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Short communication

Evaluation of folate-binding proteins and stability of folates in plant foliages

Bijesh Puthusseri[a,b], Peethambaran Divya[a,c], Lokesh Veeresh[a], Gyanendra Kumar[a], Bhagyalakshmi Neelwarne[a,*]

[a] CSIR-Central Food Technological Research Institute, Mysore 570020, India
[b] University of Texas Health Northeast, Tyler, TX 75708, USA
[c] Indian Institute of Science, Banaglore 560012, India

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ABSTRACT

The present study reports the presence of folate binding proteins (FBPs) in the plants, coriander and Arabidopsis, and their contributions toward folate enhancement. After observing that salicylic acid (SA) enhanced the accumulation of folates in coriander, a study was conducted in Arabidopsis, where twofold increase in folates occurred in foliage upon SA treatment. For obtaining insights into genes involved in SA-induced folate accumulation, microarray data of responsive genes in Arabidopsis were screened. Based on the expression profiles, 19 genes were further analysed by qPCR. The results revealed that folate biosynthetic genes were largely down-regulated, whereas a gene of a putative folate-binding protein (FPB) was up-regulated, which correlated with a significant increase of FPBs in foliage. This new information on a plant FBP appears useful for metabolic engineering of a wide range of crops to enhance the content and stability of the folates during post-harvest storage.

1. Introduction

Folate deficiency is linked to anaemia, poor health and infant mortality in several populations globally. Therefore, efforts are being made to find novel approaches that ensure the delivery of adequate levels of natural folates through plant foods. Within the plant cell, the folate turnover rate is very high, owing to a number of key roles in various metabolic processes in addition to those stated above. Increasing the levels of folates by metabolic engineering of folate biosynthetic genes (Fig. 1) has been successful in tomato fruits (de la Garza et al., 2004; de la Garza, Gregory, & Hanson, 2007) and in rice seeds (Storozhenko et al., 2007). Since the produced folates are quickly degraded in plants, it is important to understand the catabolism of plant folates and possible mechanisms through which folates are stabilized (Gorelova, Ambach, Rébeillé, Stove, & Van Der Straeten, 2017). Studies in mammalian tissues and in milk have indicated that the protein-bound nature of folates increases their stability (Ford, Salter, & Scott, 2009). Therefore, Other than the biosynthetic genes, the folate binding proteins (FBPs) are important gene-targets for folate metabolic engineering. Taking this as a lead, the current study investigates whether such a mechanism occurs in plants so that overexpression of FPBs may serve as a potential strategy for the stabilization of folates in plant foods. Transgenic expression of a bovine FBP gene in Arabidopsis resulted in 150-fold folate increase in rice (Blancquaert et al., 2015), implying the need to understand the genes involved in folate enhancement in plants and to target them for folate metabolic engineering.

Spraying foliage with an aqueous solution of SA (250 μM) resulted in a twofold increase in total folates in coriander, which rendered better folate stability during post-harvest storage (Puthusseri, Divya, Lokesh, & Neelwarne, 2012), improved bio-accessibility while reducing the pro-oxidant status in tissues (Puthusseri, Divya, Lokesh, & Neelwarne, 2013). This indicated that stabilizing factors might be playing a major role in enhancing folate accumulation. However, there are no reports on the presence of FBPs in plants, indicating a need for experimental elucidation, and leading to information on the putative gene targets for folate metabolic engineering, which is the objective of this study. Since decoded forms of genomic data are not yet available in other food crops, the current study used Arabidopsis as the model plant and analysed its genomic data for addressing all folate-related pathways to elucidate the mechanism through which higher levels of folates accumulated in the foliage. This new information would pave way for enhancing folate content in several food crops by metabolic engineering of the folate pathway.

Abbreviations: SA, salicylic acid; FBP, folate binding protein; 5-MTHF, 5-methyltetrahydrofolate; FA-EDA-FITC, folic acid conjugated with fluorescein isothiocyanate; FW, fresh weight; DW, dry weight

* Corresponding author.
E-mail addresses: bneelwarne@gmail.com, blakshmi_1999@yahoo.com (B. Neelwarne).

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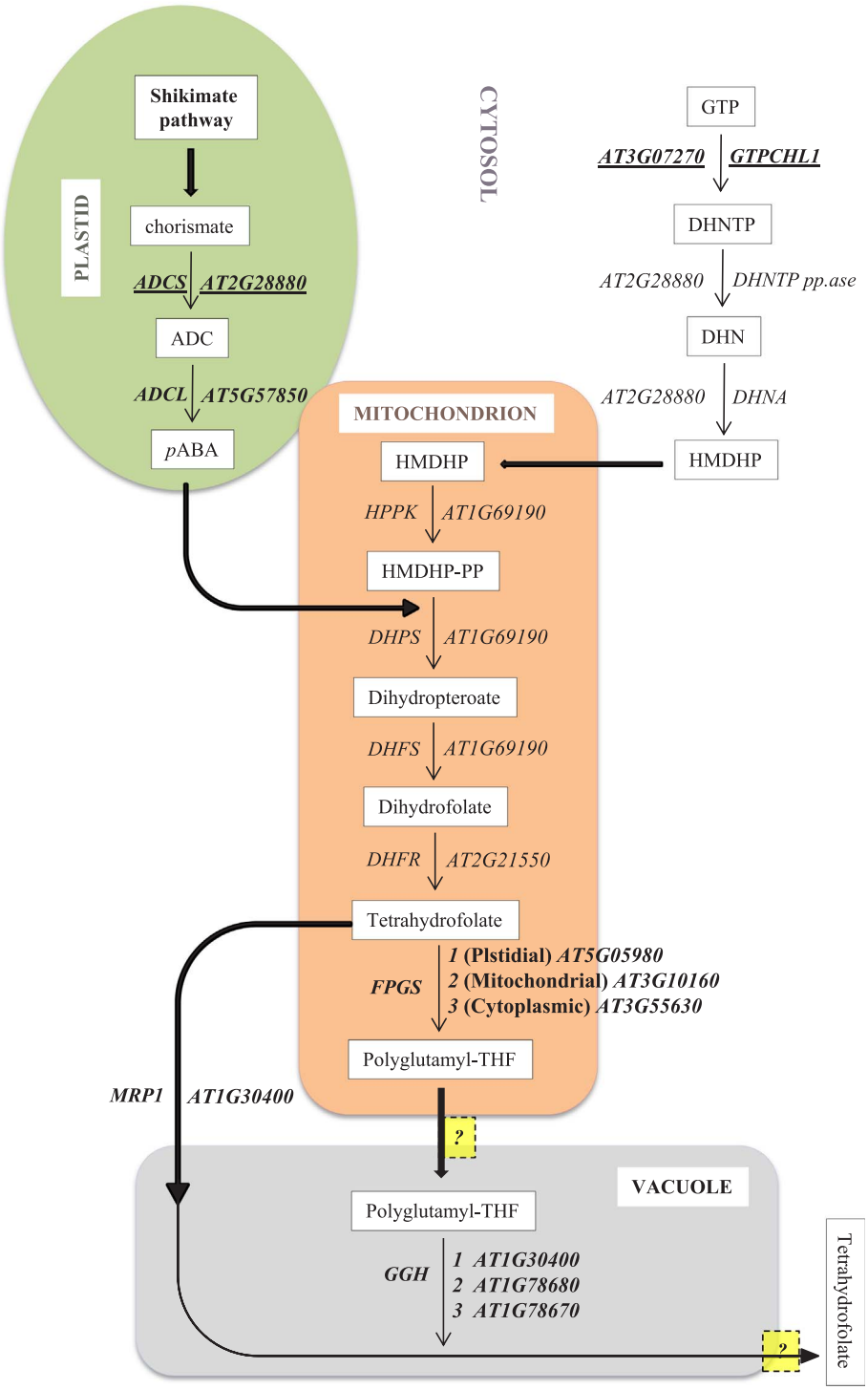

Fig. 1. Biosynthetic pathway of folates in Arabidopsis. Names of those genes underlined were targeted for folate metabolic engineering (de la Garza et al., 2004, 2007; Storozhenko et al., 2007), and those with bold fonts are used for expression analysis in the present study. ADSC, aminodeoxychorismate; DHN, dihydroneopterin; DHNTP, dihydroneopterin triphosphate; Glu, glutamate; GTP, guanosine 5′-triphosphate; HMDHP, hydroxymethyldihydropterin; HMDHP-PPi, hydroxymethyldihydropterin pyrophosphate; PPi, pyrophosphate; Pi, inorganic phosphate; ADCS, aminodeoxychorismate synthase; ADCL, aminodeoxychorismate lyase; GTPCHL1, GTP cyclohydrolase I; DHFR, dihydrofolate reductase; DHFS, dihydrofolate synthase; DHNA, dihydroneopterin aldolase; DHPS, dihydropteroate synthase; FPGS, folylpolyglutamate synthetase; HPPK, hydroxymethyldihydropterin pyrophosphokinase; MRP1, multidrugresistant protein; GGH, gammaglutamyl hydroxylase. The '?' inside the dotted boxes indicates the transporter proteins yet to be identified.

2. Materials and methods

2.1. Salicylic acid treatment and folate estimation

Taking leads from our earlier study in coriander, which was done in greenhouse conditions (Puthusseri et al., 2013), the current study in *Arabidopsis thaliana* (ecotype Columbia-0) plants was also conducted, under similar conditions, by using plants grown in separate pots. Aqueous-SA solution (300 μM) was sprayed onto the foliage of four-week-old plants. Folate extraction and estimation were done as previously reported (Puthusseri et al., 2012).

The folate content in each extract was determined by microbiological assay in microtitre plates (Horne, 1997) using *L. rhamnosus*. The plates were incubated at 37 °C for 20 h, and were read at 600 nm in an ELISA plate reader (Spectramax 340PC^{384}, Molecular Devices, Sunnyvale, USA).

2.2. RNA extraction and gene expression analysis

For this study, 19 key genes related to the folate metabolic pathway were considered; seven of folate biosynthesis, seven for catabolism and five for folate stabilization. Details of the genes and the primers used