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TRANSCRIPTOMIC PROFILING AND RNA-SEQ ANALYSIS OF SECONDARY METABOLITE BIOSYNTHESIS PATHWAYS IN MEDICINAL PLANTS UNDER STRESS CONDITIONS: FOCUS ON GENE EXPRESSION PATTERNS (MRNA) REGULATING ALKALOID

Tejaswini Prasad Babar[1], Ruchita Shrivastava[2], Nageswara Reddy Gosu[3], Debashree Kakati[4], Ranadevan Rajakumaravelu[5], V. Rajasekhar Reddy[6]**

[1]Associate Professor, Department Of Dravyaguna, Bharati Vidyapeeth (Deemed To Be University), College Of Ayurved, Pune, 411043, Maharashtra, India.
[2]Former Senior Lecturer (Jbs), Department Of Botany, Govt. Homescience Pg Lead College, Narmadapuram (Mp), Pin Code – 461001.
[3]Professor, Department Of Chemistry,Vel Tech Rangarajan Dr. Sagunthala R&D Institute Of Science And Technology, Avadi, Chennai - 600062, India.,
[4]Department Of Botany, Mangaldai College (Autonomous). Darrang, Assam, Pin Code – 784125
[5**]Assistant Professor, Department Of Biochemistry, Institute Of Medical Sciences & Sum Hospital Ii, Siksha 'O' Anusandhan (Deemed To Be University), Phulnakhara, Bhubaneswar, Odisha, India, Pin Code – 754001, Email Id - Ranadevanr@Gmail.Com,
[6]Assistant Professor Of Chemistry, Department Of H & S, Sree Dattha Institute Of Engineering & Science (Ugc Autonomous), Sheriguda (V), Ibrahimpatnam (M), Ranga Reddy District, Greater Hyderabad – 501510, Telangana, India.

ABSTRACT
Alkaloids are diverse chemical defence and signalling molecules used by medicinal plants, however, the transcriptional logic connecting environmental stress to pathway output is disassembled across species and alkaloid classes. This integrative review summarises 40 peer-reviewed studies on RNA sequencing, transcriptome resources, transcription factors, and pathway genetics in Catharanthus roseus, Papaver somniferum, Dendrobium officinale, Nicotiana species, and tropane-alkaloid systems. Evidence was organised around stress perception, hormone signalling, precursor allocation, expression of biosynthetic enzymes, transport and cellular compartmentation. Jasmonate signalling is the most reproducible upstream driver across systems and acts through COI1-JAZ derepression and MYC2-, ERF/ORCA-, bHLH- and WRKY-centred transcriptional modules. However, methyl jasmonate, ethylene, salicylic acid and elicitor treatments not only upregulate all pathway genes, but affect branch selection, timing, tissue specificity and the balance of pathway activators and repressors. Coordinated expression of iridoid and indole branches, ORCA/BIS regulators and late vindoline genes for monoterpenoid indole alkaloid output in C. roseus. Opium poppy gene clusters, fusion enzymes and laticifer-sieve-element specialisation limit benzylisoquinoline alkaloid accumulation. In tobacco, JAZ–MYC2 and NIC2-locus ERFs control nicotine genes, while in D. officinale a broader, partly unresolved alkaloid network can be seen. The synthesis proposes the minimum standards for experimental design and validation and identifies a conserved four-module circuit - signal perception, precursor reallocation, pathway activation and transport/sequestration. Future advances will rely on time-resolved, cell-type-aware, long-read and metabolite-integrated transcriptomics rather than differential expression alone.

Keywords: Alkaloid biosynthesis; medicinal plants; RNA sequencing; stress response; transcription factors; jasmonate signaling.

INTRODUCTION

Alkaloids are specialised metabolites with nitrogen, which are responsible for many of the pharmacological properties of medicinal plants. Vinblastine and vincristine from Catharanthus roseus, morphinan and noscapine alkaloids from Papaver somniferum, nicotine from Nicotiana and scopolamine-related tropanes illustrate how structurally different pathways have been recruited for defence, and then exploited as medicines or biochemical tools. The biosynthesis of these compounds involves the concerted production of precursor molecules, the participation of several classes of enzymes, trafficking that is organ- and cell-specific, and mechanisms that prevent autotoxicity (Facchini, 2001; O'Connor & Maresh, 2006; Ziegler & Facchini, 2008; Hagel & Facchini, 2013). Thus, alkaloid concentration is not a simple property of the constitutive

pathway activity. It is generated from the dynamic regulation of developmental state, tissue identity and environmental signals.

Hormone and redox networks mediate the regulation of specialised metabolism by biotic challenge, wounding, drought, salinity, oxidative stress and exogenous elicitors. Jasmonic acid (JA) and methyl jasmonate (MeJA) are especially important because perception by the COI1 complex leads to degradation of JAZ repressors and release of MYC-family transcription factors. These factors act in concert or in competition with AP2/ERF, WRKY, bHLH and MYB proteins to re-direct carbon and nitrogen to defence chemistry. Salicylic acid (SA) and ethylene add pathway specific cross-talk, while reactive oxygen species, calcium and mitogen-activated protein kinases transmit early stress information (Kazan & Manners, 2013; Wasternack & Song, 2017; Hickman et al., 2017; Isah, 2019). The resulting response is often transient and branch-selective; an elicitor may strongly induce early precursor genes, weakly affect late enzymes, or increase transcripts without a proportional gain in metabolite.

RNA sequencing (RNA-seq) revolutionised this field by allowing quantification, in a single experiment, of known biosynthetic genes, previously unannotated transcripts, splice isoforms and regulatory modules in non-model species. De novo assembly enabled the discovery of transcripts before the availability of high-quality medicinal plant genomes, and modern reference-guided analysis improves isoform resolution and cross-study comparability (Wang et al., 2009; Grabherr et al., 2011; Conesa et al., 2016). Differential-expression analysis, co-expression networks and gene–metabolite correlations can be used to prioritise candidate enzymes and transcription factors, but rigorous interpretation requires replication, normalisation, temporal sampling and biochemical validation (Love et al., 2014; Langfelder & Horvath, 2008; Higashi & Saito, 2013). This review synthesises evidence from major medicinal plant alkaloid systems, defining conserved mRNA signatures, explaining species-specific differences, and proposing a practical RNA-seq framework for future stress–alkaloid studies.

MATERIAL AND METHODS

We performed a targeted integrative literature search for peer-reviewed articles available through July 2026. Search concepts were combined using “RNA-seq,” “transcriptome,” “gene expression,” “co-expression” or “transcription factor” and “alkaloid,” “medicinal plant,” “methyl jasmonate,” “ethylene,” “salicylic acid,” “elicitor,” and the focal taxa Catharanthus, Papaver, Dendrobium, and Nicotiana. Titles, authorship, journal details and digital object identifiers were cross-checked using publisher records, PubMed-indexed entries, discipline databases and discoverability in Google Scholar. Forty references were kept to balance basic pathway biology, primary transcriptomic studies, regulatory genetics and accepted RNA-seq methodology. This is an evidence synthesis rather than a formal meta-analysis, due to substantial variation across studies in treatments, tissues, sequencing depths, reference assemblies and metabolite assays.

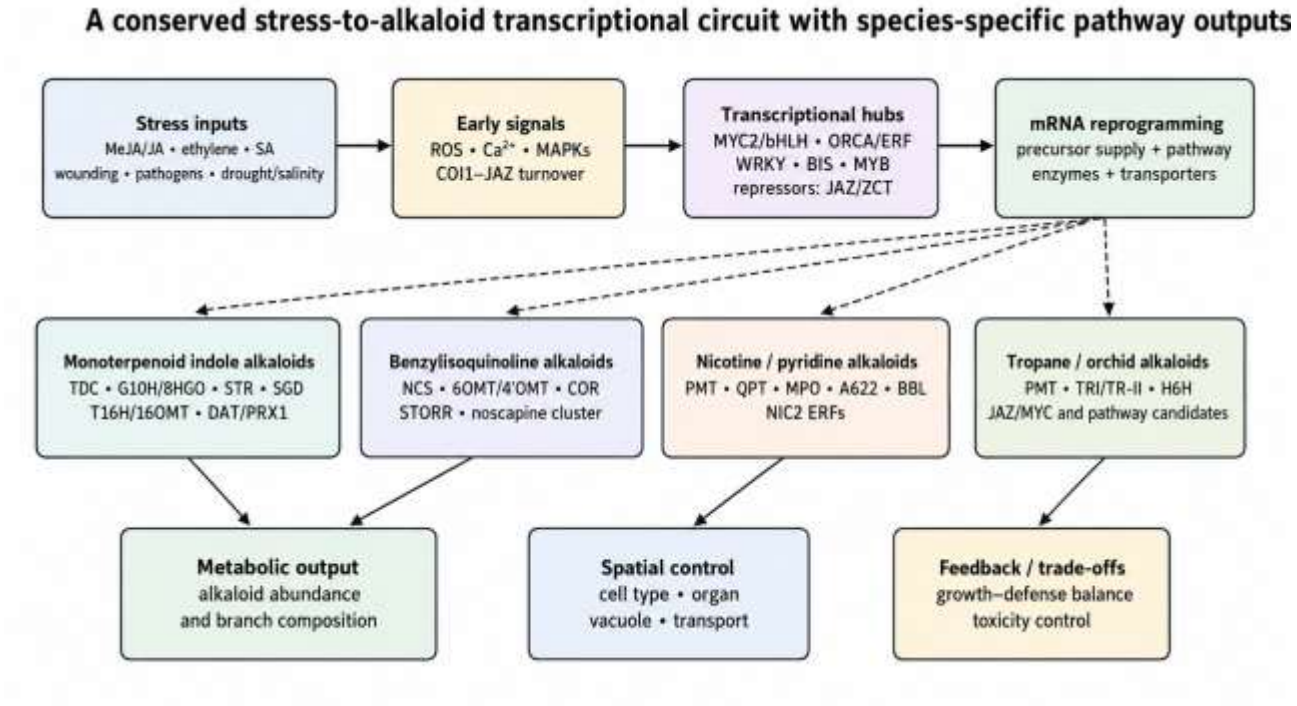
For each study, species, tissue or culture system, stress or perturbation, transcriptomic method, major differentially expressed pathway genes, regulatory factors and metabolite level validation were extracted. The evidence was organised into six modules: (1) stress perception and hormone signalling; (2) precursor and primary-metabolism reallocation; (3) core alkaloid-pathway enzymes; (4) transcriptional activators and repressors; (5) transport and cellular compartmentation; and (6) metabolite output. Each module-species combination was given a semiquantitative evidence score: 0 for no direct coverage in the selected corpus, 1 for limited or single-study coverage, 2 for replicated but incomplete evidence, and 3 for convergent evidence from multiple experimental approaches. The scores reflect literature density, not expression level. No biological measurements were pooled and no original fold changes were made.

RESULTS AND SYNTHESIS

The 40-reference corpus is dominated by C. roseus, emphasising its pharmaceutical importance, as well as its remarkably mature molecular toolkit. Transcriptome resources from multiple MIA-producing species permitted gene discovery prior to the development of complete genomes (Góngora-Castillo et al., 2012). Broad transcriptome profiling of C. roseus has identified tissue-biased genes and candidate regulators, and epidermal transcriptomics has shown that pathway steps are partitioned among specialised cell types (Murata et al., 2008; Verma et al., 2014). Gene-to-metabolite network analysis (Rischer et al., 2006) can further distinguish productive pathway modules from general stress responses by correlated expression. This concentration of resources accounts for the fact that MIA studies provide the clearest model for interpreting stress-regulated alkaloid mRNA patterns.

The most reproducible architecture across the corpus is a four-module circuit: signal perception, precursor reallocation, pathway activation and transport/sequestration. Stress hormones initially affect JAZ stability and early transcription factor activity. Primary metabolic pathways then increase or redistribute tryptophan, tryptamine, secologanin, tyrosine, putrescine or nicotinic-acid-derived precursors. Structural genes are activated in co-ordinated blocks and whether intermediates reach downstream enzymes is determined by transporters or cell-type boundaries. Figure 1 summarises this conserved circuit with separate enzyme sets for major alkaloid classes. The model also stresses the feedback and toxicity control underlying why a high transcript level for a single enzyme rarely predicts final yield.

Figure 1. Conserved stress-to-alkaloid transcriptional circuit with species-specific pathway outputs. Source: Author-generated synthesis based on Facchini (2001), Ziegler and Facchini (2008), Patra et al. (2013), Wasternack and Song (2017), Pan et al. (2018), and Jiao et al. (2022).



JA-responsive AP2/ERF regulation has been reported in C. roseus by identification of an elicitor-responsive element in the strictosidine synthase promoter and the ORCA2 regulator (Menke et al., 1999). Subsequently, the regulator ORCA3 was identified as a general regulator of primary and secondary metabolism linking the tryptophan supply to MIA genes (van der Fits & Memelink, 2000). Time course engineering showed that ORCA3 overexpression and JA treatments gave rise to different transcriptional responses, suggesting that a transcription factor and its upstream hormone are not interchangeable perturbations (Peebles et al., 2009). CrWRKY1 also provides another level of regulation and positively regulates some of the TIA genes. BIS1 regulates the iridoid pathway to secologanin (Suttipanta et al., 2011; Van Moerkercke et al., 2015). Thus, in addition to the induction of strictosidine synthase, synchronising the indole and iridoid branches is required to direct flux in a productive direction.

In addition, the finishing of pathways has changed how we interpret transcriptomic data. The identification of the seco-iridoid pathway explained the presence of the genes responsible for the formation of the monoterpene precursor of strictosidine (Miettinen et al., 2014). Later identification of missing vinblastine pathway enzymes suggests late oxidative and coupling steps could be masked by low abundance tissue-restricted transcripts (Caputi et al., 2018). Thus, increasing the read depth is not necessarily a way to improve RNA-seq studies, but choosing the right leaf age and cell layer is. Besides global transcriptional changes, tryptophan overproduction may also induce compensatory responses, as indicated by the overexpression of anthranilate-synthase in hairy roots (Sun et al., 2016). These results are compatible with models involving pathways rather than single gene explanations.

Comparative effects of hormones indicating qualitative differences in branch control. In general, MeJA induces defence and TIA-associated transcription, but ethylene can enhance, delay or re-route subsets of the same pathway. Comparative transcriptomics identified common and hormone-specific mRNA responses in C. roseus including transcription factors, precursor pathways and late TIA genes (Pan et al., 2018). SA treatment also induced a unique transcriptome with altered alkaloid-related genes and enhanced defence functions (Soltani et al., 2022). We showed that combining independent experiments to filter out platform- and lab-specific noise was beneficial, and a meta-analysis across datasets revealed recurrent MeJA- and ethylene-responsive candidates (Tabatabaeipour et al., 2024). Collectively, these studies argue against "stress induction" as a single experimental category: the branch of the pathway that is activated depends on hormone identity, dose, exposure time and tissue.

Table 1. Comparative transcriptomic evidence for stress-responsive alkaloid biosynthesis in focal medicinal-plant systems.

Plant system	Representative stress/perturbation	mRNA modules repeatedly implicated	Interpretive outcome	Key sources
C. roseus (MIA)	MeJA/JA, ethylene, SA; ORCA3 or precursor engineering	ORCA2/3, BIS1, CrWRKY1; TDC, G10H/iridoid genes, STR/SGD, late vindoline genes	Strongest evidence for coordinated branch control; hormone identity and tissue age alter output	Menke et al., 1999; Pan et al., 2018; Soltani et al., 2022
P. somniferum (BIA)	Elicitors, developmental and cell-type variation	Noscapine cluster, STORR/morphinan genes, NCS/OMT/COR modules	Gene clustering aids coordination, but laticifer–sieve-element division limits bulk-tissue interpretation	Winzer et al., 2012; Onoyovwe et al., 2013; Guo et al., 2018
Nicotiana spp. (nicotine)	MeJA/JA, wounding/herbivory models	COI1–JAZ–MYC2, NIC2 ERFs; PMT, QPT, A622, BBL	JA network is conserved; paralogs and root-to-shoot transport determine nicotine accumulation	de Boer et al., 2011; Yang et al., 2015
D. officinale (orchid alkaloids)	MeJA and precursor feeding	JAZ/MYC/bHLH/ERF candidates; amino-acid and putative alkaloid-enzyme modules	Useful candidate discovery, but pathway chemistry and enzyme identities remain incomplete	Chen et al., 2019; Jiao et al., 2022
Tropane-producing Solanaceae	Hormonal and developmental cues	PMT, tropinone reductases, H6H, acyltransferases; transport candidates	Root-biased synthesis and tissue transport require spatial validation	Kohnen-Johannsen & Kayser, 2019

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Figure 2. Composition of the curated 40-reference evidence base. Sources were assigned to one primary category for visualization; the chart is a review-corpus descriptor, not a bibliometric census.

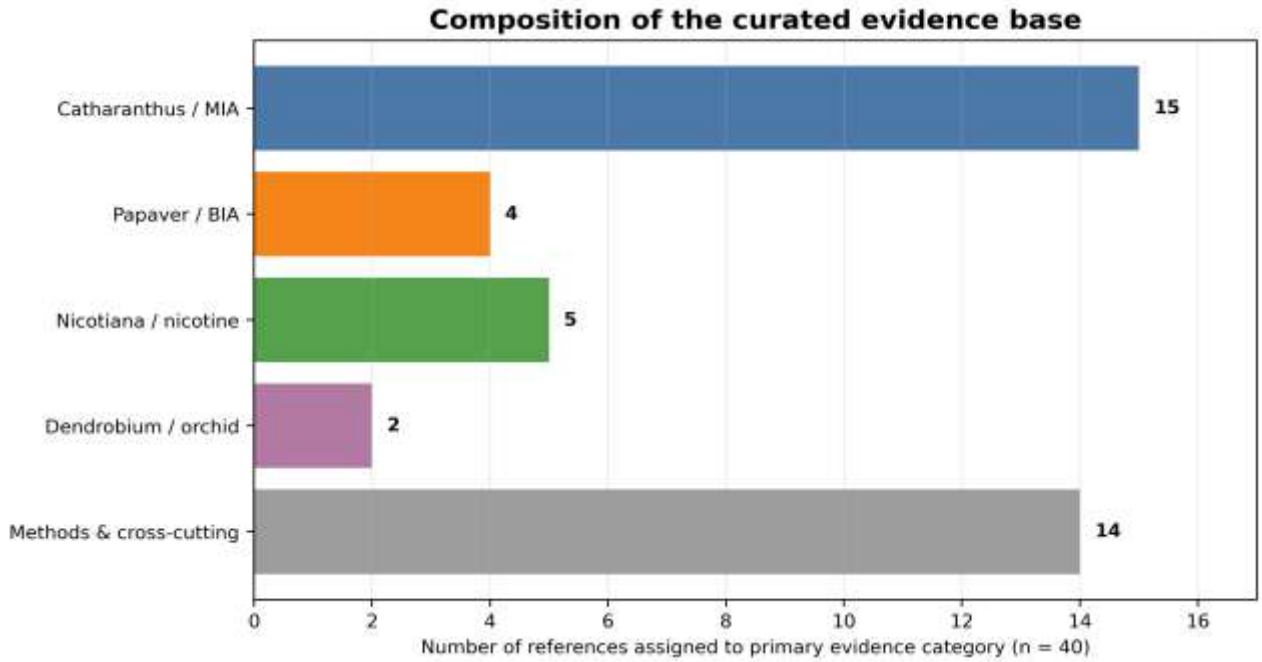

Figure 3. Literature evidence-density matrix for stress-responsive alkaloid regulation. Source: Author coding of the 40-reference corpus. Scores describe coverage and consistency (0–3) and are not gene-expression fold changes.

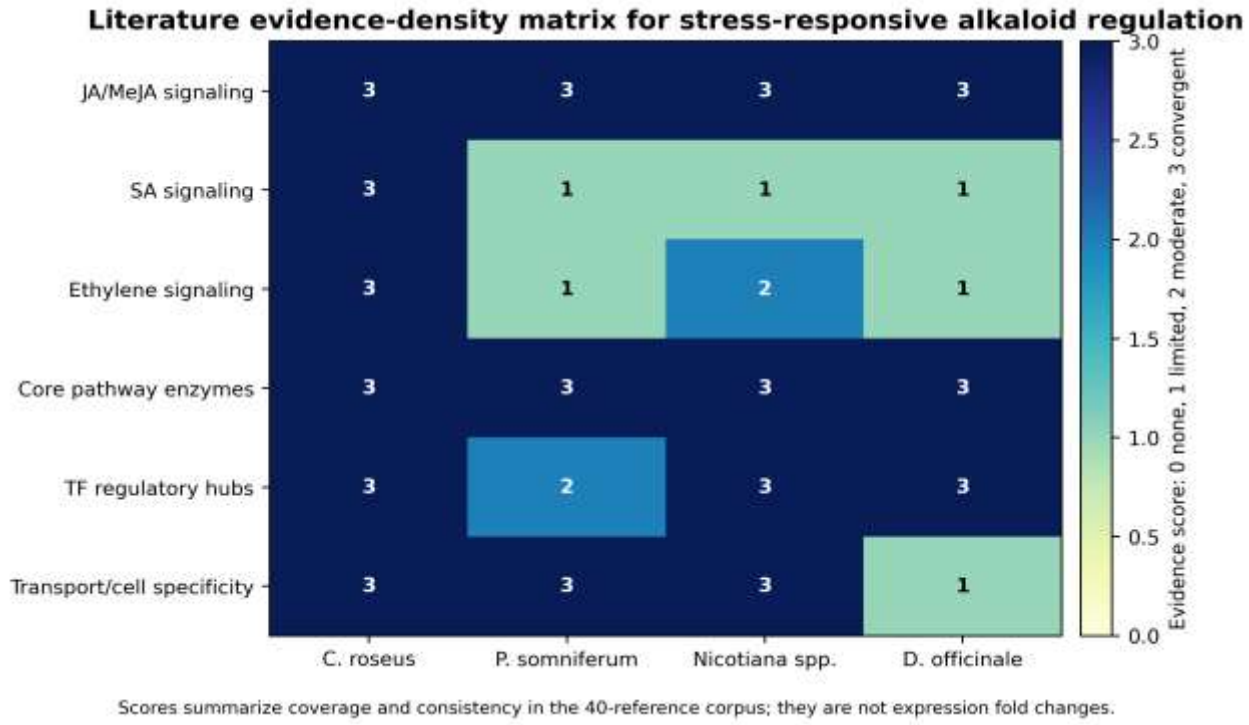

Table 2. Regulatory modules, candidate mRNA markers and validation priorities.

Module	Candidate mRNA markers	Expected interpretation	Essential validation
Stress perception / JA	COI1, JAZ family, MYC2-like bHLHs	Rapid JAZ induction can coexist with JAZ protein degradation; reflects feedback, not pathway repression alone	Protein stability or reporter assay; early time course

MIA regulation	ORCA2/3, BIS1, CrWRKY1, TDC, STR, SGD, iridoid genes	Balanced induction of indole and iridoid branches is more predictive than one structural gene	Paired metabolomics; promoter binding; tissue-specific expression
BIA regulation	NCS, OMTs, STORR, COR, noscapine-cluster genes	Cluster and fusion-gene expression must be interpreted with laticifer/sieve-element specialization	Cell-type sampling; enzyme assays; alkaloid profiling
Nicotine regulation	NtMYC2, NIC2 ERFs, PMT, QPT, A622, BBL	MYC2 and ERFs cooperate; duplicated genes may be differentially regulated	Paralog-specific qPCR; root and leaf metabolite/transport analysis
Transport/sequestration	ABC, MATE, NPF and pathway-specific transport candidates	Expression may explain transcript–metabolite discordance and toxicity avoidance	Subcellular localization; uptake/efflux assays; knockout/overexpression

Another common determinant of alkaloid yield is precursor supply. MIA biosynthesis needs the conjugation of an indole branch producing tryptamine and an iridoid branch producing secologanin. An increase of transcription of only one branch might lead to accumulation of intermediates but not an increase of the end product. Engineering of anthranilate synthase shows the ability to rewire many genes other than the target gene by manipulating tryptophan supply. Control of the iridoid branch is BIS1-dependent, suggesting the transcriptional control of the precursor balance. Tyrosine-derived dopamine and 4-hydroxyphenylacetaldehyde are both essential for benzylisoquinoline alkaloids. Nicotine and tropane alkaloid pathways compete for polyamine-derived intermediates. Stress also influences nitrogen assimilation, aromatic amino acids, redox cofactors and energy metabolism. Therefore, primary-metabolism pathways should be retained in enrichment and network models of RNA-seq analyses, instead of being filtered out as non-specific. A credible pathway model requires that precursor, cofactor and structural-gene modules change in a coherent fashion and that the direction of those changes be chemically compatible with the observed alkaloid profile.

A third layer of interpretation is the balance between activators and repressors. High expression of positive regulators such as ORCA3, CrWRKY1, BIS1 or a NIC2 ERF can be negated by JAZ proteins, ZCT-type repressors, competing transcription factors or hormone cross-talk. Conversely, induction of a repressor transcript following treatment may be homeostatic feedback rather than a failure of pathway activation. This distinction is particularly important for JA signalling, where rapid induction of JAZ gene expression follows the degradation of JAZ proteins. Correlation networks that ignore sign, time and protein turnover may therefore assign the same biological meaning to mechanistically opposed genes. Combining evidence of promoter motifs, transcription-factor binding and protein-interaction improves causal interpretation. In practice, a regulatory claim is stronger if (a) the activator precedes structural-gene induction, (b) its motif is enriched in target promoters, (c) perturbation changes both transcript and metabolite output, and (d) the response is reproduced in an independent tissue or dataset (Patra et al., 2013; Hickman et al., 2017).

Transport and sequestration are the least consistently measured but potentially most decisive module. Alkaloid intermediates may cross membranes several times, travel between neighbouring cell-types, travel through vascular tissues or be stored in vacuoles. These processes are to protect the producing plant and to bring together enzymes and substrates. However, transporter transcripts are often only discussed when structural genes fail to explain metabolite abundance. Epidermal specialisation in C. roseus, the laticifer–sieve-element division in opium poppy, and the movement of nicotine from root to shoot demonstrate that spatial organization is not an accessory feature of alkaloid biosynthesis (Murata et al., 2008; Onoyovwe et al., 2013; Wang & Bennetzen, 2015). Candidate ABC, MATE, NPF and pathway-specific transporters should therefore be included in co-expression modules and tested in localisation and transport assays. Spatial transcriptomics, laser-capture sampling or cell sorting can determine whether a transcript–metabolite mismatch is due to regulation, intercellular transfer or dilution of rare biosynthetic cells in bulk tissue.

The composition of the evidence base is presented in Fig. 2, and the respective evidence density scores are summarised in Fig. 3. All four focal systems provide strong support for JA/MeJA and core-enzyme regulation. Conversely, SA and ethylene evidence is localised in C. roseus, while transporter and cell-type evidence is strongest in C. roseus and opium poppy. The imbalance is biologically meaningful: a pathway may look transcriptionally simply because transport, spatial organization or secondary hormone responses have not yet been assayed. The research on tropane-alkaloids also indicates that PMT, tropinone reductases, H6H and downstream acylation steps are tissue-specific and controlled by developmental and hormonal context (Kohnen-Johannsen & Kayser, 2019).

DISCUSSION

Cross-species synthesis is supportive of a conserved logic, not a universal list of marker genes. JA signalling is the most transferable upstream signature, as rapid induction of JAZ genes can be used to mark pathway activation even if JAZ

proteins are repressors, because JAZ transcription is part of a negative feedback loop following proteasomal degradation. MYC2 or similar bHLH factors then couple hormone signalling to lineage-specific ERF modules. In C. roseus, ORCA factors and BIS proteins co-regulate MIA, whereas in tobacco, NIC2 ERFs interact with MYC2 and in Papaver pathway clustering and cell-type specialisation impose different constraints. The practical implication is that a transcriptomic study should test a regulatory module, repressor turnover, activator induction, structural-gene coordination and transport, rather than classify individual genes as simply up or down regulated. This modular view is in line with the general reviews on plant transcriptional control and alkaloid trafficking (Patra et al., 2013; Ziegler & Facchini, 2008).

The most reliable cross-species comparisons occur at the level of protein families and functional modules rather than one-to-one gene orthology. Alkaloid pathways have been repeatedly expanded by duplication, neofunctionalization, enzyme promiscuity, gene clustering and fusion, so that the same chemical transformation may be catalysed by unrelated or differently regulated enzymes in separate lineages. Even families that are conserved can acquire new promoter architecture or cell-type specificity. Orthogroup analysis should be supplemented by phylogeny, conserved-domain inspection, genomic neighbourhood, expression pattern and biochemical plausibility. This is especially relevant for the transfer of annotations from C. roseus to orchids or from tobacco to tropane-producing Solanaceae. A candidate derived only from sequence similarity may be involved in a different specialized-metabolite pathway. Conversely, a specialised metabolism evolves fast and may encode the missing step in lineage-specific transcripts with poor database annotations. The most defensible comparative strategy is to first identify a conserved regulatory or biochemical role and then test which local paralog fulfils it. Transcriptome resources, pathway-focused chemistry, and tissue-resolved expression together provide the evidence to distinguish true functional conservation from superficial sequence similarity (Facchini, 2001; O'Connor & Maresh, 2006; Hagel & Facchini, 2013; Góngora-Castillo et al., 2012).

Bulk RNA-seq has three main limitations. First, averaging of tissue may obscure reciprocal expression between epidermis, internal phloem-associated parenchyma, laticifers, roots and leaves. Second, steady-state mRNA levels are decoupled from metabolite abundance by translation, enzyme turnover, substrate availability, reversible conjugation, vacuolar sequestration and long-distance transport. Third, incomplete genomes and very similar paralogs can lead to ambiguous read assignment, especially in polyploids and gene clusters. De novo assemblies are important for non-model plants but can lead to transcript fragmentation and paralog merging; reference-guided workflows may also fail when annotations do not include stress isoforms. These limitations are why a statistically significant transcript change is a hypothesis-generating result, not proof of biochemical function.

Computational choices can materially change the biological story. The genes that are termed responsive are determined by low-count filtering, library-size normalisation, outlier treatment, transcript-to-gene aggregation and the statistical design formula. Multifactor models should model genotype, tissue, time, batch and relevant interactions instead of separate pairwise tests that inflate error and obscure context dependence. For non-model species, assembly completeness should be assessed with metrics including conserved-gene content, read representation, contiguity, redundancy, and evidence for full-length coding sequences; a high N50 is insufficient. Differential-expression results should contain normalised counts, effect sizes, uncertainty and adjusted probabilities; pathway enrichment should be done on an expressed-gene background. Co-expression networks need variance stabilisation and enough samples to estimate correlations reliably. Robust modules can be distinguished from pipeline artefacts using sensitivity analyses (e.g. different transcript filters, mapping parameters and network powers). These principles are particularly relevant for alkaloid pathways, which frequently exhibit paralogs, gene clusters, low-abundance cell-specific transcripts and incomplete annotations (Grabherr et al., 2011; Love et al., 2014; Conesa et al., 2016; Langfelder & Horvath, 2008), and have been established in the literature of RNA-seq best-practice and network analysis.

So, a good experimental design should couple biologically meaningful stress regimes with temporal and spatial resolution. Minimum of 3 independent biological replicates per condition, but more is better for field-grown or genetically diverse material. Early time points capture signalling and transcription-factor responses, intermediate time points capture enzyme mRNAs and later time points capture metabolite accumulation and feedback. Tissue selection should be guided by the known biosynthetic geography. Paired targeted or untargeted metabolomics should be performed on the same biological samples. Randomisation of sequencing libraries across preparation and flow cell batches is recommended. Stranded libraries are useful when anticipating antisense transcription or overlapping genes. Quality control, statistics on mapping or assembly of the reads, filtering of transcripts, thresholds for differential-expression need to be reported transparently (Conesa et al., 2016; Love et al., 2014).

Figure 4. Recommended RNA-seq-to-function workflow for alkaloid pathway studies. Source: Author-generated synthesis based on Wang et al. (2009), Conesa et al. (2016), Love et al. (2014), Langfelder and Horvath (2008), and Higashi and Saito (2013).

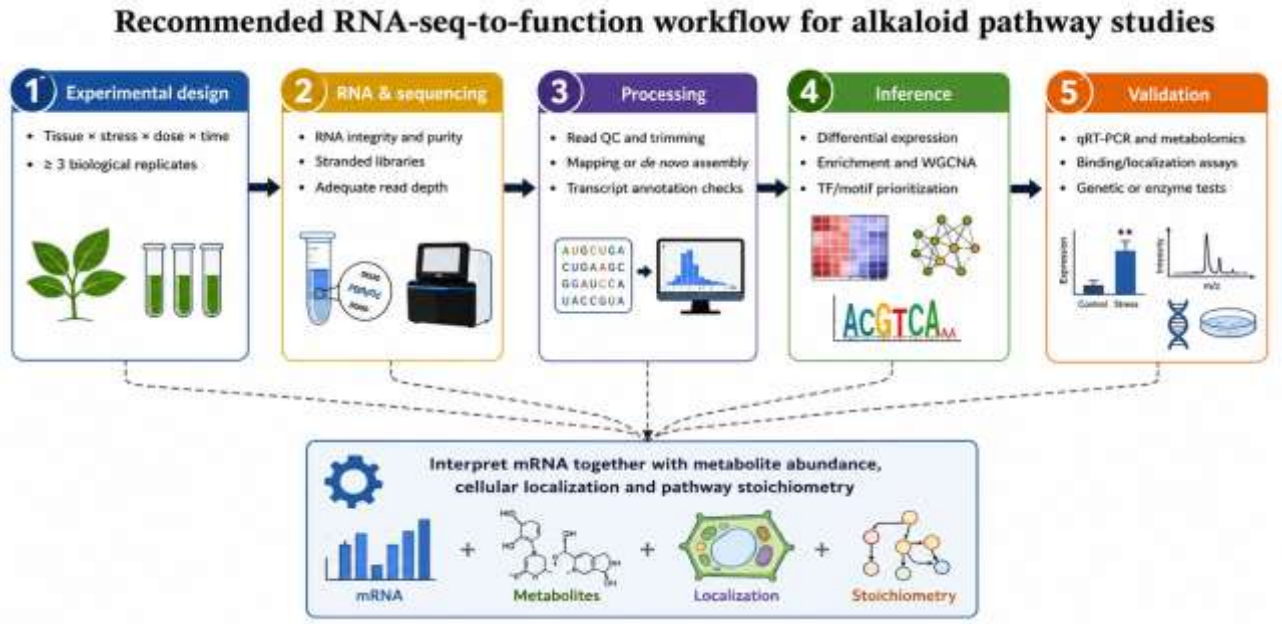

Table 3. Minimum design and reporting recommendations for stress-responsive alkaloid RNA-seq studies.

Design element	Minimum recommendation	Reason
Replication and blocking	≥3 independent biological replicates; randomize extraction and library batches	Separates treatment effects from biological and technical variance
Tissue and time	Sample biosynthetic tissues and at least early, intermediate, and late time points	Distinguishes signaling, enzyme transcription, and metabolite accumulation
Sequencing/annotation	Stranded libraries; report depth, mapping/assembly metrics, transcript filtering and versioned annotation	Reduces ambiguous isoform and paralog inference
Statistics	Pre-specified contrast, multiple-testing correction, effect sizes and count diagnostics	Prevents overinterpretation of low-count or batch-driven genes
Integration	Measure alkaloids on matched samples; use co-expression/network and pathway enrichment	Connects mRNA modules to biochemical output
Functional confirmation	qRT-PCR plus at least one binding, perturbation, localization, enzyme or isotope-tracing assay	Distinguishes causal pathway genes from correlated stress markers
Data sharing	Deposit raw reads, metadata, code and metabolite tables in stable repositories	Enables reanalysis and cross-study meta-analysis

Network analysis may take the field beyond lists of differentially expressed genes. Weighted co-expression analysis identifies modules that correlate with alkaloid concentration, treatment or tissue identity but module membership should be robust to parameter choices and independent datasets (Langfelder & Horvath 2008). Candidate regulators can then be prioritised for temporal precedence, promoter motif enrichment, network centrality and covariation with multiple enzymes. Meta-analysis can disentangle recurrent signals from experiment-specific effects, as demonstrated for hormone responses in C. roseus. However, co-expression does not give information about directionality and transcription-factor binding, so reporter assays, CRISPR or RNAi perturbation, heterologous enzyme assays and isotope-labelling experiments are still needed.

These requirements are incorporated in the recommended workflow in Figure 4. The key principle is triangulation: an mRNA candidate is strongest when it is stress responsive, co-expressed with known pathway genes, localised to the appropriate cell type, associated with the expected metabolite change, and functionally perturbed. Long-read sequencing will enhance isoform and gene-cluster annotations, and single-nucleus, single-cell and spatial transcriptomics can resolve

biosynthetic handoffs between cell types. Ribosome profiling and proteomics can identify transcripts that are being actively translated, and chromatin accessibility or transcription-factor occupancy can distinguish direct from indirect targets. The integration with metabolomics and flux analysis will be especially important for pathways where the branch competition dictates the medicinally valuable end products.

Several translational opportunities arise. These transcript-based early markers might be used to optimise stress elicitations, so that harvest can be performed prior to severe growth penalties. Instead of overexpressing individual structural genes, breeding or genome editing could target regulatory nodes that coordinate multiple enzymes. Synthetic biology can be used to build pathway segments in microbial or plant chassis, but native transcriptomic data are still required to identify missing enzymes, transporters and toxicity-management mechanisms. The most promising targets are often bottlenecks that coordinate precursor supply, cell-to-cell transfer and late pathway chemistry rather than the most highly induced transcripts.

CONCLUSION

The RNA-seq studies in medicinal plants have discovered a conserved framework in stress response, where hormone perception, JAZ-MYC regulation, lineage-specific transcription factors, structural genes, and transport processes work in concert to orchestrate alkaloid biosynthesis. The most supported upstream signal is JA/MeJA, but ethylene, SA, tissue identity and developmental timing re-shape pathway branch choice. C. roseus provides the most comprehensive regulatory model, opium poppy demonstrates the importance of gene clusters and multicellular biosynthesis, tobacco exemplifies JAZ–MYC2–ERF cooperation, and D. officinale highlights the discovery challenge in incompletely mapped pathways. Future studies should replace single time-point differential expression catalogues with replicated time courses, cell-type resolution, long-read annotation, metabolite pairing, and functional validation. Such designs will transform mRNA patterns from descriptive signatures to causal models that can support reliable metabolic engineering and stress-guided production of medicinal alkaloids.

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