Plant Physiology and Biochemistry 89 (2015) 100–106

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Enhanced production of steviol glycosides in mycorrhizal plants: A concerted effect of arbuscular mycorrhizal symbiosis on transcription of biosynthetic genes

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ARTICLE INFO

Article history:
Received 23 December 2014
Accepted 18 February 2015
Available online 19 February 2015

Keywords:
Arbuscular mycorrhizal fungi
Stevia rebaudiana
Steviol glycosides biosynthesis genes
Gene transcription
Methyl erythritol phosphate pathway
Glucosylation

ABSTRACT

Stevia rebaudiana (Bertoni) produces steviol glycosides (SGs) – stevioside (stev) and rebaudioside-A (reb-A) that are valued as low calorie sweeteners. Inoculation with arbuscular mycorrhizal fungi (AMF) augments SGs production, though the effect of this interaction on SGs biosynthesis has not been studied at molecular level. In this study transcription profiles of eleven key genes grouped under three stages of the SGs biosynthesis pathway were compared. The transcript analysis showed upregulation of genes encoding 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway enzymes viz.,1-deoxy-o-xylulose 5-phospate synthase (DXS), 1-deoxy-o-xylulose 5-phospate reductoisomerase (DXR) and 2-C-methyl-D-erytrithol 2,4-cyclodiphosphate synthase (MDS) in mycorrhizal (M) plants. Zn and Mn are imperative for the expression of MDS and their enhanced uptake in M plants could be responsible for the increased transcription of MDS. Furthermore, in the second stage of SGs biosynthesis pathway, mycorrhization enhanced the transcription of copalyl diphosphate synthase (CPPS) and kaurenoic acid hydroxylase (KAH). Their expression is decisive for SGs biosynthesis as CPPS regulates flow of metabolites towards synthesis of kaurenoid precursors and KAH directs these towards steviol synthesis instead of gibberellins. In the third stage glucosylation of steviol to reb-A by four specific uridine diphosphate (UDP)-dependent glycosyltransferases (UGTs) occurs. While higher transcription of all the three characterized UGTs in M plants explains augmented production of SGs; higher transcript levels of *UGT76G1*, specifically improved reb-A to stev ratio implying increased sweetness. The work signifies that AM symbiosis upregulates the transcription of all eleven SGs biosynthesis genes as a result of improved nutrition and enhanced sugar concentration due to increased photosynthesis in M plants.

1. Introduction

Stevia rebaudiana (Bertoni) finds an unparalleled use in the medicinal world due to singular properties of its diterpenoid steviol glycosides (SGs) — stevioside (stev) and rebaudioside-A (reb-A) that are nearly 300 times sweeter than sucrose (Lemus-Mondaca et al., 2011). These have been used for the treatment of diabetes, dental maladies, obesity, hypertension and cancer (Geuns, 2003) hence, many efforts have been propounded to increase the concentration of SGs. While certain physiological parameters such as photoperiodism are known to affect the concentration of SGs (Ceunen et al., 2012; Ceunen and Geuns, 2013; Yang et al., 2015) on the other hand, mutualistic associations such as arbuscular mycorrhizal (AM) symbiosis offer a potent, economically conducive approach to encounter the growing demand of SGs (Mandal et al., 2013). Despite of several studies connoting the potential of AMF to augment the secondary metabolite production in commercially important medicinal plants (Kapoor et al., 2002a,b, 2004, 2007; Copetta et al., 2006); their role in manipulating the biosynthesis pathways of these metabolites have not been dissected at molecular level. In a first of such attempts, it was recently reported that AM symbiosis upregulates the expression of crucial artemisinin biosynthesis genes (Mandal et al., 2014). However, more studies need to be done to optimize the mechanism based on which AM symbiosis functions to increase the secondary metabolite production.

Biosynthesis of SGs takes place in leaves (Brandle et al., 1998) in a seventeen steps process that can be categorized under three

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http://dx.doi.org/10.1016/j.plaphy.2015.02.010
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Self attested
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9/6/2017