Plant Secondary Metabolite Production: Limitations in the Field and Solutions with *Agrobacterium rhizogenes* mediated Hairy Root Culture

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In the recent era, the cultivation of medicinal plants in the field faces several challenges, including environmental dependency, limited arable land, seasonal fluctuations, and vulnerability to pests, diseases, and climate change. Additionally, overharvesting of wild medicinal plants threatens biodiversity and sustainability. Field cultivation also leads to inconsistent yields and variations in the quality and quantity of bioactive compounds due to environmental stressors. Furthermore, slow growth rates and long maturation times in many medicinal plants limit large-scale production of valuable phytochemicals.

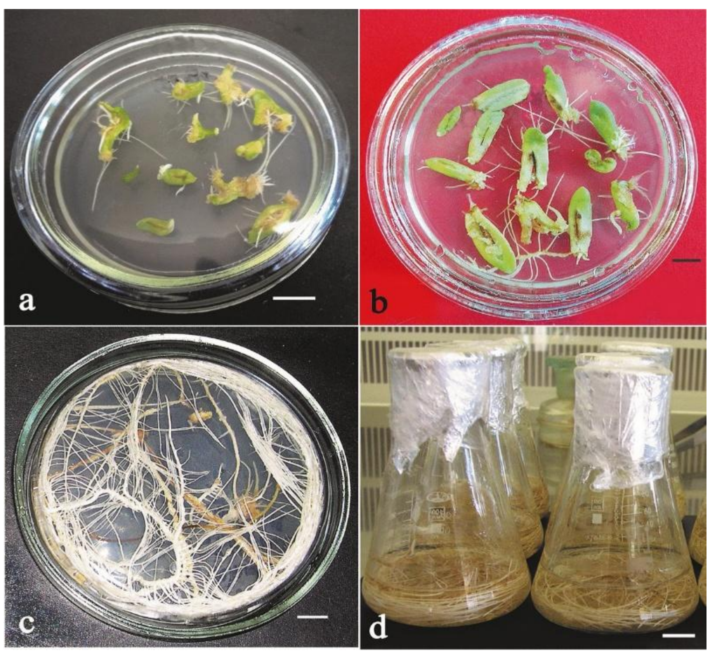

Fig.1. Outlined diagram of Agrobacterium Rhizogenes mediated infection to the plant cell

In vitro production systems utilizing *Agrobacterium rhizogenes* have emerged as a powerful alternative to overcome these challenges. It is a Gram-negative soil bacterium that produces hairy root disease in dicotyledonous plants, which are characterized by rapid growth, genetic stability, and high biosynthetic capacity for secondary metabolites. These hairy roots can be cultured under controlled laboratory conditions, independent of environmental factors, ensuring consistent and sustainable production of bioactive compounds.

Hairy root cultures eliminate the need for extensive land use and protect natural plant populations by reducing the reliance on wild harvesting. They also enable year-round production and can be optimized using elicitors, precursors, or genetic engineering to enhance metabolite yields. For example, hairy root cultures of *Withania somnifera* (ashwagandha) produce withanolides (Biswas et al., 2023), while *Artemisia annua* hairy roots enhance artemisinin production (Ikram et al., 2017). Large-scale bioreactors have been developed to further improve productivity, making the process scalable and commercially viable.

By addressing the limitations of field cultivation, *A. rhizogenes*-mediated hairy root cultures offer a sustainable, efficient, and environmentally friendly solution for the production of high-value medicinal compounds. Some research highlight represents in Table 1.

Challenges and Potential Solution for the Production of Secondary Metabolite through Hairy Root Culture

While *Agrobacterium rhizogenes*-mediated hairy root cultures offer significant advantages for the production of secondary metabolites, several challenges remain in optimizing and scaling this technology (Awere et al. 2024)

1. Variability in Transformation Efficiency

- **Challenge**: The efficiency of transformation and hairy root induction varies significantly across plant species and even among cultivars. Some plants are recalcitrant to *A. rhizogenes*-mediated transformation.

- **Potential Solutions**: Development of species-specific transformation protocols, optimization of infection parameters (e.g., bacterial strain, infection medium), and use of engineered *A. rhizogenes* strains.

2. Low Yield of Target Metabolites

- **Challenge**: In some cases, the concentration of desired secondary metabolites in hairy root cultures is lower than in naturally grown plants.
- **Potential Solutions**: Application of elicitors (biotic or abiotic stress inducers), precursor feeding, metabolic engineering of biosynthetic pathways, and co-culture systems to enhance metabolite yields.

Plant Species	Secondary Metabolite	Metabolite Class	Applications
Withania somnifera (Ashwagandha)	Withanolides	Steroidal lactones	Anticancer, anti-inflammatory, adaptogenic
Catharanthus roseus (Periwinkle)	Vincristine, Vinblastine	Alkaloids	Anticancer (leukemia, lymphoma)
Artemisia annua (Sweet Wormwood)	Artemisinin	Terpenoids	Antimalarial
Glycyrrhiza glabra (Licorice)	Glycyrrhizin	Saponins	Anti-inflammatory, antiviral, sweetener
Capsicum annuum (Chili Pepper)	Capsaicinoids	Alkaloids	Analgesic, food additive
Hypericum perforatum (St. John's Wort)	Hypericin, Hyperforin	Phenolics	Antidepressant, antiviral
Coleus forskohlii	Forskolin	Diterpenoids	Weight loss, cardiovascular health
Echinacea purpurea	Cichoric Acid	Phenolics	Immune booster, antioxidant
Solanum tuberosum (Potato)	Solanine	Glycoalkaloids	Pest deterrent, pharmacological research
Ocimum basilicum (Basil)	Rosmarinic Acid	Phenolics	Antioxidant, anti-inflammatory
Panax ginseng (Ginseng)	Ginsenosides	Triterpenoid saponins	Adaptogen, cognitive enhancement
Datura stramonium	Scopolamine, Hyoscyamine	Tropane alkaloids	Anticholinergic, motion sickness treatment
Papaver somniferum (Opium Poppy)	Morphine, Codeine	Alkaloids	Pain management, cough suppression
Rubia cordifolia (Indian Madder)	Alizarin	Anthraquinones	Natural dye, anticancer
Tinospora cordifolia (Giloy)	Tinosporaside	Glycosides	Immunomodulator, antidiabetic

Kumar et al. (2018)

3. Scalability Issues

- **Challenge**: Scaling up hairy root cultures from lab to industrial levels is difficult due to challenges in maintaining sterility, shear sensitivity of roots, and ensuring homogeneous growth in bioreactors.
- **Potential Solutions**: Development of advanced bioreactor designs (e.g., bubble column, airlift, and wave bioreactors), optimization of culture conditions, and use of immobilization techniques.

4. Instability of Cultures

- **Challenge**: Long-term maintenance of hairy root cultures can lead to genetic and metabolic instability, potentially reducing metabolite yields over time.
- **Potential Solutions**: Regular sub-culturing, cryopreservation of cultures, and monitoring metabolite profiles to ensure stability.

5. Regulatory and Safety Concerns

- **Challenge**: The use of genetically modified organisms (GMOs) like *A. rhizogenes*-induced hairy roots can raise regulatory concerns, especially for pharmaceutical or food applications.
- **Potential Solutions**: Use of non-GMO techniques like transient transformation and rigorous safety assessments to meet regulatory standards.

6. Limited Knowledge of Biosynthetic Pathways

- **Challenge**: In many plants, the biosynthetic pathways for secondary metabolites remain poorly understood, hindering the targeted enhancement of production.
- **Potential Solutions**: Genomic, transcriptomic, and metabolomics studies to elucidate pathways and identify key enzymes or regulatory genes.

7. Contamination Risks

- **Challenge**: In vitro cultures are highly susceptible to microbial contamination, which can reduce the productivity or viability of hairy roots.
- **Potential Solutions**: Improved sterile techniques, antimicrobial agents, and automated culture systems to minimize contamination.

8. High Initial Costs

- **Challenge**: Setting up in vitro systems and bioreactors requires significant capital investment, which can be a barrier for small-scale operations.
- **Potential Solutions**: Development of cost-effective culture systems and collaboration between academic and industrial sectors to share resources.

9. Difficulty in Extracting and Purifying Metabolites

- **Challenge**: Harvesting and purifying secondary metabolites from hairy root cultures can be labour-intensive and expensive.
- **Potential Solutions**: Use of optimized extraction protocols, downstream processing technologies, and solvent-free extraction methods like supercritical fluid extraction.

10. Competition from Alternative Technologies

- **Challenge**: Other technologies, such as synthetic biology and microbial fermentation, also compete for secondary metabolite production.
- **Potential Solutions**: Highlighting the unique advantages of hairy root cultures, such as species-specific metabolite production, and combining these systems with other biotechnologies.

Despite these challenges, advancements in genetic engineering, bioreactor design, and systems biology continue to address limitations, making *A. rhizogenes*-mediated production a promising and evolving field for sustainable secondary metabolite synthesis.

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