D. J. (2000). Screening of arbuscular mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. *Forest ecology and management* 126 (2): 91-95. <https://doi.org/cqr3x6>
- Ramesh, K., Singh, V., Megeji, N. W. (2006). Cultivation of stevia [*Stevia rebaudiana* (Bert.) Bertoni]: A comprehensive review. *Advances in Agronomy* 89: 137-177. <https://doi.org/dhw5v5>
- Rillig, M. C. (2004). Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecological Letters* 7: 740–754. <https://doi.org/b8x2qd>
- Rich, M. K., Schorderet, M., Reinhardt, D. (2014). The role of the cell wall compartment in mutualistic symbioses of plants. *Frontiers in Plant Science* 5: 238. <https://doi.org/f6bjj9>
- Sarmiento-López, L. G., López-Meyer, M., Sepúlveda-Jiménez, G., Cárdenas, L., Rodríguez-Monroy, M. (2021). Arbuscular mycorrhizal symbiosis in *Stevia rebaudiana* increases trichome development, flavonoid and phenolic compound accumulation. *Biocatalysis and Agricultural Biotechnology* 31: 101889. <https://doi.org/f77r>
- Simon L., Bousquet J., Levesque R. C., Lalonde M. (1993). Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature* 363: 67–69. <https://doi.org/d5p52s>
- Schüßler, A., Schwarzott, D., Walker, C. (2001). A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* 105: 1413-1421. <https://doi.org/c86djg>
- Tavarini, S., Angelini, L. G. (2013). *Stevia rebaudiana* Bertoni as a source of bioactive compounds: the effect of harvest time, experimental site and crop age on steviol glycoside content and antioxidant properties. *Journal of Science of Food and Agriculture* 93 (9): 2121–2129. <https://doi.org/f4z9zq>
- Tavarini, S., Sgherri, C., Ranieri, A.M., Angelini, L.G. (2015). Effect of nitrogen fertilization and harvest time on steviol glycosides, flavonoid composition, and antioxidant properties in *Stevia rebaudiana* Bertoni. *Journal of Agricultural and Food Chemistry* 63 (31): 7041–7050. <https://doi.org/f7nwd>
- Tavarini, S., Passera, B., Martini, A., Avio, L., Sbrana, C., Giovannetti, M., Angelini, L. G. (2018). Plant growth, steviol glycosides and nutrient uptake as affected by arbuscular mycorrhizal fungi and phosphorous fertilization in *Stevia rebaudiana* Bert. *Industrial Crops and Products* 111: 899-907. <https://doi.org/f7nwd>
- Turgeman, T., Asher, J. B., Roth-Bejerano, N., Kagan-Zur, V., Kapulnik, Y., Sitrit, Y. (2011). Mycorrhizal association between the desert truffle *Terfezia boudieri* and *Helianthemum sessiliflorum* alters plant physiology and fitness to arid conditions. *Mycorrhiza* 21 (7): 623-630. <https://doi.org/d22r45>
- Vafadar, F., Amooaghaie, R., Otrshy, M. (2014). Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. *Journal of Plant Interactions* 9 (1): 128–136. <https://doi.org/f77m>
- Vierheilig, H. (2004). Regulatory mechanisms during the plant arbuscular mycorrhizal fungus interaction. *Canadian Journal of Botany* 82 (8): 1166-1176. <https://doi.org/dkwwg87>
- Wanke, M., Skorupinska-Tudek, K., Swieczewska, E. (2001). Isoprenoid biosynthesis via 1-deoxy D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (DOXP/MEP) pathway. *Acta Biochimica Polonica* 48 (3): 663-672. <https://doi.org/f77n>
- Zubek, S., Mielcarek, S., Turnau, K. (2012). Hypericin and pseudohypericin concentrations of a valuable medicinal plant *Hypericum perforatum* L. are enhanced by arbuscular mycorrhizal fungi. *Mycorrhiza* 22 (2): 149-156. <https://doi.org/c537f8>
- Young, K.E., Zwank, P.J., Valdez, R., Dye, J.L., & Tarango, L.A. (1997). Diet of Mexican spotted owls in Chihuahua and Aguascalientes, Mexico. *Journal of Raptor Research* 31 (4): 376–380