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REVIEW

MicroRNAs Roles in Plants Secondary Metabolism

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ABSTRACT

Plant growth and development is dependent on the regulation of classes of microRNAs (miRNAs) that have emerged as important gene regulators. These miRNAs can regulate plant gene expression to function. They play an important roles in biological homeostasis and environmental response controls. A wide range of plant biological and metabolic processes, including developmental timing, tissues specific development, and differentiation, depends on miRNAs. They perpetually regulate secondary metabolite functions in different plant family lines. Mapping of molecular phylogenies shows the distribution of secondary metabolism in the plant territory. More importantly, a lot of information related to miRNA regulatory processes in plants is revealed, but the role of miRNAs in secondary metabolism regulation and functions of the metabolites are still unclear. In this review, we pinnacle some potential miRNAs regulating the secondary metabolite biosynthesis activities in plants. This will provide an alternative knowledge for functional studies of secondary metabolism.

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1. Introduction

Plants have been utilized as a source of nutrition, drug, and livelihood improvement over the year [1]. Human population development is believed to directly relate to increasing demand for plants and plant activities, which give merits to expand the yield of plants for different purposes. [2] Advanced progressive logical strategies have also predicted an increase or decrease in plant functions and capacities. With this, it is revealed that these advanced strategies can help solve many plant-related issues to help bridge the gap between humans and plants. The advance strategic inputs have also shown the influence of miRNAs in managing primary biological processes, including metabolic procedures at the post-transcriptional level. The miRNAs are non-coding small RNAs composed of 20–23 nucleotides. [3] About thousands of these miRNAs have been identified in plants. [4] In plants, they are divided into two groups: conserved miRNAs that occur in different families and species-specific miRNAs characterized by low-level expression. [5] Different works have shown that miRNAs influence plant developmental activities such as blooming of leaves, seed emergence, and root morphology [1], not forgetting the hormonal reactions in the plants. [6] They also play a critical part in a wide range of biological processes, [7] such as developmental timing, [8] cell and tissue differentiation, [9] proliferation, [10] apoptosis, [11] and metabolism. [12] Their function at the plant's biological level improves growth, development, and biological responses. At the gene expression level, they effectively obstruct the translation of their target gene (mRNAs). [13] More importantly, the miRNAs can control the expression of genes that encode transcription factors [14] and strain reaction proteins, which impact the biological processes in the plant. [15] They similarly control organic procedures in plants, such as upkeep of genome honesty, primary and secondary metabolisms, [16] development of signal transductions, [17] signaling pathways, [18] and adaptive responses to biotic and abiotic stresses. [19] Where these metabolites contribute to plants' growth and development, the nutrient and beneficial properties of plants are also owed to the presence of an abundance of these metabolites. [20] The metabolites are a group of two types, primary and secondary. [21] The secondary metabolites regulated plant phytochemicals processes related to their interactions with the environment. [22] The secondary metabolites consist of phytochemical compounds, including terpenoids, alkaloids, phenolics, glycosides, tannins, and saponins. The metabolites are predicted to function in the interactions of plants and their environment. The metabolites are predicted to protect plants from a series of environmental conditions. [23] Specifically, flavonoids, terpenoids, and carotenoids are recognized for their dynamic functional roles in plant reproduction system, growth, development, and defense mechanisms. [24] Over the years, miRNAs significant role in regulating biosynthetic activities and secondary metabolites in plants has gained much attention. [16] This review presents updated knowledge of miRNA's functions in regulating phenolypropanoid biosynthesis, terpenoid biosynthesis, alkaloid biosynthesis, chlorophyll biosynthesis, carotenoids biosynthesis, and genes targeted by miRNAs.

2. Molecular phylogenies distribution of secondary metabolites in plants

Over the years, botanists have reconstructed a tree of existence for the plant kingdom usage of nucleotide sequences, plastid, and nuclear marker genes. [25] Ensuing this, molecular phylogenies can map the distribution of secondary metabolism in the plant territory. It is suggested that plants accumulate a positive secondary metabolism type, making it probable that their

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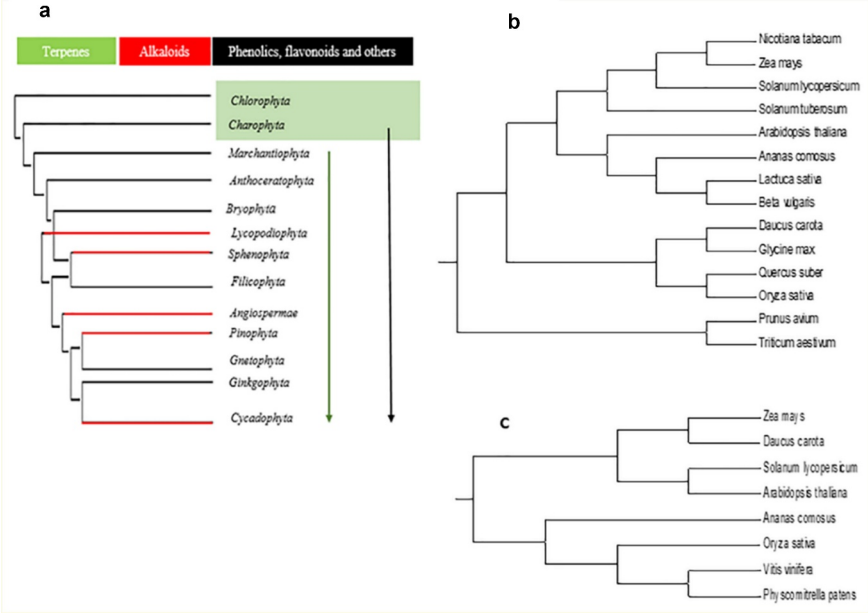

Figure 1. Molecular phylogeny of terrestrial plants. Groups of biological species that produce terpenes are marked in green, terpenoids in red and phenolics, flavonoids, and others in black (a). Molecular phylogeny of PAL (b) and CHS (c) secondary from their corresponding genes' nucleotides sequences.

synthesis and functions are genetic. This would advocate that the corresponding pathways and miRNAs have been advanced all through early growth. [26] In Figure 1, we show the molecular phylogeny of some selected significant plant families. The lines show the position of synthesizing flavonoids and other phenolics deriving from phenylalanine. This suggests that the secondary plant metabolites existed from the early ages (Figure 1 A), linking the secondary metabolites gene families to the miRNAs' possible functions in secondary metabolites (Figure 1B,C). Considering the most corresponding main enzymes, phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) have been detected in plants. [27] Terpenoids, such as mono-, sesqui-, tri-, and tetraterpenes, and steroids are also existing from lower plants to more higher plants, suggesting that the generate pathways of the main energetic substance isoprene and the cyclization of C-5 units had been present already in plants ancestry.[28,29] Alkaloids have a more excellent patchy distribution, and distinct alkaloidal sorts are usually precise for certain taxon groups; therefore, they had been regularly used as chemotaxonomic markers. Alkaloids can be detected in plants but are predominant in angiosperms. [30] Because many alkaloids are neurotoxins, which interfere with pursuit of neuronal sign transduction, we speculate that the diversification of alkaloids in plants coevolved with the rapid diversification of tertiary periods. The distribution of alkaloids provides a clear example of the perception of secondary metabolism dynamics with limited occurrences.

3. Regulatory function of miRNAs in secondary metabolism activities

Many scientific questions are asked: under what condition are secondary metabolites produced and can the production be regulated by miRNAs? A large number of miRNAs control genes (translation factors and structure genes) and biological activities including environmental change reactions that affect natural procedures in plants. [31] They function to regulate plant's biological processes such as maintenance of genome integrity, primary and secondary metabolism, development, signal transduction and pathways, homeostasis, innate and immunity, and adaptive responses to environmental change. [32] The secondary metabolites are groups of phytochemicals that regulate various processes related to the plants' interaction and their environment. [33] They are mixtures assimilated with terpenoids, alkaloids, phenolics, glycosides, tannins, saponins and shield plants from biotic and abiotic stressors. [34] Plants integrate secondary metabolism categories to help in self-protection. [35] The generation of metabolites can be overseen by the miRNAs since the miRNAs can positively or negatively regulate the production of the anticipated metabolites while limiting the production of toxic metabolites and new metabolites that can be produced. [36] Stimulated plants initiate change in quality articulation and generation of guarded metabolites, and miRNAs control these activities. For example, *Solanum tuberosum* L. under light improvement revealed that light-responsive miRNAs could significantly control alkaloid digestion, lipid biosynthesis, and cellulose catabolism. [37] In addition, transcriptomes analysis of *Swertia chirayita* shows recognizable proof that miRNAs regulate metabolite biosynthesis. [38] The miRNAs consisted of *miR-156a, miR-166a, miR-166b, miR-168, miR-11071*, and *miR-11320*, that regulates metabolites biosynthesis. [38] Moreover, in *Swertia chirayita*, it is predicted that homeobox-leucine zipper encoded a protein with a potential relationship with metabolites biosynthesis process. [34] Similarly, the funtion of *miR-774* and *miR-1126* in numerous plant biological processes includes the development of secondary metabolic pathways. [39] In summary, these illustrations indicate that specific miRNAs are associated with the secondary metabolism activities in response to the influence of plant growth and differentiation.

3.1 miRNAs/phenylpropanoid biosynthesis

Phenylpropanoids are the largest class of plant secondary metabolites. They are derived from the aromatic amino acid phenylalanine. [40] They mainly include flavonoids, monolignols, phenolic acids, stilbenes, and coumarins. [41] It is broadly disseminated in most plant realms revealing its fundamental functions in the secondary metabolites. They improve plants through cell walls, protection against high light and UV radiation, phytoalexins against pathogens, and pollinator interactions. [42] Its pathway serves as a rich source of metabolites activities in plants, which is required for the biosynthesis of lignin, flavonoids, coumarins, and hydroxycinnamic acids. [43]

Moreover, the phenylpropanoid pathway serves as a brilliant structure for developing through genetically manipulating complex natural product pathways and responds to environmental changes in plants. [44,45] An example is the overexpression of *miR-7695* to boost disease resistance to a fungal pathogen that causes blast disease in *Magnaporthe oryzae* plant. [46] Interestingly, the blocking of *miR-858a* activity can result in the upregulation of flavonoid-specific target genes like *AtMYB12* and *AtCHS1* which is involved in the phenylpropanoid pathway process. [47] Besides, *miR-159, miR-172*, and *miR-530* obtained from the roots and leaves also regulate secondary metabolite associated with mRNAs of *Chlorophytum borivilianum, Oryza sativa*, and *Arabidopsis thaliana*. [48]

Interestingly, the target genes of *miR-9662, miR-894, miR-172*, and *miR-166* are revealed to be involved in regulating the plant phenylpropanoid saponin metabolic biosynthetic process, but an activated *miR-8154* and *miR-5298b* also increase the phenylpropanoid content. [48] In summary, this phenylpropanoid is being broadly disseminated in the plant realm. It assumes a tremendous fundamental functional work in a plant, such as improving growth and cell differentiation of cell walls. The miRNAs discussed in phenylpropanoid functional activities regulate plant growth and development. With this, much attention should be given to phenylpropanoid potential miRNAs since most agronomic crops and model plants are exposed to light and other inhibitory conditions in practice.

However, specifically in flavonols, it is exceptionally unpredictable. In *Arabidopsis thaliana*, different MYB transcription factors control flavonols biosynthesis process, including *MYB11*, and *MYB12*. [49] These MYBs influence three explicit flavonols in *Arabidopsis* under which *miR-858a* is expressed extensively in all the tissues, which means that the expression of *miR-858a* may directly control the MYBs and the flavonol production in the *Arabidopsis* leaf tissue. [6] Similarly, *miR-156* regulates flavonoid biosynthesis in *Arabidopsis. miR-156-SPL9* directly influenced anthocyanin production through targeting genes encoding *AP-1-like transcription factor* (PAP1) and dihydroflavonol 4-reductase. [50] During the flavonoid expansion, *miR-160* upregulate *Auxin response factor 17* (ARF17) and downregulate *Auxin response factor 18* (ARF18), to response to different factors affecting root development. [51] *MiR-828* act as a negative regulator of anthocyanin and polyphenolics biosynthesis, regulated the conspicuous red fruit trait of *Ananas comosus* var. *Bracteatus*. It directs post-transcriptional gene silencing of MYB family members associated with UV light- and sugar-signaling. [52] Two flavonoid responsive genes, *Cryptochrome1* (CRY 1) and *Cryptochrome2* (CRY 2), is upregulated by *miR-172*, to which they mediate the expression and stimulation of *CONSTANS 67* independently during the photoperiodic flowering time. [53] In grapevine berry fruit, it is revealed that the up-regulation of *miR-3627* and *miR-4376* facilitates anthocyanin accumulation. [54] Several miRNAs including, *miR-171, miR-166i, miR-159e, miR-845, and miR-396e*, were identified to control carotenoids and flavonoids biosynthesis. [55]

3.2 miRNAs/terpenoid biosynthesis

The largest group of natural products are the terpenoids, used as aroma compounds and natural flavor enhancers. [56] The terpenoids are synthesized through two independent pathways: cytosolic mevalonic acid (MVA) and plastidial methylerythritol phosphate (MEP) pathway.[57] Terpenoid compounds are produced in high abundance under biotic stress as compared to abiotic stress. [58] In *Ananas comosus* var. *Bracteatus* leaves, it is revealed through transcription analysis that about 43 identified unigenes encoded enzymes are involved in the terpenoid backbone biosynthesis. [57] Moreover, *miR-2919, miR-5251, miR-838, miR-5021, and miR-5658* from *Ferula gummosis* control for terpene biosynthesis. However, the transcription factors *SPL7, SPL11*, and *ATHB13* from *Arabidopsis* predicted to function in terpenoid biosynthesis are putatively regulated by *miR-5021 and miR-5658*. [59] It is predicted that *miR-5021* and *miR-414* function to regulate terpenoid backbone biosynthesis and sesquiterpenoid and triterpenoid biosynthesis, respectively, whereas *miR-7540, miR-5183, miR-6449, miR-5255, miR-5491*, and *miR-6435* target downstream of catalysts in mono, sesqui-, di-, and tri-terpenoids of triterpenoid biosynthesis. [60] In the same vein, *Persicaria minor* has a high level of secondary metabolite compounds, particularly terpenoids, where *pmi-miR-6173* and *pmi-miR-6300* are downregulated, but *miR-396a* and *miR-398 f/g* are upregulated. [61]

Moreover, in *Arabidopsis thaliana* and *Pogostemon Cablin*, transcription factor, *SPL9* and *SPL10* targeted by *miR-156* are predicted to be an activator for terpene synthesis. [62] Overexpression of *miR-156* results in the downregulation of the sesquiterpenes level in *Arabidopsis thaliana* and *Pogostemon Cablin*. [62] MiRNAs are predicted to be involved in sesquiterpenes biosynthesis pathways through a computational prediction approach in *Xanthium strumarium*. [62] Similarly, the overexpression of *miR-393* in *Arabidopsis thaliana* changes the glucosinolate and camalexin levels through perturbation of auxin signal pathway. [63] Table 1 presents some miRNAs predicted to regulate terpenoid biosynthesis in some plant species. These miRNAs will be very useful in terpenoid biosynthesis, regulating these plant species' metabolic and biological processes.

3.3 miRNAs/alkaloids biosynthesis

Alkaloids are nitrogen-containing low molecular-weight compounds. They are mostly derived from amino acids. [71] Alkaloids are recognized to play a significant role in plant defense against pathogens. [72] Over the years, about 12,000 alkaloids have been characterized in these highly diverse and

Table 1. Probable miRNAs regulation in terpenoid biosynthesis with target gene and their functions.

MicroRNAs	Targets	Functions	Ref.
miR-164a-d miR-528 miR-319e miR-159	*4-hydroxy-3-methylbut-2-enyl-diphosphate synthase.*	It is essential for chloroplast development and required for the salicylic acid (SA)-mediated disease resistance to biotrophic pathogen.	64
miR-9662a-p miR-894	*Heptaprenyl diphosphate synthase*	Supplies heptaprenyl diphosphate, the precursor for the side chain of the isoprenoid quinone menaquinone	48,58,65
miR-319a miR-166 J miR-156 g miR156m miR-156e	*Diphosphomevalonate decarboxylase and SPL transcription factor*	Accumulation of anthocyanins, whereas reduced *miR156* activity results in high levels of flavonols	48,55,66
miR-171a-3p	*Phosphomevalonate kinase*	Transfers a chemical group, e.g., a methyl group, to another compound (acceptor) and catalyzes phosphate transfer to a second substrate.	48,58
miR-172d-3p	*1-deoxy-D-xylulose-5-phosphate synthase*	A limiting enzyme for plastidic isoprenoid biosynthesis and essential for chloroplast development.	48,58,67
miR-172 c	*Farnesyl diphosphate synthase and geranylgeranyl diphosphate synthase*	Geranylgeranyl diphosphate (GGPP) synthase activity. The enzyme catalyzes the synthesis of GGPP from farnesyl diphosphate and isopentenyl diphosphate.	48,55,68
miR-167 g-5p miR-167 c miR-167 f-5p	*Isopentenyl-diphosphate Delta-isomerase*	It is involved in the pathway chlorophyll biosynthesis, which is part of Porphyrin-containing metabolism compounds.	48,55,69
miR-167 c miR-159 c	*Hydroxymethylglutaryl-CoA synthase*	Condenses acetyl-CoA with acetoacetyl-CoA to form HMG-CoA, which is the substrate for HMG-CoA reductase	61,70
miR-396-3p miR-894 miR-9662a miR-159 miR-172 c	*Farnesyl-diphosphate synthase and geranylgeranyl diphosphate synthase*	Flavonoid biosynthesis	55
miR396-3p miR-894	*Trans-nonaprenyl-diphosphate synthase*	Flavonoid biosynthesis	55

heterogeneous secondary metabolites. [73] The alkaloids consist of indole, piperidine, tropane, purine, pyrrolizidine, imidazole, quinolizidine, isoquinoline, and pyrrolidine compounds. [72] These compounds are created through different metabolic pathways in the alkaloids. [74] However, genome-based technological advancement has added to our current study's understanding of alkaloid biosynthetic pathways and functions. [34] Consistent information on miRNAs' functional roles regulating alkaloid biosynthesis and its accumulation in plant's monarchy is on the rise. [34] Research conducted in 2014 revealed different miRNAs regulating alkaloid biosynthesis pathway in *Opium poppy* plant (species of flowering plant in the family of Papaveraceae), [75] including *pso-miR-13, pso-miR-2161*, and *pso-miR-408*. The *pso-miRNA-2161* acts as an intermediate molecule in benzylisoquinoline alkaloids biosynthesis targeted with mRNA encoding *S-adenosyl-L-methionine, 30-hydroxy -N-methylcoclaurine*, and *40-O-methyltransferase* enzymes which convert S-norcoclaurine into S-reticuline. [76] Correspondingly, *pso-miR-13* is evaluated to target mRNA *7-O-methyltransferase*, which is predicted to convert S-reticuline to morphinan alkaloids. [34]

Interestingly, most of the endogenous targets of miRNAs obstruct the function of corresponding miRNAs by inhibiting miRNAs' binding with their authentic target genes. [77] For example, in poisonous *Taxus baccata*, [78] two predicted paclitaxel biosynthetic genes, *Taxane 13α hydroxylase* and *Taxane 2α-O-benzoyltransferase*, cleavage target of *miR-164* and *miR-171* to obstructs the poisonous content. [79] *Mentha* is revealed to have *miR-156, miR-414*, and *miR-5021* responsible for regulating essential oil biosynthesis. However, *miR-156* is specially revealed to participate in flavone and alkaloids biosynthesis. [80] A better understanding of miRNAs regulating alkaloid and molecular regulation of these predicted miRNAs can improve plant growth, such as integrated by plants, to protect self-protection.

3.4 miRNAs/chlorophyll biosynthesis

The chlorophyll content is critical for plant pigments involved in light engagement and energy transfer during photosynthesis. [81] They are essential for generating reactive oxygen for plant species, essential for plant growth and development. [82] The precursors of chlorophyll biosynthesis involve oxygen, which enables the activity of secondary metabolite in a specific plant. [82] It has been well documented that multiple pathways regulate chlorophyll biosynthesis at transcriptional and post-transcriptional levels, which means that chlorophyll biosynthesis is involved in the secondary and primary metabolic

Table 2. The roles of chlorophyll biosynthetic miRNAs in plants and their target genes.

MicroRNAs	Regulation	Species	Target	Phenotypes	Target Function	Ref.
miR-171b	Up	Broccoli	*SCL*	Dark green	Plant information transmission and signal transduction	92
miR-171b	Up	*Arabidopsis thaliana*	*SCL*	Silique	Signal transduction	93
miR-127 *miR-160* *miR-161* *miR-163*	Down	*Ananas comosus* var. *bracteatus*	*DVR add function*	White	Catalyzes the conversion of divinyl chlorophyllide to monovinyl chlorophyllide	86
miR-171	Down	*Arabidopsis thaliana*	*POR*	Pale Green	Catalyzes the photoreduction of protochlorophyllide (Pchlide) to chlorophyllide.	94 91

activities. [83] Most studies have revealed that diploid and autotetraploid plant leaves can decrease chlorophyll content to activate the secondary and primary metabolic activities.[84]

Meanwhile, some miRNAs that are predicted to target chlorophyll biosynthesis can increase the superoxide dismutase activity, solubility protein content, relative conductivity, sugar content, proline contents, and malondialdehyde contents.[85] In *Ananas comosus var. bracteatus*, it is revealed that *Ab-miR-124* is predicted to target the trihelix transcription factor, which acts as a molecular switch in response to light signals and related to chlorophyll biosynthesis.[86] More importantly, *miR-124* is revealed to be significantly down-regulated in the white leaves of *Ananas comosus* var. *bracteatus* leaves. Furthermore, *miR-127, miR-160, miR-161*, and *miR-163* are predicted to target *divinyl chlorophyllide a 8-vinyl-reductase* (DVR) which is down-regulated in the complete white leaves in var. *Bracteatus*.[86] *miR-156* is revealed to participate in the vegetative changing phase by down-regulating several chlorophyll *Squamosa promoter binding protein-like* (SPL) transcription factors.[87] *MiR-159* targets *Proto-oncogene like 1* transcription factor, which is required for further development in light. *Phytochrome interacting factor 3* (PIF3) is a negative regulator in chloroplast development and decreases the expression level of chlorophyll biosynthetic and photosynthetic gene activities. Moreover, *Phytochrome interacting factor 1* (PIF1) also represses chlorophyll biosynthesis. [88] Another study has revealed that the chlorophyll biosynthetic and photosynthetic genes could be directly targeted by *PIF3*. [89] These findings further support the theory that *PIF3* acts as a negative regulator in chloroplast development by directly repressing chlorophyll biosynthetic and photosynthetic genes.[89] The transcriptional level of *SCL* genes is regulated by miRNAs.[90] In *Arabidopsis, miR-171* is predicted to regulate chlorophyll biosynthesis activities positively in the light but down-regulate chlorophyll biosynthesis when targeting *Scarecrow-Like 6* (SCLs6).[91] However, the overexpression of *bol-miR171b* in transgenic *broccoli* exhibited dark green leaves with a high chlorophyll content.[92] *miR-171* upregulates *Protochlorophyllide oxidoreductase* (POR), a key enzyme in chlorophyll biosynthesis, as shown in Table 2, but the *SCLs* gene in different plant species functions differently, notwithstanding its general functions in plants. [94] Similarly, overexpression of *miR-171 c* targeting *scl6, scl22*, and *scl27* showed higher chlorophyll content and *POR* expression levels.[94] In addition, *miR-171*, targeting *rSCL27*, expresses to reduce the chlorophyll content and *POR* expression levels. On the other hand, the downregulation of *POR* expression reduces the chlorophyll content.[95] *MiR-171-SCL* module is also predicted to mediate gibberellin-dependent effects on chlorophyll biosynthesis due to its regulation of DELLA proteins and *POR* expression in the light, but not in the dark. Finally, *SCLs* induce the expression of *miR-171* genes, revealing a regulatory feedback loop.[96] This demonstrates that the overexpression of *miR-171* could affect the chlorophyll content in transgenic plants. In the table, we put together miRNAs that regulate chlorophyll biosynthetic activities in some plant species and their target gene function.

3.5 miRNAs/carotenoids biosynthesis

Plants are natural chemical factories that synthesize health-promoting micronutrients like carotenoids.[97] They are lipophilic compounds that play a critical role in pigmentation,[98] photosynthesis,[99] and development.[100] Carotenoids promote health, sexual behavior in plant reproduction, forming colors and flavors.[22] In plants, carotenoids help capture light, offer photoprotection, and signal control over gene expression.[101] Carotenoid biosynthetic nature is required throughout the plant's life cycle. The structures and levels of carotenoids are exceptionally tuned to the environment and the stages of development.[22] Carotenoids are combined and sequestered in

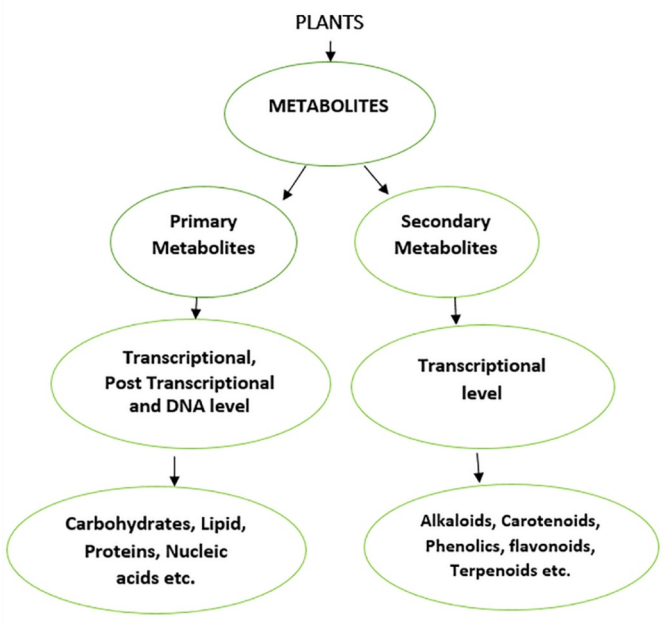

Figure 2. Systematic level occurrences of primary and secondary metabolites. The secondary metabolites through transcriptional level, give rise to different metabolites. The primary metabolites consist of transcriptional and post-transcriptional level through which proteins, lipids and carbohydrates are observed.

plastid organelles, for example, dim developed etioplasts, light-developed chloroplasts, leucoplasts from roots, amyloplasts from seeds, and chromoplasts from natural products.[102] It has been proven by research that most plants have high carotenoid content. [103] The carotenoid content in complete green leaves of *Ananas comosus var. bracteatus* is significantly higher than in the complete white leaves in three leaf developmental stages.[86]

Moreover, *SET Domain Group 8* (SDG8) varies in *histone-3lysine-4 trimethylation* surrounded by *Carotenoid isomerase (CRTISO)* gene and lutein biosynthesis. [104] The *sedge* and *CRTISO* reduced lutcin in leaves but tend to accumulate *cis*-carotenoids in etiolated seedlings. Overexpression of *miRNA-156* enhanced lutein and β carotene in *Brassica napus* seeds and shoots reproduction. [105] In *Arabidopsis, miR-156* regulates carotenoids SPL families, which further regulates other genes through complex gene regulation. Microarray analysis revealed that *Histone lysine methyltransferase* could control the expression of *SPL-15*. [106] *miRNA-156* is upregulated by 24-epibrassinolide, a high-lightening link between *SDG8* regulation, shot branching, and carotenoids.[32,105] Another analysis of the lycopene β-cyclase gene targeted by *miR-1857* is involved in carotenoid biosynthesis. [107]

5. MicroRNAs FORWARD FOR SECONDARY AND PRIMARY METABOLITES ACTIVITIES

It is meaningful to look into the intertwining functions of the primary metabolites and secondary metabolites in the plant to give a clear distinction of their activities through cell differentiation, growth, and development. Primary metabolites differed from secondary metabolites because they are required at every stage of the plant's growth process.[108] The precursor molecules for secondary metabolites biosynthesis are a channel from primary metabolites. [109] The primary metabolite biosynthetic regulation pathway is pressing advantage over the secondary metabolite for that the primary metabolite biosynthetic pathway is well explored at the transcriptional, post-transcriptional, and DNA levels. Still, the secondary metabolic pathway is narrowed at the transcriptional level. [110] The secondary metabolic pathways' activities are recently on the rise at the post-transcriptional level, which is the miRNAs interaction. As shown in Figure 2, we display the explored direction of the two metabolic pathways from the plants to their specific metabolites. Consequently, most of the research has focused on miRNAs' role during the primary metabolism of growth and development. Moreover, these miRNAs of primary metabolism and some other miRNAs have been reported for their essential role during secondary metabolism, for instance, the *SPL-miRNA-156* activities in secondary metabolites. [34]

Similarly, *miR-4995* targets *3-deoxy-7-phosphoheptulonate synthase* in the initial step of the phenylpropanoid pathway for picrosides-I biosynthesis. [34] *The 3-deoxy-7-phosphoheptulonate* enzyme also initiates the phenylpropanoid pathway process. This enzyme holds the key to the pathway's progress as its regulation of cinnamic acid production, which affects picrosides-I content in plants. [34] Regarding these regulatory roles, the modification in the miRNAs' expression level would help control the biosynthesis activities of secondary metabolites in plants. For example, *SPL9* and *TCP3* transcription factors play a significant role in secondary metabolism regulation, [16] miRNAs targeting these genes would be ideal candidates for such an approach. [34] However, identifying and understanding the three-dimensional and temporal expression of other miRNAs might also regulate the effort at the branch point of primary and secondary metabolic pathways.

6. Conclusion

Owing to the wide range of the biosynthetic pathways of the secondary metabolism, biological significance in plants is essential. Secondary metabolites as written and their regulatory miRNAs functions unveiled are a helpful biological research approach. However, on the other hand, computational deep sequencing technology methods for miRNAs predictions have huge statistics, which can also be considered. With all these, the knowledge of miRNAs regulation of secondary metabolism in the plant will increase plants' physical and biological functional knowledge. These studies will help the entire biosynthetic pathway's metabolic studies to give a clear understanding of biosynthetic phytochemicals such as secondary metabolites.

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Disclosure statement

The authors declare no conflict of interest in this research.

Author contributions

MOA conceived the idea and designed the manuscript. ZX, MM, and FR collected literatures and contributed to the writing of the manuscript. MJ critically advised and evaluated the manuscript for further correction.

Data Availability

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