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Enhanced production of tropane alkaloids in transgenic *Scopolia parviflora* hairy root cultures over-expressing putrescine *N*-methyl transferase (PMT) and hyoscyamine-6β-hydroxylase (H6H)

Y. M. Kang · D. J. Park · J. Y. Min · H. J. Song · M. J. Jeong · Y. D. Kim · S. M. Kang · C. S. Karigar · M. S. Choi

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Abstract *Scopolia parviflora* adventitious roots were metabolically engineered by co-expression of the two gene putrescine *N*-methyl transferase (PMT) and hyoscyamine-6β-hydroxylase (H6H) cDNAs with the aid of *Agrobacterium rhizogenes*. The transformed roots developed into morphologically distinct *S. parviflora* PMT1 (Sp*PMT1*), *S. parviflora* PMT1 (Sp*PMT2*), and *S. parviflora* H6H (Sp*H6H*) transgenic hairy root lines. Consequent to the introduction of these key enzyme genes, the production of the alkaloids hyoscyamine and scopolamine was enhanced. Among the transgenic hairy root lines, Sp*PMT2* line possessed the highest growth index. The treatment of transgenic hairy roots with growth regulators further enhanced the production of scopolamine. Thus, the results suggest that PMT1, PMT2, and H6H genes may not only be involved in the metabolic regulation of alkaloid production but also that these genes may play a role in the root development.

Keywords Hyosamine-6β-hydroxylase (H6H) · Putrescine *N*-methyl transferase (PMT) · Hairy roots · Phytohormones · *Scopolia parviflora* · Tropane alkaloids

Y. M. Kang
College of Agricultural and Life Sciences, Cornell University,
United States Department of Agriculture,
308 Sturtevant Hall,
Geneva, NY 14456, USA

D. J. Park · H. J. Song · M. J. Jeong · M. S. Choi (✉)
Division of Environmental Forest Science,
Gyeongsang National University,
Jinju 660-701, South Korea
e-mail: mschoi@gnu.ac.kr

J. Y. Min
Sancheong Oriental Medicinal Herb Institute,
Sancheong-gun, Gyeongnam 666-931, South Korea

Y. D. Kim
Institute of the Hadong Green Tea, Hadong-gun,
Gyeongnam 667-804, South Korea

S. M. Kang
Department of Forest Research,
Gyeongsangnam-do Forest Environment Research Institute,
Jinju 660871, South Korea

C. S. Karigar
Department of Biochemistry, Bangalore University,
Bangalore 560001, India

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Introduction

Hyoscyamine and scopolamine are two of the most important tropane alkaloids (TA) found as secondary metabolites in the *Solanaceae* family members (Hashimoto and Yamada 1987). Since these alkaloids possess various therapeutic properties, they are used in different medical applications. The general route for the production of such secondary metabolites on a commercial scale is to employ plant cells and their tissue cultures. Such approaches, however, have found limited success owing to low yields (Cusido *et al.* 1999). The metabolic engineering strategies thus can be developed to overcome the limitations of the traditional *in vitro* methods (Dixon 2001). Recently, efforts along these lines have been conducted, and a TA biosynthetic pathway has been manipulated for enhanced metabolite productions (Sato *et al.* 2001; Kang *et al.* 2005).

The amino acids arginine and ornithine are the starting substrates for the biosynthesis of TA. The critical step