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ALKALOID PRODUCTION BY TRANSFORMED ROOT (HAIRY ROOT) CULTURES OF CATHARANTHUS ROSEUS

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ABSTRACT

Catharanthus roseus (Madagascar periwinkle) is a medicinal plant well known for producing terpenoid indole alkaloids (TIAs) such as vinblastine and vincristine, which are used as chemotherapeutic agents. Since natural accumulation of these metabolites is low and total chemical synthesis is impractical, in vitro systems—particularly transformed (hairy) root cultures induced by Agrobacterium rhizogenes—have emerged as viable alternatives for research and production. This paper reviews the biological basis of TIA biosynthesis, outlines experimental approaches to establish hairy root cultures, and discusses strategies such as elicitation, precursor feeding, and genetic engineering to enhance alkaloid yields. Prospects for metabolic engineering and large-scale cultivation are also highlighted.

1. INTRODUCTION

Catharanthus roseus synthesizes more than 130 different TIAs, among which vinblastine and vincristine are the most pharmaceutically important due to their anticancer properties (Goklany et al., 2009). However, in planta yields are extremely low, often less than 0.0005% of dry weight, and the multistep chemical synthesis of bisindole alkaloids is economically unfeasible. Hence, alternative systems for their production are crucial.

Hairy root cultures induced by A. rhizogenes represent one such system. These roots grow rapidly without exogenous hormones, show genetic stability, and are capable of producing specialized metabolites at levels comparable to or higher than field-grown plants (Lee-Parsons et al., 2008). The present study synthesizes literature on the establishment of hairy root cultures in C. roseus, evaluates strategies to increase alkaloid accumulation, and outlines analytical methods for gene expression and metabolite profiling.

Keywords:

Catharanthus roseus; Hairy root culture; Terpenoid indole alkaloids (TIAs); Vinblastine; Vincristine; Agrobacterium rhizogenes; Metabolic engineering; Jasmonate elicitation; Precursor feeding; Secondary metabolites; Plant biotechnology; Gene expression; Alkaloid quantification

2. LITERATURE REVIEW

Hairy root technology

When A. rhizogenes infects plant tissue, it transfers T-DNA from its root-inducing (Ri) plasmid into the plant genome, resulting in fast-growing and highly branched roots known as hairy roots (Travesset & Lee-Parsons, 2012). These roots are stable over generations and have been widely exploited for the production of secondary metabolites in numerous medicinal plants.

TIA biosynthesis in C. roseus

The TIA pathway begins with condensation of tryptamine, produced from tryptophan by tryptophan decarboxylase (TDC), and secologanin, derived from the terpenoid pathway, to yield strictosidine via strictosidine synthase (STR). Strictosidine is then converted by strictosidine β-glucosidase (SGD) into reactive intermediates that give rise to monomeric alkaloids such as catharanthine, ajmalicine, and vindoline (Paul et al., 2014). Bisindole alkaloids like vinblastine are produced by coupling of catharanthine and vindoline, a process tightly regulated at tissue and cellular levels. Transcription factors such as ORCAs and CrMYC2 mediate jasmonate-responsive expression of pathway genes (Zhang et al., 2011; Van der Fits & Memelink, 2000).

Strategies to enhance alkaloid yields

Multiple approaches have been developed to improve TIA accumulation in hairy roots:

Elicitation with methyl jasmonate (MeJA), salicylic acid, or yeast extract significantly upregulates biosynthetic genes (Gantet et al., 1998).

Precursor feeding with tryptamine or loganin enhances substrate availability (Goklany et al., 2009).

Genetic engineering involving overexpression of transcription factors such as ORCA3 or pathway genes like TDC and STR increases alkaloid levels (Paul et al., 2015; Van Moerkercke et al., 2010).

Culture optimization through manipulation of sucrose concentration, nitrogen sources, pH, and aeration has also been shown to modulate productivity (Fréderich et al., 2020).

3. OBJECTIVES

1. Establish hairy root cultures of C. roseus using A. rhizogenes.
2. Confirm transformation using molecular markers (rol genes) and morphological traits.
3. Quantify baseline alkaloid production and compare with non-transformed controls.
4. Assess elicitation, precursor feeding, and medium optimization strategies to enhance alkaloid yields.
5. Analyze expression of key biosynthetic and regulatory genes under different treatments.

4. Materials and Methods

Plant material: Sterile seedlings of C. roseus.

Transformation: Leaf or stem explants inoculated with A. rhizogenes and co-cultivated. Hairy roots excised and maintained on hormone-free medium.

Confirmation: PCR amplification of rol genes and absence of vir genes.

Treatments: MeJA (100–200 µM), yeast extract (0.1–1%), tryptamine/loganin feeding, and media modifications.

Analysis: HPLC/LC–MS for alkaloids; qRT-PCR for gene expression (TDC, STR, SGD, ORCA3, CrMYC2).

Statistics: ANOVA and correlation analysis between gene expression and alkaloid content.

5. Expected Results

Hairy root induction confirmed by rol gene PCR and typical plagiotropic growth (Travesset & Lee-Parsons, 2012).

Higher alkaloid levels in hairy roots compared with callus cultures (Lee-Parsons et al., 2008).

MeJA treatment induces strong upregulation of TDC and STR, leading to increased catharanthine and ajmalicine levels (Paul et al., 2014).

Precursor feeding enhances downstream metabolite accumulation, particularly when combined with elicitors (Goklany et al., 2009).

6. DISCUSSION

Hairy root cultures provide several advantages, including genetic stability and high biosynthetic capacity. However, the compartmentalized biosynthesis of bisindole alkaloids limits vinblastine accumulation. Metabolic engineering targeting both structural genes and regulatory factors has shown promise (Paul et al., 2015; Zhang et al., 2011). Bioprocess optimization, elicitation regimes, and combinatorial precursor feeding further increase yields.

Nonetheless, full reconstitution of bisindole biosynthesis may require co-culture or synthetic biology approaches (Van Moerkercke et al., 2010).

7. CONCLUSION

Hairy root cultures of C. roseus are a reliable system for producing TIAs and studying their biosynthetic regulation. Although large-scale commercial production of vinblastine and vincristine remains challenging, combining metabolic engineering with advanced bioprocessing offers a feasible strategy for future applications.

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