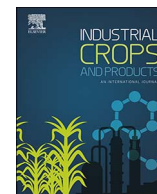




Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Plant growth, steviol glycosides and nutrient uptake as affected by arbuscular mycorrhizal fungi and phosphorous fertilization in *Stevia rebaudiana* Bert

Silvia Tavarini^a, Barbara Passera^a, Andrea Martini^a, Luciano Avio^a, Cristiana Sbrana^b, Manuela Giovannetti^{a,c}, Luciana G. Angelini^{a,c,*}

^a Department of Agriculture, Food and Environment, University of Pisa, Italy

^b Institute of Agricultural Biology and Biotechnology, National Research Council, Pisa, Italy

^c Interdepartmental Research Center “Nutraceuticals and Food for Health”, University of Pisa, Italy

ARTICLE INFO

Keywords:

Biofertilizers

Diterpene glycosides yield

Nutrient use efficiency

Rhizoglyphus irregularis

ABSTRACT

The increasing demand for products based from *Stevia rebaudiana* Bertoni (both leaves and purified steviol glycosides) makes of interest the research on sustainable production systems, in order to guarantee secure availability and high quality of agricultural raw materials. Arbuscular mycorrhizal fungi (AMF) symbiosis represents an interesting tool for increasing crop production and quality, especially thanks to improved nutrient absorption, particularly phosphorus (P). In the present study, *Stevia rebaudiana* Bert. plants were exposed to different levels of P fertilization (0, 25 and 50 mg P₂O₅ kg⁻¹ soil) with or without *Rhizoglyphus irregularis* inoculation, in order to evaluate root colonization, plant growth and productive parameters, steviol glycosides (SVglys) yield, as well as nitrogen (N) and P concentrations and uptake. A nutrient balance was also carried out and the nutrient use efficiency was evaluated. Stevia roots were highly colonized by *Rhizoglyphus irregularis*, especially in the absence of P fertilization. During the whole vegetative growth, the AMF symbiosis, in association with the P supply, benefitted stevia growth, especially with regard to leaf dry biomass production and SVglys yield. Arbuscular mycorrhizal fungi symbiosis was able to modify the growth habit of stevia plants, with increased branching and a reduced plant height. At the end of the vegetative growth, mycorrhizal plants reached the highest leaf dry yield, together with the highest SVglys production. The application of 25 mg P₂O₅ kg⁻¹ soil in association with AMF symbiosis seemed to be the most effective treatment in improving stevia SVglys yield and P uptake together with P nutrient use efficiency.

1. Introduction

Stevia rebaudiana Bert. (hereafter stevia), a perennial semi-shrub of *Asteraceae* family, has been long used as a sweetener and herbal remedy by the Guaraní people (Ramesh et al., 2006; Madan et al., 2010). This steviol glycoside-rich plant now represents an economic opportunity, especially after the approval of the use of steviol glycosides (SVglys) as a food additive in many countries (Angelini et al., 2016), and the very recent recognition, in Europe, of stevia leaves as “traditional food” in tea, herbal and fruit infusions (Novel Food Catalogue, European Commission, 2017).

More than 30 SVglys have been detected in stevia leaf extracts (Wölwer-Rieck, 2012), of which the most abundant are stevioside and rebaudioside A, followed by rebaudiosides B-E, dulcoside A and steviolbioside (De et al., 2013; Pal et al., 2015; Tavarini et al., 2015). The

sweetness of SVglys ranges between 250 and 300 times that of sucrose (Crammer and Ikan, 1986). In addition to the sweet taste, a recent review assessed the health-promoting properties of steviol glycosides and other active principles of stevia (Marcinek and Krejpcio, 2016).

Stevia leaves present a unique composition in terms of the presence of several biologically important secondary metabolites, such as labdanes, flavonoids, phenolic acids, sterols, triterpenoids, chlorophylls, organic acids, mono-disaccharides, and inorganic salts (Gardana et al., 2010; Tavarini et al., 2015; Tavarini and Angelini, 2013). The global market for stevia has grown considerably (Mintel, 2014): consumers increasingly tend to opt for products with a natural origin, and their concerns derived from the use of synthetic sweeteners (Soffritti et al., 2006, 2016; Chiozzotto et al., 2011; Suez et al., 2014; Kuk and Brown, 2016). As the agricultural production of stevia is still problematic and insufficient to meet such a growing global demand, its cultivation could

* Corresponding author at: Department of Agriculture, Food and Environment, University of Pisa, Italy.
E-mail address: luciana.angelini@unipi.it (L.G. Angelini).

<http://dx.doi.org/10.1016/j.indcrop.2017.10.055>

Received 16 June 2017; Received in revised form 28 September 2017; Accepted 29 October 2017
0926-6690/ © 2017 Elsevier B.V. All rights reserved.

represent a great opportunity for farmers.

Identifying the main pre-harvest factors that affect the phytochemical profile of stevia is key to improve its productivity and the amounts of beneficial active compounds in its leaves. One of the main challenges in stevia production is the use of arbuscular mycorrhizal fungi (AMF) which are ubiquitous beneficial root symbionts which promote plant growth and affect the production of health-promoting secondary metabolites (Sbrana et al., 2014; Pedone-Bonfim et al., 2015). Sharma et al. (2015) highlighted the lack of information on the use of AMF as biofertilizers in stevia, as useful tools for modern agriculture that can reduce chemical inputs as well as the impact on the environment.

Arbuscular mycorrhizal symbioses may increase mineral nutrients and water uptake, and photosynthetic rate (Gosling et al., 2006). The improvement in P absorption in mycorrhizal plants is widely recognized, together with AMF activity as bioenhancers, biostimulants and biocontrol agents (Smith and Read, 2008; Smith et al., 2011; Rouphael et al., 2015; Bücking and Kafle, 2015; Corrêa et al., 2014). However, studies on the effects of mycorrhizal symbiosis on the quantitative and qualitative production of stevia are limited (Portugal et al., 2006; Mandal et al., 2015) and do not consider the interaction between AMF and P fertilization on the biosynthesis of the different steviol glycosides.

The aim of this study was to evaluate the effects of AMF inoculation, P fertilization levels (P) and their reciprocal interaction (AMF $\times$ P) on stevia root colonization, the main biometric and productive parameters, and SVglys yield, throughout the vegetative growth. In addition, nitrogen and phosphorus concentrations in three different plant organs (leaves, stems and roots) and the nutrient uptake and partitioning within the plant were analysed at harvest.

2. Material and methods

2.1. Chemicals

The HPLC-grade solvents, acetonitrile, formic acid and water were purchased from J. T. Baker (Phillipsburg, NJ, USA). Common Stevia Glycosides Standards Kit (steviolbioside, dulcoside A, rebaudioside B, stevioside, rebaudioside A and rebaudioside C) were purchased from Chromadex (LGC Standards S.r.l., Milan, Italy). All solvents and water were thoroughly degassed prior analyses.

2.2. Plant material and experimental conditions

A pot trial was carried out at the Experimental Centre of the Department of Agriculture, Food and Environment (DAFE) (Central Italy, Pisa, 43° 40' N; 10° 19' E), during the 2015 growing season. The stevia plants were obtained from a high rich-rebaudioside C genotype, belonging to the DAFE germplasm collection, through stem cuttings to ensure the production of uniform plant material. In December 2014, apical portions were cut from the mother plants grown in greenhouse conditions, and transferred on sterile peat-based growing media, into plug trays, for elongation and rooting. The derived plantlets were maintained under controlled conditions in the greenhouse until the beginning of the trial. At the beginning of May 2015, uniform sized (5–7 cm height) plants were selected and transplanted to 3 L pots with 2 kg of autoclaved soil in each pot. The substrate used was a mixture of 9/10 sandy loam soil (sand 75%; silt 22%; clay 3%; organic matter 15 g kg⁻¹; pH 8.1; total nitrogen 0.6 g kg⁻¹; available phosphorus 11.9 mg kg⁻¹; exchangeable potassium 107.1 mg kg⁻¹) and 1/10 peat-based growing media (VALCOFERT S.r.l, Empoli, Italy). The substrate obtained was mixed and autoclaved twice (121 °C for 1 h), with a 24 h gap between one cycle and the next, in order to kill naturally occurring AMF propagules.

The trial was conducted in open-air conditions, from May to the first ten days of September. A weather station located near the experimental

site was used to record any changes in minimum, maximum and mean air temperatures and total rainfall. Mean maximum and minimum temperatures in the growing season were 28.8 °C and 15.8 °C, respectively, with 283.8 mm of total rainfall.

The plants were exposed to six treatments, consisting in three phosphorus doses (0, 25 and 50 mg P₂O₅ kg⁻¹ of soil) with (M) and without (NM) mycorrhizal inoculum: NM + 0P (without AMF inoculum and without P fertilization); NM + 25P (without AMF inoculum with 25 mg P₂O₅ kg⁻¹ of soil); NM + 50P (without AMF inoculum with 50 mg P₂O₅ kg⁻¹ of soil); M + 0P (mycorrhizal without P); M + 25P (mycorrhizal with 25 mg P₂O₅ kg⁻¹ of soil); M + 50P (mycorrhizal with 50 mg P₂O₅ kg⁻¹ of soil).

According to Tavarini et al. (2015), a randomized block design with two treatment factors (phosphorous, mycorrhizal inoculum) occurring in a factorial structure was used. Three harvests were carried out at 69 (16 July 2015), 89 (5 August 2015) and 123 (8 September 2015) days after transplanting (DAT) sampling 3 replicates for each treatment. Phosphorus, as triple superphosphate, was added at the beginning of the trial. One month after the beginning of the trial, nitrogen fertilization was supplied to each plant (0.25 g N pot⁻¹, as ammonium nitrate). The plants were well-watered through the experiment (75–80% of field capacity) thanks to a drip irrigation system.

On the last sampling date, the plants were at the beginning of the reproductive stage, when the SVglys leaf concentration reaches the maximum (Sumida, 1980; Xiang, 1983; Ramesh et al., 2006). At each sampling date, plant height, branch number, total fresh above- and below-ground biomass were measured. The plants were then air-dried in a ventilated oven from 30 °C to 40 °C until constant weight, for dry weight determination of the leaves, stems and roots. Root to shoot ratio was also measured as: root dry mass/(leaf + stem) dry mass. The various dry plant parts were ground to a fine powder, by a laboratory mill (IKA universal grinder M20), and used for subsequent analyses.

2.3. AMF inoculum and mycorrhizal colonization

Mycorrhizal treatments were set up using inoculum consisting of mycorrhizal roots and soil containing spores and extraradical mycelium of the AMF species *Rhizoglyphus irregularis* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl (syn. *Rhizophagus irregularis* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüssler, formerly known as *Glomus intraradices*), isolate IMA6. The inoculum was obtained from *Medicago sativa* L. and *Zea mays* L. pot cultures in a mixture of sandy loam soil and calcinated clay (OILDRI, Chicago, IL, USA) (1:1 v/v), kept at the Microbiology laboratory, Department of Agriculture, Food and Environment (DAFE), University of Pisa, Italy. After excision of the shoots from the host plants, the substrate was air dried at room temperature, roots were ground, carefully mixed with the soil, and stored until use.

All pots received 50 mL of a filtrate, obtained by sieving the mycorrhizal inoculum through a 50 µm pore diameter sieve and then through Whatman paper no. 1 (Whatman International Ltd, Maidstone, Kent, UK), to ensure a common microflora to all treatments. In the mycorrhizal treatment, stevia plants were inoculated with 130 mL of inoculum, while NM plants received the same volume of a mock inoculum, prepared by steam-sterilization of the whole inoculum. The inoculation occurred just before transplanting.

One month after the transplant, root samples from three pots per treatment were collected for the determination of mycorrhizal colonization. The method was based on clearing and then staining with 0.05% Trypan blue in lactic acid root samples (Phillips and Hayman, 1970). The percentage of colonized AMF root length was assessed on representative root samples from each plant, using the gridline intersect method (Giovannetti and Mosse, 1980).

Download English Version:

<https://daneshyari.com/en/article/8880882>

Download Persian Version:

<https://daneshyari.com/article/8880882>

[Daneshyari.com](https://daneshyari.com)