Chapter

# MicroRNAs Associated with Secondary Metabolites Production

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## Abstract

MicroRNAs (miRNAs) are noncoding RNAs that play an important role in the regulation of the genetic expression in animals and plants by targeting mRNAs for cleavage or translational repression. Several miRNAs regulate the plant development, the metabolism, and the responses to biotic and abiotic stresses. Characterization of an miRNA has helped to show its role in fine tuning the mechanisms of posttranscriptional gene regulation. Although there is a lot of information related to miRNA regulation of some processes, the role of miRNA involved in the regulation of biosynthesis of secondary plant product is still poorly understood. In this chapter, we summarize the identification and characterization of miRNAs that participate in the regulation of the biosynthesis of secondary metabolites in plants and their use in the strategies to manipulate a controlled manipulation.

**Keywords:** alkaloids, flavonoids, flavonols, gene expression, isoprenoids

## 1. Introduction

### 1.1 Definition of miRNA

mRNAs are noncoding single-stranded RNA molecules that range from a length of 18 to 28 nucleotides. These molecules play an important role in post-transcriptional regulation by the inhibition of the expression of target genes by binding to mRNA [1]. Eukaryotic organisms, such as plants and animals, and some viruses express this type of molecules [2, 3]. Lin-4 is the first miRNA that was identified in *Caenorhabditis elegans*, although later in the same organism were found 22-nt lin-4 and 21-nt let-7; currently, more than 18,226 miRNAs are reported [4]. Although miRNA:target-gene interactions are widely conserved, this process is limited between kingdoms [5]. miRNAs are distributed in genome as single or clusters expressed as polycistronic units and share function relationships [6]. In plants, most miRNAs are encoded by their own primary transcript; few examples of miRNA cluster are reported (i.e., miR395). Introns are the main hotspots for their origination [7]. Some of the mechanisms where they act are the development of time and host-pathogen interactions, as well as cell differentiation, proliferation, apoptosis, and tumorigenesis.

### 1.2 Mechanism of action of miRNAs

The biogenesis of miRNAs in plants and animals presents some differences. In both plants and animals, the precursors of miRNAs are polyadenylated caps and RNAs, and transcribed as for any coding RNA by RNA polymerase II (RNAPII) [5, 8]. However, in the plants, the primary transcript (pri-miRNA) that gives rise to the miRNA is produced by the nuclear RNAase dicer-like 1 (DCL1) and its accessory proteins SERRATE (SE) and hyponastic leaves (HYL1) [8]. Also, Drosha gene is absent in plants [5].

In *Arabidopsis thaliana*, DCL1 and hyponastic leaves 1 (HYL1) cleave the pri-miRNA in the nucleus of the cell, which gives rise to the precursor-miRNA (pre-miRNA) dsRNA. Subsequently, there is another cleavage by the action of DCL1 and HYL1 to release the miRNA; the two nucleotide 3′ overhangs are methylated by the action of the sRNA-specific methyltransferase HUA enhancer1 (HEN1). When the mature single-stranded miRNA is found in the cytoplasm, it is loaded onto AGO1 that is present in RNA-induced silencing complexes (RISC), repressing the expression by mRNA cleavage [9]. 3′ untranslated region (3′ UTR) is the union region of the miRNAs to its target mRNAs which allows it to be repressed [10].

The expression of miRNAs is regulated by transcription factors. Negative on TATA less 2 (NOT2) promotes the transcription of protein miRNA genes and facilitates efficient DCL1 recruitment in miRNA biogenesis [11]. Cell division cycle 5 (CDC5) acts as a positive transcription factor associating with miRNA genes [12]. Pleiotropic regulatory locus 1 (PRL1) has the ability to bind to DCL1 and pri-miRNAs. The miRNA duplex is transported to the cytoplasm by nuclear export factor *Drosophila* Exportin-5 ortholog HASTY (HST).

The miRNA target genes can be a single member of a gene family or regulate a multiple family members. Thus, multiple miRNA genes could be targeting a single member, with tissues and stage specificity, and/or a single miRNA gene could be regulating multiple family members. The spatial and temporal expression and abundance of mature miRNAs are tightly regulated; they vary greatly among different miRNAs; and the abundance also varies depending on the tissue types or developmental stages [13].

### 1.3 Regulatory processes involving miRNAs

In the cytoplasm of cells, the miRNAs regulate the expression of genes at the posttranscriptional level via mRNA degradation and/or translational repression [14]. Unlike animals, in plants, there is a perfect complementarity between miRNA and target mRNA [14]. To carry out the silencing, a ribonucleoprotein RNA-induced silencing complex (RISC) is formed [15]. AGO1, AGO2, AGO4, AGO7, and AGO10 slicer activity has been reported, even though AGO1 is associated with most miRNAs [16]. AGO1-catalyzed RNA cleavage (slicing) represses miRNA targets [17].

## 2. miRNAs and secondary metabolism in plants

In plants, miRNAs control the expression of genes that encode transcription factors, stress response proteins, and others, which have an impact on biological processes. The miRNAs regulate the biological processes in the plans such as maintenance of genome integrity, primary and secondary metabolism, development, signal transduction, signaling pathways, homeostasis, innate immunity, and adaptive responses to biotic and abiotic stress [18]. Secondary metabolites are a group

of phytochemicals that regulate various processes related to the interaction of the plant with its environment [19]. These compounds include terpenoids, alkaloids, phenolics, glycosides, tannins, and saponins, and defend plants from several biotic an abiotic stressors [20]. Even though these types of compounds are synthesized by plants to help in self-defense, they have diverse industrial uses such as insecticides, dyes, flavoring compounds, and nutraceuticals having a positive effect on human health. Commercial importance has resulted in a great interest in studying possibilities of enhancing its production [21]. It is known that miRNAs control several biological processes at the posttranscriptional level. Currently, some studies reveal the role that miRNAs have in the regulation of secondary metabolic pathways [20]. Therefore, the production of compounds derived from secondary metabolism can be managed through the miRNAs. Since they are positively or negatively regulated, the production of desired metabolites can be induced, the production of toxic metabolites can be limited, and new metabolites can be produced [22].

Computational analysis carried out in two transcriptomes of Swertia resulted in the identification of miRNAs associated to secondary metabolites biosynthesis; miR-156a, miR-166a, miR-166b, miR-168, miR-11071, and miR-11320 targeting metabolic enzymes, such as aspartate aminotransferase, ribulose-phosphate 3-epimerase, acetyl-CoA acetyltransferase, phosphoglycerate mutase, and premnaspirodiene oxygenase, also include a gene encoding a homeobox-leucine zipper protein (HD-ZIP) with a possible association in secondary metabolites biosynthesis in *Swertia chirayita* [23]. Elicited or infected plants induce change in gene expression and production of defensive metabolites and these might be regulated by miRNAs. *Solanum tuberosum* L. under light stimulus found light-responsive miRNAs that are important regulators in alkaloid metabolism, UMP salvage, lipid biosynthesis, and cellulose catabolism [24]. Cadmium stress in oilseed rape (*Brassica napus* L.) reported miRNAs involved in the regulations of TFs, biotic stress defense, ion homeostasis, and secondary metabolism synthesis [25]. *Nicotiana tabacum* plants infected with tobacco mosaic virus (TMV), at the early stage of infection (5 dpi), show a cluster of miRNAs with down-accumulation, while most of the miRNAs were upregulated at 15 and 22 dpi, including both miRNAs and miRNA targets [26].

### 2.1 Flavonoids

Flavonoids are secondary metabolites that possess a polyphenolic structure. Those compounds consist of hydroxylated phenolic substances having a benzo-$\gamma$-pyrone structure and derived of phenylpropanoid pathway [27]. Within the subgroups of the flavonoids are flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins, and chalcones [28]. For plants, this type of compounds is synthesized as a result of the interaction with the environment, other plants, and microorganisms. They have diverse biological functions as anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties, which are structure dependent [28]. The above makes flavonoids a compound with nutraceutical, pharmaceutical, medicinal, and cosmetic applications [28]. The production of secondary metabolites is found in cases regulated by the miRNAs (**Table 1**). Little is known about the miRNAs involved in flavonoid biosynthesis. In *Helianthus*, 323,318 ESTs were computationally screened for the miRNAs identification of them, and a miR911 family was found related to the biosynthesis of tocopherols. Gou et al. [51] demonstrate that accumulation of anthocyanins in the stems of *A. thaliana* is under the regulation of miR156-targeted squamosa promoter binding protein-like (SPL) genes. High miR156 activity promotes accumulation of anthocyanins and activity-induced of flavonols. This study also demonstrates that SPL9 negatively regulates anthocyanin accumulation through

| Plant species | miRNA | Target | Function | References |
|---|---|---|---|---|
| *Flavonoids* | | | | |
| Sunflower | miR2911 | Gamma-tocopherol methyl transferase | Tocopherols biosynthesis | [29] |
| *A. thaliana* | miR156 | SPL transcription factor | Accumulation of anthocyanins, whereas reduced miR156 activity results in high levels of flavonols | [51] |
| *Diospyros kaki* | miR395p-3p and miR858b | bHLH and MYB, respectively | Proanthocyanidin biosynthesis | [30] |
| *Lonicera japonica* | miRNAs (U436803, U977315, U805963, U3938865 and U4351355) | R2R3-MYB transcription factors | Flavonoid biosynthesis | [2, 3] |
| *A. thaliana* | MicroRNA858a | R2R3-MYB transcription factors | Flavonoid biosynthesis | [31] |
| *Halostachys caspica* | miR6194 and miR5655 | Flavanone 3-hydroxylase | Flavonols, anthocyanidins proanthocyanidins synthesis | [32] |
| *Podophyllum hexandrum* | miR1873/miR5532 | Dihydroflavonol 4-reductase C/-hydroxyisoflavanone dehydratase | Flavonoid/isoflavonoid biosynthesis | [33] |
| *Alkaloids* | | | | |
| Opium poppy (*Papaver somniferum)* | pso-miR13, pso-miR2161, and pso-miR408 | 7-O-methyltransferase, *S*-adenosyl-L-methionine:3′-hydroxy-*N*-methylcoclaurine 4′-O-methyltransferase 2/ 4′-O-methyltransferase2 (4-OMT)/FAD-binding and BBE domain-containing protein, also known as reticuline oxidase-like protein | Benzylisoquinoline alkaloids | [34] |
| Tobacco | miRX17, miRX27, miRX20, and miRX19 | QPT1, QPT2, CYP82E4, and PMT2 | Nicotine biosynthesis and catabolism | [35] |
| *T. baccata* | miR164 and miR171 | Taxane 13α hydroxylase and taxane 2α-O-benzoyltransferase | Paclitaxel biosynthetic genes | [36] |
| *R. serpentina* | miR396b | Targets kaempferol 3-O-beta-D-galactosyltransferase | Flavonol glycosides | [37] |
| *Mentha* spp. | miR156 | Basic helix-loop-helix (bHLH) | Flavone/flavonol biosynthesis | [38] |
| *Terpenoids* | | | | |
| *P. kurroa* | iRNA-4995 | 3-Deoxy-7-phosphoheptulonate synthase (DAHP synthase) | Terpenoid biosynthesis ultimately affecting the production of picroside-I | [39] |

| Plant species | miRNA | Target | Function | References |
|---|---|---|---|---|
| Korean ginseng (*Panax ginseng* Meyer) | miR854b and miR854c | 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR), farnesyl diphosphate synthase (FPS), geranyl-diphosphate synthase, squalene synthase, and squalene epoxidase (SE) | | [40] |
| *C. roseus* | mir-5021 | MYB transcription factor, geranyl diphosphate synthase, GCPE protein, UDP-glucose iridoid glucosyltransferase | Primary and secondary metabolism, Isoprenoid/terpenoid biosynthesis iridoid production in higher plants | [41] |
| *Xanthium strumarium* L. | miR7539, miR5021, and miR1134 | Nontranscriptional factor proteins, such as DXS, HMGR, IDS, and IDI, essential to produce IPP and DMAPP | Terpenoid biosynthesis | [42] |
| *Xanthium strumarium* L. | miR7540, miR5183, miR6449, miR5255, miR5491, and miR6435 | R-linalool synthase, gibberellin 3-oxidase, ent-kaurene synthase, squalene epoxidase, beta-amyrin synthase, and germacrene A oxidase | Mono-, sesqui-, di-, and tri-terpenoids biosynthesis | [42] |
| *Ferula gummosa* | miR2919, miR5251, miR838, miR5021, and miR5658 | SPL7, SPL11, and ATHB13 TFs | Terpene biosynthesis | [43] |
| *Pogostemon cablin* | miRNA156 | Squamosa promoter binding protein-like (SPL) | Sesquiterpene biosynthesis | [44] |
| *A. thaliana* | miR156 | SPL transcription factor | Modulate sesquiterpene synthase gene TPS21 responsible for the biosynthesis of (E)-β-caryophyllene | [44] |
| *Mentha* spp. | miR156, miR414, and miR5021 | Basic helix-loop-helix (bHLH) geranyl di-phosphate synthase subunit alpha-like protein (NACA), respectively | Terpenoid backbone biosynthesis, sesquiterpenoid and triterpenoid biosynthesis | [38] |
| *Others* | | | | |
| *S. chirayita* | miR-168, miR-11320, miR-166a, miR-11071, miR-156a and miR-166b | Acetyl-CoA acetyltransferase (AACT), aspartate aminotransferase (PHAT), premnaspirodiene oxygenase (PSO), ribulose-phosphate 3-epimerase (RPE), phosphoglycerate mutase (PGM), and a gene encoding homeobox-leucine zipper protein (HD-ZIP) | Secondary metabolites biosynthesis | [23] |
| *A. thaliana* | miR163 | Family of small molecules of methyltransferases | Secondary metabolism | [45] |
| *A. thaliana* | miR393 | Auxin receptors (TIR1, AFB2 and AFB3) | Increase of glucosinolate and decrease of camalexin | [46] |

| Plant species | miRNA | Target | Function | References |
|---|---|---|---|---|
| Potato | mirn79 | AP2/ERF transcription factor | JA-responsive secondary metabolites | [24] |
| *S. rebaudiana* | miRstv_7 | UDP-glycosyltransferase 76G1 (ugt76g1), kaurenoic acid hydroxylase (KAH), and kaurene oxidase (KO) | Steviol glycoside biosynthesis | [47] |
| *Arabidopsis thaliana* | miR826 and miR5090 | AOP2 | Glucosinolate biosynthesis | [48] |
| *A. thaliana* | miR826 | Alkenyl hydroxyalkyl producing 2 | Glucosinolate synthesis | [49] |
| *Salvia miltiorrhiza* | miR5072 | Acetyl-CoA C-acetyl transferase | Tanshinones biosynthesis | [50] |

**Table 1.**
*miRNA related to secondary metabolite production.*

destabilization of a MYB-bHLH-WD40 transcriptional activation complex. *Diospyros kaki* fruits collected at two examined stages (15 and 20 WAF) showed differential expression of the mRNAs, indicating that these miRNAs might regulate PA synthesis during development, and some of them are miR858 and miR156, which regulate PA synthesis. miR858 positively regulates the genes responsible for the production of PA, while miR156 does so in a negative way. miR395 is another miRNA that has an influence on PA biosynthesis [30]. Some miRNAs (U436803, U977315, U805963, U3938865, and U4351355) regulate fatty acid and flavonoid biosynthesis in *Lonicera japonica* [2, 3]. The characterization in *A. thaliana* shows that miR858a targets MYB transcription factors that are involved in flavonoid biosynthesis, growth, and development. Over-expression of miR858a downregulates several MYB transcription factors, and the higher expression of MYBs in MIM858 lines leads to the redirection of the metabolic flux toward the synthesis of flavonoids [31]. Yang et al. [32] indicate that salt stress conditions regulate miRNAs; some salt stress-related biological pathways includes calcium signaling pathway, MAPK signaling pathway, plant hormone signal transduction, and flavonoid biosynthesis [32]. Himalayan mayapple (*Podophyllum hexandrum*), miR1438 target caffeoyl-CoA O-methyltransferase and is related to phenylalanine metabolism, phenylpropanoid biosynthesis, flavonoid biosynthesis, stilbenoid, diarylheptanoid, and gingerol biosynthesis. miR1873 targets dihydroflavonol 4-reductase C related to flavonoid biosynthesis. miR5532 2-hydroxyisoflavanone dehydratase is related to isoflavonoid biosynthesis [33].

### 2.2 Alkaloids

Alkaloids are naturally compounds that have one or more of their nitrogen atoms. Alkaloids are classified into different groups: indole, piperidine, tropane, purine, pyrrolizidine, imidazole, quinolizidine, isoquinoline, and pyrrolidine alkaloids [52]. Because of their toxicity, alkaloids act as defense compounds against diverse pathogens or herbivores. Understanding the regulation of alkaloid biosynthesis is crucial for its production. Target transcript identification analyses in Opium poppy (*Papaver somniferum*) revealed that pso-miR13, pso-miR2161, and pso-miR408 (**Table 1**) might be involved in BIA biosynthesis. pso-miR13 might cleave 7-OMT transcript, involved in the conversion of S-reticuline to morphinan alkaloids. 4-OMT is the target of pso-miR216 and mediates the production of S-reticuline that is also an intermediate molecule in BIA biosynthesis. On the other hand, pso-miR408 possibly targets mRNA from reticuline oxidase-like protein in charge of the conversion of S-reticuline to (S)-scoulerine in the BIA pathway [34]. Studies in tobacco (*Nicotiana tabacum*), identified four unique tobacco-specific miRNAs miRX17, miRX27, miRX20, and miRX19 that were predicted to target key genes of the nicotine biosynthesis and catabolism pathways, QPT1, QPT2, CYP82E4, and PMT2 genes, respectively [35]. In *Taxus baccata*, two paclitaxel biosynthetic genes, taxane 13α hydroxylase and taxane 2α-O-benzoyltransferase, are the cleavage targets of miR164 and miR171, respectively [36]. *In silico* analysis reveals that miR396b in *Rauwolfia serpentina* targets kaempferol 3-O-beta-D-galactosyltransferase whose activity as transferase activity, transferring hexosyl groups is essential for formation of flavonol glycosides [37]. A computational approach in *Mentha* spp., revealed that miR156, miR414, and miR5021 are essential for regulation of essential oil biosynthesis. miR156 participates in flavone, flavonol biosynthesis, and terpenoid backbone biosynthesis [38].

### 2.3 Terpenoids

Plant terpenoids secondary metabolites are synthesized from C5 precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). They are

classified according to the number of carbon atoms as monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), carotenoids (C40), and polyprenols (>45) [53]. Like the alkaloids and the flavonoids, the biological characteristics and the applications of interest in the industry. Computational identification of miRNAs was done in six transcriptomes of *Picrorhiza kurroa* revealed that miRNA-4995 has a regulatory role in terpenoid biosynthesis (**Table 1**), ultimately affecting the production of picroside-I [39]. *In silico* profiling of microRNAs (miRNAs) in Korean ginseng (*Panax ginseng* Meyer) indicate that 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR), farnesyl diphosphate synthase (FPS), geranyl-diphosphate synthase, squalene synthase, and squalene epoxidase (SE) were predicted been regulated by miR854b and miR854c, especially SE [40]. miR-5021 was identified in *Catharanthus roseus* which targets two enzymes involved in biosynthesis of terpenoid indole alkaloids (TIAs), GCPE protein, and Terpenoid cyclase [41]. miR7539, miR5021, and miR1134 might be involved in regulating terpenoid biosynthesis by targeting upstream terpenoid pathway genes; nontranscriptional factor proteins, such as DXS, HMGR, IDS, and IDI, essential to produce IPP and DMAPP, the common precursors for all the downstream end terpenoids [42]. miRNAs miR7540, miR5183, miR6449, miR5255, miR5491, and miR6435 target downstream enzymes in the biosynthesis of mono-, sesqui-, di-, and tri-terpenoids; they were R-linalool synthase, gibberellin 3-oxidase, ent-kaurene synthase, squalene epoxidase, beta-amyrin synthase, and germacrene A oxidase [42]. miR2919, miR5251, miR838, miR5021, and miR5658 were found to be related to the pathway of terpene biosynthesis in *Ferula gummosa*. SPL7, SPL11, and ATHB13 TFs are putatively regulated by miR1533, miR5021, and miR5658, respectively [43]. miRNA156-targeted squamosa promoter binding protein-like (SPL) intervenes in the temporal space regulation of sesquiterpene biosynthesis [44]. miR5021 is also involved in terpenoid backbone biosynthesis and miR414 is related to sesquiterpenoid and triterpenoid biosynthesis [38].

### 2.4 Other secondary metabolites

miRNAs were identified from *in vitro* culture of roots and leaves tissues of the transcriptome of *Withania somnifera*; miR159, miR172, miR5140, and miR5303 in root tissue and miR477, miR530, miR1426, and miR5079 of leaf tissue. These miRNA were associated in the regulation of secondary metabolites. Endogenous miRNAs (miR159 and miR5140 from roots, miR477 and miR530 from leaves) may be help to increase the metabolites (withanoides) yield. Also, miR159, miR172 from roots, and miR530 from leaves were involved in the regulation of secondary metabolite associated with mRNAs [54]. *Chlorophytum borivilianum*, *Oryza sativa*, and *Arabidopsis thaliana* target gene prediction indicate that miR9662, miR894, miR172, and miR166 might be involved in regulating saponin biosynthetic pathway [55]. miR8154 and miR5298b increase taxol, phenylpropanoid, and flavonoid biosynthesis in subcultured Taxus cells [56]. *In silico* analysis indicate that miRstv_7* target ugt76g1, KAH, KO, for steviol glycoside biosynthesis [47]. In *Arabidopsis thaliana*, miR826 and miR5090 share the target AOP2, which encodes a 2-oxoglutarate-dependent dioxygenase that is involved in glucosinolate biosynthesis [48]. *Salvia miltiorrhiza* miR5072 targets acetyl-CoA C-acetyl transferase that is involved in the biosynthesis of tanshinones [50]. miR826 targets alkenyl hydroxyalkyl producing 2 oxoglutarate dioxygenase, which is involved in glucosinolate synthesis [49].

## 3. Conclusion

miRNAs are small molecules associated with developmental processes controlling gene expression. The mechanisms involved posttranscriptional and

transductional processes. The miRNA secondary metabolism control is a relative new field of study; the knowledge of the regulation of secondary metabolism in plants will help to understand the production of these products in controlled systems. Some of these products have an important economical value because of their use in agricultural, food, and cosmetic industries making these areas (miRNA regulation) very attractive.

## Acknowledgements

The authors thank Universidad De la Salle Bajío campus Campestre for financial support. MVH thank to CONACyT for postdoctoral fellowship (288773).

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## References

[1] Treiber T, Treiber N, Meister G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. Nature Reviews. Molecular Cell Biology. 2018;**19**(12):808. DOI: 10.1038/ s41580-018-0059-1

[2] Liu SR, Zhou JJ, Hu CG, Wei CL, Zhang JZ. MicroRNA-mediated gene silencing in plant defense and viral counter-defense. Frontiers in Microbiology. 2017;**8**:1801. DOI: 10.3389/fmicb.2017.01801

[3] Liu J, Yuan Y, Wang Y, Jiang C, Chen T, Zhu F, et al. Regulation of fatty acid and flavonoid biosynthesis by miRNAs in *Lonicera japonica*. RSC Advances. 2017;**7**(56):35426-35437. DOI: 10.1039/ C7RA05800D

[4] Melo CA, Melo SA. MicroRNA biogenesis: Dicing assay. Methods in Molecular Biology. 2014;**1182**:219-226. DOI: 10.1007/978-1-4939-1062-5_20

[5] Millar AA, Waterhouse P. Plant and animal microRNAs: Similarities and differences. Functional and Integrative Genomics. 2005;**5**:129-135. DOI: 10.1007/s10142-005-0145-2

[6] Bruscella P, Bottini S, Baudesson C, Pawlotsky JM, Feray C, Trabucchi M. Viruses and miRNAs: More friends than foes. Frontiers in Microbiology. 2017;**8**:824. DOI: 10.3389/ fmicb.2017.00824

[7] França GS, Hinske LC, Galante PA, Vibranovski MD. Unveiling the impact of the genomic architecture on the evolution of vertebrate microRNAs. Frontiers in Genetics. 2017;**21**(8):34. DOI: 10.3389/fgene.2017.00034. eCollection 2017

[8] Achkar NP, Cambiagno DA, Manavella PA. miRNA biogenesis: A dynamic pathway. Trends in Plant Science. 2016;**21**(12):1034-1044. DOI: 10.1016/j.tplants.2016.09.003. Epub 2016 Oct 25

[9] Eamens A, Wang MB, Smith NA, Waterhouse PM. RNA silencing in plants: Yesterday, today, and tomorrow. Plant Physiology. 2008;**147**(2):456-468. DOI: 10.1104/pp.108.117275

[10] O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Frontiers in Endocrinology. 2018;**9**:402. DOI: 10.3389/fendo.2018.00402

[11] Wang L, Song X, Gu L, Li X, Cao S, Chu C, et al. NOT2 proteins promote polymerase II-dependent transcription and interact with multiple microRNA biogenesis factors in *Arabidopsis*. The Plant Cell. 2013;**25**(2):715-727. DOI: 10.1105/ tpc.112.105882. Epub 2013 Feb 19

[12] Zhang S, Xie M, Ren G, Yu B. CDC5, a DNA binding protein, positively regulates posttranscriptional processing and/or transcription of primary microRNA transcripts. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(43):17588-17593. DOI: 10.1073/pnas.1310644110. Epub 2013 Oct 7

[13] Jagadeeswaran G, Zheng Y, Sumathipala N, Jiang H, Arrese EL, Soulages JL, et al. Deep sequencing of small RNA libraries reveals dynamic regulation of conserved and novel microRNAs and microRNA-stars during silkworm development. BMC Genomics. 2010;**11**:52. DOI: 10.1186/1471-2164-11-52

[14] Catalanotto C, Cogoni C, Zardo G. MicroRNA in control of gene expression: An overview of nuclear functions. International Journal of Molecular Sciences. 2016;**17**(10):E1712. DOI: 10.3390/ijms17101712

[15] Iwakawa HO, Tomari Y. The functions of microRNAs: mRNA decay and translational repression. Trends in Cell Biology. 2015;**25**(11):651-665. DOI: 10.1016/j.tcb.2015.07.011. Epub 2015 Oct 1

[16] Singh RK, Gase K, Baldwin IT, Pandey SP. Molecular evolution and diversification of the Argonaute family of proteins in plants. BMC Plant Biology. 2015;**15**:23. DOI: 10.1186/s12870-014-0364-6

[17] Arribas-Hernández L, Marchais A, Poulsen C, Haase B, Hauptmann J, Benes V, et al. The slicer activity of ARGONAUTE1 is required specifically for the phasing, not production, of trans-acting short interfering RNAs in *Arabidopsis*. The Plant Cell. 2016;**28**:1563-1580. DOI: 10.1105/tpc.16.00121

[18] Djami-Tchatchou AT, Sanan-Mishra N, Ntushelo K, Dubery IA. Functional roles of microRNAs in agronomically important plants-potential as targets for crop improvement and protection. Frontiers in Plant Science. 2017;**8**:378. DOI: 10.3389/fpls.2017.00378. eCollection 2017

[19] Mahajan V, Mahajan A, Pagoch SS, Bedi YS, Gandhi SG. MicroRNA mediated regulation of plant secondary metabolism: An *in silico* analysis. Journal of Natural Science, Biology and Medicine. 2011;**2**(Suppl S1): 44-45

[20] Gupta OP, Karkute SG, Banerjee S, Meena NL, Dahuja A. Contemporary understanding of miRNA-based regulation of secondary metabolites biosynthesis in plants. Frontiers in Plant Science. 2017;**8**:374. DOI: 10.3389/fpls.2017.00374

[21] Tiwari R, Rana C. Plant secondary metabolites: A review. International Journal of Engineering Research and General Science. 2015;**3**:661-670

[22] Sabzehzari M, Naghavi M. Phyto-miRNAs-based regulation of metabolites biosynthesis in medicinal plants. Gene. 2019;**682**:13-24. DOI: 10.1016/j.gene.2018.09.049. Epub 2018 Sep 26

[23] Padhan JK, Kumar P, Sood H, Chauhan RS. Prospecting NGS transcriptomes to assess regulation of miRNA-mediated secondary metabolites biosynthesis in *Swertia chirayita*, a medicinal herb of North-Western Himalayas. Medicinal Plants—International Journal of Phytomedicines and Related Industries. 2016;**8**:219-228. DOI: 10.5958/0975-6892.2016.00029.0

[24] Qiao Y, Zhang J, Zhang J, Wang Z, Ran A, Guo H, et al. Integrated RNA-seq and sRNA-seq analysis reveals miRNA effects on secondary metabolism in *Solanum tuberosum* L. Molecular Genetics and Genomics. 2017;**292**:37-52. DOI: 10.1007/s00438-016-1253-5. Epub 2016 Sep 27

[25] Jian H, Yang B, Zhang A, Ma J, Ding Y, Chen Z, et al. Genome-wide identification of microRNAs in response to cadmium stress in oilseed rape (*Brassica napus* L.) using high-throughput sequencing. International Journal of Molecular Sciences. 2018;**19**:1431. DOI: 10.3390/ijms19051431

[26] Bazzini A, Manacorda C, Tohge T, Conti G, Rodriguez MC, Nunes-Nesi A, et al. Metabolic and miRNA profiling of TMV infected plants reveals biphasic temporal changes. PLoS One. 2011;**6**:e28466. DOI: 10.1371/journal.pone.0028466

[27] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Scientific World Journal. 2013;**2013**:162750. DOI: 10.1155/2013/162750. eCollection 2013

[28] Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. Journal

of Nutritional Science. 2016;**5**:e47. DOI: 10.1017/jns.2016.41. eCollection 2016

[29] Barozai MY, Baloch IA, Din M. Identification of microRNAs and their targets in *Helianthus*. Molecular Biology Reports. 2012;**39**:2523-2532. DOI: 10.1007/s11033-011-1004-y. Epub 2011 Jun 14

[30] Luo Y, Zhang X, Luo Z, Zhang Q, Liu J. Identification and characterization of microRNAs from Chinese pollination constant nonastringent persimmon using high-throughput sequencing. BMC Plant Biology. 2015;**15**:11. DOI: 10.1186/s12870-014-0400-6

[31] Sharma D, Tiwari M, Pandey A, Bhatia C, Sharma A, Trivedi PK. MicroRNA858 is a potential regulator of phenylpropanoid pathway and plant development. Plant Physiology. 2016;**171**:944-959. DOI: 10.1104/pp.15.01831

[32] Yang R, Zeng Y, Yi X, Zhao L, Zhang Y. Small RNA deep sequencing reveals the important role of microRNAs in the halophyte *Halostachys caspica*. Plant Biotechnology Journal. 2015;**13**:395-408. DOI: 10.1111/pbi. 12337

[33] Biswas S, Hazra S, Chattopadhyay S. Plant Gene. 2016;**6**:82-89. DOI: 10.1016/j.plgene.2016.04.002Identification of conserved miRNAs and their putative target genes in *Podophyllum hexandrum* (Himalayan Mayapple)

[34] Boke H, Ozhuner E, Turktas M, Parmaksiz I, Ozcan S, Unver T. Regulation of the alkaloid biosynthesis by miRNA in opium poppy. Plant Biotechnology Journal. 2015;**13**:409-420. DOI: 10.1111/pbi.12346

[35] Li F, Wang W, Zhao N, Xiao B, Cao P, Wu X, et al. Regulation of nicotine biosynthesis by endogenous target mimicry of microRNA in tobacco. Plant Physiology. 2015;**169**:1062-1071. DOI: 10.1104/pp.15.00649

[36] Hao DC, Yang L, Xiao PG, Liu M. Identification of Taxus microRNAs and their targets with high-throughput sequencing and degradome analysis. Physiologia Plantarum. 2012;**146**:388-403. DOI: 10.1111/j.1399-3054.2012.01668.x. Epub 2012 Jul 25

[37] Prakash P, Rajakani R, Gupta V. Transcriptome-wide identification of *Rauvolfia serpentina* microRNAs and prediction of their potential targets. Computational Biology and Chemistry. 2016;**61**:62-74. DOI: 10.1016/j.compbiolchem.2015. 12.002

[38] Singh N, Srivastava S, Shasany A, Sharma A. Identification of miRNAs and their targets involved in the secondary metabolic pathways of *Mentha* spp. Computational Biology and Chemistry. 2016;**64**:154-162. DOI: 10.1016/j.compbiolchem.2016.06.004

[39] Vashisht I, Mishra P, Pal T, Chanumolu S, Singh T, Chauhan R. Mining NGS transcriptomes for miRNAs and dissecting their role in regulating growth, development, and secondary metabolites production in different organs of a medicinal herb, *Picrorhiza kurroa*. Planta. 2015;**241**:1255-1268. DOI: 10.1007/s00425-015-2255-y. Epub 2015 Feb 7

[40] Mathiyalagan R, Subramaniyam S, Natarajan S, Kim YJ, Sun MS, Kim SY, et al. *In silico* profiling of microRNAs in Korean ginseng (*Panax ginseng* Meyer). Journal of Ginseng Research. 2013;**37**:227-247. DOI: 10.5142/jgr.2013.37.227

[41] Pani A, Mahapatra RK. Computational identification of microRNAs and their targets in *Catharanthus roseus* expressed sequence tags. Genome Data. 2013;**1**:2-6. DOI: 10.1016/j.gdata.2013.06.001

[42] Fan R, Li Y, Li C, Zhang Y. Differential microRNA analysis of glandular trichomes and young leaves in *Xanthium strumarium* L. reveals their putative roles in regulating terpenoid biosynthesis. PLoS One. 2015;**25**(10):e0139002. DOI: 10.1371/journal.pone.0139002. eCollection

[43] Sobhani NA, Naghavi MR. Mining *Ferula gummosa* transcriptome to identify miRNAs involved in the regulation and biosynthesis of terpenes. Gene. 2018;**1**(645):41-47. DOI: 10.1016/j.gene.2017.12.035. Epub 2017 Dec 19

[44] Yu Z, Wang L, Zhao B, Shan C, Zhang Y, Chen D, et al. Progressive regulation of sesquiterpene biosynthesis in *Arabidopsis* and *Patchouli* (*Pogostemon cablin*) by the miR156-targeted SPL transcription factors. International Journal of Engineering Research and General Science. 2015;**8**:98-110. DOI: 10.1016/j.molp.2014.11.002

[45] Ng D, Zhang C, Miller M, Palmer G, Whiteley M, Tholl D, et al. Cis- and trans-regulation of miR163 and target genes confers natural variation of secondary metabolites in two *Arabidopsis* species and their allopolyploids. The Plant Cell. 2011;**23**:1729-1740. DOI: 10.1105/tpc.111.083915. Epub 2011 May 20

[46] Seilaniantz A, MacLean D, Jikumaru Y, Hill L, Yamaguchi S, Kamiya Y, et al. The microRNA miR393 re-directs secondary metabolite biosynthesis away from camalexin and towards glucosinolates. The Plant Journal. 2011;**67**:218-231. DOI: 10.1111/j.1365-313X.2011.04591.x. Epub 2011 Jun 24

[47] Saifi M, Nasrullah N, Ahmad M, Ali A, Khan J, Abdin M. *In silico* analysis and expression profiling of miRNAs targeting genes of steviol glycosides biosynthetic pathway and their relationship with steviol glycosides content in different tissues of *Stevia rebaudiana*. Plant Physiology and Biochemistry. 2015;**94**:57-64. DOI: 10.1016/j.plaphy.2015.05.009

[48] He H, Liang G, Li Y, Wang F, Yu D. Two young microRNAs originating from target duplication mediate nitrogen starvation adaptation via regulation of glucosinolate synthesis in *Arabidopsis thaliana*. Plant Physiology. 2014;**164**(2):853-865. DOI: 10.1104/pp.113.228635. Epub 2013 Dec 23

[49] Liang G, He H, Yu D. Identification of nitrogen starvation-responsive microRNAs in *Arabidopsis thaliana*. PLoS One. 2012;**7**:e48951. DOI: 10.1371/journal.pone.0048951. Epub 2012 Nov 14

[50] Xu X, Jiang Q, Ma X, Ying Q, Shen B, Qian Y, et al. Deep sequencing identifies tissue-specific microRNAs and their target genes involving in the biosynthesis of tanshinones in *Salvia miltiorrhiza*. PLoS One. 2014;**9**:e111679. DOI: 10.1371/journal.pone.0111679

[51] Gou JY, Felippes FF, Liu CJ, Weigel D, Wang JW. Negative regulation of anthocyanin biosynthesis in *Arabidopsis* by a miR156-targeted SPL transcription factor. The Plant Cell. 2011;**23**:1512-1522. DOI: 10.1105/tpc.111.084525

[52] Roy A. A review on the alkaloids an important therapeutic compound from plants. Plant Biotechnology Journal. 2017;**3**:1-9. DOI: 10.1021/acs.jpcb.5b10121

[53] Jiang Z, Kempinski C, Chappell J. Extraction and analysis of terpenes/terpenoids. Current Protocols in Plant Biology. 2016;**1**:345-358. DOI: 10.1002/cppb.20024

[54] Swati-Srivastava, Sanchita, Singh R, Srivastava G, Sharma A. Comparative study of withanolide biosynthesis-related miRNAs in root and leaf tissues of *Withania somnifera*. Applied Biochemistry and Biotechnology. 2018;**185**:1145-1159. DOI: 10.1007/s12010-018-2702-x. Epub 2018 Feb 24

[55] Kajal M, Singh K. Small RNA profiling for identification of miRNAs involved in regulation of saponins biosynthesis in *Chlorophytum borivilianum*. BMC Plant Biology. 2017;**17**:265. DOI: 10.1186/s12870-017-1214-0

[56] Zhang M, Dong Y, Nie L, Lu M, Fu C, Yu L. High-throughput sequencing reveals miRNA effects on the primary and secondary production properties in long-term subcultured Taxus cells. Frontiers in Plant Science. 2015;**6**:604. DOI: 10.3389/fpls.2015.00604