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Research article

Chromium-induced tropane alkaloid production and H6H gene expression in *Atropa belladonna* L. (Solanaceae) *in vitro*-propagated plantlets

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ABSTRACT

Hyoscyamine and scopolamine tropane alkaloids found in several solanaceous plants are anticholinergic drugs. Hyoscyamine 6β-hydroxylase (H6H) catalyzes two consecutive oxidation reactions. The first reaction is the hydroxylation of hyoscyamine to 6β-hydroxyhyoscyamine and the second is epoxidation of 6β-hydroxyhyoscyamine yielding scopolamine that is the final metabolite in the tropane alkaloid biosynthetic pathway. The effects of trivalent chromium as $KCr(SO4)_2$ on the production of tropane alkaloids and the expression of hyoscyamine 6β-hydroxylase gene (h6h) were studied in micro-propagated *Atropa belladonna* L. plantlets. The results showed that chromium treatment decreased the growth parameters (weights and lengths of the plantlets) and chlorophyll contents and increased proline contents. Moreover, semiquantitave RT-PCR analysis showed that the transcript level of H6H increased under chromium treatment. This treatment also increased hyoscyamine and scopolamine contents as shown by HPLC analysis. Changes of scopolamine contents correlate with the expression levels of h6h gene under different concentrations of chromium.

1. Introduction

Solanaceaeous plants such as *Atropa*, *Datura*, *Duboisia*, *Hyoscyamus* and *Scopolia* exclusively produce the hyoscyamine and scopolamine tropane alkaloids and are widely used as anticholinergic drugs, which act on the parasympathetic nervous system [1]. Hyoscyamine is usually the main alkaloid in many of these plants while scopolamine is often produced in small amounts. Scopolamine is more valuable and is preferable to hyoscyamine in the pharmaceutical market because of its higher physiological activity and fewer side effects. There is currently a tenfold higher commercial demand for scopolamine (in the N butylbromide form) than for both hyoscyamine and atropine (the racemic mixture of D and L Hyoscyamine) [2]. In *Atropa belladonna*, these two tropane alkaloids mostly synthesize in young root cells and translocate to aerial parts of the plant [3].

The biosynthesis of scopolamine is a complex process requiring many distinct enzymatic steps and several genes of these enzymes have now been cloned. The last step of scopolamine biosynthesis is catalyzed by Hyoscyamine 6β-hydroxylase (H6H, EC 1.14.11.11). H6H is a member of the 2-oxoglutarate-dependent dioxygenase family. The enzyme catalyzes two consecutive oxidation reactions, i.e. the hydroxylation of hyoscyamine to 6β-hydroxyhyoscyamine and the epoxidation of 6β-hydroxyhyoscyamine yielding scopolamine which is the final biosynthetic metabolite [4–8]. In addition to the alkaloid substrate H6H requires Fe^{+2}, 2-oxoglutarate, O_2 and ascorbate for catalysis [4,8].

Among the strategies employed to enhance plant secondary metabolite production an outstanding one is the use of biotic and abiotic elicitors. An elicitor is a compound that not only induces accumulation of antimicrobial phytoalexins in plants but also stimulates any type of defense responses [9]. Vernay et al. [10] found that exogenous chromium induces an increase in the scopolamine synthesis from hyoscyamine in *Datura innoxia*. An increase in the expression of h6h gene can considerably enhance the production of scopolamine in organ cultures of *A. belladonna*, *H. niger* and *A. baetica* [2], which proves that metabolic engineering may be a feasible approach to improve scopolamine production.

In this study, we have investigated the effects of trivalent chromium [Cr (III)] on growth, chlorophyll and proline contents

Abbreviations: H6H, Hyoscyamine 6β –Hydroxylase; IAA, Indol Acetic Acid; BA, Benzyl Adenine; PMT, Putrescine N-methyltransferase; TR (I and II), Tropinon Reductase (I and II).

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