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Co-expression network analysis for identification of key long non-coding RNA and mRNA modules associated with alkaloid biosynthesis in *Catharanthus roseus*

Original paper

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Abstract: *Catharanthus roseus*, produces a diverse array of specialized metabolites known as monoterpene indole alkaloids (MIAs) through an extensive and intricately branched metabolic pathway. It is imperative to unravel the intricate regulatory networks and relationships between the genes involved in the production of these metabolites. Long non-coding RNAs (lncRNAs) are emerging as significant regulatory factors in various biological processes. In this study, 4303 out of 86726 transcripts were identified as potential lncRNAs in *C. roseus*. Subsequently, we identified coding genes that exhibited a high correlation with CrlncRNA, designating them as potential target genes for collectively modulating the MIA pathway using Weighted Gene Co-Expression Network Analysis (WGCNA), leading to the identification of crucial gene clusters associated with the biosynthesis of MIAs. Based on the findings, three modules (dark turquoise, magenta, and orange) and hub genes were pinpointed as being linked to MIAs. Additionally, the most prominent known coding genes were 10-hydroxygeraniol oxidoreductase, GATA-like transcription factor (*GATA1*), 7-deoxyloganetic acid UDP-glucosyltransferase (*7DLGT*), desacetoxyvindoline 4-hydroxylase (*DH4*), *MYC2*, and *MPK6*. The unknown target genes were related to stress response and the intricate process of hormone transduction. *ORCA*, *MYC2*, and *GATA1* are crucial in regulating the MIA pathway, likely requiring cooperation with CrlncRNAs.

Keywords: lncRNAs, specialized metabolites, transcription factors (TFs), medicinal plants, gene modules.

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Introduction

Isoprenoids, also known as terpenoids, are a diverse group of about 30,000 unique compounds found in plants. They perform a range of functions in plants, including acting as essential components of primary metabolism, such as photosynthetic pigments, hormones, and redox cofactors. They also include specialized metabolites that play a role in attracting pollinators and defending plants against pathogens and herbivores (Bouvier et al., 2005; Gershenzon and Dudareva, 2007). Monoterpene indole alkaloids are plant secondary metabolites, also known as specialized metabolites, mainly found in the Gentianales, consisting of more than 3,000 MIAs derived from their central precursor strictosidine, which exhibits a remarkable structural diversity and pharmacological activities (O'Connor and Maresh, 2006; Brown et al., 2015).

Plants of the Apocynaceae, Nyssaceae, Loganiaceae and Rubiaceae families are known to synthesize MIAs as part of their defense mechanisms (O'Connor and Maresh, 2006; De Luca et al., 2014).

The medicinal plant *Catharanthus roseus* (L.) G. Don produces dimeric (bis-indole) MIAs, serving aseither anticancer agents (vinblastine and vincristine) (Martino et al., 2018) or precursors (anhydrovinblastine) for the synthesis of natural and semi-synthetic anticancer alkaloids (e.g. vinorelbine) (Ngo et al., 2009). Vincristine and vinblastine have been listed by the US Food and Drug Administration as anticancer drugs with shortages in 2019-2020 (Fox and Unguru, 2020). *C. roseus* synthesizes MIAs through an extensive and intricately branched 31-step metabolic pathway. Over the past few decades, 38 MIA pathway genes including structural genes and several transcription factors involved in the MIA biosynthesis pathway have been discovered using co-expression analysis of tissue-derived omics datasets and biochemical knowledge of the reactions (Figure 1) (Li et al., 2022).

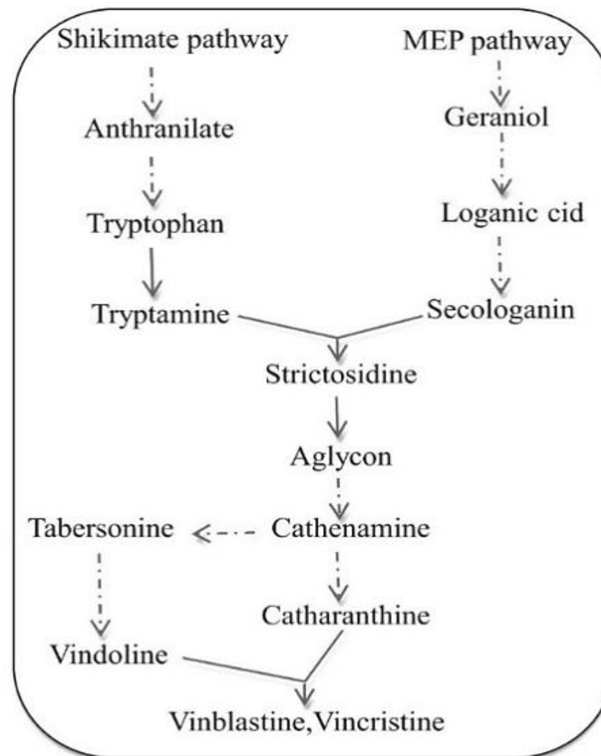

Figure 1. Schematic of the MIA pathway in *C. roseus*. The biosynthesis of MIA starts with the coupling of secologanin (terpenoid moiety) and tryptamine (indole moiety). The terpenoid moiety originates from geraniol, which produced by the mevalonate-independent pathway (MEP). The indole part is derived via anthranilate from the shikimate pathway.

The first transcription factors identified in *C. roseus* belonged to the AP2-ERF ORCA (octadecanoid derivative- responsive catharanthus apetala 2-domain) family. These transcription factor gene clusters, including *ORCA2*, *ORCA3*, *ORCA4*, *ORCA5* and *ORCA6*, are able to upregulate the transcription of key enzyme-encoding genes in the downstream biosynthetic pathway of MIA, such as *STR*, *TDC*, *IS* and *G10H*, and consequently MIAs accumulation (Menke et al., 1999; Van der Fits and Memelink, 2000; Paul et al., 2017; Yang et al., 2023). The basic helix-loop-helix (bHLH) transcription factor CrMYC2 regulates the *TDC* gene, a key gene for tryptamine biosynthesis, and STR and MIA accumulation. Upon perception of jasmonic acid (JA) (Zhang et al., 2011), the MAP kinase cascade, consisting of CrMAPK3/6, CrMAPKKK1, CrMAPKK1 and phosphorylates CrMYC2 and subsequently the CrMYC2 activates the *ORCA* gene cluster and the *TIA* pathway genes (Paul et al., 2017; Singh et al., 2020).

Other transcription factors involved in the regulation of different parts of the pathway including bHLH iridoid synthesis (BIS), such as *BIS1*, *BIS2* and *BIS3* genes, regulate the expression of genes involved in the secoiridoid pathway (Van Moerkercke et al., 2015). CrWRKY1 regulates the serpentine branch of MIA biosynthesis pathway by activating TDC and repressing *ORCA* genes (Suttipanta et al., 2011). CrGATA1 has been identified and confirmed to be involved in vindoline production in response to light (Liu et al., 2019).

In *C. roseus*, transcription repressors have also been identified, suggesting their involvement in the modulation of MIA biosynthesis. Two G-box binding factor (GBF) proteins, CrGBF1 and CrGBF2, may act as transcriptional repressors of MIA biosynthesis (Sibéril et al., 2001). They act as antagonists of CrMYC2 and modulate gene expression in MIA biosynthesis (Sui et al., 2018). Zinc finger proteins such as ZCT1, ZCT2 and ZCT3 act as transcriptional repressors of TDC and STR promoters (Pauw et al., 2004; Rizvi et al., 2016; Paul et al., 2017). RMT1 binding to the ORCA3 promoter, a target of MYC2, suggesting that RMT1 represses the *ORCA3* gene by competing with CrMYC2 for binding to the same cis-element (Patra et al., 2018). Furthermore, the involvement of microRNAs (miRNAs) in regulating MIA pathway has been investigated in *C. roseus* (Prakash et al., 2015; Shen et al., 2017). Conversely, no study has reported the involvement of long non-coding RNAs in the regulation of MIA biosynthesis, especially in *C. roseus*.

RNA transcripts longer than 200 nt with no or minimal coding potential or lacking an ORF encoding >100 amino acids are classified as lncRNAs (Yu et al., 2019). They can affect all elements of genes, including exons, introns, promoters, untranslated regions and terminators, and control gene expression at multiple levels, including modification of chromatin accessibility, translation, transcription and splicing. The integrity of the genome is protected by certain lncRNAs, while others react to environmental cues such as drought, temperature, nutrients and pathogens. The majority of lncRNAs are investigated in the context of protein coding gene regulation and may, therefore, have a functional relationship with mRNA expression (Wierzbicki et al., 2021). The expression of most reported plant lncRNAs is regulated by environmental conditions. They respond to developmental or environmental cues (Yu et al., 2019). Over the last decade, several lncRNAs have been identified along with their potential targets and mechanisms of action that contribute to the growth, development and stress response in plants. In the case of prolonged cold exposure, a process known as vernalization COLDAIR, COLDWRAP and COOLAIR lncRNAs are required to effectively silence FLC transcription which accelerates flowering (Swiezewski et al., 2009; Heo and Sung, 2011; Csorba et al., 2014; Kim and Sung, 2017). APOLO regulates the expression of *PID* gene and auxin signal transduction (Ariel et al., 2020). IPS1 controls the expression of the target gene *PHO2* under Pi starvation, facilitating its uptake phosphate homeostasis (Franco-Zorrilla et al., 2007). The key role of ASCO in regulating alternative splicing (Rigo et al., 2020), SUF in female identity (Hisanaga et al., 2019), 1GOD in seed dormancy (Fedak et al., 2016), ELENA1 in enhancing immunity to *Pseudomonas syringae* PV. (Seo et al., 2019), SVALKA in freezing response (Kindgren et al., 2018), slylnc0195 and slylnc1077 lncRNAs in TYLCV infection response (Wang et al., 2015), DRIR in enhanced drought tolerance (Borah

et al., 2018), ENOD40 in root nodulation (Sousa et al., 2001; Campalans et al., 2004; Rohrig et al., 2004) has been demonstrated.
In this research, we aimed to identify the regulatory function of lncRNAs in collaboration with key coding genes associated with the MIA biosynthesis pathway in *C. roseus*.

Materials and Methods

Data collection

Transcriptomics and metabolomics data of *C. roseus* were obtained from two different databases. The transcriptomics data were retrieved from the Medicinal Plant Genomics Resource (MPGR) database (http://mpgr.uga.edu) (Gongora-Castillo et al., 2012; Kellner et al., 2015). Additionally, the metabolomics data were obtained from the plant/eukaryotic and microbial systems resource database (https://metnetweb.gdcb.iastate.edu/PMR/).

Prediction of candidate lncRNAs

The assembled transcripts of *C. roseus* were acquired from MPGR. To distinguish lncRNAs from all the assembled transcripts, transcripts with a length less than 200 nt and an open reading frame (ORF) longer than 100 amino acids were excluded (Yu et al., 2019). The remaining transcripts underwent a BLASTX search (E-value cutoff of 1E-5) against Pfam, TAIR, Uniprot, and NCBI databases to remove those with probable coding proteins. Further evaluation of coding potential was performed using the coding potential calculator (CPC) and coding-non-coding index (CNCI). Transcripts with a CPC score less than −1 and a CNCI score less than 0 were retained. The transcripts that passed the above-mentioned stringent filtering pipeline were considered as candidate lncRNAs and were considered for the next step of the analysis.

Co-expression network construction

WGCNA package of the R software analysis was applied to construct the *C. roseus* mRNA-lncRNA co-expression network and to identify clusters of transcripts exhibiting similar expression patterns (Langfelder and Horvath, 2008). A similarity matrix was generated by calculating Pearson correlations between each pair of transcripts. The resulting matrix was transformed into an adjacency matrix using a power function (β=12). Subsequently, the topological overlap matrix (TOM) was calculated for hierarchical clustering analysis. The dynamic tree cut algorithm was then utilized to detect distinct modules within the network. These WGCNA modules were subsequently correlated with metabolites to uncover relevant networks. Modules displaying significant gene-metabolite correlations (Pearson's correlation coefficient) were singled out for further investigation. Hub genes were identified by evaluating intramolecular connectivity. Finally, the visualization of module's network was accomplished using Cytoscape software version 3.6.1.

Target gene prediction and enrichment analysis

The identified protein-coding transcripts were annotated using BLASTX against the TAIR database. In addition, genes that co-occurred with lncRNAs in the same module were considered potential targets of those lncRNAs. To gain a deeper insight into the functions of these coding transcripts, GO enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed using the gProfiler tool.

Results

Identification of key lncRNAs

To identify lncRNAs in the *C. roseus*, we conducted an analysis of assembled transcripts obtained from the MPGR database. Our filtering criteria excluded transcripts with a length of less than 200 base pairs and an open reading frame (ORF) length exceeding 100 amino acids. Additionally, we checked for sequence similarity to known protein sequences by blastx against various databases. The remaining transcripts were subjected to further assessment using CPC and CNCI programs to determine their protein-coding potential. Following stringent filtration, a total of 4303 transcripts were identified as potential lncRNAs (Supplementary Table S4).

Construction of co-expression modules

LncRNAs and coding genes correlated to MIAs were screened out by WGCNA package implemented in R (Langfelder and Horvath, 2008). By utilizing the WGCNA algorithm the coding and non-coding contigs with similar co-expression

patterns and correlation to metabolites are classified into a set of modules.

A total of 22 modules were constructed, each distinguished by different colors (Figure 2). The number of genes within each module varied, ranging from 32 genes (module palevioletred3) to 3513 genes (dark turquoise). Three modules were found to be highly correlated with the biosynthesis of alkaloids. The results indicated that the dark turquoise and orange modules were correlated with catharanthine, serpentine, and secologanin. Additionally, the orange module showed a correlation with vindoline, the magenta module was associated with catharanthine and vindoline. Furthermore, the number of coding and non-coding genes in each module differed. The orange module comprised 2644 coding genes and 671 lncRNAs, the dark turquoise module had 3097 coding genes and 416 lncRNAs finally, the magenta module contained 633 coding genes and 43 lncRNAs.

Functional annotation

The Gene Ontology (GO) analysis showed significant enrichment on genes of the three selected modules. Specifically, the orange module was enriched with 112 biological processes (BPs), 37 molecular functions (MFs), and 49 cell components (CCs) (Supplementary Table S1). The dark turquoise module showed enrichment in 128 BPs, 43 MFs, and 47 CCs (Supplementary Table S3).

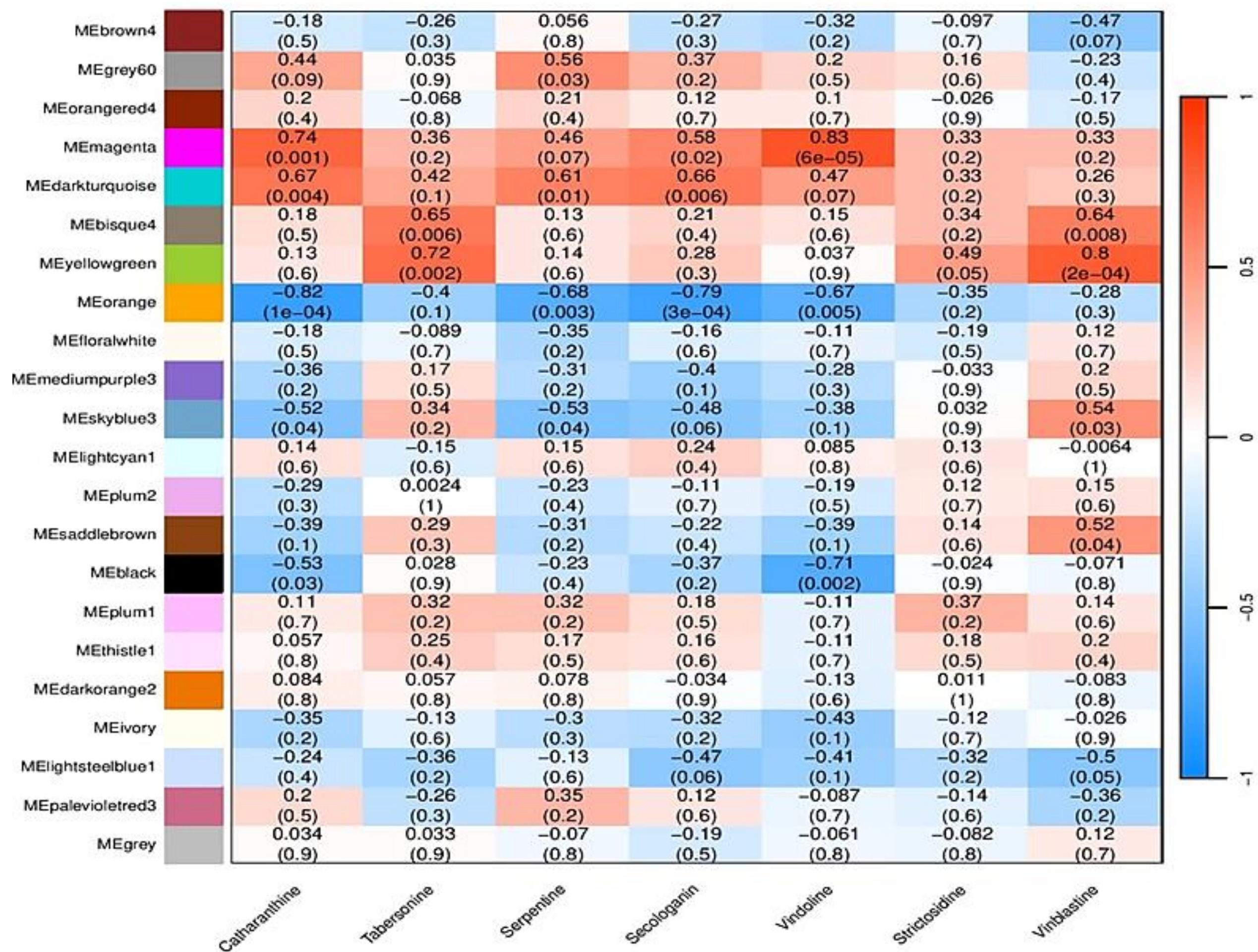

Figure 2. Correlation analysis between eigengene modules and MIAs. Each row shows a module eigengene, each column shows a secondary metabolite. The number in each box represents the correlation value with the numbers in brackets representing correlation *p-values.* Each module contained both coding and non-coding elements (lncRNAs) that correlated with MIAs.

Table 1. Prediction of the function of the top twenty hub genes by BLASTX search against TAIR .

Hub genes	Type	Function
cra_locus_2149	Protein coding	RIBOSOMAL RNA PROCESSING 4
cra_locus_5284	Protein coding	ELKS/Rab6-interacting/CAST family protein, chromosome condensation
cra_locus_8209	Protein coding	MACPF protein which promotes pathogen resistance by activating SA signaling
cra_locus_3069	Protein coding	Hypothetical protein, RNA metabolic process, regulation of gene expression
cra_locus_134	Protein coding	Acyl acid amido synthetases catalysing the conjugation of IAA to amino acids.
cra_locus_10800	Protein coding	Not available
cra_locus_8643	Protein coding	Not available
cra_locus_3578	Protein coding	Structural polyprotein
cra_locus_9321	Protein coding	MAIN-LIKE 1, Encodes aminotransferase like protein containing a plant mobile domain
cra_locus_9332	Protein coding	Protein phosphatase 2C family protein
cra_locus_5559	Protein coding	HEXOKINASE-LIKE 3, response to various abiotic stresses including UV, drought, osmotic, heat and cold.
cra_locus_10506	Protein coding	Encodes a subunit of RNA polymerase I
cra_locus_7967	Protein coding	Eukaryotic translation initiation factor 2B complex
cra_locus_4093	Protein coding	Not available
cra_locus_2086	Protein coding	Endoplasmic reticulum vesicle transporter protein
cra_locus_7687	Protein coding	Zinc finger protein, regulation of gibberellic acid mediated signaling pathway
cra_locus_123151	LncRNA	-
cra_locus_29870	LncRNA	-
cra_locus_123996	LncRNA	-
cra_locus_47564	LncRNA	-

Finally, the magenta module displayed enrichment in 100 BPs, 21 MFs, and 59 CCs (Supplementary Table S2). In the orange module, there was a significant enrichment of genes associated with organic substance metabolic process, cellular process and metabolic process processes. While, the dark turquoise module primarily consisted of genes enriched in cellular process and establishment of localization and response to chemicals. Meanwhile, the genes in the magenta module were associated with the photosynthesis-related functions (Figure 3 and Supplementary Tables S1-3).

The selected modules 'pathway enrichment analysis demonstrated that the 'metabolic pathways' primary metabolism, 'nitrogen compound metabolic process' and ' biosynthesis of secondary metabolites' were the top significantly enriched pathways (Supplementary Tables S1-3).

Identification of hub genes of interested modules

To discover the central and key genes associated with the MIAs, we determined genes with high connectivity within each module, and the top 20 candidate hub genes were selected based on their connectivity levels. Intramodular connectivity of orange modules are visualized in Figure 4.

Of the twenty first hub genes, 4 were lncRNAs and 16 were coding genes. The transcript sequences of

the coding genes were searched against the Arabidopsis genome database (TAIR). The results identified the transcripts as MACPF protein which involved in pathogen resistance enhancing, HEXOKINASE-LIKE 3 involved in various abiotic responses, MACPF protein promotes pathogen resistance by activating SA signaling, Indole-3-Acetic Acid–Amido synthetase that maintains auxin homeostasis, zinc finger transcription factor responsible for gibberellic acid homeostasis and ribosomal RNA processing proteins (Table 1).

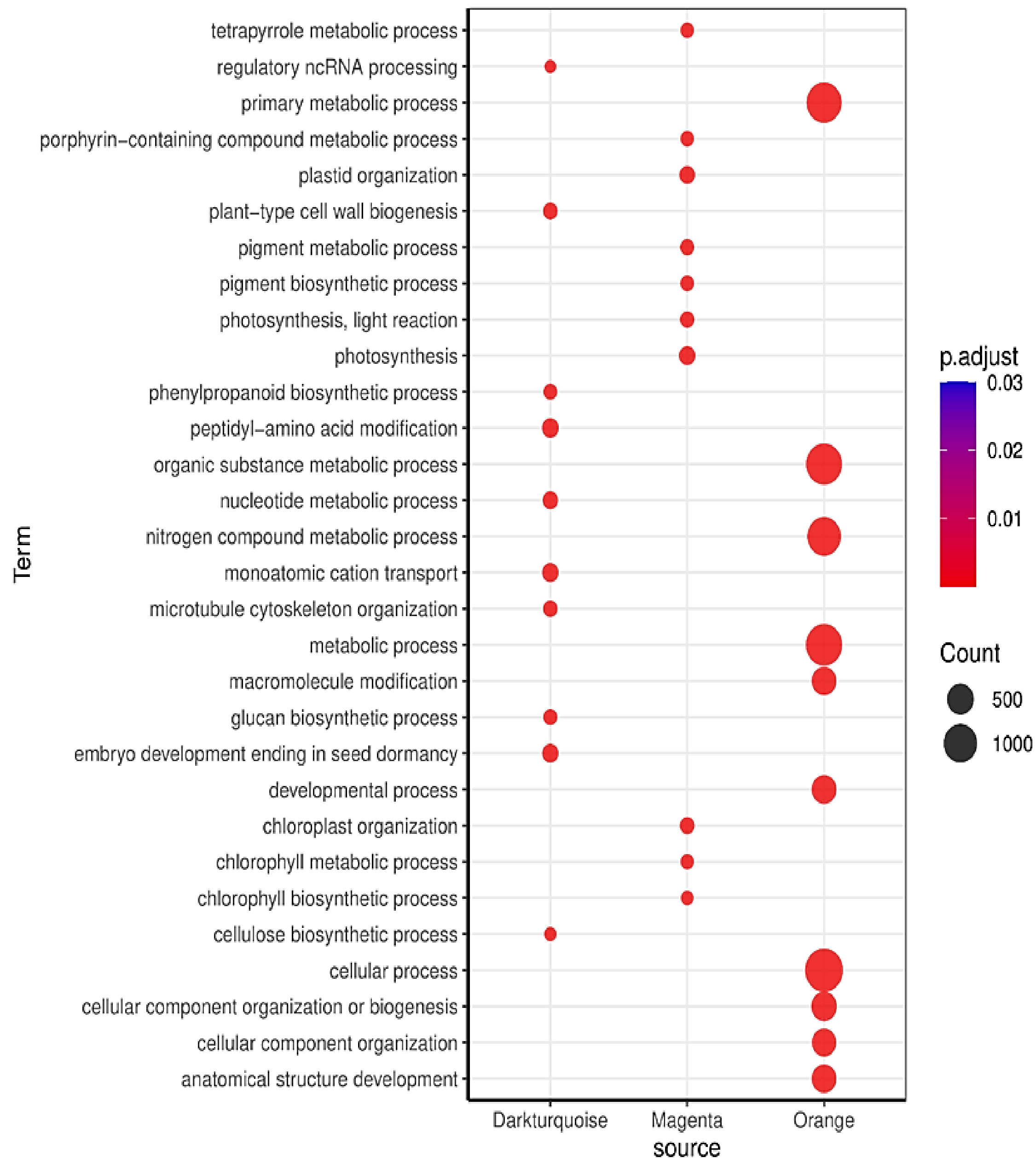

Figure 3. Gene ontology (GO) enrichment analysis of the genes of selected modules. Top GO terms were selected based on the adjusted $p < 0.05$ for categories of biological process. The genes of three selected modules were significantly enriched in terms including primary metabolic process, metabolic process, nitrogen metabolic compound, organic substance metabolic process and cellular process.

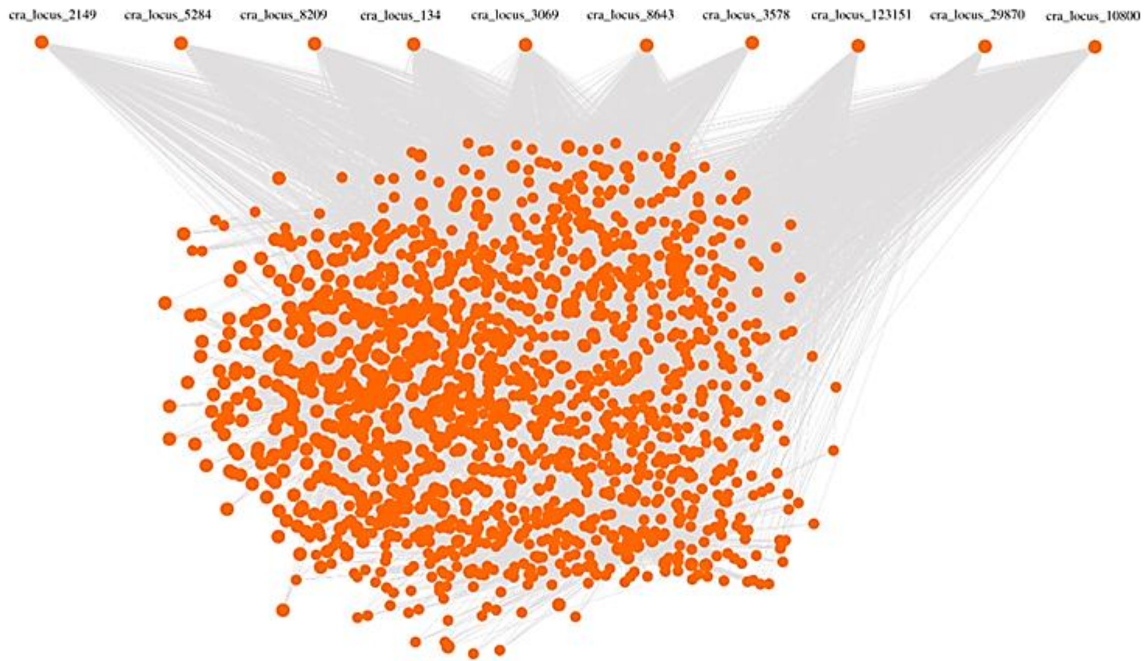

Figure 4. The gene-gene interaction network in orange module.

Among hub genes, we identified known MIA biosynthetic genes including 10-hydroxygeraniol oxidoreductase (*10HGO*), GATA-like transcription factor (*GATA1*), 7-deoxyloganetic acid UDP-glucosyltransferase (*7-DLGT*), desacetoxyvindoline 4-hydroxylase (*CrDH4*), MYC2 and MPK6.

Discussion

C. roseus produces a wide range of specialized metabolites of the MIA class via a complex, long and highly branched metabolic pathway. Reconstitution of the natural product pathway in plants is a unique challenge due to the number of enzymatic steps, the complex tissue and subcellular localization of the intermediates, and the intricate regulatory networks, as exemplified by the vinblastine biosynthesis pathway (Yamamoto et al., 2019). Tissue metabolome and transcriptome datasets in plants are necessary for metabolic pathway gene discovery, where the genes correlated with the molecule of interest or phenotype, together with extensive knowledge of enzymatic biochemical reactions, are important for gene discovery (Li et al., 2022).

In recent years, significant progress has been made in the isolation and characterization of genes encoding key biosynthetic enzymes, transporters and transcription factors in the MIA pathway (Colinas et al., 2020; Singh et al., 2020; Li et al., 2022) however, the potential regulatory role of lncRNAs has not been investigated. In the present study, the key roles of coding and non-coding (lncRNAs) genes associated with MIA pathway biosynthesis were investigated. The coding genes that correlate with lncRNAs can be considered as their targets where they exert their regulatory role by altering the transcriptional and post-transcriptional situation of their coding target genes (Wang et al., 2019; Yu et al., 2019; Zhang et al., 2019) and possibly the accumulation of MIAs.

In the present study, we focused on identifying lncRNAs in *C. roseus*. This endeavour was achieved through the implementation of a bioinformatics pipeline, resulting in the successful detection of a total of 4303 lncRNAs. In addition, our investigation extended to the use of co-expression network analysis, a tool that enabled us to pinpoint both the relevant coding genes and the lncRNAs involved in the intricate process of alkaloid biosynthesis.

This analytical approach unveiled the presence of three distinct modules: orange, dark turquoise, and magenta. Remarkably, these modules exhibited a robust correlation with the alkaloid biosynthesis pathway. Notably, the orange module displayed an enrichment of C2H2, C3H, Homeobox, bHLH and AP2-EREBP TF families. Turning our attention to the dark turquoise module, our examination uncovered a range of TFs such as C3H, bHLH, C2H2, bZIP and Homeobox. Among the TFs found to correlate with MIAs in this study, the regulatory function of the bHLH-CrMYC2 and AP2-ERF ORCA TFs has been demonstrated in previous studies (Van der Fits and Memelink, 2000; Zhang et al., 2011; Pan et al., 2012; Sui et al., 2018; Singh et al., 2020; Yang et al., 2023). CrMYC2 acts upstream of ORCA3, directly regulating it by binding to its promoter and indirectly controlling ORCA4 and ORCA5. CrMYC2, activates ORCA3, which in turn induces the expression of several MIA biosynthetic genes (Zhang et al., 2011) in the tryptamine or vindoline branches, including cytochrome p450 reductase (*CPR*), anthranilate synthase (*AS*), D-1-deoxyxylulose 5 -phosphate synthase (*DXS*), tryptophan decarboxylase (*TDC*), STR and desacetoxyvindoline 4-hydroxylase (*D4H*) but not those in the seco-iridoid branch, such as iridoid synthase (*IS*) and geraniol 10-hydroxylase (*G10H)* (Van der Fits and Memelink, 2000; Van Moerkercke et al., 2015). The top five TF families detected in magenta module were bHLH, C2H2, MYB, bZIP, C2C2-Gata. Interestingly the GATA TF was detected at a high frequency in the magenta module, which this module showed a high correlation with vindoline (0.83, p-value 0.00006). The GATA TF regulates light-induced vindoline biosynthesis in *C. roseus*. Expression of 5 out of 7 genes in the vindoline pathway is significantly induced by GATA TF in response to light (Liu et al., 2019).

Based on the pathway analysis conducted on the hub genes contained within the selected modules, our observations revealed substantial enrichment in pathways denoted as metabolic pathways, porphyrin metabolism, photosynthesis and biosynthesis of secondary metabolites. Furthermore, we conducted a screening process to identify hub genes, revealing that the orange module's most prominent hub genes were *G10H*, GATA-like TF (*GATA1*), 7-deoxyloganetic acid UDP-glucosyltransferase (*7-DLGT*), *D4H* and *MYC2* TFs. Similarly, the dark turquoise module showed MPK6 as its central hub gene, while the magenta module's key hub gene was UDP-glucosyltransferase. The catalytic or regulatory role of the identified hub genes in the MIA pathway will be described. A previous study demonstrated that the CrMAPK6, as a member of the CrMAPKK1-MAPK3/6 cascade, acts upstream of the *ORCA* gene cluster and CrMYC2 TFs to modulate MIA pathway gene expression and MIA accumulation (Paul et al., 2017).

The iridoid pathway begins with the enzyme G10H, a cytochrome P450 monooxygenase that hydroxylates geraniol to 10-hydroxygeraniol. Production of several MIAs enhanced by overexpression of G10H in hairy roots (Wang et al., 2010; Peebles et al., 2011). G10H is regulated by BIS1 transcription factor (Van Moerkercke et al., 2015). Overexpression of *ORCA3* and a structural gene *G10H* results in increased accumulation of strictosidine, vindoline, catharanthine and ajmalicine in *C. roseus* plants. (Pan et al., 2012). *7-DLGT* catalyses the glucosylation of 7-deoxyloganetic acid to 7-deoxyloganic acid in the secoiridoid pathway. The endpoint of the iridoid or secoiridoid branch is secologanin, which couples with tryptamine to form strictosidine, the universal and important MIA precursor in plants. (Miettinen et al., 2014). D4H is involved in the conversion of tebersonine to vindoline. Vinblastine, the end product of the MIA biosynthetic pathway, is derived from the coupling of vindoline and catharanthine (Liu et al., 2019).

Furthermore, hub genes that play crucial roles in stress response and the intricate process of hormone transduction were identified. These genes were highly correlated with hub CrlncRNAs such as *Crlnc808*, *Crlnc758*, *Crlnc316* and *Crlnc1046*. Because of the mutual molecular mechanisms between stress responses and the specialized metabolites biosynthesis these coding sequences can be considered as the targets of CrlncRNAs that possibly involved in regulating MIA biosynthesis.

A collaboration model, involving BPL3-nalcFL7-FL7 to coordinate plant immunity, exists as a result of interaction among lncRNA and code genes. In this cascade, the lncRNA (nalncFL7) negatively

regulates resistance to *Phytophthora capsica* by suppressing the accumulation of FL7 (FORKED-LIKE7) transcripts, which positively regulate plant immunity to *P. capsica* (Ai et al., 2022). In another example, lncRNA and transcription factors working together to regulate the stress response, it has been shown that lncRNA33732, activated by WRKY1, induces RBOH expression to increase H_2O_2 accumulation in the induced defense in tomato plants against *P. infestans* and enhances tomato resistance to *P. infestans* (Cui et al., 2019).

Highly correlated CrlncRNAs with known MIA-associated genes, including catalytic enzymes and transcription factors, as well as MIAs, suggest that CrlncRNAs represent a new layer of regulatory elements of the MIA pathway. In addition, we introduce the correlated genes with CrlncRNAs as their possible coding target genes, which CrlncRNAs can regulate MIAs accumulation by modulating their targets.

Conclusion

C. roseus produces an extensive variety of specialized metabolites belonging to the MIA class through a highly complex and branched pathway. This implies a complex regulation by specific transcription factors, of which several families have been discovered in the last few decades. Conversely, no study has reported the involvement of lncRNAs in the regulation of MIA biosynthesis. In the current study, WGCNA was used to directly integrate transcriptome and metabolome data to construct gene networks that reflect the relationships between lncRNAs and metabolites. The next step was to identify coding genes that correlated with candidate CrlncRNAs and MIAs. Among the known MIA pathway genes, *10HGO*, *GATA1*, *7-DLGT*, *D4H*, *MYC2*, and *MPK6* were tightly correlated with candidate lncRNAs and MIAs. Therefore they can be proposed as coding targets of lncRNAs that modulate MIAs production in a cooperative manner. In addition, these findings further highlight the regulatory role of the MPK6-MYC2-ORCA cascade in the MIA pathway.

Supplementary Materials

The Supplementary Material for this article can be found online at: https://www.jpmb-gabit.ir/article_709096.html.

Supplementary Table S1. The GO enrichment results of orange module.

Supplementary Table S1. The GO enrichment results of magenta module.

Supplementary Table S3. The GO enrichment results of darkturquoise module.

Supplementary Table S3. Identified lncRNAs associated with MIAs in *C. roseus*.

Author Contributions

Conceptualization, H.M. and A.T.; methodology, F.A., A.T.; software, A.T., F.A. and A.S.; formal analysis, F.A., B.S.K.; investigation, H.M.; data curation, F.A., A.T.; writing—original draft preparation, F.A.; writing—review and editing, A.S. and A.T.; visualization, F.A. and B.S.K.; supervision, H.M.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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مقاله پژوهشی

آنالیز شبکه هم‌بیانی جهت شناسایی ماژول‌های mRNA و long non-coding RNA کلیدی مرتبط با بیوسنتز آلکالوئیدها در گیاه *Catharanthus roseus*

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چکیده: گیاه *Catharanthus roseus*، طیف متنوعی از متابولیت‌های تخصصی به نام مونوترپن ایندول آلکالوئیدها (MIAs) را از طریق یک مسیر متابولیکی گسترده و پیچیده تولید می‌کند. بنابراین شناسایی شبکه‌های تنظیمی پیچیده و روابط بین ژن‌های دخیل در تولید این متابولیت‌ها ضروری بنظر می‌رسد. RNAهای طولانی غیر کدکننده (lncRNAs) اخیرا به عنوان عوامل تنظیم‌کننده مهم در فرآیندهای زیستی متنوع ظهور پیدا کرده‌اند. در این مطالعه، ۴۳۰۳ از ۸۶۷۲۶ رونوشت به عنوان lncRNA بالقوه در گیاه *C. roseus* شناسایی شدند. متعاقباً، ژن‌های کدکننده بسیار همبسته با CrlncRNA شناسایی شدند و آن‌ها به‌عنوان ژن‌های هدف بالقوه دخیل در تنظیم مسیر MIA با استفاده از تجزیه و تحلیل شبکه هم‌بیانی ژن وزنی (WGCNA) تعیین شدند که منجر به شناسایی خوشه‌های ژنی مهم مرتبط با بیوسنتز MIA شد. بر اساس یافته‌ها، سه ماژول (dark turquoise، magenta و orange) و همچنین ژن‌های هاب مرتبط با MIA مشخص شدند. به‌علاوه، مهمترین ژن‌های کدکننده شناخته‌شده مسیر شامل 10-hydroxygeraniol oxidoreductase، فاکتور رونویسی *GATA1*، 7-deoxyloganetic acid UDP-glucosyltransferase (*7-DLGT*)، desacetoxyvindoline 4-hydroxylase (DH4)، *MYC2* و *MPK6* با lncRNAs و متابولیت‌های MIA همبستگی داشتند. ژن‌های هدف ناشناخته، با فرآیندهایی مانند پاسخ به استرس و انتقال هورمون مرتبط بودند. نتایج بدست آمده نشان دهنده اهمیت ژن‌های *ORCA*، *MYC2* و *GATA1* در تنظیم مسیر MIA است و به احتمال زیاد این ژن‌ها نقش خود را در همکاری CrlncRNAs ایفا می‌کنند.

کلمات کلیدی: LncRNAs، متابولیت‌های تخصصی، فاکتورهای رونویسی، گیاهان دارویی.

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