International Biodeterioration & Biodegradation 106 (2016) 127–132

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

The function of an installed photosynthetic formaldehyde-assimilation pathway containing dihydroxyacetone synthase and dihydroxyacetone kinase enhanced formaldehyde removal from solution in transgenic geranium leaves

Hao Tan [a, b, 1], Suqin Xiao [b, 1], Shuang Han [b], Xiuxia Xuan [b], Zhen Sun [b], Kunzhi Li [b], Limei Chen [b, *]

[a] Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Chenggong, Kunming, 650500, China
[b] Faculty of Life Science and Biotechnology, Chenggong Campus, Kunming University of Science and Technology, Chenggong, Kunming, 650500, China

ARTICLE INFO

Article history:
Received 20 June 2015
Received in revised form
18 October 2015
Accepted 18 October 2015
Available online 30 October 2015

Keywords:
Transgenic geranium leaves
DAS/DAK HCHO-Assimilation pathway
Liquid HCHO stress
HCHO uptake
HCHO metabolism

ABSTRACT

In a previous study, we created transgenic geranium leaves installed with a photosynthetic formaldehyde (HCHO)-assimilation pathway containing dihydroxyacetone synthase (DAS) and dihydroxyacetone kinase (DAK). In the current study, DAS and DAK expression was confirmed in the leaves of three selected transgenic lines via RT-PCR analysis. ^{13}C NMR analysis showed that the actions of the DAS/DAK pathway completely inhibited the role of citrate-produced pathway during HCHO metabolism in transgenic geranium leaves treated with 2 mM $H^{13}CHO$ for 4 h. Further analysis indicated that HCHO uptake from this solution was always higher in the leaves of the three transgenic lines than in WT (untransformed) leaves during a 48-h treatment period. This result suggests that the role of the installed DAS/DAK pathway enhanced HCHO uptake from solution in transgenic geranium leaves. Moreover, the relationship between HCHO uptake and treatment time approached a linear function during the first 40-h treatment period due to the function of the installed pathway. Based on the $H^{13}CHO$ metabolic profile and the HCHO uptake curves, $H^{13}CHO$ metabolic pathways in transgenic leaves treated with 2 mM $H^{13}CHO$ were postulated and the contribution of the DAS/DAK pathway to HCHO uptake by transgenic leaves was quantitatively evaluated.

1. Introduction

In developing countries, the majority of decorative items are still made using formaldehyde (HCHO) as an adhesive. The continuous release of non-crosslinked, free HCHO from these materials (Flyvholm and Andersen, 1993) results in poor air quality in public places and homes. People living and working in these environments generally exhibit a syndrome that has been associated with HCHO pollution (Main and Hogan, 1983). The use of physical and chemical methods to remediate HCHO pollution is not only expensive but also produces secondary pollution.

Accumulating evidence has shown that microorganisms (Korpi et al., 1997; Yurimoto et al., 2005; Di Maiuta et al., 2009; Luo et al., 2014) and plants (Giese et al., 1994; Schmitz et al., 2000; Song et al., 2013; Zeng et al., 2014; Zhang et al., 2014; Sun et al., 2015) can absorb HCHO and convert it into cellular components. Therefore, many researchers have attempted to develop technologies using microorganisms and plants to remediate HCHO pollution in air (Xu and Hou, 2010; Sibirny et al., 2011; Lu et al., 2012). However, due to the limited ability of plants to metabolize HCHO, the stress induced by HCHO toxicity gradually reduces their HCHO absorption efficiency (Schmitz et al., 2000). Therefore, several plant species have been engineered with genes related to HCHO degradation (Tada et al., 1996; Achkor et al., 2003; Nian et al., 2013) or assimilation (Chen et al., 2010; Song et al., 2010; Xiao et al., 2012) from other plants or microorganisms. The manipulation improves HCHO metabolism in the engineered plants, thereby enhancing their HCHO absorption efficiency.

Geranium (*Pelargonium* sp. *frensham*) is a perennial ornamental

* Corresponding author.
E-mail address: chenlimeikm@126.com (L. Chen).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ibiod.2015.10.012
0964-8305/

that is often grown as a greening plant. More importantly, geranium is easily transformed via leaf petioles (KrishnaRaj et al., 1997). Dihydroxyacetone synthase (DAS) and dihydroxyacetone kinase (DAK) are two key enzymes in the HCHO-assimilation pathway in methylotrophic yeasts (Yurimoto et al., 2005). DAS catalyzes the condensation of HCHO with xylulose 5-phosphate to produce dihydroxyacetone (DHA) and glyceraldehyde 3-phosphate. DAK converts DHA into dihydroxyacetone phosphate. We previously overexpressed the genes encoding DAS and DAK in tobacco chloroplasts (Xiao et al., 2012) to construct a photosynthetic HCHO-assimilation pathway (the DAS/DAK pathway). In a recent study, we also modified geranium plants using these genes (Zhou et al., 2015). ^{13}C NMR analysis showed that this pathway can function simultaneously with native HCHO metabolic pathways in transgenic geranium leaves under gaseous $H^{13}CHO$ stress. This dual functioning enhances the ability of transgenic plants to purify gaseous HCHO pollution from the environment (Zhou et al., 2015). In the ribulose monophosphate pathway in methylotrophic bacteria, 3-hexulose-6-phosphate synthase (HPS) and 6-phosphate-3-hexuloisomerase (PHI) play key roles in HCHO fixation. An HPS/PHI fusion protein expressed in *Escherichia coli* possesses both HPS and PHI enzyme activities. We previously introduced a photosynthetic HCHO-assimilation pathway into geranium using this HPS/PHI fusion protein. The transgenic geranium leaves exhibited different kinetics for liquid and gaseous HCHO removal (Song et al., 2010). These differences may have resulted from the distinct HCHO removal mechanisms that existed in the transgenic leaves under liquid and gaseous HCHO stress conditions. Moreover, distinct HCHO metabolism mechanisms have been observed in petunia leaves treated with liquid HCHO (Zhang et al., 2014) versus gaseous HCHO (Sun et al., 2015). Therefore, the present study investigated the kinetics of HCHO removal from solution in transgenic geranium leaves containing the DAS/DAK HCHO-assimilation pathway. The mechanism underlying HCHO metabolism in the transgenic geranium leaves following treatment with $H^{13}CHO$ solution was also analyzed using ^{13}C NMR.

2. Materials and methods

2.1. Growth and maintenance of transgenic plants

To aseptically grow geranium, the shoots of transgenic lines selected by Zhou et al. (2015) and untransformed plants (WT) were transferred into MS agar medium (pH 5.7) containing 3% (w/v) sucrose and grown in a growth chamber at 25 °C under constant light (100 μmol m^{-2} s^{-1}). After two months of growth, leaves were collected for subsequent experiments.

2.2. RT-PCR analysis

Total RNA was isolated from the leaves of transgenic and WT geranium plants using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RNA concentration was quantified using spectrophotometry. For reverse transcription (10 μl reaction), the total RNA samples (3 μg) were incubated with reagents from a RevertAid™ M-MuLV Reverse Transcriptase Kit (Fermentas). An aliquot of 2 μl cDNA was used for each RT-PCR reaction. RT-PCR was performed using Ex-Taq DNA polymerase (TaKaRa). The *tubulin* transcript (EF202092) was used as an internal control. The PCR primer sequences were as follows: 5′-CAT TAT CTA GAC ATG AAG TTC CAC-3′ (forward) and 5′-TAA ATG ATT TTG ATC ATG TTT TGG-3′ (reverse) for *das* cDNA, 5′-CTG AAG GAA AGC TTG ATC TC-3′ (forward) and 5′-CTA CAA CTT GGT TTC AGA TTT G-3′ (reverse) for *dak* cDNA, and 5′-TCC TCC CGG GAT CCC AGC ATA C-3′ (forward) and 5′-GCC CTT AAT TCC ATC TCG TC-3′ (reverse) for *tubulin*.

2.3. Measurement of HCHO removal from HCHO solution

Transgenic and WT geranium leaves (2 g fresh weight, FW) were treated with 2 mmol l^{-1} HCHO. The treatment was performed as described by Song et al. (2013). The same treatment solution without leaves was used under the same conditions to monitor HCHO volatilization. This served as the control (CK). Autoclaved leaves (dead leaves) were treated using the same method to detect HCHO adsorption. The concentrations of residual HCHO in the treatment solutions were measured at 8, 16, 24, 32, 40 and 48 h using an assay published by Nash (Nash, 1953). The percentage of HCHO volatilized from the treatment solutions was calculated using Equation (1): volatilized HCHO (%) = initial HCHO% − residual HCHO% in the CK solution. The percentage of HCHO adsorption was calculated using Equation (2): adsorbed HCHO (%) = initial HCHO % − residual HCHO% in the treatment solution with dead leaves − the volatilized HCHO% from the CK solution. and the HCHO absorption was calculated using the Equation (3): absorbed HCHO (%) = initial HCHO% − residual HCHO% in the treatment solution with living leaves − the volatilized HCHO% from the CK solution − the adsorbed HCHO% by dead leaves. The percentage of HCHO absorption were calculated using Equation (3): absorbed HCHO (%) = initial HCHO% − residual HCHO% in the treatment solution with living leaves − volatilized HCHO% from the CK solution − HCHO% adsorbed by dead leaves.

2.4. $H^{13}CHO$ and $NaH^{13}CO_3$ labeling experiments

$H^{13}CHO$ and $NaH^{13}CO_3$ were provided by Cambridge Isotopes Laboratories (Andover, MA). $H^{13}CHO$ and $NaH^{13}CO_3$ labeling experiments were performed as described by Xiao et al. (2012). For $H^{13}CHO$ treatment, transgenic and WT geranium leaves (2 g FW) were soaked in 70 ml of 2 mmol l^{-1} $H^{13}CHO$ solution containing 5 mmol l^{-1} $KHCO_3$ and 0.1% MES (w/v) at pH 5.7 for 4 h. For $NaH^{13}CO_3$ treatment, WT geranium leaves (2 g FW) were soaked in 5 mmol l^{-1} $NaH^{13}CO_3$ solution (containing 0.1% MES) and then incubated under constant light (100 μmol m^{-2} s^{-1}) at 25 °C for 4 h with shaking (100 rpm). After the incubation, the leaves were washed, frozen in liquid nitrogen and stored at −80 °C.

2.5. ^{13}C NMR analysis

Soluble metabolites were extracted from frozen leaves as described by Song et al. (2013). For ^{13}C NMR, 500 μl of extract was transferred into a 5-mm NMR tube, and ^2H_2O was added to lock the signal. Formamide (10 mM) was included as a reference (Ref). ^{13}C NMR was performed at 500 MHz in a Bruker DRX 500 spectrometer (Bruker Biosciences Corporation, Billerica, MA). The acquisition parameters used were described in previous publications (Chen et al., 2010; Song et al., 2013). ^{13}C NMR spectra were calibrated to the Ref at 166.85 ppm to facilitate the comparison of interesting peaks in different samples. Resonance peaks were assigned by running a series of spectra of authentic compounds. The relative content of each metabolite was estimated by integration of its target peaks relative to the Ref, which was set to 1.

2.6. Statistical analysis

Significant differences were determined using DPS software ($P < 0.05$). These values are reported as the means ± SD ($n = 3$). Different letters were used to represent significant differences at $P < 0.05$. Values annotated with the same letters exhibited no significant differences.