Production of tropane alkaloids by metabolic engineering of *Hyoscyamus niger* H6H(hyoscyamine 6β-hydroxylase) gene introduced *Scopolia parviflora* hairy root

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Abstract

The *Hyoscyamus niger* hyoscyamine 6β-hydroxylase (H6H, EC 1.14.11.11) gene was introduced into the genome of a *Scopolia parviflora* by the binary vector system using the disarmed *Agrobacterium rhizogenes* strain KCTC 2703. Expression of H6H enzyme which are involved in alkaloids pathway by western blot analysis using proteins extracted from leaf, stem, flower, branch root and main root were examined. The enzyme expression was found only in the roots, with no expression in leaf, stem and flower. The alkaloids contents were the most higher in root and then leaf and stem has very small amount of alkaloid contents were analyzed by HPLC. The expression level of H6H in transgenic plants were two or more times than wild type plants. In transgenic plant which constitutively expresses H6H enzyme, high concentration of scopolamine was accumulated.

Introduction

Hyoscyamine and scopolamine, which are found mainly in *Hyoscyamus, Duboisia, Atropa* and *Scopolia* species, are used medicinally as anticholinergic agents that act on the parasympathetic nerve system. Several *Solanaceae* species have been used, as the commercial sources of these alkaloids, but the scopolamine content in these plants often are much lower than those of hyoscyamine. As this reason, there has been long-standing interest in increasing the scopolamine content of cultivated medicinal plant. Large-scale cultures of hairy roots of several *Solanaceae* species has been reported so far . In order to overcome the low production yield, studies in plant cell cultures focused on the optimization of culture condition and high yielding cell lines. Also, genetic engineering of secondary metabolites pathway aims to either increase or decrease the quantity of a certain

compound or group of compounds. In this study, *Hyoscyamus niger* H6H genes were introduced into *Scopolia parviflora*, a typical scopolamine-rich plant, and expressed constitutively in hairy roots. We reported the introduction of *Hyoscyamus niger* H6H gene into *Scopolia parviflora*, and the characterization of the resulting transgenic cultures.

Material and Methods

We have used the cDNA encoding *Hyoscyamus niger* which was kindly provided by T. Hashimoto of NAIST in Japan. The XbaI and BamHI fragment of the polymerase chain reaction (PCR) products H6H cDNA insert was isolated from PBlueH6H, and after BamHI site filled-in with Klenow, the fragment was subcloned to SmaI in pBE2113 vector between Ω seguence and nopaline synthase terminator. The pBE2113 vector was transferred to *Agrobacterium rhizogenes* strain KCTC 2703 by direct transfer method. Exconjugants were used to transform *S. parviflora* leaf explants. Western blot analysis of total H6H extracts (20μg/lane) from wild-type and transgenic plant transformed with H6H were subjected to SDS-PAGE and immunoblot analysis were performed using monoclonal rabbit antibody. Hairy root were harvested, weighed, and analysed using by HPLC (MeCN:50 mM K_2HPO_4=22:78, 4.6x25 cm TSK gel ODS column, and UV 215 nm.

Results and Discussion

To analyze the H6H enzyme activity which regulates alkaloids biosynthesis, we extracted tropane alkaloids from transgenic plants grown on culture medium, purified and then quantified by HPLC. H6H enzyme expression was found only in the roots, no expressed in leaf, stem, and flower. As parallel results, the contents of hyoscyamine and scopolamine in hairy roots were almost two times higher than those of another tissues. Transgenic hairy roots clones were induced after infection of *S. parviflora* with *Agrobacterium rhizogenes*. Kanamycin resistant plants were cultured and proliferated them. Western blot analysis using H6H specific monoclonal antibody showed that the H6H polypeptide of about 36KD was abundant in several hairy root lines. In addition, the expression level of H6H protein in transgenic plants was more times than that of wild type plants (Fig 1). By the overexpression of the gene encoding H6H in *S. parviflora* hairy root cultures, a 2 and 3 fold higher than that of wild type plants (Fig 1). In this results, we proposed that overexpression of plant affects an inducible promoter and optimal culture condition. This results applied successfully for mass production of tropane alkaloids.

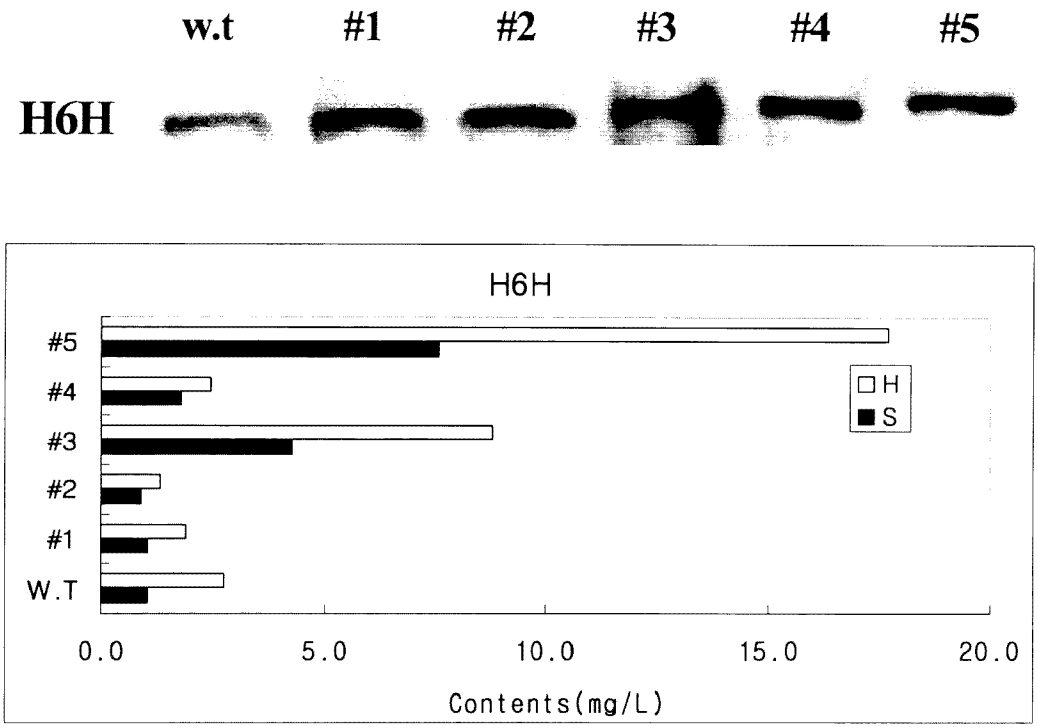

Fig. 1. Contents of tropane alkaloids and over-expression of H6H in transgenic lines and wild type of *S. parviflora* hairy roots

Above: Western blot analysis of H6H enzyme

Below: Alkaloids contents of wild type plant and transgenic lines

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