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A claim-specific evidence-boundary model for plant UDP-glycosyltransferases: from substrate candidates to native function

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UDP-glycosyltransferase (UGT) research now generates far more candidate genes and metabolites than can be assigned experimentally. This Hypothesis and Theory article introduces a claim-specific evidence-boundary model developed from potential inferential transitions encountered when interpreting published UGT evidence; no new experimental data are presented. The model separates two entry filters from two complementary but non-interchangeable claim axes. Experimental accessibility determines which UGT–acceptor–donor combinations can be tested, whereas predictive prioritization orders testing and can redirect efforts to obtain compounds or donors. Once a defined combination enters an experiment, enzyme-dependent product detection and structural characterization delimit the chemical claim, while heterologous reconstruction and native perturbation delimit biological claims. We hypothesize that new evidence will preferentially increase confidence in the claim directly addressed by that evidence, whereas cross-axis evidence alone will not resolve an outstanding chemical or biological claim. The model extends identification and reporting guidance to UGT reaction and native-function claims. It predicts uneven confidence gains across the chemical and biological axes and identifies transitions where claims may outpace evidence. The model generates three testable predictions. Aligned evidence should produce larger confidence gains for the corresponding claim than for a non-aligned claim. Claim inflation should be enriched at prediction-to-reaction, feature-to-structure, and non-native-to-native transitions relative to other evaluable inferential transitions. Boundary-specific reporting should improve inter-evaluator agreement and reduce misclassification when factual information is held constant. The framework may support study design, peer review, and reusable UGT records.

KEYWORDS

functional attribution, machine learning, metabolite annotation, non-model plants, specialized metabolism, structural identification, substrate discovery, UDP-glycosyltransferases

1 Introduction: from candidate genes to credible plant function

Plant UDP-glycosyltransferases (UGTs) contribute extensively to the chemical diversity of specialized metabolism by modifying flavonoids, terpenoids, phenolic acids, alkaloids, phytohormones, and other metabolites. Glycosylation can alter solubility, stability, transport, storage, and biological activity, with consequences for defense, development, and environmental responses (He et al., 2022; Louveau and Osbourn, 2019; Vasudevan and Lee, 2020). The central challenge is no longer finding candidate UGT genes. It is assigning substrates and biological functions with evidence sufficient for the claim.

This challenge has grown with expanding genome and transcriptome resources. Individual plant genomes can contain hundreds of candidate UGTs. For example, 158 UGT genes were annotated in *Panax notoginseng* (Burkill) F.H. Chen and 339 members were reported in *Epimedium pubescens* Maxim (Jiang Z.Q. et al., 2021; Yao et al., 2022). These study-specific counts illustrate the scale of candidate discovery rather than a directly comparable census across species. Only a small fraction of plant UGTs has been experimentally linked to defined reactions (He et al., 2022; Liu et al., 2023). Functional assignment therefore depends on defensible links among candidate genes, metabolites, enzyme reactions, and biological effects.

Several inferential boundaries separate candidate discovery from function. Chemical and donor availability define what can be tested. Metabolomics expands candidate selection but does not resolve every chemical identity (Bönisch et al., 2014; Saint-Vincent et al., 2023; Wu et al., 2022; Zhang et al., 2022). Product detection raises a separate structural question because reaction-associated signals, mass-spectrometric features, and orthogonally confirmed products support different chemical claims (Zhang et al., 2020; Lethe et al., 2024).

These problems are especially important in non-model plants. Here, the term refers operationally to species in which stable transformation, mutant collections, curated metabolite databases, authentic standards, or pathway-specific genetic resources are limited or unevenly available. Many medicinal plants, specialty crops, wild relatives, and chemically rich lineages fit this definition. Previous reviews have covered plant glycosylation pathways, natural-product diversification, UGT mining, and enzyme engineering (He et al., 2022; Louveau and Osbourn, 2019; Lu et al., 2023; Vasudevan and Lee, 2020).

This article addresses the evidential gap between candidate discovery and supported functional claims with a claim-specific evidence-boundary model. The model was developed conceptually from potential points of inferential overreach in the published UGT studies cited throughout the article; it is not a systematic evidence synthesis and introduces no new experimental dataset. Representative studies illustrate the evidence transitions and alternative explanations that motivate the model rather than establish the frequency of claim inflation or provide an inventory of all characterized plant UGTs. A claim-specific evidence boundary is the evidential limit for a specified conclusion; it marks the point beyond which that conclusion is not supported. The central hypothesis is that new evidence will preferentially increase confidence in the claim directly addressed by that evidence, whereas cross-axis evidence alone will not resolve an outstanding chemical or biological claim. From this hypothesis, three predictions are derived concerning claim-specific confidence transfer, the distribution of claim inflation, and the reproducibility gained through boundary-specific reporting.

2 The claim-specific evidence-boundary model

The model separates two entry decisions from two post-test claim axes (Box 1). Accessibility asks whether a proposed UGT–acceptor–donor combination can be tested; prioritization asks which combination should be tested first. These entry filters interact iteratively: a high-priority but inaccessible candidate can redirect compound preparation, donor acquisition, or assay development. After testing, the chemical axis addresses reaction occurrence and product identity, while the biological axis addresses heterologous cellular feasibility and native contribution. Each axis contains claim nodes, meaning the specific questions to which evidence is assigned. These distinctions describe the organization of the model, not successive confidence levels. Figure 1 presents the architecture before Sections 3–5 examine its components.

The chemical axis addresses whether a defined reaction occurred and what product formed; the biological axis addresses whether the reaction is feasible in cells and contributes in the source plant. Evidence on one axis cannot substitute for evidence on the other: an identified *in vitro* product does not establish native function, and a native metabolic change does not by itself identify the affected compound. "Partially dependent" refers to a limit on interpretation, not a requirement that the axes advance together. If perturbing a UGT changes only an unresolved liquid chromatography–mass spectrometry (LC-MS) feature, the native claim concerns that feature or its pathway context; it cannot name a specific glycoside beyond the available structural evidence. Identifying the feature later may change how the perturbation is interpreted in its

Abbreviations: 1D, one-dimensional; 2D, two-dimensional; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; FAIR, findable, accessible, interoperable, and reusable; GFP, green fluorescent protein; GWAS, genome-wide association study; HRMS/MS, high-resolution tandem mass spectrometry; LC-HRMS, liquid chromatography coupled to high-resolution mass spectrometry; LC-HRMS/MS, liquid chromatography coupled to high-resolution tandem mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; LC-MS/MS, liquid chromatography coupled to tandem mass spectrometry; MD, molecular dynamics; MRM, multiple reaction monitoring; MSI, Metabolomics Standards Initiative; MS/MS, tandem mass spectrometry; m/z, mass-to-charge ratio; NMR, nuclear magnetic resonance; PRM, parallel reaction monitoring; QM/MM, quantum mechanics/molecular mechanics; RNA, ribonucleic acid; RNAi, RNA interference; T-DNA, transfer DNA; UDP, uridine diphosphate; UDP-Glc, uridine diphosphate glucose; UGT, UDP-glycosyltransferase; VIGS, virus-induced gene silencing; WGCNA, weighted gene co-expression network analysis.

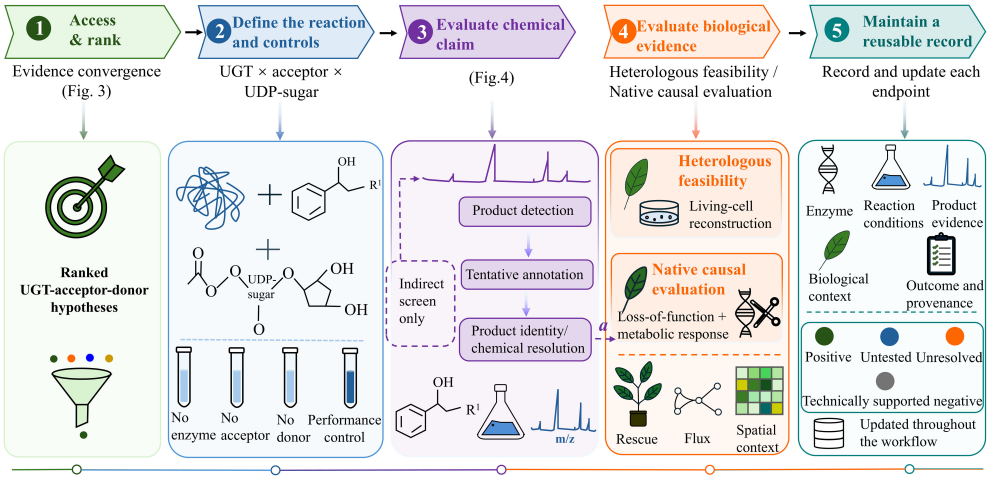


FIGURE 1
UGT evidence assessment and the interpretive dependency between chemical and biological claims. Accessibility and prioritization determine which UGT–acceptor–donor combination enters testing. Defined assays and controls support assessment of reaction occurrence, while product detection, annotation, and structural evidence delimit product identity and chemical resolution. The model has four claim nodes: reaction occurrence, product identity, heterologous cellular feasibility, and native contribution. In the biological panel, "Heterologous feasibility" denotes the heterologous cellular feasibility node, while "Native causal evaluation" denotes assessment of the native contribution node. Living-cell reconstruction, loss-of-function and metabolic responses, rescue, flux, and spatial context are evidence components supporting these questions, not additional claim nodes. Product detection and tentative annotation are chemical evidence endpoints, not additional claim nodes. Gain-of-function evidence, discussed in Section 5.3 and Table 3, also informs native contribution but requires attention to dosage and ectopic expression. The purple dashed arrow (a) links product identity and chemical resolution to native biological evaluation. It denotes two interpretive constraints: pathway-claim specificity cannot exceed the chemical resolution of the affected feature, and chemical identity can alter the biological interpretation of a perturbation. An unresolved feature can support a feature-level response but cannot by itself justify attribution to a specific metabolite or pathway step. Chemical identity alone does not establish native causation, and biological perturbation alone does not resolve product structure. The dashed arrow does not represent a causal effect, an estimated probability, or a required experimental sequence. Records retain evidence, experimental context, provenance, and boundary-specific outcomes throughout the assessment. UGT, UDP-glycosyltransferase. The numbered panels and solid connectors summarize an illustrative workflow; they do not require both claim axes to advance in a fixed sequence. Reaction outcomes shown in the record panel are condition-specific assay states, not structural-identification categories. m/z, mass-to-charge ratio.

pathway context, but chemical identification alone does not show that the UGT caused the native change. Figure 1 shows this one-way interpretive constraint with a dashed link.

The central hypothesis is that new evidence will preferentially increase confidence in the claim directly addressed by that evidence, whereas cross-axis evidence alone will not resolve an outstanding chemical or biological claim. The novelty of the model lies not in asserting that stronger evidence is preferable. It lies in separating entry decisions from post-test claims, predicting asymmetric confidence transfer across partially dependent axes, and locating transitions at which claim inflation should be enriched. These propositions make the model open to empirical challenge rather than treating it only as a reporting prescription.

Box 1 consolidates the terminology by layer. Claim-specific confidence transfer means the change in evaluator confidence for the claim directly addressed by new evidence; cross-axis evidence addresses a question on the other claim axis. Boundary-specific reporting organizes evidence and unresolved questions by claim node. Entry filters, claim axes, and claim nodes describe the model's structure. Evidence states describe the observations and their experimental context; claim categories and claim strength describe the conclusion being advanced. Evaluator confidence and calibration describe its assessment. Inflation and deflation are the two directions of miscalibration, not additional evidence states or claim nodes. For a tested UGT–acceptor–donor combination, the evidence state is assessed against the category and stated strength of the claim. An evaluator judges its support, while calibration compares its wording with what the evidence warrants. Evaluator confidence can change without the claim becoming better calibrated. The directed schematic accompanying Box 1 shows these assessment dependencies for each claim node. Claim category and strength together describe the stated claim; the evidence state and stated claim inform evaluator confidence and calibration separately. The schematic is acyclic but does not specify a fitted Bayesian network, conditional probabilities, or statistical conditional independence.

Table 1 maps the scope of the Metabolomics Standards Initiative (MSI), the Schymanski confidence levels, and the FAIR (findable, accessible, interoperable, and reusable) principles onto the entry filters, claim nodes, and evaluation fields defined in Box 1. MSI provides minimum reporting standards for chemical analysis, including sample preparation, quality control, and metabolite identification (Sumner et al., 2007). The Schymanski levels communicate confidence in a proposed small-molecule structure from high-resolution mass spectrometry (Schymanski et al., 2014). These frameworks inform the chemical evidence state, especially product identity; neither specifies criteria for attributing a reaction to a particular UGT or establishing its native contribution. FAIR addresses the findability, accessibility, interoperability, and reusability of data and metadata (Wilkinson et al., 2016). It therefore applies

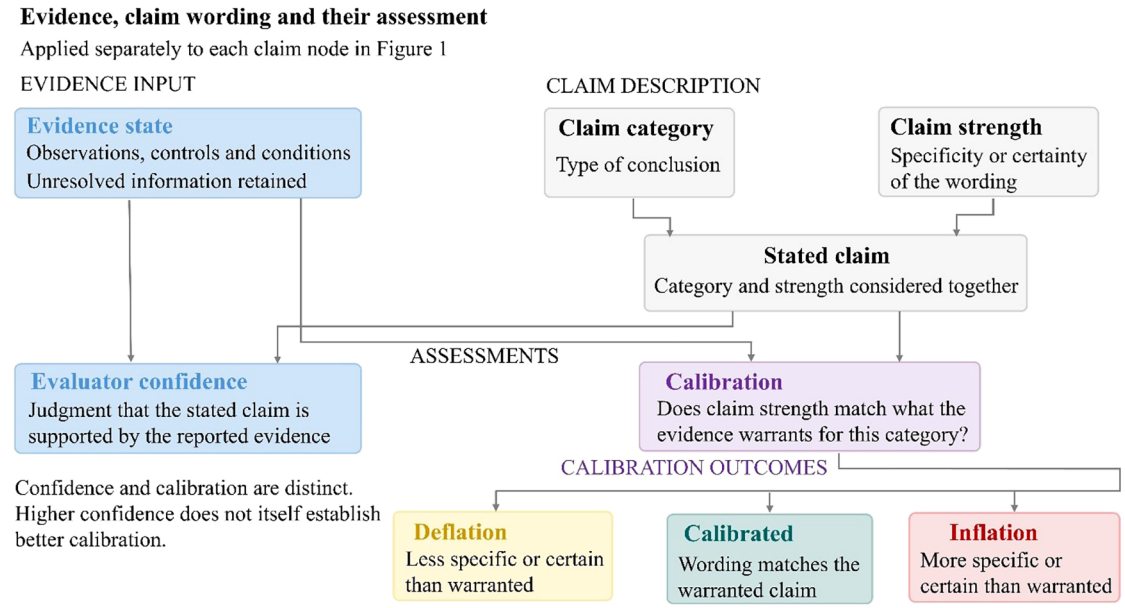
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BOX 1 Consolidated definitions by layer in the claim-specific evidence-boundary model.

Layer	Term	Definition	Illustrative UGT example
Model structure	Entry filters	Accessibility determines what can be tested; prioritization determines what is tested first. Neither establishes activity.	An unavailable plant metabolite is prioritized from co-expression, but the assay awaits compound isolation and the relevant UDP-sugar.
Model structure	Claim axes	The chemical axis concerns reaction occurrence and product identity; the biological axis concerns heterologous feasibility and native contribution.	A purified UGT forms a product *in vitro*, while its role in the source plant remains untested.
Model structure	Claim nodes	Specific questions within an axis: did the reaction occur; what product formed; is production feasible in a heterologous host; and does the UGT contribute in the source plant? Nodes are not evidence types.	An enzyme-dependent product feature addresses reaction occurrence; identifying that product addresses a separate chemical node.
Evidence description	Evidence state	The observations, controls, conditions, and unresolved information available for a particular claim node.	An enzyme-dependent LC-HRMS/MS feature appears with a defined acceptor and UDP-Glc, but no authentic product standard is available.
Claim description	Claim category	The type of conclusion stated about a node, such as reaction activity, product identity, heterologous feasibility, or native contribution.	"This UGT forms a product *in vitro*" is a reaction claim; "it supplies that metabolite in planta" is a native-contribution claim.
Claim description	Claim strength	The specificity or certainty of the wording within a claim category.	"A hexoside-like product" is less structurally specific than naming a particular glucoside and linkage.
Claim evaluation	Evaluator confidence	An evaluator's judgment that the stated claim is supported by the reported evidence; it is not a new observation or a measure of calibration.	A reader rates support for the named product as low when only accurate mass is reported.
Claim evaluation	Calibration	Correspondence between stated claim strength and what the evidence warrants for that category.	A controlled product feature is reported as enzyme-dependent formation of an unresolved glycosylated product.
Claim evaluation	Inflation	Upward miscalibration: a claim is more specific or certain than the evidence warrants.	Co-expression alone is described as proof that a UGT catalyzes a particular plant reaction.
Claim evaluation	Deflation	Downward miscalibration, or under-claiming: a claim is less specific or certain than the evidence warrants.	After authentic-standard agreement under identical conditions, the authors still call the product only an unknown feature.

The examples are hypothetical illustrations, not new UGT experiments. LC-HRMS/MS, liquid chromatography coupled to high-resolution tandem mass spectrometry; UDP-Glc, uridine diphosphate glucose. A claim node specifies the question being assessed; a claim category classifies the conclusion stated about that question. Evaluator confidence is a judgment of support, whereas chemical-identification confidence is part of the chemical evidence state. The structural terms and the descriptive and evaluative fields belong to different layers, not competing classifications of the model.

Directed assessment dependencies.



Arrows: conceptual assessment dependencies. No causal effects or conditional probabilities are specified. Definitions: Box 1.

The schematic applies separately to reaction occurrence, product identity, heterologous cellular feasibility, and native contribution in Figure 1. Category and strength together describe the stated claim; this grouping is not an additional claim node. The evidence state and stated claim inform evaluator confidence and calibration separately. Deflation, calibrated wording, and inflation are calibration outcomes, not additional evidence states. Arrows denote conceptual assessment dependencies, not causal effects or an experimental sequence. Crossing lines without a junction do not connect. No conditional probabilities or statistical conditional independence are specified; this is not a fitted Bayesian model. Definitions and hypothetical UGT examples are given in the preceding glossary.

TABLE 1 Mapping established frameworks to the components of the UGT evidence-boundary model.

Model component	MSI	Schymanski	FAIR	Proposed model: contribution and unresolved questions (not coded)
Entry filters: accessibility and prioritization	N	N	N	Separates physical testability from testing priority; a prediction remains untested until experimentally examined.
Chemical node: reaction occurrence	N	N	N	Assesses UGT-dependent reaction occurrence under stated conditions; product identity and native contribution may remain open.
Chemical node: product identity	D	D	N	Links identification evidence to the product-identity claim while retaining unresolved structural ambiguity.
Biological node: heterologous cellular feasibility	N	N	N	Assesses production in the tested host and conditions; source-plant contribution remains unresolved.
Biological node: native contribution	N	N	N	Assesses native contribution while retaining limits from chemical resolution, perturbation design, and biological context.
Claim strength, evaluator confidence, and calibration	N	N	N	Separates claim wording, evaluator confidence, and calibration; predicted confidence transfer and transition-specific inflation remain untested.
Reusable records across nodes	P	N	D	Links evidence and context to each claim node; record reuse does not itself validate a functional claim.

Coverage code: D, directly addressed; P, explicitly addressed in part; N, no component-specific criteria. D denotes explicit criteria for the component as defined in Box 1; P denotes explicit requirements for a specified subset of that component; N denotes no component-specific criteria, even when a framework can support the underlying data or reporting. General applicability, chemical identification, or data reuse alone does not establish coverage of a different UGT claim node or of evaluator confidence and calibration.
Coding concerns the scope of MSI chemical-analysis reporting standards (Sumner et al., 2007), Schymanski identification levels (Schymanski et al., 2014), and FAIR principles (Wilkinson et al., 2016), rather than their general usefulness. MSI and Schymanski directly address product identification. MSI receives P for reusable records because its chemical-analysis metadata requirements cover a defined subset of records, rather than records across all claim nodes; Schymanski identification levels do not specify record-reuse requirements and receive N for that row. FAIR directly addresses reusable data and metadata, but specifies no criteria for the entry filters or functional claim nodes. The evaluation row concerns UGT claim strength, human evaluator confidence, and calibration as defined in Box 1, rather than chemical-identification confidence levels. N indicates a difference in scope, not a deficiency. FAIR digital accessibility differs from physical experimental accessibility. The final column is descriptive and is not assigned D/P/N codes; the proposed model's predictions remain untested.

across records supporting all four claim nodes, but does not determine which functional conclusion those records warrant. FAIR accessibility concerns access to digital objects; the model's accessibility filter concerns whether a UGT–acceptor–donor combination can be tested. For example, identifying an *in vitro* product by comparison with an authentic standard can strengthen a product-identity claim without establishing the UGT's contribution in the source plant. Making that experiment's data FAIR supports retrieval and reuse without supplying the missing native evidence. The proposed model adds an explicit assessment of support at each claim node and predicts asymmetric changes in evaluator confidence and enrichment of inflation at specified inferential transitions. These predictions remain to be tested. The hypothetical cumulative ladder is a separate comparator that ranks a study by its furthest result and treats earlier uncertainty as resolved; it is not an interpretation attributed to MSI, Schymanski, or FAIR. Table 1 codes only the three established frameworks; its final, uncoded column describes the proposed model's contribution and unresolved questions. D denotes explicit criteria for the component as defined in Box 1; P denotes explicit requirements for a specified subset of that component; N denotes no component-specific criteria, even when a framework can support the underlying data or reporting. General applicability, chemical identification, or data reuse alone does not establish coverage of a different UGT claim node or of evaluator confidence and calibration.

Box 1 is the common reference for the terms used below. Sections 3–5 examine the two entry filters and the two claim axes. Section 6 applies these definitions to experimental decisions and boundary-specific outcome states, Section 7 considers reusable records, and Section 8 specifies three tests of interpretive construct validity, the distribution of claim inflation, and reporting utility. The examples motivate and pressure-test the architecture; they do not predetermine the outcomes of those tests.

3 Accessibility and prioritization as entry filters

Only a fraction of plant chemical space reaches an enzyme assay: sampling, detection, structural annotation, compound supply, and UDP-sugar availability all restrict the tested combinations. An observed substrate profile thus reflects access as well as enzyme chemistry (Figure 2).

Figure 2 summarizes these restrictions. Sampling and analytical response determine which features are recorded; annotation and statistical selection determine which receive further attention. Routes such as purchase, plant isolation, chemical synthesis, and microbial production then determine which proposed acceptors can be tested, while UDP-sugar availability independently constrains donor choice. Additional literature examples are grouped by boundary in Supplementary Table S1.

3.1 Accessibility defines the testable reaction space

Commercial panels support reproducible screening but favor available, stable compounds. Broad phenolic screens in *Vitis vinifera* L. illustrate the gain in coverage within a defined panel, not coverage of untested plant metabolites (Bönisch et al., 2014). Multiplexing increases throughput without changing this boundary (Sirirungruang et al., 2025). Studies of tea UGTs involved in the

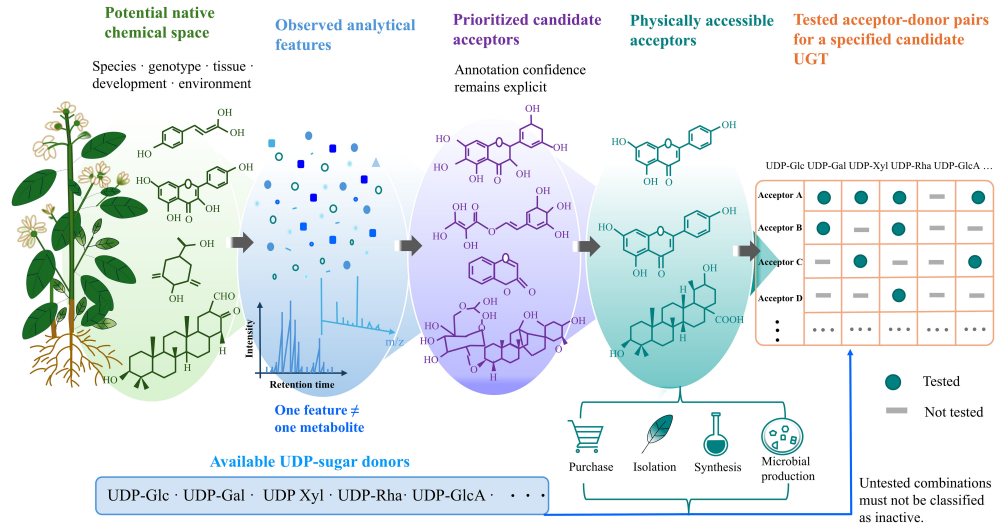


FIGURE 2
Evidence boundaries defining the accessible reaction space in plant UGT substrate discovery. Sampling, analytical detection, candidate prioritization, physical substrate access, and UDP-sugar availability progressively restrict the combinations testable for a specified candidate UGT. Analytical features are not equivalent to unique metabolites, and untested combinations should not be classified as inactive. UGT, UDP-glycosyltransferase. Circles indicate tested combinations; gray bars indicate combinations not tested and do not denote inactivity. UDP-Gal, uridine diphosphate galactose; UDP-Xyl, uridine diphosphate xylose; UDP-Rha, uridine diphosphate rhamnose; UDP-GlcA, uridine diphosphate glucuronic acid; UDP-Glc, uridine diphosphate glucose; m/z, mass-to-charge ratio.

biosynthesis of galloylated catechins and flavonol glycosides illustrate the same boundary: recombinant assays establish activity toward the acceptors tested, but do not establish coverage of untested endogenous substrates (Cui et al., 2016).

Plant metabolomics can redirect attention to endogenous candidates: *Arabidopsis thaliana* (L.) Heynh. *UGT76B1* mutants and overexpression lines revealed isoleucine-derived metabolites for biochemical follow-up (von Saint Paul et al., 2011). The initial observation is an analytical feature defined by mass-to-charge ratio (m/z), retention time, and signal intensity, rather than a purified structure. Such a feature may guide prioritization but cannot establish product identity.

Glycoside-focused workflows extract further information from neutral losses, precursor labeling, and fragmentation patterns (Wu et al., 2022). They may reveal a shared aglycone, related glycosides, or successive modifications of one scaffold, particularly when authentic standards are unavailable. The result is a chemically informed candidate relationship; the proposed acceptor must still be isolated, synthesized, or produced before direct enzyme testing.

Sampling design shapes discovery. A native acceptor may be restricted to one cell type, developmental phase, or stress response; bulk extraction can dilute it, and rapid turnover can keep free abundance low despite substantial flux. Rankings based on abundance therefore favor stable metabolites. Candidates entering assays retain their sampling context and annotation confidence.

Isolation can preserve the stereochemical form present in the source plant; flavonoids isolated from *Glycyrrhiza uralensis* Fisch. ex DC. were used directly in recombinant UGT assays (Jiang D. et al., 2021). Chemical synthesis can instead provide positional isomers, analogues, or defined series for testing regioselectivity (Bidart et al., 2022; Guo et al., 2022). Both routes are limited by abundance, instability, purification losses, stereochemical complexity, or multistep chemistry.

Engineered microorganisms can supply scarce acceptors when relevant pathway steps are known (Wang et al., 2020). Further examples are summarized in Supplementary Table S1. Material supply does not establish product identity; purification and structural assessment remain necessary.

A negative assay with uridine diphosphate glucose (UDP-Glc) alone does not exclude a plausible acceptor: some family 1 UGTs use alternative UDP-sugars (Gharabli and Welner, 2024). Donor choice is therefore part of the tested combination, not a background condition. The construction of a UDP-arabinose regeneration system for triterpenoid arabinosylation illustrates how donor regeneration can expand the reactions accessible to testing; however, the resulting evidence remains specific to the donor and acceptor conditions examined (Sun et al., 2023).

Arabidopsis UGT89A2 makes this boundary concrete. The Col-0 isoform strongly favors UDP-xylose, whereas the C24 isoform can use UDP-glucose and UDP-xylose (Chen and Li, 2017). A UDP-glucose-only screen could incorrectly rank these closely related variants.

The first screen need not include every possible UDP-sugar. Sugar residues already present in the plant metabolome provide a stronger basis for donor selection. Accumulation of rhamnosides or xylosides, particularly when supported by phylogenetic position or donor-binding residues, justifies testing the corresponding donors. Expanding the panel without metabolic or structural guidance increases cost and produces more reactions of uncertain relevance.

Donor choice also affects interpretation of a positive result. Formation of a glucoside under an imposed UDP-Glc condition demonstrates chemical feasibility under that condition. Native relevance is less certain when the plant accumulates a different sugar conjugate or when the supplied donor is unlikely to occur in the same cellular setting as the enzyme and acceptor.

Source-species chemistry can guide acceptor acquisition and donor selection. Marginal hits are best reported as catalytic capacity under the stated conditions unless kinetics, tissue chemistry, or genetic evidence supports a native substrate relationship (Sirirungruang et al., 2025). Failure to detect a product defines only the tested acceptor–donor space.

3.2 Predictive evidence orders experimental testing

Possible UGT–acceptor–donor combinations exceed experimental capacity. Prioritization therefore determines which compounds are obtained, proteins expressed, and analytical resources used. It is iterative: predictive support can justify investment in making an inaccessible candidate testable, while failed acquisition can return a candidate to the ranking stage.

Covariation, phylogeny, structural modeling, and machine learning offer complementary reasons to select a combination for testing (Chen et al., 2025; Saint-Vincent et al., 2023; Yang et al., 2018). Covariation identifies spatial or temporal pathway associations; phylogenetic placement supplies an evolutionary prior from characterized relatives. Structural modeling addresses active-site compatibility, whereas spectral or authentic-standard matching addresses chemical identity. These inputs are not interchangeable, and none of the predictive inputs directly establishes a reaction. Published studies illustrate these complementary sources of candidate-prioritization evidence. Combined transcriptomic and metabolomic analysis has been used to investigate genes associated with triterpenoid biosynthesis in Cyclocarya paliurus (Chen et al., 2024). Genome-wide UGT analysis in peach examined family composition, expression patterns, and functional features (Wu et al., 2017). A genome-wide study in pomelo characterized UGT family evolution and expression profiles (Wu et al., 2020). Such analyses can guide candidate selection, but do not by themselves establish a specific UGT-catalyzed reaction.

Metabolite–gene covariation can reflect shared regulation, neighboring reactions, or mixed cell populations rather than catalysis. A misannotated feature also misdirects the proposed substrate name; its identifier, m/z, retention time, adduct, and annotation level should remain traceable.

Lack of correlation is also inconclusive because acceptor turnover, delayed glycoside accumulation, or constitutive UGT expression can uncouple transcript and metabolite abundance. Covariation is most informative when spatial and temporal patterns fit the proposed pathway.

Formal UGT nomenclature and phylogenetic grouping are different systems. Recommended nomenclature uses sequence divergence to define families and subfamilies, commonly applying about 40% amino acid identity for family membership and 60% for subfamily membership (Mackenzie et al., 1997). Plant UGT phylogenies provide evolutionary context rather than direct functional labels, including when groups beyond A–N are considered (Li et al., 2001; Wilson and Tian, 2019).

Close sequence similarity does not guarantee identical acceptor ranges or regioselectivity. Family 1 UGTs share a conserved fold and donor-binding region, but acceptor recognition depends on local variation in pocket geometry and residues. Duplication and divergence can separate paralog functions, while more distant enzymes can converge on similar chemistry.

Docking can reject grossly incompatible poses but cannot measure catalytic activity. Pocket conformation, protonation, UDP-sugar placement, and reactive geometry affect its interpretation; favorable scores remain prioritization evidence.

Model quality limits interpretation. Experimental structures generally provide the strongest starting geometry. Homology and AlphaFold models are less certain around flexible loops, side chains, and ligand-induced rearrangements. Water, ionic conditions, protonation, and inclusion of the UDP-sugar can also alter rankings. Reproducibility depends on reporting these choices.

Molecular dynamics (MD) can test whether a proposed pose remains stable and whether catalytic distances fluctuate over time. Quantum mechanics/molecular mechanics (QM/MM) approaches can examine reaction pathways and energetic barriers. These methods are especially informative after activity has been observed, when the question concerns regioselectivity, catalytic residues, or mutation effects (Bi et al., 2022; Li et al., 2024). Combined modeling, mutagenesis, and product analysis can then support a mechanistic interpretation.

Training data often limit machine learning. Published records favor available acceptors, common UDP-sugars, and well-studied UGTs; reliable negatives are scarce. Apparent negatives may reflect poor protein expression, unsuitable donors, inadequate sensitivity, or unsuitable conditions.

GT-Predict combined experimental activity data with bioinformatic features to predict glycosyltransferase activity (Yang et al., 2018). Protein-language-model and structural representations have also been used to classify broader substrate preferences across UGT sequence space (Chen et al., 2025). Such models can rank far more candidate relationships than can be tested directly.

Data splits based on sequence-cluster, lineage, source-study, or acceptor scaffold better assess transfer to unfamiliar cases than random splits. Predicted substrate class should not be expanded into a site- or isomer-specific claim without corresponding product evidence.

Predictive convergence is informative only when inputs are not redundant. Outputs derived from the same sequence, samples, or structural model do not constitute independent confirmations, and predictive support cannot exceed the structural confidence of the metabolite candidate.

Disagreement among inputs can identify what needs checking. Strong metabolite–gene covariation with poor structural compatibility may indicate an annotation error, an incorrect UGT candidate, or a deficient structural model. Phylogenetic support without detection of the relevant metabolite may instead reflect sampling or analytical limitations.

A traceable matrix retains covariation, phylogenetic context, structural compatibility, machine-learning output, annotation confidence, and compound or donor availability as separate inputs. Conflicts and missing information remain flags, not evidence. The output is a finite set of testable UGT–acceptor–donor hypotheses requiring controlled biochemical and product-level evidence. Figure 3 shows how these inputs converge without being counted as independent confirmation.

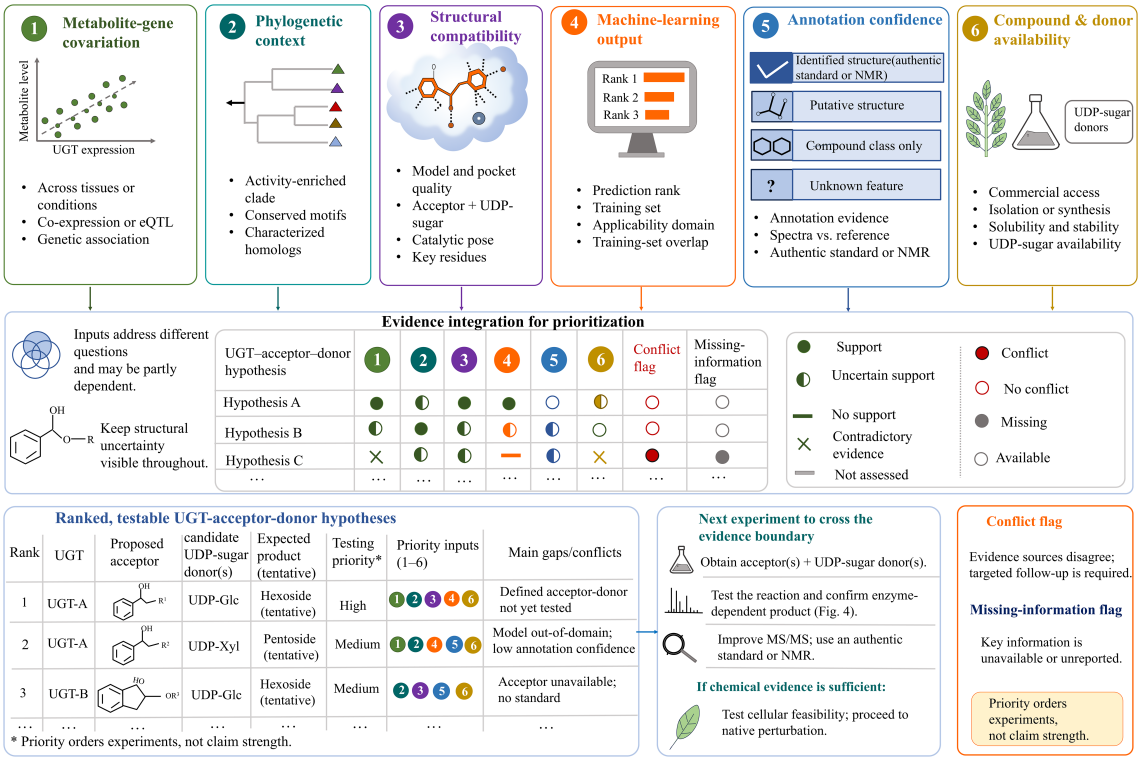


FIGURE 3
Evidence convergence for prioritizing testable UGT–acceptor–donor hypotheses. Metabolite–gene covariation, phylogenetic context, structural compatibility, machine-learning output, metabolite-annotation confidence, and candidate-compound and UDP-sugar availability are retained as separate inputs. These inputs may be partially dependent and should not be counted as independent confirmations. Conflict and missing-information flags identify disagreements and critical gaps without constituting additional evidence sources. Integration of the available information informs testing priority, not claim strength or substrate assignment. Prioritized hypotheses still require enzyme-dependent product detection with appropriate controls and structural evidence matched to the claim. UGT, UDP-glycosyltransferase. The lower-right panel lists possible follow-up experiments, rather than a required sequence: heterologous reconstruction and native perturbation address different biological questions and may be selected independently. Colors identify the numbered input sources, not additional confidence levels. eQTL, expression quantitative trait locus; MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance.

4 Chemical claim axis: from product features to defensible structures

Once a defined UGT–acceptor–donor combination enters an *in vitro* assay, the result may appear as a chromatographic peak, an ion feature, a fragmentation spectrum, or a change in a reaction-coupled signal. UDP release or a pH shift indicates that the reaction mixture has changed, whereas an enzyme-dependent mass-spectrometric feature provides more direct evidence of product formation. Product naming requires additional evidence.

Quantification addresses a separate question. Multiple reaction monitoring (MRM) and parallel reaction monitoring (PRM) can measure a sufficiently defined product with high sensitivity, but quantitative precision does not resolve structural uncertainty. Product detection, tentative structural annotation, structural confirmation or elucidation, and quantitative analysis should therefore remain distinct. Table 2 makes this distinction explicit by linking

TABLE 2 Analytical endpoints in glycosyltransferase product analysis and their evidential boundaries.

Endpoint	Typical readout	Supported claim	Boundary
Reaction signal	UDP, pH, or radiometric assay	Transfer-associated signal	Product unidentified
Product feature	LC-HRMS(/MS)	Enzyme-dependent glycosylated feature	Sugar, linkage, and stereochemistry unresolved
Tentative annotation	HRMS/MS; library or network match	Glycoside class or candidate structure	Isomers may remain unresolved
Quantification	MRM or PRM	Amount of a defined analyte	No added structural confidence
Confirmation/elucidation	Standard matching; isolation and NMR	Known product confirmed or novel structure elucidated	Absolute sugar or chirality may remain

Analytical endpoints are classified by the question they answer rather than by instrument performance. Greater sensitivity or resolving power does not automatically increase structural confidence. LC-HRMS(/MS), liquid chromatography coupled to high-resolution mass spectrometry, with or without tandem mass spectrometry; HRMS/MS, high-resolution tandem mass spectrometry; MRM, multiple reaction monitoring; PRM, parallel reaction monitoring; NMR, nuclear magnetic resonance; UDP, uridine diphosphate.

each analytical endpoint to the inference it supports and the structural boundary that remains. Figure 4 places these endpoints along the chemical claim axis, from indirect reaction signals to structural confirmation or elucidation.

4.1 Product detection requires enzyme-dependent features and proper controls

An expected mass alone is insufficient. A product feature should depend on the complete reaction and be checked against no-enzyme, heat-inactivated-enzyme, no-acceptor, and no-donor controls where applicable (Guo et al., 2022; Jiang D. et al., 2021).

Accurate mass restricts possible molecular formulas. Isotope patterns and relationships among protonated, deprotonated, metal-adduct, and ammonium-adduct ions help determine whether several signals arise from one compound. Further support comes from tandem mass spectrometry (MS/MS) fragments related to the acceptor and a plausible neutral loss corresponding to an attached sugar residue (Ren et al., 2025; Speeckaert et al., 2020). Together, these observations support an enzyme-dependent product feature but may not establish its complete chemical identity.

Weak ionization, matrix suppression, instability, co-elution, acquisition windows, or restrictive processing can obscure a product. "Not detected" therefore describes the stated analytical conditions, not necessarily inactivity of the UGT–acceptor–donor combination.

4.2 MSI levels separate structural annotation from identification

MSI distinguishes identified metabolites from putative annotations, compound classes, and unknowns; Schymanski et al. offer a complementary, non-equivalent scale for high-resolution MS identification (Sumner et al., 2007; Schymanski et al., 2014). Accurate mass and MS/MS alone generally warrant a qualified annotation, not an unambiguous structure.

One compound may generate several analytical features through adducts, isotope peaks, dimers, or in-source fragments, while different compounds may share the same elemental composition and precursor mass. Positional isomers, stereoisomers, and glycosides containing different monosaccharides may therefore remain indistinguishable without orthogonal evidence.

Neutral-loss analysis can narrow the class of attached residue. Losses of 162, 146, and 132 Da are commonly consistent with hexose, deoxyhexose, and pentose residues, respectively (Wu et al., 2022; Zhang et al., 2022). These losses do not establish exact sugar identity: 162 Da does not distinguish glucose from galactose or mannose, 146 Da does not establish rhamnose, and 132 Da does not separate xylose from arabinose.

Fragments derived from the aglycone can connect a product to the starting acceptor. Characteristic acceptor ions combined with a plausible sugar-loss pathway strengthen the case for glycosylation of that scaffold. Fragmentation may also help distinguish *O*-glycosides

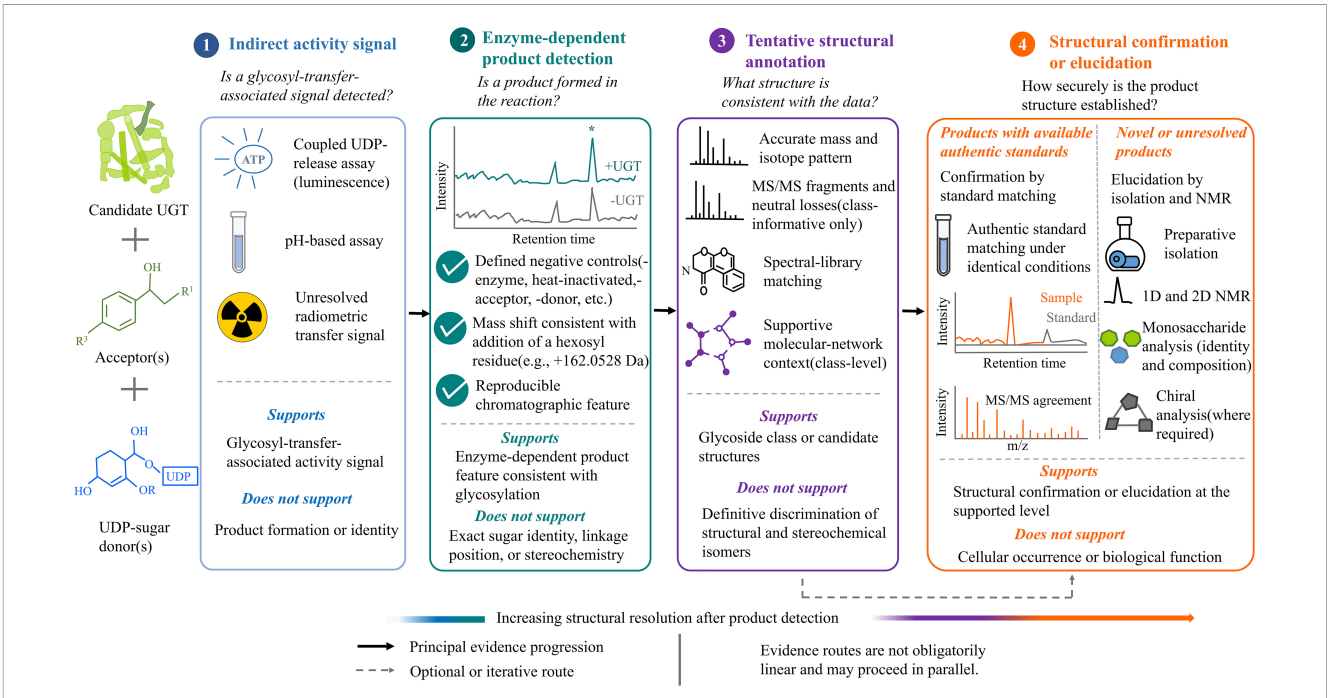


FIGURE 4
Evidence boundaries from indirect activity signals to structural confirmation or elucidation of UGT reaction products. Indirect assays detect glycosyl-transfer-associated signals but do not establish product formation. Enzyme-dependent chromatographic features provide direct product evidence, while accurate mass, isotope patterns, MS/MS, and spectral-library matching support tentative structural annotation. Products with available authentic standards can be confirmed by retention-time and MS/MS agreement under identical conditions; novel or unresolved products may require preparative isolation, 1D and 2D NMR, and sugar or chiral analysis. Structural claims should not exceed the analytical endpoint reached. UGT, UDP-glycosyltransferase. MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance; 1D, one-dimensional; 2D, two-dimensional. In the illustrative chromatogram, the asterisk marks the enzyme-dependent candidate product feature; +UGT and –UGT denote reactions with and without the UGT, respectively. The +162.0528 Da mass increment is consistent with addition of a hexosyl residue and does not identify glucose. ATP, adenosine triphosphate; UDP, uridine diphosphate; m/z, mass-to-charge ratio.

from *C*-glycosides, although the pattern depends on scaffold, ionization mode, and collision energy (Qian et al., 2025; Wu et al., 2022). Interpretation should therefore rely on the overall spectrum and relevant standards or related compounds rather than a single apparently diagnostic fragment.

Spectral libraries and molecular networking are useful when datasets contain many related products. They can reveal clusters sharing an aglycone or differing through successive modifications (Wu et al., 2022; Zhang et al., 2022). Network proximity indicates similar fragmentation behavior, not structural identity, enzymatic origin, or pathway order. Species-specific glycosides absent from reference libraries may consequently remain annotated only at the level of molecular formula or compound class.

Annotation errors propagate into co-expression, weighted gene co-expression network analysis (WGCNA), and metabolite–gene relationships. These analyses associate a UGT with the measured feature, not an incorrectly assigned structure. Traceable records retain the original feature identifier, precursor ion, adduct, retention time, major fragments, and annotation level (Xian et al., 2024).

For known products, authentic standards provide the strongest practical route to confirmation. Co-elution alone can be coincidental; agreement in retention behavior, accurate mass, and MS/MS fragmentation is more persuasive. This combination was used to distinguish mono- and diglycosylated flavonols in *Eriobotrya japonica* (Thunb.) Lindl (Qian et al., 2025).

4.3 Indirect assays and targeted quantification answer different questions

In a UGT71G1 screen, buprenorphine raised the UDP signal about 8.8-fold, but the corresponding glycoside was not detected by LC-MS/MS (Lethe et al., 2024). This directly tests the boundary between an indirect signal and product formation.

UDP luminescence and pH-based assays can compare many enzymes, acceptors, donors, or reaction conditions with relatively little analytical effort (Lethe et al., 2024; McGraphery and Schwab, 2020). Radiometric transfer assays serve a similar screening role when the radiolabeled product is not separated or resolved at the product level. In that form, the readout reflects UDP release, pH change, or radiolabel transfer rather than direct structural observation of the product.

The relationship between signal and product can be disturbed by nonspecific UDP-sugar hydrolysis, interference from buffer components or acceptors, sequential glycosylation, or formation of unexpected products. Indirect assays are therefore most useful for triage, identifying reactions that warrant chromatographic and mass-spectrometric follow-up.

Negative indirect signals are also conditional. Weak activity may fall below the assay threshold, or the reaction may require a different donor, pH, incubation time, or substrate concentration. Non-detection in an indirect assay should therefore be interpreted within the conditions tested.

Targeted LC-MS/MS becomes useful once the analyte is defined sufficiently to select meaningful precursor–product ion pairs. MRM and PRM can compare product abundance among enzymes, mutants, treatments, or sampling times and may support kinetic measurements (Jiang D. et al., 2021; Ren et al., 2025). Reliable quantification requires retention-time confirmation, validated transitions, appropriate calibration, and an internal standard where possible. Quantitative precision does not compensate for inadequate structural evidence.

4.4 Orthogonal evidence is required for novel or position-specific glycosides

The analytical standard should increase with the specificity of the claim. A novel glycoside, unreported linkage position, rare sugar residue, or defined stereoisomer requires more than a plausible precursor mass and fragmentation pattern. High-resolution MS and MS/MS constrain composition, aglycone identity, and residue class, but exact linkage and configuration often require orthogonal evidence.

Preparative isolation enables one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectroscopy. NMR can establish connectivity, locate the glycosylation site, and support anomeric or relative configuration. Sugar hydrolysis, derivatization, chiral analysis, or reliable standards may still be required for absolute sugar identity and configuration (Zhang et al., 2020). Structural confidence depends on agreement among these data.

Material supply may limit this process. Preparative reactions can be constrained by enzyme instability, acceptor solubility, UDP-sugar cost, product inhibition, or competing side reactions. Repeated enrichment, larger *in vitro* reactions, and production in engineered microorganisms can increase yield (Bi et al., 2022; Hao et al., 2025; Li et al., 2021). Products generated in heterologous hosts still require purification because host metabolism may produce closely related compounds.

When only trace amounts of product are available, NMR may remain impractical. A tentative annotation can still be reported if its evidential basis is transparent. Control chromatograms, precursor ions, major fragments, retention behavior, and the basis of the proposed structure should be shown, and unresolved elements should remain explicit in the chemical name and discussion.

5 Functional attribution *in planta*: from biochemical competence to native contribution

Chemical identity and native function remain distinct. Heterologous recombinant expression is central to biochemical characterization: it enables acceptor and donor screening, kinetic comparisons, product preparation, and analysis of catalytic residues. Living-cell reconstruction further tests cellular feasibility, while native perturbation tests source-plant contribution (Table 3). Native genetic manipulation is not a prerequisite for useful UGT characterization, particularly when stable transformation or editing is difficult in medicinal and other non-model plants.

5.1 Heterologous reconstruction tests cellular feasibility

Transient expression in *Nicotiana benthamiana* and yeast reconstruction place a candidate UGT in a living cell with

TABLE 3 Evidence types for attributing function *in planta* to glycosyltransferases and their inferential boundaries.

Evidence	Key requirement	Supported claim	Boundary
In vitro product	Defined acceptor–donor pair and direct product evidence	Biochemical competence	Not native occurrence
Heterologous reconstruction	UGT-dependent product in a living host	Cellular feasibility	Host differs from source plant
Native loss of function	Validated perturbation and compatible metabolic change	Native contribution or requirement	Redundancy and compensation may obscure effects
Gain of function or rescue	Product increase or phenotype restoration	Sufficiency or rescue	Dosage or ectopic-expression effects
Context or flux	Spatial overlap, pathway changes, or isotope incorporation	Spatial or pathway compatibility	No direct catalysis by itself

Evidence types are classified by the biological question they address. Biochemical competence and heterologous cellular feasibility are substantial conclusions in their own right. Native attribution requires evidence of source-plant contribution; native perturbation is not a prerequisite for every useful UGT characterization.

endogenous cofactors, membranes, transport, and competing metabolism. Upstream enzymes or supplied acceptors can support pathway reconstruction (Reed and Osbourn, 2018). These systems provide substantial evidence of cellular feasibility under the tested conditions. A study combining computationally guided UGT discovery with heterologous expression in engineered Escherichia coli to produce lauryl glucoside illustrates how candidate selection and host-based production can establish catalytic feasibility under defined conditions. Such evidence does not, by itself, establish the enzyme's native physiological role in its source organism (Promubon et al., 2024).

A positive result requires UGT-dependent product accumulation relative to empty-vector, no-UGT, and, where feasible, catalytically inactive controls. Acceptor feeding can strengthen attribution. In multistep reconstructions, the proposed acceptor must also be verified because failure upstream can mimic lack of UGT activity.

Heterologous hosts differ from the source plant in UDP-sugar pools, endogenous enzymes, transport, compartmentation, and expression level. Acceptor absence, modification, or separation may prevent production; strong expression may expose the UGT to non-native metabolites. Empty-vector, unfed, and acceptor-only controls distinguish host products from UGT-dependent products, whose identity still requires the structural assessment described in Section 4.

5.2 Native perturbation tests contribution in the source plant

In poplar, *UGT71L1* disruption altered salicinoids in hairy roots and later in whole plants, connecting the gene to native metabolism beyond recombinant activity (Fellenberg et al., 2020; Gordon et al., 2022).

Gene knockout, genome editing, RNA interference (RNAi), and virus-induced gene silencing (VIGS) move the analysis beyond association. They test whether loss of a candidate UGT changes the proposed conversion in the native genetic background. The clearest pattern is reduced or absent product together with a compatible change in the acceptor or nearby pathway metabolites. A reciprocal rise in the acceptor is not always expected because unstable intermediates may be diverted rapidly into competing branches.

Transient silencing is often the most practical route in medicinal and other non-model plants. UGT86C11 in *Andrographis paniculata* (Burm. f.) Nees provides one example and is examined across the evidence boundaries in Section 5.6 (Srivastava et al., 2021). In *Camellia sinensis* (L.) Kuntze, recombinant *UGT91Q2* activity, expression patterns, metabolic changes, and suppression of *UGT91Q2* together supported its contribution to nerolidol glycoside formation during low-temperature responses (Zhao et al., 2020).

Silencing efficiency and specificity limit interpretation. A 50% decrease in transcript abundance does not imply a 50% contribution to pathway flux. Closely related UGTs may compensate, and a silencing fragment may affect more than one family member. Transcript reduction, likely off-target genes, the proposed acceptor, target glycoside, and nearby pathway metabolites should be examined in the same biological material.

A weak metabolic response after gene disruption does not necessarily refute the proposed reaction. Redundant paralogs, condition-specific expression, or pathway rerouting may mask the effect. Conversely, a strong developmental or defense phenotype can arise from altered hormone balance, UDP-sugar use, or broader network effects. The metabolic conversion itself should remain central to the causal interpretation.

5.3 Complementation and overexpression address different causal questions

Cross-species complementation with the *Petunia* × *hybrida* (Hook.) Regel gene *UGT79B31* illustrates what such experiments can establish. Introduction of *UGT79B31* into the corresponding *Arabidopsis* loss-of-function background restored a specific flavonol diglycoside (Knoch et al., 2018). The enzyme could perform the reaction in a plant cellular and metabolic context, although the experiment did not define its native expression domain or physiological role in petunia.

Complementation is most persuasive when reintroduction of the wild-type allele restores a metabolic defect in independent loss-of-function lines. A catalytically inactive variant can further test whether rescue depends on enzyme activity and reduce concern about unrelated transformation effects.

Overexpression asks whether increased UGT abundance is sufficient to shift metabolism. An increase in the proposed product,

especially with depletion of its acceptor, is consistent with the reaction. The result is stronger when it mirrors the opposite pattern in loss-of-function material.

Gene dosage can create chemistry that is uncommon under native expression. Strong or ectopic promoters may place the UGT in tissues, developmental phases, or compartments where it normally has little access to the proposed acceptor. Expression magnitude and location should therefore be considered alongside the metabolic response.

Research on the *A. thaliana* gene *UGT76B1* illustrates the value of comparing genetic directions. Knockout and overexpression materials showed opposing changes in isoleucine-derived metabolites, followed by recombinant testing (von Saint Paul et al., 2011). Later transfer DNA (T-DNA) insertion and CRISPR/Cas9-mediated genome-editing studies linked *UGT76B1* to *N*-hydroxypipecolic acid and salicylic acid homeostasis (Mohnike et al., 2021).

UGT76B1 also shows that a physiologically relevant UGT need not have one exclusive substrate. Condition-specific metabolomics and bidirectional genetic evidence help identify which reactions within a broader *in vitro* profile matter biologically.

EkCGT1 provides a non-model medicinal-plant example. In *Euscaphis konishii* Hayata, overexpression lines accumulated more chromone *C*-glycosides than RNAi lines and had larger leaves and expanded vascular bundles (Liu et al., 2026). This combines biochemical evidence with native genetic perturbation. The metabolic and morphological claims remain separate: the reported comparison does not by itself show whether altered morphology reflects endogenous EkCGT1 function, increased or ectopic expression, or downstream metabolic changes. Assigning the morphology specifically to glycoside accumulation would require evidence distinguishing those alternatives, such as expression-matched complementation and a catalytically inactive control.

5.4 Expression, localization, and metabolomics define biological plausibility

A native reaction requires an opportunity for contact. Expression of a UGT in a tissue rich in the proposed glycoside, or induction before product accumulation, helps identify where and when the pathway may operate. These patterns are useful when selecting material for silencing, gene editing, or metabolomic analysis.

Temporal overlap is not always straightforward. A UGT transcript may peak before the product accumulates, especially when the glycoside is stable or stored for long periods. The free acceptor may appear only briefly. Expression data therefore help define where and when a proposed reaction is biologically plausible.

Subcellular localization imposes another constraint. A cytosolic UGT cannot act on an acceptor that remains permanently confined elsewhere. Contact may still occur if an intermediate passes through the cytosol before transport to a vacuole or another storage compartment. Proposed relationships should be considered alongside upstream-enzyme localization, transport routes, and likely metabolite compartmentation.

Green fluorescent protein (GFP) fusions are widely used to examine localization. The fusion can alter targeting or activity, and transient overexpression may spread fluorescence beyond the native compartment. Free GFP, established compartment markers, and confirmation of catalytic activity where possible provide useful controls.

Co-localization supports spatial compatibility within the resolution of the method. Spatial metabolomics, mass spectrometry imaging, cell sorting, subcellular fractionation, and spatial transcriptomics can refine that context, each with trade-offs in redistribution, sensitivity, or annotation. Their main value is to identify where causal experiments should be performed.

5.5 Pathway-level evidence refines native attribution

Native perturbation is most informative when analysis extends beyond a single glycoside. Targeted measurements should follow the proposed acceptor and product, while untargeted metabolomics can reveal alternative conjugates and pathway rerouting. Directionally compatible changes across independent lines and relevant conditions are more informative than an idealized one-to-one stoichiometric response. Genetic evidence does not remove structural uncertainty; chemical wording remains bounded by the analytical evidence.

Stable-isotope tracing can strengthen pathway-level inference by testing flux through the proposed conversion, although uptake, transport, dilution, and turnover still matter. Physiological phenotypes extend the biological interpretation only after the relevant chemical and genetic links are supported.

5.6 A worked example across the evidence boundaries

In *A. paniculata*, expression and metabolite patterns first prioritized UGT86C11. Recombinant enzyme converted andrograpanin at C19, with assay-product mass spectra consistent with a neoandrographolide standard (Srivastava et al., 2021).

Source-plant VIGS lowered *UGT86C11* transcript and neoandrographolide while increasing andrograpanin. This strengthens a bounded native contribution under the tested conditions; localization and expression provide context rather than independent proof.

5.7 Non-detection and null responses are boundary-specific

Non-detection is informative only when the corresponding test could have revealed the predicted response. In a purified-enzyme assay, interpretation depends on protein quality, controls, donor choice, reaction conditions, and analytical sensitivity. When these prerequisites are adequate, lack of product can provide evidence against the tested UGT–acceptor–donor combination under those conditions.

Null responses in living systems require different alternatives to be considered. Heterologous non-production can reflect expression, substrate supply, compartmentation, turnover, or detection limits. A native perturbation with no metabolic change may instead reflect incomplete perturbation, redundancy, compensatory flux, or an

inappropriate tissue or condition. Failure to resolve product structure is a structural uncertainty rather than a biological negative.

6 Operational decisions and boundary-specific outcome states

6.1 Claim-specific decisions and boundary-specific outcomes

The next experiment should address the unresolved claim. If enzyme-dependent product formation has not been established, a controlled reaction assay is required. If a product feature is reproducible but its identity remains uncertain, orthogonal structural evidence is the relevant next test. When the chemical reaction is sufficiently defined but biological relevance is unresolved, heterologous reconstruction can test cellular feasibility and native perturbation can test contribution in the source plant. Either route may be chosen according to the biological question and available tools; neither is a mandatory precursor to the other.

Outcome language should also follow the boundary. Reaction assays should use four basic states: positive under the stated conditions, technically supported negative under the stated conditions, unresolved, or not tested. A technically supported negative requires validated protein quality, appropriate controls, a justified donor and acceptor combination, and sufficient analytical sensitivity, whereas an unresolved result indicates that one or more of these conditions prevented a valid judgment. Not tested means that the corresponding reaction experiment was not performed. Structural work requires a different vocabulary: confirmed or elucidated, tentatively annotated, unresolved, or not assessed. Here, not assessed means that a product feature or record exists but the corresponding level of structural evaluation was not undertaken; it is therefore distinct from an unperformed reaction test. Heterologous and native perturbation experiments should likewise record the tested response together with the validation status and conditions.

6.2 Prospective design and retrospective audit

Prospectively, the model directs effort toward the claim that most limits the intended conclusion. Consider a medicinal-plant study in which transcript–metabolite covariation prioritizes a UGT and a controlled assay with UDP-Glc and a purified triterpene acceptor yields an enzyme-dependent LC-HRMS/MS feature. Without an authentic product standard or NMR-quality material, the product name remains qualified. If native perturbation is also unavailable, the study can support biochemical activity and the demonstrated level of structural annotation, but not native pathway contribution. Structural and biological claims can later be strengthened independently.

Retrospective audit uses the same logic without grading the study as a whole. A published report may contain strong biochemical evidence but over-specific product naming, or strong native perturbation evidence while leaving some metabolite structures unresolved. Each claim is recalibrated against the evidence that directly addresses it. Conversely, complementary biochemical, structural, and native evidence can justify a stronger bounded statement when all components support the stated conversion. The model therefore identifies both claim inflation and claim deflation; these directions are recorded separately. Table 4 gives concise examples of upward and downward claim miscalibration and evidence-matched wording.

7 Reusable evidence records: turning single-enzyme studies into cumulative plant biology

UGT studies are difficult to compare when the reported substrate name is detached from reaction conditions, product evidence, compound provenance, or unsuccessful tests. Reusable records should preserve enough context to reconstruct what was tested, what was observed, and which claim remained unresolved.

TABLE 4 **Common claim-calibration errors in plant glycosyltransferase substrate discovery and evidence-matched alternatives.**

Miscalibrated claim	Why	Evidence-matched wording
162 Da loss confirms glucose	Hexose isomers remain	Consistent with a hexoside
UDP release confirms a glycoside	Product is unidentified	Transfer-associated signal detected
Co-expression identifies substrate	Correlation is not causation	Candidate prioritized for testing
Docking proves activity	Compatibility is not catalysis	Pose supports prioritization
Heterologous production proves native function	Host context differs	Cellular feasibility supported
No product means inactive	Technical causes remain possible	Not detected under tested conditions
Phylogeny assigns specificity	Relatives can diverge	Phylogeny supports a substrate hypothesis
Co-localization proves catalysis	Overlap is not conversion	Spatial compatibility supported
Validated native perturbation and rescue of a resolved metabolic conversion show only association	Native metabolic changes and rescue support contribution to the tested conversion	UGT contributes to the tested conversion in the tested native background

Safer statements align claim strength with the available evidence.

BOX 2 Worked example of assessing UGT claims at four claim nodes.

Claim node	Evidence state	Claim category	Claim wording	Evaluator confidence	Calibration assessment
Chemical node: reaction occurrence	*In vitro* assay with recombinant UGT, defined acceptor and UDP-Glc; enzyme-dependent LC-HRMS/MS peak observed, no authentic standard	Reaction activity	This UGT forms an enzyme-dependent product feature *in vitro*	Medium	Calibrated for reaction occurrence; product identity remains unresolved
Chemical node: product identity	Exact mass matches predicted hexoside; no reference standard, no NMR	Product identity	This product is a specific glucoside at the C-3 position	Low	Inflation; only mass evidence, no structural proof
Biological node: heterologous cellular feasibility	Transient expression in *N. benthamiana*; a chemically identified product accumulates relative to matched no-UGT controls	Heterologous feasibility	This UGT can produce the metabolite in heterologous plant cells	High	Calibrated for cellular feasibility in the tested heterologous host
Biological node: native contribution	Co-expression correlation only; no knockout/overexpression perturbation	Native contribution	This UGT is responsible for this metabolite in the native plant	Low	Strong inflation; co-expression alone cannot prove native function

This worked example illustrates separate assessments at the four claim nodes. Authors can align claims with evidence and unresolved questions, reviewers can assess calibration at each node, and curators can preserve evidence, conditions, and provenance. The first-row claim is calibrated for enzyme-dependent product-feature formation *in vitro*; unconfirmed identity does not inflate this reaction-occurrence claim. Product identity remains unresolved at its separate node. The second-row position-specific structural claim is inflated because exact mass alone cannot establish that structure. These are hypothetical examples; confidence ratings are illustrative judgments, not measured scores. A study need not resolve every claim node to be complete for its stated question.

A minimum UGT record should link the enzyme sequence, acceptor, donor, assay conditions and controls, analytical endpoint, structural confidence, biological context, provenance, and a boundary-specific outcome state. This structure is compatible with MSI reporting and FAIR principles (Sumner et al., 2007; Wilkinson et al., 2016).

7.1 Experimental context and outcome states

Reaction labels are interpretable only with the experiment that produced them. Expression system, protein quality, substrate and donor concentrations, buffer, temperature, time, controls, and analytical method can all change the observed outcome. Kinetic values likewise require the donor identity, substrate range, incubation period, quantification method, and fitting procedure.

Outcome fields should preserve the boundary-specific distinctions defined in Section 6.1. A reaction assay should record positive under the stated conditions, technically supported negative under the stated conditions, unresolved, or not tested. Structural records should retain confirmed or elucidated, tentatively annotated, unresolved, or not assessed states. Heterologous and native perturbation records should retain the tested response, experimental context, and validation status. Missing, untested, or unresolved observations should not be converted into inactive-reaction labels (Chen et al., 2025; Yang et al., 2018).

7.2 Chemical and spectral provenance

Chemical records should retain a structural identifier, known stereochemistry, source, purity, preparation route, and reference evidence. This is especially important for plant-isolated or synthesized metabolites and uncommon UDP-sugars, whose availability, stability, or purity can alter apparent activity or donor preference.

Product names should remain linked to chromatographic and mass-spectrometric evidence, including precursor ion, adduct, retention time, major fragments, controls, and authentic-standard status. Raw spectra should be accessible where possible. A feature annotated only as a flavonoid hexoside should remain at that level until stronger evidence supports a more specific identity (Wu et al., 2022).

High-throughput screens gain long-term value when weak reactions, donor-dependent effects, and unexpected products are retained rather than only strong hits (Sirirungruang et al., 2025). Updated annotations should remain linked to the original feature and spectra so that revision does not erase provenance.

7.3 Provenance for model-ready datasets

Predictive models inherit the composition and labeling errors of their training data. Current UGT records overrepresent common acceptors, UDP-glucose, and well-studied lineages, while unusual donors and reliable reaction negatives are sparse. Generalization outside familiar chemical space is therefore uncertain.

Model-ready datasets should retain source study, assay design, analytical and structural endpoints, outcome state, sequence cluster, plant lineage, acceptor scaffold, donor class, and source laboratory. These fields allow train–test splits to separate biological and chemical relatives and reduce information leakage (Chen et al., 2025; Yang et al., 2018). Chemical labels must also preserve the structural resolution of the underlying data.

Prediction, experiment, and curation can then operate iteratively without collapsing uncertainty. Models rank candidates;

experiments return boundary-specific outcomes; revised structural or biological evidence updates the record while preserving its provenance.

8 Discussion: implications, limitations, and testable propositions

The central hypothesis predicts preferential confidence gain for the directly addressed claim, without cross-axis evidence alone resolving an outstanding chemical or biological claim. Accessibility and prioritization govern entry into experimentation; chemical and biological evidence govern post-test claims. Predictive evidence orders experiments but is not reaction evidence. A study may define a biochemical conversion while leaving native relevance unresolved, or support a native pathway contribution while retaining uncertainty in metabolite identity. These asymmetries generate three testable predictions.

Proposition 1 — Evidence gains are claim-specific. This is a proposed test, not an experiment conducted here. Matched cases varying structural and native evidence would test whether each evidence component changes confidence more strongly in its aligned claim. Prespecified contrasts separate those differences from evidence-by-evidence interactions. Comparable aligned and cross-axis effects, or confidence that fails to track external correctness criteria, would challenge the proposition. Supplementary Material S2 specifies the factorial design, blinding, ratings and analysis considerations.

Proposition 2 — Claim inflation should cluster at specific transitions. This is a proposed retrospective audit, not an audit conducted here. It would compare inflation at prediction-to-reaction, feature-to-structure and non-native-to-native transitions with other evaluable transitions. Absence of the predicted enrichment, or reproducible enrichment elsewhere, would challenge the boundary map. Supplementary Material S2 specifies sampling, blinded coding, subsequent transition assignment and the primary outcome.

The primary audit corpus must exclude all studies used to develop, motivate or illustrate the model anywhere in this manuscript or its supplements, including the EkCGT1 and cytokinin examples. Reports reusing their experimental evidence and records used to develop or pilot the rubric must also be excluded. Search, eligibility, exclusion and sampling rules must be fixed before coding. Illustrative examples cannot contribute to the primary enrichment estimate (Supplementary Material S2).

Proposition 3 — Boundary-specific reporting should improve interpretive reproducibility. This is a proposed reporting-format trial, not a trial conducted here. Evaluators would classify supported claims from information-matched records presented in different formats. The primary outcomes are agreement and error against an independently adjudicated reference. Little or no prespecified improvement would weaken the proposed reporting utility. Supplementary Material S2 specifies allocation, avoidance of repeat exposure, scoring, reference adjudication and analysis considerations.

These propositions distinguish the model from a hypothetical cumulative-ladder interpretation. Such an interpretation assumes that one later stage subsumes earlier uncertainty and that all studies move toward the same endpoint. UGT studies often ask different questions. A structural study may legitimately stop after product elucidation, whereas a pathway study may prioritize native perturbation and retain a coarser annotation for secondary metabolites. The relevant standard is therefore completeness for the stated claim, not progress toward a universal final level.

The model has defined limits. Its basic record is a UGT–acceptor–donor combination under specified conditions. Promiscuous enzymes, multiple donors or competing acceptors, sequential glycosylation, enzyme combinations, and tissue- or condition-dependent substrate switching therefore require linked records rather than one collapsed assignment. Native claims may also differ by tissue or condition. Genetic redundancy, incomplete perturbation, compartmentation, rapid turnover, inaccessible standards, and incomplete sampling can leave a claim unresolved even when the experiment is technically sound. New analytical technologies may shift a boundary by improving structural or spatial resolution, but they do not remove the need to state which question was answered. The framework is least useful when the goal is purely descriptive and no enzyme–reaction or native-function claim is made.

Generality across substrate classes remains to be tested. The model concerns plant family 1 UGTs acting on small molecules. Its distinction between reaction, product identity and native contribution should also be relevant to hormone conjugation and small-molecule *N*-glycosylation. Cytokinin *N*-glucosylation by UGT76C1 and UGT76C2 provides a chemically distinct example (Hou et al., 2004). Hormone claims nevertheless require attention to active hormone pools, conjugates, tissue context and feedback. Equal calibration performance or the same distribution of inflation across substrate classes is not assumed; future tests should report results by substrate class and linkage type. Protein *N*-glycosylation mediated by oligosaccharyltransferase is a different process (Koiwa et al., 2003). Extending this particular UGT–acceptor–donor record to that process would require additional definitions and separate validation.

A competing interpretation is that expert judgment already performs these distinctions without a formal framework. If so, blinded audits should show high agreement even without boundary-specific labels, and the proposed reporting fields should add little. The model is justified only if it improves consistency, exposes recurrent claim transitions, or makes incomplete evidence more reusable. Its value is therefore empirical rather than rhetorical. Resources such as pUGTdb could support such tests by linking reported reactions to richer provenance and confidence metadata, without assuming that every record must reach the same endpoint (Liu et al., 2023).

9 Conclusion

The model separates the decision to test a UGT–acceptor–donor combination from the chemical and biological conclusions drawn afterward. It predicts that new evidence will change

confidence most strongly in the claim it directly addresses, that overstatements will cluster at the prediction-to-reaction, feature-to-structure, and non-native-to-native transitions, and that boundary-specific reporting will improve agreement among evaluators. These remain hypotheses for the tests proposed in Section 8. In practice, the model asks authors to state what was tested and what remains unresolved, reviewers to judge each conclusion against its own evidence, and curators to preserve the conditions and provenance needed to revisit that judgment (Box 2).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

YQY: Data curation, Investigation, Writing – original draft. HYH: Conceptualization, Supervision, Writing – review & editing. YQL: Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was used in the creation of this manuscript. During the preparation of this manuscript, the authors used DeepSeek V3 (DeepSeek Inc., Hangzhou, Zhejiang, China) for English language polishing, structural refinement, and consistency checking. The authors reviewed and edited all outputs generated by the model. All academic concepts, literature synthesis, evidence frameworks, and figure and table designs were developed and fully verified by the authors, who take full responsibility for the content of the manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2026.1981660/full#supplementary-material

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