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Hairy root technology: a biotechnological platform for plant secondary metabolite production

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INTRODUCTION

Hairy roots developed after infection of plants with Agrobacterium rhizogenes, a symbiotic bacterium currently taxonomically renamed as Rhizobium rhizogenes, have been used for the production of economically valuable, diverse plant-based metabolites. R. rhizogenes, a gram-negative soil bacterium inhabiting near plant roots causes the 'hairy root syndrome', characterized by the development of nongeotropic branching roots at the site of infection. Hairy roots are fast growing, free from pathogen and herbicide contamination and genetically stable. Due to these properties, these have been considered as ideal systems for production of specialized macromolecules and secondary metabolites useful for both pharmaceutical and industrial applications.

What is hairy root technology

Hairy roots were discovered by Chilton in 1982 by Chilton and his co-workers (Chilton et al. 1982). A. rhizogenes harbours a Ri Plasmid that contains T-DNA carrying genes for hairy root formation. Once A. rhizogenes detects a wounded plant tissue, it activates its vir gene system, facilitated by phenolic compounds such as acetosyringone. The vir genes complex stimulates the excision and transfer of T-DNA from the bacteria into the plant genome via a type IV secretion system (T4SS). The T-DNA is transported to the nucleus where it gets integrated into the host genome. The T-DNA contains genes (rolA, rolB, rolC, and rolD) that induce hairy root phenotype by regulating plant hormonal balance by modulating auxin and cytokinin homeostasis. In addition, aux1 and aux2 genes are present which encode enzymes involved in auxin biosynthesis, increasing indole-3-acetic acid (IAA) levels to further enhance root development.

Advantages of hairy roots

Hairy roots have been used in plant regeneration, production of specialized metabolites, environmental remediation, biotransformation and production of recombinant proteins. Hairy root cultures offer several key benefits over traditional methods of metabolite production:

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- 1. Hairy roots have a faster growth rate than normal roots in culture
- 2. Hairy roots have a hormone-independent growth pattern, i.e. they do not need plant growth regulators to multiply
- 3. The production of metabolites in hairy roots remains consistent over time
- 4. They produce equal or higher levels of compounds than the natural plant
- 5. They can be maintained under sterile, controlled conditions throughout the year

Hairy roots have been used for the production of a large number of secondary metabolites such as alkaloids, anthocyanins, flavonoids, ginsenosides, phenolics, stilbenes, lignans, and terpenoids. Hairy roots have also been used for the production of protein-based products such as vaccines, monoclonal antibodies, and therapeutic proteins.

Applications of hairy root technology



- Plant secondary metabolite production

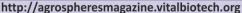
Hairy root cultures have been established in many medicinal and aromatic plants to produce compounds such as flavonoids, terpenoids, phenolic compounds, and saponins. For example: Catharanthus roseus hairy roots are used to produce anti-cancer alkaloids like vincristine and vinblastine. Hairy roots have been induced in *Panax ginseng* for the production of ginsenosides, important for their tonic and antioxidant properties. Similarly, hairy roots in Withania somnifera (Ashwagandha) have been induced to produce withanolides, which have anti-stress and anti-inflammatory effects. The polyphenol curcumin used in the management of oxidative and inflammatory conditions has been induced in the hairy roots of Atropa belladonna. The production Tanshinones, which have antioxidant activity, anti-inflammatory activity. cardiovascular effects, and antitumor activity has been increased using Salvia miliorrhiza hairy roots. Hairy root cultures have been induced in black carrots

(Daucus carota), a major anthocyanin accumulator.

There are several other examples where hairy root cultures have been used for the production of secondary metabolites.

- Phytoremediation

Roots of plants are important for phytoremediation as they absorb contaminants from soil and phytotransformation, the metabolic degradation of the contaminant. Hairy roots have helped in supplementing this role as they increase the root biomass thereby increasing the contaminant uptake rate or they have been used to express a transgene in the hairy roots that assists in phytotransformation. For example, hairy root cultures of Helianthus annuus (sunflower) have been found to remove the antibiotics tetracycline oxytetracycline from aqueous Similarly, *Armoracia rusticana* (horseradish) hairy roots have the ability to acetaminophen. Lycopersicon esculentum hairy roots have shown to remove phenol from media



in the presence of 5 mm hydrogen peroxide, while, hairy roots of *Brassica napus* have been used to phytoremediate 2,4-dichlorophenol (2,4-DCP).

- Systems biology research

Hairy roots are also valuable model systems for studying plant metabolic pathways. They serve as reliable models to investigate gene regulation, enzyme activity, and metabolic flux in the production of specific phytochemicals. Genetic engineering techniques can be used to insert or overexpress key biosynthetic genes, thereby boosting metabolite synthesis or enabling the production of novel compounds. Hairy roots can also be used to develop and test gene circuits. wood-related genes: Cinnamoyl-CoA Reductase1 (CCR1), a key lignin biosynthetic and IAA9A an auxin dependent transcription factor of the Aux/IAA family were edited using CRISPR/Cas9 Technology in Eucalyptus hairy roots. Similarly, steroid 16α hydroxylase St16DOX gene was edited using CRISPR/Cas9 in potato, resulting in the generation of α-solanine-free hairy roots. In another study, overexpression of GmNAC15 in soybean hairy roots resulted in enhanced salt tolerance.

CONCLUSION

In summary, hairy root technology combines the benefits of plant genetic transformation, metabolic stability, and controlled culture conditions. The technology can be applied to rare, valuable, threatened, or endemic medicinal species for production of commercially valuable metabolites. Moreover, they can be used as tools for studying biosynthesis of plant-derived molecules and upscaling of bioproduction systems. By combining the advantages of Agrobacterium rhizogenes-mediated stable growth, transformation, and high biosynthetic potential, hairy root cultures provide excellent alternative to conventional cultivation and chemical synthesis. With further advances metabolic engineering, bioprocess optimization, hairy root technology will play a greater role in sustainable production and plant biodiversity conservation.

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