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In Vitro Production of Secondary Metabolites through Plant Cell and Organ Cultures (with Biomedical Relevance)

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Abstract

Secondary metabolites of plant origin—alkaloids, terpenoids, phenolics, and naphthoquinones—remain indispensable leads and adjuvants in modern therapeutics. Conventional field cultivation faces bottlenecks (low yields, seasonal/geographical variability, overharvesting). In vitro plant cell, tissue, and organ culture platforms offer controlled, year-round production and enable targeted pathway manipulation, aligning well with biomedical pipelines that require batch-to-batch consistency, traceability, and cGMP-amenable scale-up. This paper synthesizes current methods for producing high-value metabolites using callus and cell suspensions, organ cultures (shoots, embryos), and Agrobacterium rhizogenes–induced hairy roots. We review elicitation and process optimization (biotic/abiotic cues, precursor feeding, medium design, light/pH), metabolic engineering (pathway genes, transcription factors, CRISPR), bioreactor strategies (stirred-tank, wave, air-lift, disposable systems), and downstream analytics (extraction; HPLC/LC–MS quantification). Case studies cover clinically relevant molecules and their biomedical contexts: paclitaxel from *Taxus* systems (oncology), artemisinin from *Artemisia annua* (antimalarial), vincristine/vinblastine from *Catharanthus roseus* (oncology), and shikonin from *Lithospermum erythrorhizon* (anti-inflammatory/wound healing). We highlight translational links to animal models (zebrafish, murine xenografts, toxicity profiling) and discuss techno-economics, sustainability, and regulatory considerations. Finally, we identify challenges—genetic drift, scale-dependent oxygen transfer, elicitation reproducibility—and propose future directions including systems-guided design, single-use intensified bioprocesses, and hybrid plant–microbial routes. Collectively, plant cell/organ factories can de-risk supply chains for essential medicines while reducing pressure on biodiversity, provided that engineering control, rigorous analytics, and quality systems converge.

Keywords: Plant cell culture; Hairy root culture; Elicitation; Metabolic engineering; Bioreactors; HPLC/LC–MS; Biomedical models.

Introduction

Plant secondary metabolites (SMs) are specialized molecules not essential for primary

growth but vital for defense and signaling; many are cornerstone drugs or drug precursors. Field production is constrained by low titres, ecological variability, and conservation risks. In vitro cell, tissue, and organ cultures enable controlled production independent of climate, and permit fine manipulation of metabolic pathways and product quality—attributes prized in biomedical manufacturing. Recent reviews underscore the maturation of these strategies for pharmaceutically important SMs.

Callus and Cell Suspension Cultures

Dedifferentiated callus on auxin/cytokinin-balanced media provides explants for cell suspensions, which are suited to scale-up and batch control. Suspensions support rapid mass transfer and are compatible with continuous or fed-batch operation, though genetic/epigenetic drift can reduce productivity over passages; therefore, line selection, cryopreservation, and periodic re-cloning are standard QA practices. Organ cultures maintain differentiated biosynthetic capacity (e.g., glandular trichomes, secretory ducts) and often show greater metabolic stability than dedifferentiated cells. Somatic embryos can be synchronized for stage-specific metabolite profiling and scale-ready for temporary immersion systems.

Agrobacterium rhizogenes transforms root tissues, generating fast-growing, hormone-independent hairy roots with stable pathways—particularly attractive for alkaloids and phenylpropanoids. Hairy roots typically exhibit high genetic/biochemical stability, strong sink metabolism, and are amenable to bioreactors. Recent syntheses detail their progress toward commercial readiness and engineering toolkits.

Elicitation & Optimization

Elicitors (biotic: yeast extract, chitosan; abiotic: MeJA, SA, heavy metals), precursor feeding, medium composition (carbon/nitrogen ratio, phosphate), light quality/photoperiod, and pH/oxygen collectively reprogram flux through target pathways. MeJA and SA are consistently powerful triggers across taxa, but responses are line-specific; design-of-experiments (DoE) accelerates optimal set-point discovery. Mechanistic reviews and meta-analyses document how JA/SA signaling networks upregulate defense-linked biosynthesis, raising titres several-fold in many systems.

Process tips:

- Stabilize redox (antioxidants) to curb browning; control shear with low-shear impellers or wave motion.
- Use perfusion or ATF filters in suspensions to decouple residence time from growth.
- Apply in situ extraction (two-phase systems) for hydrophobic products (e.g., shikonin), reducing product feedback inhibition.

Metabolic Engineering (pathway genes, TFs, CRISPR)

Modern toolkits—overexpression/silencing of structural genes, TFs (e.g., AP2/ERFs, bHLH), and CRISPR editing—enable flux redirection and bottleneck relief. For artemisinin, multi-step rewiring and regulatory tuning (e.g., amorpha-4,11-diene synthase, CYP71AV1) have elevated yields; analogous strategies are applied to taxanes and TIAs (terpenoid indole alkaloids). Hairy root platforms are increasingly synthetic-biology-ready, supporting stable multi-gene stacks.

Plant cells require gentle mixing, high kLa, and tight temperature/DO/pH control. Modern configurations span air-lift, stirred-tank with marine impellers, wave-mixed single-use bags (up to ~1–2 m^3), and specialized root bioreactors (mist, trickle, nutrient sprinkle). Reviews describe the engineering envelope and cGMP-compatible single-use options that reduce contamination risk and cleaning validation.

Scale-up levers: biomass density management, carbon feeding (sucrose), controlled

shear, perfusion, and PAT (off-gas CO/O, capacitance probes). Recent overviews from plant-cell "factory" programs emphasize reproducibility and integration with downstream purification—key translational needs for biomedical lots.

Downstream Processing & Analytics (extraction; HPLC/LC–MS)

Hydrophobic products (taxanes, shikonin) benefit from two-phase extraction (e.g., n-hexadecane) or supercritical CO; polar alkaloids often require ion-pair or solid-phase cleanup prior to HPLC/LC–MS(/MS). Validated targeted LC–MS/MRM methods quantify families of alkaloids/terpenoids at low ng ·g{¹ levels; imaging MS and untargeted LC–MS support discovery and QC fingerprinting.

Case Studies (Biomedical Focus)

Paclitaxel (Taxol): *Taxus* Cell/Organ Systems

Taxus suspensions and organ cultures produce paclitaxel and related taxanes. Advances in pathway elucidation (taxadiene synthase; downstream P450s), elicitation (MeJA), and cell-line selection have supported industrial processes; complementary plant and microbial routes are being pursued to stabilize supply.

Artemisinin remains central to antimalarial therapy; plant yields are naturally low. In vitro cultures, metabolic and regulatory engineering, and optimized extraction collectively raise titres and reduce field-supply volatility.

Vinca Alkaloids (Vincristine/Vinblastine): *Catharanthus roseus*

TIAs are classic oncology drugs; hairy roots and cambial meristematic cells provide robust chassis. Systems biology and engineering (precursors, TFs, compartmentalized flux) continue to improve titres; bioreactor demonstrations show path toward scale.

Shikonin: *Lithospermum erythrorhizon*

Shikonin (naphthoquinone) displays anti-inflammatory/wound-healing activities. In vitro systems are highly responsive to elicitation and in situ solvent extraction; bioreactor runs with two-phase systems tripled titres relative to controls.

Bioactivity & Toxicology in Animal Models

For translation, pharmacodynamics (e.g., microtubule inhibition by vinca alkaloids; antimitotic action of paclitaxel), pharmacokinetics, and safety must be validated in animals. Zebrafish screens facilitate rapid cardiotoxicity/angiogenesis assays; murine xenografts and syngeneic models assess antitumor efficacy; rodent malaria models validate artemisinin derivatives. Plant-cell–derived actives are chemically identical to field-derived counterparts when purity is verified by orthogonal analytics (NMR, LC–MS/MS), enabling substitution in preclinical protocols subject to GMP documentation. (General biomedical linkage supported by paclitaxel and artemisinin reviews.)

Sustainability & Economics

In vitro production decouples supply from land/wild harvest, curbs biodiversity pressure, and can be life-cycle-advantaged if media recycling and energy efficiency are optimized. Single-use systems reduce water/cleaning chemicals, though plastic waste and bag cost must be balanced against reduced contamination risk and faster changeovers.

Challenges & Future Directions

Challenges: genetic instability in suspensions; elicitation reproducibility; oxygen transfer/shear constraints at scale; metabolite feedback inhibition; regulatory alignment for botanical APIs. Directions: (i) Design-build-test-learn cycles integrating multi-omics with model-based optimization; (ii) CRISPR multiplexing and TF engineering for durable high-flux lines; (iii) intensified single-use bioprocesses (perfusion, PAT); (iv) hybrid routes combining plant culture and microbial bioconversion; (v) greener

downstream (SFE-CO, recyclable solvents).

Conclusion

Plant cell and organ cultures now constitute credible biomedical supply platforms for essential secondary metabolites. With engineered stability (especially hairy roots), smart elicitation, and modern single-use bioreactors plus rigorous LC–MS analytics, titles can approach or exceed field benchmarks while ensuring quality and traceability. Continued convergence of systems biology and bioprocess engineering will determine how broadly these "green factories" secure the pipelines of oncology, anti-infective, and regenerative therapeutics.

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