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Choosing Between Callus, Suspension Cells, Hairy Roots, and Regenerated Plants for Specialized-Metabolite Production Through Pathway Localization and Cellular Differentiation

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ABSTRACT

Selecting a plant in-vitro production platform is often treated as a practical comparison among biomass growth, metabolite titre, transformability, and scale. That framing is incomplete when specialized-metabolite biosynthesis depends on differentiated cell identity, intercellular pathway partitioning, tissue-specific transport, or developmentally regulated competence. This original comparative decision article evaluates callus, suspension cells, hairy roots, and regenerated plants as biologically distinct production states rather than interchangeable culture formats. The analysis integrates evidence on cell-type-specific specialized metabolism, multicellular compartmentation, tissue culture, metabolic engineering, regeneration, and spatial metabolomics. The central argument is that platform selection should begin with the biological requirements of the target pathway and only then consider process convenience. Callus and suspension cultures can provide accessibility, rapid manipulation, and scalable cell-based processing, yet dedifferentiation may alter regulatory or spatial features required by some pathways. Hairy roots retain root-like organization and high genetic accessibility, but transformation itself can modify metabolic state. Regenerated plants can restore broader tissue context, although regeneration does not guarantee chemical fidelity or process efficiency. We therefore propose a conditional decision architecture that separates biosynthetic compatibility, spatial-pathway requirements, manipulation, stability, and scale instead of collapsing them into a single platform ranking. The framework also identifies cases in which parallel or staged use of more than one platform is more defensible than premature commitment. Its principal limitation is that current evidence remains strongly species-, pathway-, and protocol-dependent; prospective matched cross-platform validation is still required.

Keywords: Specialized metabolism, Plant cell culture, Hairy roots, Pathway localization, Cellular differentiation, Metabolic engineering

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Introduction

Specialized metabolites are frequently discussed as chemical outputs of species, organs, or biosynthetic pathways, but current plant biology increasingly treats their production as a question of where, how, and under which biological state the pathway operates [1]. This shift matters for biotechnology because a production platform does not merely provide biomass. It creates a particular cellular organization, regulatory environment, developmental state, and transport context in which a pathway must function.

Medicinal-plant pathways are governed by interacting transcriptional, enzymatic, developmental, and signaling layers rather than by precursor availability alone [2]. Consequently, transferring a target pathway from an intact plant into callus, suspension cells, transformed roots, or a regenerated plant may preserve some determinants of

biosynthetic competence while modifying others. The relevant comparison is therefore not simply whether each system can produce a compound, but whether its biological state retains the regulatory and spatial requirements needed for reliable production.

Plant in-vitro biotechnology already encompasses multiple production configurations, each with distinct advantages and limitations [3]. Callus cultures are accessible and experimentally flexible; suspension cells intensify that accessibility and can be integrated with controlled bioprocessing; hairy roots combine transformation with a differentiated root phenotype; and regenerated plants re-establish broader tissue organization at the cost of slower, more complex handling. These categories should not be interpreted as an ordinal ladder from "simple" to "advanced." Their usefulness depends on the pathway being asked to function within them.

The same platform can therefore be appropriate for one product and poorly matched to another even within the same species. A metabolite synthesized within one broadly competent cell type poses a different engineering problem from a pathway whose enzymes, transport steps, or storage functions are separated across tissues. Likewise, a system that delivers high short-term accumulation may still be a weak choice if the productive state is unstable or cannot be reproduced during scale transition. These distinctions motivate comparison at the level of pathway requirements rather than platform reputation.

Plant cell cultures can also serve as engineerable production hosts, making them attractive when pathway reconstruction, elicitation, or genetic intervention is a primary objective [4]. Yet engineering convenience and biological fidelity are separable properties. This article therefore compares the four systems through a pathway-first lens. It asks which biological features a target pathway appears to require, which platform can plausibly retain or recreate those features, and when stability, manipulability, or scale should overrule an otherwise biologically attractive choice. The resulting decision architecture is proposed as an evidence-bounded framework rather than a validated universal ranking.

Why culture-system choice depends on pathway biology

The first selection question should be whether the target chemistry is compatible with a largely cell-autonomous state or depends on differentiated cellular identities. Cell-type-specific control of specialized metabolism can arise through transcriptional programs, enzyme localization, post-translational regulation, and the unequal distribution of biosynthetic capacity among neighboring cells [5]. A culture system that removes the relevant identity may therefore remain viable and rapidly growing while becoming less suitable for the pathway of interest.

This issue becomes sharper when biosynthesis is multicellular. Specialized pathways can partition reactions, intermediates, or regulatory functions across distinct cell types rather than completing the entire sequence within one homogeneous population [6]. Such organization creates a platform constraint: suspension cells may be excellent hosts for pathways whose essential chemistry can be reconstructed within individual cells, whereas a pathway requiring intercellular exchange may demand retained tissue organization or deliberate re-engineering of transport and cellular specialization.

Single-cell transcriptomics has made this distinction experimentally visible by resolving pathway-gene expression across cell populations that bulk measurements would average together [7]. In medicinal plants, single-cell multi-omics in *Catharanthus roseus* further demonstrated that biosynthetic functions relevant to monoterpenoid indole alkaloid metabolism are distributed among distinct cellular states [8]. These studies do not establish that every specialized pathway requires intact tissue, but they show why total transcript or metabolite abundance can conceal biologically decisive compartmentation.

More generally, plant cell types maintain distinctive metabolic programs [9]. Pathway biology is therefore better viewed as a continuum of organizational dependence than as a binary distinction between "cellular" and "whole-plant" metabolism. Some routes may need predominantly intracellular enzyme sets, whereas others may depend on specialized storage, secretion, precursor exchange, or developmentally restricted partner cells. Where evidence does not resolve that dependence, uncertainty itself supports comparative testing rather than an assumption that the simplest culture will suffice.

Culture-system choice should consequently be framed as a compatibility problem between pathway requirements and cellular state. The practical implication is not that differentiated systems are always superior, but that dedifferentiation, transformation, and regeneration should each be treated as biological interventions capable of changing biosynthetic competence rather than as neutral methods of maintaining plant material.

Comparative characteristics of plant production platforms

Hairy roots are attractive when the target pathway is compatible with root identity because they retain organized root-like growth while providing a genetically accessible transformed system [10]. Their value lies in this combination rather than in any presumption of universal chemical fidelity. They can support specialized-metabolite production and pathway engineering, although productivity and scale remain dependent on species, construct, culture conditions, and reactor design [11].

Suspension cultures occupy a different design space. Their dispersed growth facilitates medium control, elicitation, sampling, and metabolic engineering, making them strong candidates when cell-autonomous biosynthesis or reconstructable pathway logic outweighs the need for intact tissue organization [12]. Plant cell culture also has established bioprocess precedents, showing that cell-based production can move beyond exploratory flask culture in appropriate product classes [13]. Such precedent, however, does not mean that any metabolite will transfer successfully to suspension production.

Callus is often treated only as a precursor to suspension or regeneration, yet metabolomic analysis can reveal substantial specialized-metabolic capacity in dedifferentiated tissue [14]. Its limitation is therefore not inevitable inactivity, but uncertainty about how closely its metabolic state corresponds to the native pathway context. Regenerated plants provide the opposite advantage: broader tissue organization can be restored, but chemical performance must be assessed together with genetic stability rather than presumed from morphology alone [15]. Regeneration can also be integrated with in-vitro metabolite production, as demonstrated experimentally in medicinal-plant systems [16].

The four platforms therefore differ along several dimensions that cannot be collapsed into nominal product accumulation. Biological organization, accessibility to manipulation, compatibility with controlled bioprocessing, likelihood of retaining native pathway context, and the burdens of regeneration or transformation can point in different directions. No individual attribute establishes overall superiority.

These contrasts are organized in **Table 1** as platform properties rather than a fixed ranking.

Table 1. Biological and process identities of four plant specialized-metabolite production platforms

Platform	Defining biological state	Principal production advantage	Main uncertainty	Decision interpretation
Callus	Dedifferentiated, often heterogeneous tissue	Rapid establishment and experimental flexibility	Native pathway organization may be altered	Useful when chemical competence persists without strict tissue architecture
Suspension cells	Dispersed or aggregated cultured cells	High accessibility to medium, elicitors, sampling, and engineering	Stability and multicellular pathway functions may be difficult to retain	Favored for cell-autonomous or reconstructable pathways
Hairy roots	Transformed, differentiated root-like tissue	Root-associated organization combined with genetic manipulability	Transformation and scale can modify expected behavior	Favored when root-associated competence is biologically relevant
Regenerated plants	Re-established organ and tissue organization	Broad restoration of developmental context	Slower handling and uncertain chemical fidelity after regeneration	Favored when pathway function depends on higher-order organization

Note: Decision interpretations are proposed synthesis rather than validated universal rules.

Cellular differentiation and biosynthetic competence

Differentiation should not be treated as a binary proxy for metabolite productivity. Specialized-metabolic regulation can draw on regulatory machinery that is neither wholly pathway-specific nor reducible to a single developmental "on/off" switch [17]. A useful comparison must therefore ask which aspects of differentiated state are functionally relevant: transcription-factor combinations, enzyme localization, transport capacity, precursor supply, storage structures, or interactions among these features.

Combinatorial transcriptional control provides one mechanism through which particular cellular states can become biosynthetically competent [18]. Dedifferentiation may disrupt such combinations, but it may also expose

new stress-responsive or experimentally inducible states. Conversely, regeneration may restore developmental programs without reproducing the exact chemical phenotype of the donor plant. For this reason, the article uses biosynthetic competence as a proposed analytical concept: the set of cellular and tissue conditions sufficient for a target pathway to operate at the required degree of completeness, persistence, and controllability.

Tissue-culture transitions themselves involve extensive developmental reprogramming. During somatic embryogenesis, shifts among non-embryogenic, embryogenic, and developing states are accompanied by major hormonal and transcriptional changes [19]. These observations support treating culture state as molecularly substantive rather than merely morphological. They do not, however, justify assuming that any specific differentiation trajectory will increase specialized-metabolite output.

Single-cell analysis of tea leaves provides a stronger connection between developmental state and specialized metabolism by linking cellular trajectories with catechin-ester pathway organization [20]. This evidence also argues against describing differentiation simply as "more" or "less." The relevant question is whether a platform reproduces the particular identity, regulatory configuration, cellular cooperation, transport environment, or storage context needed by the target pathway.

This distinction separates native from engineered competence. A culture lacking part of the original tissue context may still become useful if a missing regulatory or transport function can be reconstructed, whereas a morphologically differentiated system may remain unsuitable if the necessary molecular state is absent. Biosynthetic competence is therefore treated here as pathway-specific and testable, not as a permanent property assigned to one culture class.

Table 2 separates the principal differentiation-related determinants from the interpretations that they do—and do not—support.

Table 2. Differentiation-related determinants of biosynthetic competence and their platform-selection implications

Determinant	What it can alter	Why it matters for platform choice	Interpretation boundary
Cell identity	Regulatory and metabolic program	Some pathways depend on particular cell states	Identity alone does not establish pathway flux
Transcriptional configuration	Coordinated enzyme expression	Culture state may preserve or disrupt pathway control	Transcript abundance is not metabolite production
Developmental state	Hormonal and regulatory environment	Regeneration or dedifferentiation can alter competence	Greater differentiation is not automatically superior
Cellular cooperation	Distribution of pathway functions	Multicellular biosynthesis may require organized tissue	Not all specialized pathways are multicellular
Transport and storage context	Intermediate movement and sequestration	Missing compartments can constrain otherwise intact chemistry	Localization may represent accumulation rather than synthesis

Note: "Biosynthetic competence" is used as a proposed integrative decision construct, not an experimentally validated platform score.

Figure 1 integrates these distinctions into a conditional architecture in which pathway biology, culture-state properties, localization requirements, process considerations, and validation boundaries remain analytically separable before a platform choice is made.

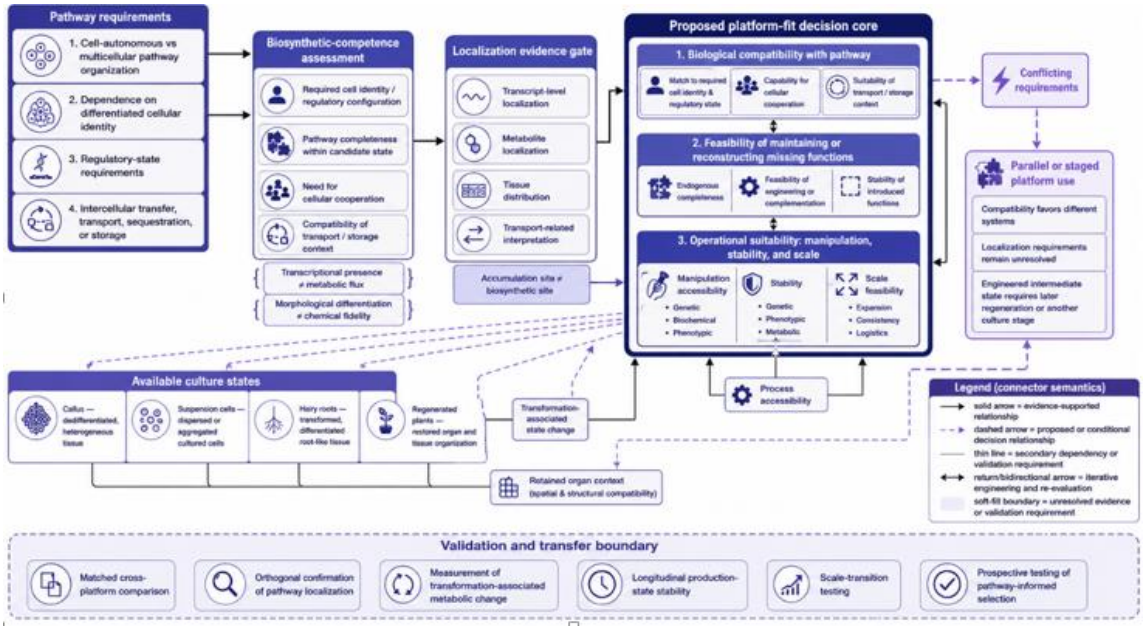

Figure 1. From pathway-dependent biosynthetic competence to conditional culture-system choice

Pathway localization matters because metabolite presence in an organ does not reveal where each biosynthetic step occurs. Single-cell metabolomics in *Catharanthus* showed pronounced intercellular localization of monoterpenoid indole alkaloids [21]. Platform selection must therefore consider whether synthesis, intermediate transfer, sequestration, and accumulation are spatially separated.

High-resolution mass-spectrometry imaging of *Rauvolfia tetraphylla* resolved spatial organization of alkaloid-related chemistry [22]. In *Salvia miltiorrhiza*, integrated metabolomics and imaging connected metabolite distributions with biosynthesis-oriented spatial interpretation [23]. Such approaches strengthen pathway mapping, but spatial colocalization alone cannot establish flux or transport direction; accumulation sites may differ from synthesis sites.

The constraint also extends beyond pathway enzymes. Tissue-specific metabolite and protein signatures, together with exudate composition, show that tissue identity can affect intracellular metabolism and export [24]. Contemporary mechanistic syntheses likewise place transport and localization alongside biosynthetic regulation [25]. A platform may reproduce core enzymatic reactions yet remain biologically incomplete if required transport, sequestration, or partner-cell functions are absent.

Localization evidence must be interpreted through analytical limitations. Plant metabolic imaging reveals distributions invisible to bulk analysis, but annotation confidence, ionization behavior, spatial resolution, and quantitative comparability remain method-dependent [26]. Platform decisions should therefore distinguish metabolite localization, transcript localization, and inferred transport. When localization is central, orthogonal confirmation is preferable to a single modality. Organized tissue is not automatically required; rather, localization dependence should be treated as a constraint to test.

A decision logic for platform selection

The proposed decision logic begins with pathway requirements rather than a preferred culture technology. Because specialized metabolism can depend on cell identity, metabolic state, and regulatory context [5, 9, 17], the first question is whether the pathway can remain complete in a dedifferentiated or dispersed state. If multicellular partitioning or tissue-restricted functions are important, the next question is whether those requirements can be preserved, reconstructed, or experimentally bypassed.

Biological compatibility must then be distinguished from fidelity after intervention. Hairy roots illustrate this gate because *Rhizobium*-mediated transformation can alter transcriptomic and metabolic state relative to untransformed roots [27]. Transformation is therefore part of the production phenotype, not a neutral route to root biomass.

The burden of evidence should also rise with pathway complexity. Cannabinoid production illustrates how tissue specialization, precursor management, pathway reconstruction, and process constraints can interact [28]. This

does not establish that complex pathways are unsuitable for cultured cells; it shows why early titre alone is insufficient evidence of robust platform fit.

The framework therefore separates biological compatibility, reconstructability, and operational suitability. A candidate can be retained if the pathway functions naturally in that state, if missing functions can be engineered without unacceptable instability, or if the platform is useful as one stage in a larger workflow. No numerical weights are assigned because the importance of differentiation, manipulation, stability, and scale is pathway-specific.

Table 3 converts this logic into conditional decisions without imposing a universal ranking.

Table 3. Proposed pathway-first decision logic for selecting or retaining a production platform

Decision question	Favorable evidence	Unfavorable or unresolved evidence	Interpretation boundary
Is biosynthesis largely cell-autonomous?	Callus or suspension remains plausible	Preserve or recreate multicellular organization	Bulk abundance does not establish autonomy
Is the required differentiated state retained?	Hairy roots or regenerated plants may fit	Test whether competence can be engineered	Morphology is not chemical fidelity
Are localization and transport requirements reproduced?	Proceed to process evaluation	Validate spatially or use a more organized state	Accumulation may differ from synthesis
Does transformation preserve metabolic state?	Continue engineered development	Reassess construct, host state, or platform	Transformation is part of the phenotype
Can missing functions be reconstructed stably?	Retain the simpler platform	Escalate to another or staged platform	Engineering feasibility is pathway-specific
Is the biologically compatible state process-suitable?	Advance stability and scale testing	Consider parallel or sequential platforms	Biological fit is not scale readiness

Note: The decision sequence is proposed and requires prospective validation.

Figure 2 summarizes the decision architecture and the points at which uncertainty should trigger validation, switching, or staged use rather than forced selection.

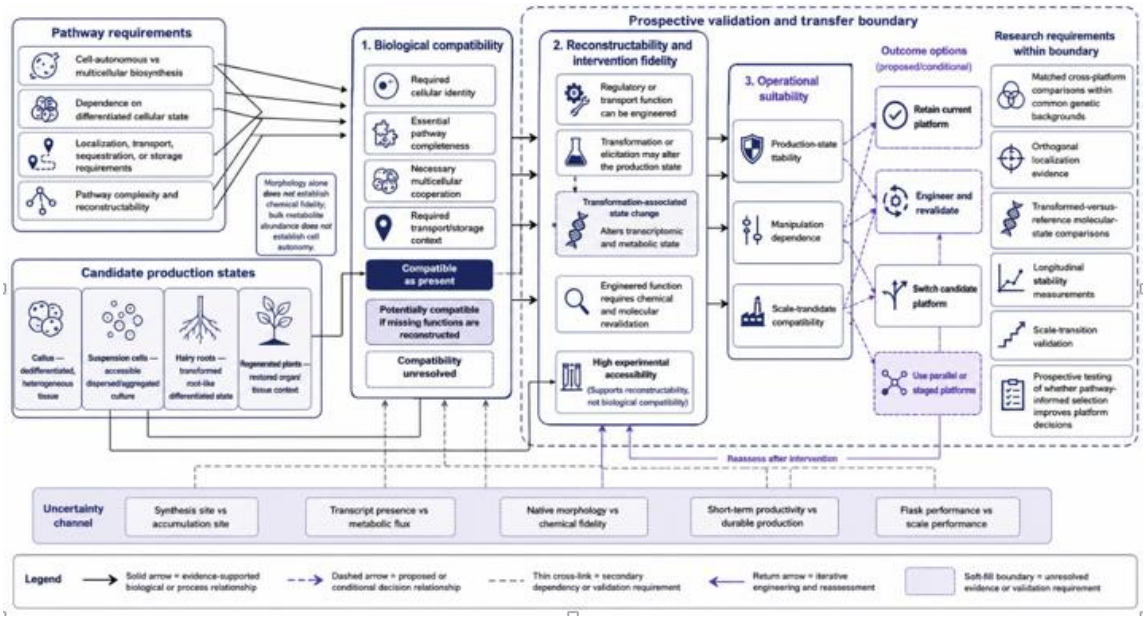

Figure 2. Conditional platform selection across biological fit, reconstructability, and process readiness

Production stability, scalability, and manipulation

Platform fit remains provisional until the productive state can be controlled through time and process change. Hairy roots can support inducible engineering of specialized metabolism, showing that differentiated root cultures can remain actively manipulable [29]. This advantage matters only when the engineered state is reproducible.

Scale is a separate constraint. Plant-cell bioreactors provide several cultivation strategies, but hydrodynamics, mass transfer, morphology, aggregation, and reactor configuration can alter performance during scale transition [30]. Small-scale productivity therefore demonstrates biochemical feasibility, not scalable manufacture.

Long-term suspension production of triterpenic acids has been demonstrated in *Thymus persicus* [31], showing that durable cell-based production is possible in particular systems. Suspension output can also remain strongly elicitation-responsive, as shown for gymnemic-acid production [32]. A productive phenotype may consequently be constitutive, inducible, transient, or adaptation-dependent.

Manipulability additionally depends on transformation architecture. *Rhizobium rhizogenes* strains, vectors, inserted sequences, and host genotype influence transformed-root establishment and behavior [33]. Biological compatibility, long-term stability, and scalability are therefore distinct dimensions rather than a single measure of platform performance [12, 30, 31].

Table 4 separates these operational boundaries from biological platform fit.

Table 4. Operational boundaries requiring validation after biological platform fit

Boundary	Demonstration needed	Interpretation to avoid	Decision consequence
Production stability	Reproducible chemistry over relevant culture duration	One high-producing harvest proves stability	Continue longitudinal testing
Manipulation response	Predictable engineering or elicitation effect	Responsiveness proves platform superiority	Evaluate intervention dependence
Transformation fidelity	Intended change without unacceptable state drift	Transformed tissue equals native tissue	Compare transformed and reference states
Scale transition	Productive culture under altered process conditions	Flask productivity predicts reactor performance	Re-optimize or reconsider platform
Process reproducibility	Controlled variation among batches or lines	Mean titre alone captures robustness	Define acceptable variability experimentally
Biological-process alignment	Pathway requirements remain satisfied during production	Scale and biology optimize independently	Reassess fit after process change

Note: No universal thresholds are proposed; validation criteria should be pathway- and process-specific.

Situations in which no single platform is optimal

Some pathways create requirements that pull platform choice in different directions. Hairy-root engineering in *Beta vulgaris* shows that genetic intervention and elicitation can be layered in one transformed-root system [34], but this does not establish that one culture state will remain optimal from discovery through production.

Transformed roots more broadly provide versatile routes to value-added products while retaining host-, transformation-, morphology-, and scale-related constraints [35]. The same logic applies to regenerated plants, callus, and suspensions: the best system for pathway discovery may differ from that for reconstruction or production.

This creates a role for staged or parallel workflows. Callus or suspension may support rapid perturbation and pathway testing, whereas organized roots or regenerated plants can test whether spatial or developmental requirements have been lost. Conversely, organized systems may reveal dependencies that are later reconstructed in a more processable cellular host. These are proposed strategies, not established superior workflows.

A “no single platform” conclusion is most defensible when spatial dependence remains unresolved, platform creation alters metabolic state, and process requirements conflict with the biology that made the platform attractive. Comparative testing can then identify which functions belong to which development stage rather than forcing premature commitment.

Research priorities

The strongest validation design would compare platforms within the same genetic background, pathway, and analytical workflow wherever biologically feasible. Current comparisons often differ simultaneously in species, genotype, medium, elicitor, developmental stage, and laboratory history. Matched studies would separate platform effects from protocol effects more effectively than literature-level yield comparisons.

Pathway localization should also become an explicit experimental input. Single-cell transcriptomics can identify candidate cellular specialization [7], whereas direct metabolite localization can reveal distributions that

transcription alone does not resolve [21]. Because imaging itself has modality-specific limits [26], high-stakes localization claims should use orthogonal evidence where feasible.

Transformation-associated metabolic drift deserves routine measurement. Direct comparisons demonstrate that transformation can reshape transcriptome and metabolome [27], while transformation technology itself has host- and vector-dependent determinants [33]. Engineered cultures should therefore include reference states capable of distinguishing intended pathway modification from broader reprogramming.

Stability and scale require longitudinal rather than snapshot evidence. Reactor transition can alter the physical environment experienced by cultured cells [30], and long-term suspension production, although possible, remains system-specific [31]. Time-course chemical, morphological, and molecular measurements would help distinguish durable production states from transient ones.

Finally, the proposed framework should be tested prospectively. Its value would lie in helping investigators reject biologically mismatched platforms earlier, identify when additional localization evidence is needed, and recognize when staged development is more defensible than single-platform commitment.

Conclusion

Callus, suspension cells, hairy roots, and regenerated plants are not interchangeable containers for specialized-metabolite pathways. Each combines cellular differentiation, spatial organization, manipulability, stability, and process accessibility differently. The central contribution of this article is therefore a proposed pathway-first decision logic that asks what biological functions the target pathway requires before asking which culture technology is most convenient.

This approach avoids two simplifications. Dedifferentiated systems should not be dismissed because they lack intact tissue organization; they may retain substantial biosynthetic capacity and provide exceptional control. Differentiated or regenerated systems should likewise not be assumed chemically faithful because their morphology resembles native tissue. Transformation, developmental reprogramming, transport, storage, and production conditions can alter the productive state.

A defensible choice consequently separates biological compatibility, reconstructability, operational stability, and scalability. Where these dimensions align, one platform may suffice. Where they conflict, parallel testing, staged development, or switching may be appropriate. These propositions remain pathway-specific and require matched comparisons, orthogonal localization evidence, longitudinal stability measurements, and prospective evaluation before the proposed architecture can be treated as predictive.

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