Contemporary Understanding of miRNA-Based Regulation of Secondary Metabolites Biosynthesis in Plants

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Plant's secondary metabolites such as flavonoids, terpenoids, and alkaloids etc. are known for their role in the defense against various insects-pests of plants and for medicinal benefits in human. Due to the immense biological importance of these phytochemicals, understanding the regulation of their biosynthetic pathway is crucial. In the recent past, advancement in the molecular technologies has enabled us to better understand the proteins, enzymes, genes, etc. involved in the biosynthetic pathway of the secondary metabolites. miRNAs are magical, tiny, non-coding ribonucleotides that function as critical regulators of gene expression in eukaryotes. Despite the accumulated knowledge of the miRNA-mediated regulation of several processes, the involvement of miRNAs in regulating secondary plant product biosynthesis is still poorly understood. Here, we summarize the recent progress made in the area of identification and characterizations of miRNAs involved in regulating the biosynthesis of secondary metabolites in plants and discuss the future perspectives for designing the viable strategies for their targeted manipulation.

Keywords: miRNAs, terpenoids, alkaloids, flavonoids, phenolics, glycosides

INTRODUCTION

Since the age of human civilization, plants are used as a source of nutrition and medicine, which is evidenced by the numerous texts from China and India (Kirtikar and Basu, 1918; Tang and Eisenbrand, 1992). The nutritional and medicinal properties of the plants are due to the presence of numerous metabolites. These metabolites are of two types: primary and secondary. Unlike primary metabolites, secondary metabolites are a huge group of phytochemicals, which are not directly involved in plant's vital processes such as growth, development, and reproduction (Fraenkel, 1959) but they are major components in defense mechanism of plants in order to protect them from any possible harm in the ecological environment (Stamp, 2003) and other interspecies protection (Samuni-Blank et al., 2012). Humans have exploited secondary metabolites in the form of flavoring agents, fragrances, insecticides, dyes, drugs, etc., More than 100,000 phytochemicals have been isolated from different plant sources so far (Mahajan et al., 2011). These secondary metabolites are broadly categorized as terpenoids, alkaloids, phenolics, glycosides, tannins, and saponins (Verpoorte, 1998). These phytochemicals are synthesized in the plants for a specialized need in a specific set of ecological conditions as their biosynthesis are highly energy consuming. This kind of biosynthesis and accumulation behavior of secondary metabolites in plants is the
result of tight regulation of their biosynthetic machinery. Metabolic engineering may further pave a way for enhancing biosynthesis of economically important phytochemicals or for producing desired combinations of such chemicals. One of the ways to tinker with biosynthetic pathways is through modulating miRNA levels as miRNAs are the ultimate regulators in plants.

miRNAs are small (21–24 nucleotides), non-coding, riboregulators that regulate gene expression in eukaryotes (Jones-Rhoades et al., 2006). miRNA is transcribed by RNA polymerase II as a precursor RNA known as the primary miRNA (pri-miRNA), which is subsequently processed by DICER-LIKE 1 (DCL1) to release the mature miRNAs. These mature miRNAs are then loaded into the RISC complex to bind mRNAs for cleavage (Jones-Rhoades et al., 2006). miRNAs are well-known molecules for their role in regulating various plants processes under biotic and abiotic stresses (Gupta et al., 2014a,b; Shriram et al., 2016). Recently, various reports suggested their roles in regulating the biosynthesis and accumulation of secondary metabolites in plants (see review Bulgakov and Avramenko, 2015). In the present review, we have updated the knowledge about present understanding on miRNAs based regulation of biosynthesis and accumulation of secondary metabolites in plants.

ROLE OF miRNAs IN FLAVONOID BIOSYNTHESIS

Flavonoids such as flavonols, flavones, isoflavones, anthocyanins, proanthocyanidins, and phlobaphene pigments are low molecular weight phenylpropanoid compounds which are widely distributed throughout the plant kingdom (Taylor and Grotewold, 2005; Lepiniec et al., 2006; Buer et al., 2010). These polyphenolic metabolites play a variety of significant biological roles such as protection against UV radiation, as signaling molecules, as phytoalexins in plant-microbe interaction, and as regulators of phytohormones such as auxin transport in plants (Santelia et al., 2008; Buer et al., 2010). The flavonoid backbone is synthesized by the central phenylpropanoid pathway and different flavonoid metabolites share common enzymes and substrates. Phenylpropanoid pathway is one of the most extensively studied pathways of secondary metabolites for transcriptional regulation in plants (Quattrocchio et al., 2006; Stracke et al., 2007; Li, 2014). In the past few years, scientific endeavors are directed toward understanding the post-transcriptional regulation of this pathway involving miRNAs. The schematic representation of the general phenylpropanoid pathway leading to major branches of flavonoid biosynthesis and their possible interaction with miRNAs has been depicted in Figure 1A.

About 17 SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) proteins are encoded by the Arabidopsis genome (Riese et al., 2007). These SPL transcription factors are reported to affect numerous processes of plant growth and development, such as vegetative phase transition by enhancing the expression of miRNA172, flowering induction by LEAFY and MADS box genes, embryonic development, cell size, trichome formation, and fertility (Wu et al., 2009; Yamaguchi et al., 2009; Xing et al., 2010; Yu et al., 2010). In addition, miR156 targeted SPL9 protein has been shown to regulate the metabolic flux during flavonoid biosynthetic pathway. Anthocyanins accumulate in an acropetal manner in Arabidopsis stems, with the highest level at the junction between the stem and the rosette leaves. This array of anthocyanin accumulation is regulated by the miR156 targeted SPL9 gene in Arabidopsis (Gou et al., 2011). The tissues having high anthocyanin concentration accumulate higher levels of miRNA156 leading to reduced SPL activity which in turn enhance the expression of F3’H, DFR, and other anthocyanin biosynthetic genes. As a result, dihydroflavonols are directed into the anthocyanin branch. On the other hand, expression of SPLs gradually increases along the growing stem because miR156 levels decline as the plant progresses during development (Gou et al., 2011). Therefore, increased accumulation of SPL leads to decreased expression of anthocyanin biosynthetic genes resulting in the increased production of flavonols by FLS. It has been demonstrated that MYB-bHLH-WD40 transcriptional activation complex is destabilized by SPL9, a target of miRNA156, by competing with bHLHs for their binding to PAP1 which in turn inhibits expression of anthocyanin biosynthetic genes (anthocyanidin synthase, flavanone 3-hydroxylase, dihydroflavonol reductase, and UDP-glucosyl transferase 75C1 etc.) influencing anthocyanin accumulation in Arabidopsis (Gou et al., 2011). Similarly, miR156-SPL9 pair influences anthocyanin production by targeting dihydroflavonol 4-reductase (Cui et al., 2014). Therefore, an antagonistic relationship exists between anthocyanin and flavonol biosynthesis in Arabidopsis. Recently, Biswas et al. (2016) have computationally identified several miRNAs such as miR172i, miR829.1, miR1438, miR1873, and miR5532 targeting miRNAs coding for enzymes of phenylpropanoid pathway, such as 4-coumarate-CoA ligase, Chalcone synthase, Caffeoyl-CoA O-methyl transferase, Dihydroflavonol 4-reductase C, 2-hydroxyisoflavone dehydratase respectively in Podophyllum hexandrum (Table 1). Overexpression of miR8154 and miR5298b in sub-cultured Taxus cell lines revealed their crucial role in the regulation of taxol, phenylpropanoid, and flavonoid biosynthesis pathways (Zhang et al., 2015). Similarly, several other miRNAs of phenylpropanoid pathway, such as miR395p-3p/ targeting bHLH mRNA in D. kaki (Luo et al., 2015), miR396b and miR828a targeting miRNAs coding for Kaempferol 3-O-beta-D-galactosyltransferase and anthocyanin regulatory C1 protein respectively in R. serpentina (Prakash et al., 2016), miR858a targeting R2R3-MYB mRNA in A. thaliana ( Sharma et al., 2016), miR6194 targeting Flavanone 3b-hydroxylase mRNA (F3H) in H. caspica (Yang et al., 2015), miR1061-3p and miR1318 in pear fruit (Wu et al., 2014) etc., (Table 1) have been reported.

Further, the use of advanced computational tools complementing the experimental methods has accelerated the accumulation of reports on new as well as existing miRNAs implying their regulatory role during flavonoid pathway in plants. Therefore, further work on functional characterization of these tiny miRNAs-target networks using reverse genetic approach would certainly pave a way for understanding post-transcriptional regulatory mechanism of the flavonoid pathway.
A. Flavonoid biosynthesis

1. Trihydroxychalcone
2. Liquiritigenin
3. Isoflavones (diadzein and genistein)
4. 2’-hydroxy iso flavone
5. Isoflavonoids (medicarpin)
6. Condensed tannins (proanthocyanidin)
7. Resveratrol (stilbene)
8. Dihydroflavonols (dihydrokaempferol, dihydroquercetin, dihydromyricetin)
9. Flavan-3,4-diols (leucoanthocyanidin)
10. Anthocyanidin
11. Flavonols
12. Flavonol glycosides

B. Terpenoid biosynthesis

1. Acetyl CoA
2. Acetoacetyl CoA
3. HMG CoA
4. Mevalonate (MVA)
5. MVA-5-phosphate
6. MVA-5-diphosphate
7. Dimethylallyl diphosphate
8. Geranyl diphosphate
9. Farnesyl diphosphate
10. MVA Pathway
11. MEP Pathway
12. Pyruvate + glyceraldehyde-3-phosphate
13. 1-deoxy-D-xylulose-5-phosphate
14. 2-C-methyl-D-erythritol 4-phosphate
15. 4-(cytidine 5’-diphospho)-2-C-methyl-D-erythritol phosphate
16. 4-(cytidine 5’-diphospho)-2-C-methyl-D-erythritol phosphate
17. 2-C-methyl-D-erythritol 2,4-cyclodiphosphate
18. (E)-4-hydroxy-3-methylbut-2-enyl diphosphate

FIGURE 1 | (A) Schematic representation of the general phenylpropanoid pathway leading to major branches of flavonoid biosynthesis and their possible interaction with miRNAs. Phe ammonia-lyase (PAL); cinnamate-4-hydroxylase (C4H); 4-coumaroyl-CoA-ligase (4CL); chalcone reductase (CHR), chalcone synthase (CHS); (Continued)
This information could further be used for metabolic engineering of the entire pathway for human benefits.

**ROLE OF miRNAs IN TERPENOID BIOSYNTHESIS**

Owing to their numerous biological roles, isoprene (C5), monoterpenes (C10), and sesquiterpenes (C15) establish the biggest class of plant volatile compounds. In plants, these volatile compounds act as defense molecules against biotic stresses, attracts pollinators and seed disseminators, and help improve thermo-tolerance (Dudareva et al., 2006). In addition, they are used as aroma compounds and natural flavor enhancers which have the beneficial impact on human health (Wagner and Elmadfa, 2003). Considering the importance of these compounds, understanding the regulatory schema of their biosynthetic pathway and accumulation stands on priority. These volatile compounds are synthesized from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are derived from two alternate biosynthetic pathways localized in different subcellular compartments. During the past several years, there has been a significant progress in identification and characterization of genes and enzymes involved in the biosynthesis of volatile terpenoids (Figure 1B), determination of their spatiotemporal expression and compartmentalization, and metabolic engineering. However, the regulatory role of miRNAs in their biosynthesis and accumulation is poorly understood, which opens a new window for further investigations.

Terpene synthases (TPSs) Catalyse the conversion of farnesyl diphosphate (FPP) into sesquiterpenes (C15). Transcription factor SPL9, the target of miRNA156, directly binds to and activates promoter of terpene synthases 21 (TPS21) gene and positively regulates its transcription thereby regulating the biosynthesis of sesquiterpenoids (IDS), and isopentenyl diphosphate isomerase (IDI) were predicted to be targeted by miR7539, miR5021, and miR1134 (Fan et al., 2015). The complete list of miRNAs and their target genes have been provided in Table 1. Most recently, bioinformatics approaches have been utilized to mine miRNAs involved in terpenoid metabolism in *Mentha spp.* (Singh et al., 2016a), *Ginger* (Singh et al., 2016b), *C. roseus* (Pani and Mahapatra, 2013), and *P. hexandrum* (Biswas et al., 2016; Table 1).

**THE ROLE OF miRNAs IN THE REGULATING BIOSYNTHESIS OF ALKALOID AND OTHER N-CONTAINING METABOLITES**

Alkaloids are nitrogen containing low molecular-weight compounds which are mostly derived from amino acids. They are known to play significant roles in defense against herbivores and pathogens and are being widely used as pharmaceuticals, stimulants, narcotics, and poisons. Unlike other secondary metabolites, this class is highly diverse and heterogenous in nature and around ∼12,000 alkaloids have been characterized till date (Ziegler and Facchini, 2008). These compounds are synthesized through diverse metabolic pathways. Recent genome based technological advancement have led us to add to on our current understanding of their biosynthetic pathways and regulation. However, knowledge on the role of miRNAs during alkaloid biosynthesis and accumulation in plant kingdom has just started to proliferate.

Boke and his coworkers in 2014 have extensively worked on regulation of the alkaloid biosynthesis by miRNA in opium poppy. They identified pso-miR13, pso-miR2161, and pso-miR408 as potential miRNAs involved in the alkaloid biosynthetic pathway. Pso-miRNA2161 targets the mRNA of gene encoding S-adenosyl-L-methionine: 30-hydroxy-N-methylcoclaurine 40-O-methyltransferase 2 (40 MT) enzyme which converts S-norcoclaurine into S-reticuline, an intermediate molecule in benzylisoquinoline alkaloids (BIA) biosynthesis. Similarly, pso-miR13 targets mRNA of 7-O-methyltransferase (7O MT) gene, which converts S-reticuline to morphinan alkaloids. pso-miR408 targets mRNA of reticuline...
| Sr. no. | miRNA | Plant species | Target | Target function | Phytochemical biosynthesis | Validation/detection | References |
|--------|-------|---------------|--------|-----------------|--------------------------|---------------------|------------|
| 1.     | miR156* | *A. thaliana* | SPL9 | Destabilizes MYB-bHLH-WD40 transcriprional activation complex | Anthocyanin biosynthesis | Transgenic approach | Gou et al., 2011 |
| 2.     | miR172i | *P. hexandrum* | 4-coumarate-CoA ligase | Catalyses the activation of 4-coumarate and other 4-hydroxycinnamates to the respective thiol esters | Flavonoid biosynthesis | Computational | Biswas et al., 2016 |
| 3.     | miR395p-3p/* | *D. kaki* | bHLH | Regulates genes of proanthocyanidin biosynthetic pathway | Proanthocyanidin biosynthesis | Illumina | Luo et al., 2015 |
| 4.     | miR396b | *R. serpentina* | Kaempferol 3-O-beta-D-galactosyltransferase | Transferase activity, transferring hexosyl groups | Flavonol glycoside | Computational | Prakash et al., 2016 |
| 5.     | miR828a | *R. serpentina* | Anthocyanin regulatory C1 protein | DNA/chromatin binding | Anthocyanin biosynthesis | Computational | Prakash et al., 2016 |
| 6.     | miR829.1 | *P. hexandrum* | Chalcone synthase | Catalyses the conversion of 4-coumaroyl-CoA and malonyl-CoA to naringenin chalcone | Flavonoid biosynthesis | Computational | Biswas et al., 2016 |
| 7.     | miR858a* | *A. thaliana* | R2R3-MYB transcription factors | Regulate genes of flavonoid biosynthetic pathway | Flavonoid biosynthesis | Transgenic approach | Sharma et al., 2016 |
| 8.     | miR858b* | *D. kaki* | MYB protein | Regulates genes of proanthocyanidin biosynthetic pathway | Proanthocyanidin biosynthesis pathway | Illumina | Luo et al., 2015 |
| 9.     | miR1438 | *P. hexandrum* | Caffeoyl-CoA O-methyltransferase | Catalyzes methylation of caffeoyl-CoA to produce feruloyl-CoA. | Lignin biosynthesis | Computational | Biswas et al., 2016 |
| 10.    | miR1873 | *P. hexandrum* | Dihydroflavonol 4-reductase | Catalyses the conversion of flavanone into dihydroflavonol | Flavanoid biosynthesis | Computational | Biswas et al., 2016 |
| 11.    | miR5532 | *P. hexandrum* | 2-hydroxyisoflavanone dehydrogenase | Catalyses conversion of 2,7,4'-trihydroxyisoflavanone into diadzein | Isoflavonoid biosynthesis | Computational | Biswas et al., 2016 |
| 12.    | miR6194 | *H. caspica* | Flavanone 3b-hydroxylase (F3H) | Catalyzes the conversion of flavanone into dihydroflavonol | Biosynthesis of flavonols, anthocyanidins and proanthocyanidins | HISEQ deep sequencing | Yang et al., 2015 |
| 13.    | miR6194 | *G. max* | Chalcone synthase | Catalyses the conversion of 4-coumaroyl-CoA and malonyl-CoA to naringenin chalcone | Flavanoid biosynthesis | Transgenic approach | Ono et al., 2013; Tutu et al., 2009 |
| 14.    | miR1061-3p | *Pyrus spp* | Naringenin 3-dioxygenase | Catalyses the 3-beta-hydroxylation of 2S-flavanones to 2R,3R-dihydroflavonols | Flavanoid biosynthesis | Computational | Wu et al., 2014 |

**FLAVONOIDS**

**TERPENOIDS**

(Continued)
| Sr. no. | miRNA   | Plant species | Target | Target function | Phytochemical biosynthesis | Validation/detection | References |
|---------|---------|---------------|--------|-----------------|----------------------------|----------------------|------------|
| 18.     | miR838  | Z. officinale | CYP71  | Menthofuran synthase activity | Terpenoid metabolism      | Computational         | Singh et al., 2016b |
| 19.     | miR5021 | P. hexandrum  | Diphosho malonate decarboxylase | Conversion of mevalonate diphosphate (MVAPP) into isopentenyl diphosphate (IPP) | Computational           | Biswas et al., 2016 |
| 21.     | miR5072 | S. miltiorrhiza | Acetyl-CoA C-acetyl transferase | Conversion of acetyl-CoA into acetocacetyl-CoA | Tanshinones (abietane-type norditerpenoid quinones) | Illumina | Xu et al., 2014 |
| 2.      | miR5183 | X. strumarium | Gibberellin 3-oxidase | Catalyses the conversion of precursor GAs to their bioactive forms | Diterpenoid | Illumina | Fan et al., 2015 |
| 24.     | miR5255 | X. strumarium | Squalene epoxidase | Oxidize squalene to 2,3-oxidosqualene | Triterpenoid | Illumina | Fan et al., 2015 |
| 26.     | miR5538 | P. hexandrum  | Protein-S-isoprenylcysteine O-methyltransferase | Catalyses the post-translational methylation of isoprenylated C-terminal cysteine residues | Terpenoid backbone biosynthesis | Computational | Biswas et al., 2016 |

(Continued)
| Sr. no. | miRNA     | Plant species | Target                          | Target function                                                                 | Phytochemical biosynthesis | Validation/detection | References        |
|--------|-----------|---------------|---------------------------------|---------------------------------------------------------------------------------|----------------------------|---------------------|-------------------|
| 27     | miR6435   | X. strumarium | Germacrene A oxidase            | Oxidations of germacrene A to produce germacrene A acid                         | Sesquiterpenoid            | Illumina            | Fan et al., 2015  |
| 28     | miR6449   | X. strumarium | Ent-kaurene synthase            | Catalyses bidirectional conversion of ent-copalyl diphosphate into ent-kaurene | Diterpenoid                | Illumina            | Fan et al., 2015  |
| 29     | miR7539   | X. strumarium | 1-deoxy-D-xylulose 5-phosphate synthase (DXS) | Catalyses conversion of 1-deoxy-D-xylulose 5-phosphate into pyruvate and D-glyceraldehyde 3-phosphate | Terpenoid backbone         | Illumina            | Fan et al., 2015  |
| 30     | miR7540   | X. strumarium | R-linalool synthase             | Catalyses the bidirectional conversion of geranyl diphosphate into (3R)-linalool | Monoterpenoid              | Illumina            | Fan et al., 2015  |
| 31     | miRstv-7* | S. rebaudiana | UDP-glycosyl transferase 76G1 (UGT76G1) | Stevioside to Rebaudioside-A                                                   | Steviol glycoside biosynthesis | Computational | Saifi et al., 2015 |
|        |           |               | Kaurenoic acid hydroytase (KAH) | Kaurene to Kaurenoic Acid bil                                       | Steviol glycoside biosynthesis | Computational | Saifi et al., 2015 |
|        |           |               | Kaurene Oxidase (KO)           | Kaurene Oxidase Hydroxylase (KOH)                                           | Steviol glycoside biosynthesis | Computational | Saifi et al., 2015 |
| 32     | miR13     | P. somniferum | 7-O-methyltransferase (7-OMT)   | Conversion of S-reticuline to morphinan alkaloids                            | BIA biosynthesis           | Illumina            | Boke et al., 2015  |
| 33     | miRX13*   | N. tabacum    | Putrescine methyltransferase 2 (PMT2) | Converts putrescine into N-methylputrescine                                   | Nicotine biosynthesis      | Illumina            | Li et al., 2015   |
| 34     | miRX17*   | N. tabacum    | Quinolinate phosphoribosyl-transferase 1 (QPT1) | Converts quinolinic acid into NAMN                                          | Nicotine biosynthesis      | Illumina            | Li et al., 2015   |
| 35     | miRX20*   | N. tabacum    | Cytochrome P450 monoxygenase (CYP82E4) | Converts nicotine into nornicotine                                             | Nicotine biosynthesis      | Illumina            | Li et al., 2015   |
| 36     | miRX27*   | N. tabacum    | Quinolinate phosphoribosyl-transferase 2 (QPT2) | Converts quinolinic acid into NAMN                                          | Nicotine biosynthesis      | Illumina            | Li et al., 2015   |
| 37     | miR408    | P. somniferum | FAD-binding and BBEm domain-containing protein, also known as reticuline oxidase-like protein | Conversion of S-reticuline to (S)-scoulerine                                    | BIA biosynthesis           | Illumina            | Boke et al., 2015  |
| 38     | miR2161   | P. somniferum | 4′-O-methyltransferase 2 (4-OMT) | Conversion of S-norcoclaurine into S-reticuline                               | BIA biosynthesis           | Illumina            | Boke et al., 2015  |
| 39     | miR5021   | C. roseus     | UDP-glucose iridoid glucosyltransferase | Transferase activity                                                           | Indole alkaloids as well as quinoline alkaloids | Computational | Pani and Mahapatra, 2013 |
| 40     | miRn24    | N. tabacum    | Branched-chain amino acid transaminase 3 (SCAT3) | Catalyse the synthesis or degradation of the branched-chain amino acids | Glucosinolate biosynthesis | Computational | Gou et al., 2011  |
| 41     | miR826    | A. thaliana   | Alkanyl hydroxalkyl Producing 2 (AOP2) | Side chain modification of Met- derived glucosinolates                      |                           |                     | Liang et al., 2012 |
| 42     | miR5090*  |              |                                 |                                 |                           |                     | He et al., 2014    |

**Notes:**
- In the column number 2 indicates that these miRNAs have been validated for their effect on metabolite accumulation in the plants.

**Columns:**
- **Sr. no.**: Serial number
- **miRNA**: miRNA name
- **Plant species**: Name of the plant species
- **Target**: Gene name
- **Target function**: Function of the gene
- **Phytochemical biosynthesis**: Type of phytochemical biosynthesis
- **Validation/detection**: Method of validation/detection
- **References**: Reference for the study
oxidase-like protein which converts S-reticuline to (S)-scoulerine in the BIA pathway. Endogenous target mimicry (eTM) of miRNAs disturbs the function of corresponding miRNAs by inhibiting binding of miRNAs with their authentic target genes (Franco-Zorrilla et al., 2007). Therefore, Li and his co-workers in 2015 have demonstrated that nta-eTMX27 inhibits the expression and function of nta-miRX27 which targets mRNA of quinolinate phosphoribosyl transferase 2 (QPT2) genes leading to enhanced nicotine biosynthesis in the topping treated tobacco. The most recent report by Mao et al. (2017) shows the regulatory role of miR156 targeting SPL9 in the biosynthesis of glucosinolates, which are secondary metabolites functioning as defense metabolites against insect herbivores and pathogens. The SPL9 interacts with JA ZIM-domain (JAZ) proteins, including JAZ3 to control jasmonate synthesis. Increased level of jasmonate further promotes the biosynthesis of glucosinolates. In addition, several other workers have reported numerous miRNAs along with their target genes involved in the alkaloid biosynthetic pathway in P. hexandra (Biswa et al., 2016), R. serpentina (Prakash et al., 2016), and C. roseus (Pani and Mahapatra, 2013) using computational approaches.

MODULATING SECONDARY METABOLITES VS. PRIMARY METABOLITE BIOSYNTHETIC PATHWAYS THROUGH miRNAs

Unlike secondary metabolites, primary metabolites are required by plants at every stage of their growth and development. And also, the precursor molecules for secondary metabolite biosynthesis are channelized from primary metabolites. Regulation of primary metabolite biosynthetic pathways is well explored at transcriptional, post-transcriptional and now at DNA level, but secondary metabolic pathway are limited at the transcriptional level and recently at post-transcriptional level (miRNA). Therefore, till now, most of the work has focussed on the role of miRNAs during primary metabolism of growth and development. Recently, these miRNAs of primary metabolism along with some other miRNAs are being reported for their crucial role during secondary metabolism, for example, the SPL-miRNA156 system (Gou et al., 2011). Similarly, miR-4995 targets 3-deoxy-7-phosphohexulonate synthase gene involved in the first step of phenylpropanoid pathway for picrosides I biosynthesis (Vashisht et al., 2015). Being the first enzyme of the pathway, this enzyme holds the key to the progress of pathway as its down-regulation can affect the production of cinnamic acid, thereby affecting picrosides I content. Taking into account the regulatory roles of miRNAs, modification in the expression of such miRNAs would be a promising approach to modulate the biosynthesis of secondary metabolites in plants. SPL9 and TCP3 transcription factors play a major role in secondary metabolism regulation (Gou et al., 2011; Li and Zachgo, 2013) and therefore miRNAs targeting these genes would be an ideal candidate for such approach (Bulgakov and Avramenko, 2015). Nevertheless, identifying and understanding the spatial and temporal expression schema of other miRNAs that might regulate the flux movement at the branch point of primary vs. secondary metabolic pathway and/or secondary metabolic pathway alone would help designing better strategies to favor the biosynthesis of economically important secondary metabolites.

CONCLUSION AND FUTURE DIRECTIONS

Owing to the diversity of the biosynthetic pathway of the secondary metabolites and their biological significance in both plants and human, exploring the regulatory schema of the pathway is crucial. Despite the role of miRNAs during different biotic and abiotic stresses and plant developmental processes, their role in regulating the biosynthesis of secondary metabolites had just started accumulating and it further requires intense and focussed work. Studies on identification of miRNAs and their targets at all possible steps of the pathway and characterizing significant miRNAs-target pairs using reverse genetics is one prime area to decipher the functions of miRNAs. Deep sequencing technologies and the modern computational approaches for miRNA predictions has resulted in the accumulation of huge data on miRNAs. Despite the availability of many computational algorithms, miRNA target identification is still a major challenge. Many miRNA targets which have miRNA binding sites with seed mismatches could not be identified due to the inability of computational tools (Doran and Strauss, 2007). Presently, most of the miRNA target predictions, consider mRNA 3′ UTRs and therefore the genes that are regulated by miRNA through binding in the region other than 3′ UTRs could not be identified (Place et al., 2008; Tay et al., 2008). The miRNAs are part of complex regulatory networks where a single miRNA control lots of genes. Thus, modulation of single miRNA expression could result in complicated biological consequences (Lee et al., 2014). This complexity makes functional validation by knock-out or overexpression of these predicted miRNAs a challenging issue.

Furthermore, understanding the DNA methylation profiles of plant genomes and their interaction with miRNAs and self-regulation of miRNAs would be an interesting future area of research. In addition, Work on the potential of herbal medicine-derived miRNAs in regulating human health or targeting genes associated with diseases are another emerging area. Such studies would help metabolic engineering of the entire biosynthetic pathway for generating novel phytochemicals or for producing desired combinations of such secondary metabolites.

AUTHOR CONTRIBUTIONS

OG and AD conceived the idea and designed the manuscript, OG, SB, SK, NM collected literature and wrote the manuscript, OG, SK, and AD critically evaluated the manuscript. All authors approved the manuscript.

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