Plant Physiology and Biochemistry 106 (2016) 46–53

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Promoting scopolamine biosynthesis in transgenic *Atropa belladonna* plants with *pmt* and *h6h* overexpression under field conditions

Ke Xia [a, 1], Xiaoqiang Liu [a, 1], Qiaozhuo Zhang [a, 1], Wei Qiang [a], Jianjun Guo [b], Xiaozhong Lan [c], Min Chen [d], Zhihua Liao [a, *]

[a] Key Laboratory of Eco-environments in Three Gorges Reservoir Region (Ministry of Education), SWU-TAAHC Medicinal Plant Joint R&D Center, School of Life Sciences, Southwest University, Chongqing, 400715, China
[b] Institute of Entomology, The Provincial Key Laboratory for Plant Pest Management of Mountainous Region, Guizhou University, Guiyang, 550025, China
[c] TAAHC-SWU Medicinal Plant Joint R&D Center, Tibetan Collaborative Innovation Center of Agricultural and Animal Husbandry Resources, Agriculture and Animal Husbandry College, Tibet University, Nyingchi of Tibet, 860000, China
[d] SWU-TAAHC Medicinal Plant Joint R&D Center, College of Pharmaceutical Sciences, Southwest University, Chongqing, 400715, China

ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form
16 April 2016
Accepted 18 April 2016
Available online 20 April 2016

Keywords:
Atropa belladonna
Hyoscyamine 6β-hydroxylase
Putrescine *N*-methyltransferase
Metabolic engineering
Tropane alkaloids

ABSTRACT

Atropa belladonna is one of the most important plant sources for producing pharmaceutical tropane alkaloids (TAs). T1 progeny of transgenic *A. belladonna*, in which putrescine *N*-methyltransferase (EC. 2.1.1.53) from *Nicotiana tabacum* (NtPMT) and hyoscyamine 6β-hydroxylase (EC. 1.14.11.14) from *Hyoscyamus niger* (HnH6H) were overexpressed, were established to investigate TA biosynthesis and distribution in ripe fruits, leaves, stems, primary roots and secondary roots under field conditions. Both *NtPMT* and *HnH6H* were detected at the transcriptional level in transgenic plants, whereas they were not detected in wild-type plants. The transgenes did not influence the root-specific expression patterns of endogenous TA biosynthetic genes in *A. belladonna*. All four endogenous TA biosynthetic genes (*AbPMT*, *AbTRI*, *AbCYP80F1* and *AbH6H*) had the highest/exclusive expression levels in secondary roots, suggesting that TAs were mainly synthesized in secondary roots. T1 progeny of transgenic *A. belladonna* showed an impressive scopolamine-rich chemotype that greatly improved the pharmaceutical value of *A. belladonna*. The higher efficiency of hyoscyamine conversion was found in aerial than in underground parts. In aerial parts of transgenic plants, hyoscyamine was totally converted to downstream alkaloids, especially scopolamine. Hyoscyamine, anisodamine and scopolamine were detected in underground parts, but scopolamine and anisodamine were more abundant than hyoscyamine. The exclusively higher levels of anisodamine in roots suggested that it might be difficult for its translocation from root to aerial organs. T1 progeny of transgenic *A. belladonna*, which produces scopolamine at very high levels (2.94 −5.13 mg g^{-1}) in field conditions, can provide more valuable plant materials for scopolamine production.

1. Introduction

The tropane alkaloids (TAs), including hyoscyamine, anisodamine and scopolamine, are used medicinally as anticholinergic agents (Poupko et al., 2007; Yun et al., 1992), which are generally produced by the plant species of the Solanaceae family, including *Atropa belladonna*, *Hyoscyamus niger*, *Datura* species, and others (Yamada and Hashimoto, 1982). Among them, *A. belladonna* is one of the most important plant sources for TA production (Wang et al., 2011). Scopolamine is the most valuable TA because of more potent pharmaceutical activity and lesser side effects relative to those of hyoscyamine (Jaremicz et al., 2014; Wang et al., 2011). However, the scopolamine content in *A. belladonna* plants is much lower than that of hyoscyamine (Yun et al., 1992). Therefore, the development of scopolamine-rich *A. belladonna* is a common goal of the scopolamine industry. Over the past decades, traditional methods, including genetic breeding, polyploid breeding and radiation

Abbreviations: ADC, arginine decarboxylase; CYP80F1, littorine mutase/monooxygenase; H6H, hyoscyamine 6β-hydroxylase; HPLC, high performance liquid chromatography; MPO, *N*-methylputrescine oxidase; ODC, ornithine decarboxylase; PGK, phosphoglycerate kinase; PMT, putrescine *N*-methyltransferase; qPCR, quantitative PCR; TAs, tropane alkaloids; TRI, tropinone reductase I; TRII, tropinone reductase II.

* Corresponding author.
E-mail addresses: zhliao@swu.edu.cn, zhihualiao@163.com (Z. Liao).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plaphy.2016.04.034
0981-9428/

breeding, have failed to develop scopolamine-rich plants of *A. belladonna* (Wang et al., 2011). With the elucidation of several important TA biosynthetic steps at molecular and biochemical levels, the genes encoding some rate-limiting enzymes that can be used to genetically modify TA biosynthesis *in planta* have been functionally identified.

Putrescine *N*-methyltransferase (PMT) catalyzes the methylation of putrescine to form *N*-methylputrescine as the precursor for TA biosynthesis (Fig. 1). It is generally considered to be the first rate-limiting enzyme involved in TA biosynthetic pathway (Hibi et al., 1992; Zhang et al., 2007). Hyoscyamine 6β-hydroxylase (H6H) is the last rate-limiting enzyme involved in scopolamine biosynthesis, which catalyzes the 6β-hydroxylation of hyoscyamine to anisodamine, as well as the epoxidation of anisodamine to scopolamine (Fig. 1) (Kai et al., 2011; Matsuda et al., 1991; Zhang et al., 2004). Thus, *PMT* and *H6H* are often used to study TA biosynthesis using transgenic technology in hairy root cultures or plants. When a single *PMT* gene was overexpressed in transgenic hairy root cultures or plants of *A. belladonna*, the TA content was not altered (Rothe et al., 2003). However, overexpression of the same *PMT* gene in *Datura metel* resulted in TA contents that were significantly increased (Moyano et al., 2003). When *H6H* was overexpressed, biosynthesis of scopolamine was definitively enhanced because H6H efficiently converts hyoscyamine to scopolamine (Yun et al., 1992). Furthermore, simultaneous overexpression of both *PMT* and *H6H* coordinately promoted biosynthesis of scopolamine and made the scopolamine content very high in transgenic hairy root cultures of *H. niger* (Zhang et al., 2004). We established transgenic plants of *A. belladonna* in which both *PMT* and *H6H* were overexpressed for the first time and found that scopolamine biosynthesis was also strongly enhanced (Wang et al., 2011).

To study biosynthesis and translocation of TAs in the offspring of transgenic *A. belladonna* (T0 progeny), we generated T1 progeny of *NtPMT-HnH6H*-overexpressing plants of *A. belladonna*. In field conditions, we analyzed the tissue profiles of the two transgenes

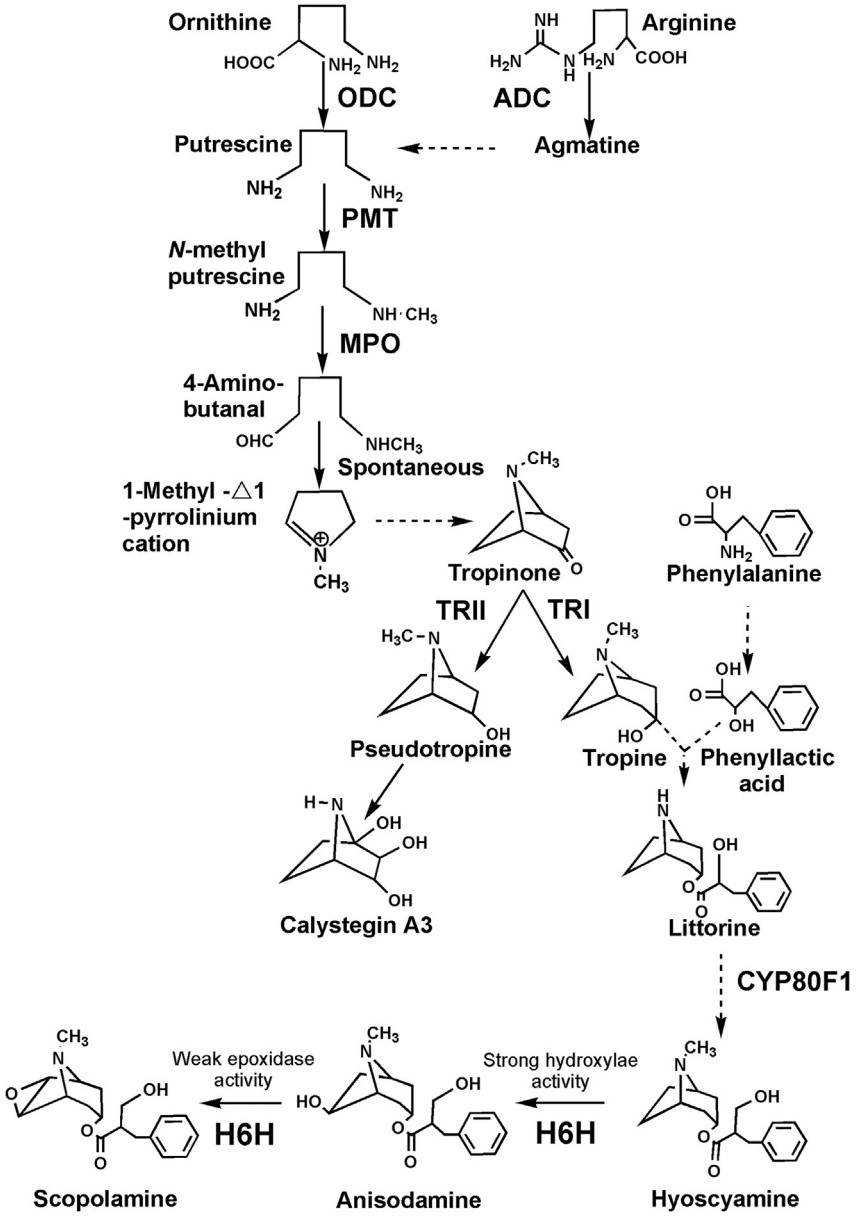

Fig. 1. The biosynthetic pathway of tropane alkaloids in Solanaceae.